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IL-22 producing "T22" T-cells account for up-regulated IL-22 in atopic dermatitis (AD), despite reduced IL-17 producing Th17 T-cells

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Abstract

Background—Psoriasis and atopic dermatitis (AD) are common inflammatory skin diseases. Up-regulated Th17/IL-23 pathway was demonstrated in psoriasis. Although potential involvement of Th17 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/Th17 signal between these diseases and establish relative frequencies of T-cell subsets in AD.

Methods—Skin biopsies and peripheral blood were collected from chronic AD (n=12) and psoriasis (n=13) patients. Relative frequencies of $CD4^+$ and $CD8^+$ T-cell subsets within these two 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 Th1 and Th17 T-cells compared with AD, while Th2 T-cells were significantly elevated in AD. Distinct IL-22 producing $CD4^+$ and $CD8^+$ T-cell populations were significantly increased in AD skin, compared to 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,

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Clinical Implication: 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.

Capsule Summary: T-cells could independently express IL-22 although IL-17 expression is reduced. This argues for 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 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.

Keywords

Atopic dermatitis; psoriasis; Th17; IL-17; IL-22; T22

Introduction

Psoriasis and atopic dermatitis (AD) are common inflammatory skin diseases previously described as opposite poles of the Th1/Th2 paradigm. Recently, interleukin (IL)-17 producing Th17 T-cells have challenged this classic paradigm, and are implicated in a growing number of inflammatory diseases. Our group and others have showed increased Th17 T-cells in psoriasis, and enhanced expression of IL-17 and IL-23 in lesional psoriatic skin.[1, 2] The IL-23/Th17 pathway is thought to contribute to the pathogenesis of psoriasis and to the distinct features of the disease (i.e. 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 majority of the $CD4^+$ Th17 cells were observed in the psoriatic dermis, $IL\text{-}17^+CD8^+$ T-cells were mostly in psoriatic epidermis.[4]

In murine systems, Th17 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 co-synthesis of IL-17 or IFN-γ, was previously identified.[3] In the skin, IL-22 mediates keratinocyte proliferation and epidermal hyperplasia by down-modulating 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]

While the role of $IL\text{-}17^+$ T-cells is well defined in psoriasis, their role in AD is still unclear. A potential involvement of Th17 T-cells in AD has been suggested, at least during onset of the disease.[11] Toda et al. has demonstrated by IHC that $IL\text{-}17^+$ cells are increased in acute, but not chronic, AD lesions compared to uninvolved or normal skin.[11] Koga et al, confirmed the IHC 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 Th17 T-cells with increased severity of the disease.[12] In contrast, our group found decreased expression of IL-23, IL-17, and IL-17-induced innate defense molecules in chronic AD lesions compared to psoriasis that suggest a relative deficiency of IL-23/Th17 pathway in AD.[13]

While IHC 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. As 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 chronic AD and psoriasis patients using intracellular cytokine staining and flow cytometry.

We confirmed that psoriasis was polarized towards a Th1/Th17 phenotype, while AD was polarized towards Th2 with relatively deficient Th17 activity. Interestingly, we found that AD skin had upregulated expression of IL-22, associated with increased frequencies of distinct IL-22-producing $CD4^+$ and $CD8^+$ T cells. This up-regulation of IL-22, that is independent from Th17 cells, might explain the discordance between reactive epidermal hyperplasia and deficient IL-17-related innate defense molecules observed in chronic AD.

Materials and Methods

Study design

Peripheral blood and skin biopsies were collected from 12 AD patients (ages 23-48; median 34) and 13 psoriasis patients (ages 28-60; median 52) under a Rockefeller University IRB-approved protocol (Table 1). Psoriasis patients with moderate to severe psoriasis and >10% body surface area involvement, and patients with acute exacerbation of chronic AD (SCORAD between 21-72), were included in the study. A therapeutic washout period of 4 weeks for oral medication and 2 weeks for topical treatment was implemented prior to specimen collection.

Skin Samples

Skin punch biopsies (5 mm diameter) were incubated at 4°C overnight in dispase (Invitrogen Life Technologies). After peeling off the epidermis, the dermis was cultured for 48h at 37°C in RPMI 1640 (Gibco-BRL Life Technologies) supplemented with 10% pooled human serum (Mediatech Inc.), 0.1% gentamicin reagent solution (Gibco-BRL Life Technologies), and 1% 1M Hepes buffer (Sigma). Single cell suspensions were obtained from culture media, washed with PBS, and analyzed by intracellular cytokine staining and FACS.

Peripheral blood samples

Peripheral blood mononuclear cells were obtained by gradient centrifugation with Ficoll-Paque Plus (Pharmacia), collected at the interface, and washed with PBS prior to intracellular cytokine staining and FACS analysis.

FACS

For intracellular cytokine staining, cells were activated for 4 hours using 25 ng/ml phorbol myristate acetate (PMA) 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. Ethylenediaminetetraacetic acid (2 mM, Fisher Scientific) was added for 10 min. at 37°C to stop activation. Cells were then incubated in aqua marina live/dead dye (Invitrogen) for 30 mins for dead cell discrimination then fixed with 4% paraformaldehyde (BD Biosciences) for 20 mins. The cells were washed, blocked in 1:100 mouse serum (BD Biosciences), permeabilized in FACSPerm (BD Biosciences), incubated for 30 mins with fluorochrome conjugated mAbs to cell surface molecules and intracellular cytokines, washed, and collected. Cells were stained with antibodies listed in Supplementary Table 1. Samples were acquired using LSR-II flow cytometer (BD Biosciences) and analyzed with FlowJo software (Treestar). All incubation steps were done on ice. Appropriate isotype controls were used.

Real-time PCR

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

Immunofluorescence

Frozen tissue sections from AD (n=5) patients were fixed with acetone and blocked in 10% normal goat serum (Vector Laboratories) for 30 minutes. The primary antibody anti-CD8-FITC (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 using

the appropriate filters of Zeiss Axioplan 2 widefield fluorescence microscope with Plan Neofluar 10 × 0.30 N.A. lens and a Hamamatsu Orca ER-cooled CCD camera, controlled by METAVUE software (MDS Analytical Technologies)

Statistical analysis

Two-tailed Students t test was used to compare values obtained from AD and psoriasis patients. The p-values were designated as $p<0.05$ (*), $p<0.01$ (**), and $p<0.005$ (***). Linear regression was utilized to analyze T cell frequencies and disease activity.

Results

Adult patients with active lesions of chronic AD (n=12) and psoriasis (n=13), having clinical and demographic characteristics described in Table 1 were studied.

IL-22 expression is significantly up-regulated in AD compared to psoriasis skin lesions

To examine the cytokine microenvironment in AD and psoriasis skin, we quantified IFNγ, IL-13, IL-17 and IL-22 mRNA expression in lesional skin compared to normal skin by real time RT-PCR. As expected, Th1-associated IFNγ mRNA was significantly upregulated in psoriasis lesions compared to AD ($p<0.05$) and normal skin ($p<0.005$) (Pso 42.37±12.83, AD 27.94±11.22, Norm 3.46±1.91), while Th2 cytokine IL-13 was highly expressed in AD lesions and absent in psoriatic and normal skin (Pso 0.00±0.00, AD 46.65±14.37, Norm 0.00±0.00; $p<0.005$). The expression of Th17-defining cytokine, IL-17, was significantly up-regulated in psoriasis lesions compared to AD and normal skin (Pso 66.31±17.56, AD 3.75±2.43, Norm 0.00±0.00; $p<0.005$). As expected, IL-22 was significantly up-regulated in psoriasis compared to normal skin ($p<0.01$). Unexpectedly, IL-22 was also up-regulated in AD lesional skin compared to normal skin ($p<0.005$), with a higher expression in AD than in psoriatic lesions (Pso 3.02±0.91, AD 16.62±8.10, Norm 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 helper ($CD3^+CD4^+$) and T cytotoxic ($CD3^+CD8^+$) cells in peripheral blood from AD and psoriasis patients by intracellular cytokine staining and flow cytometry (Figure 2A). We defined IFNγ-producing, IL-4-producing and IL-17-producing T helper cells as Th1, Th2 and Th17 cells, respectively. In the same manner, IFNγ-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 Th1, Th2 or Th17 T-cells did not differ significantly between the AD and psoriasis patients ($CD4^+$/Th1 Psor 8.24%±1.11, AD 9.15% ±1.34; Th2 Psor 1.34%±0.20, AD 1.18%±0.15; Th17 Psor 0.39%±0.08, AD 0.50%±0.09) (Fig. 2B). Similarly, the percentages of Tc1, Tc2 and Tc17 T-cells in peripheral blood did not differ significantly between the two diseases ($CD8^+$/Tc1 Psor 26.54%±5.24, AD 24.17%±3.82; Tc2 Psor 2.07%±0.832, AD 0.55%±0.09; Tc17 Psor 0.11±0.02, AD 0.06%±0.02). The frequencies of IL-22-producing $CD4^+$ and CD8+ T-cells in peripheral blood also did not differ significantly between psoriasis and AD patients ($CD4^+IL\text{-}22^+$ Psor 0.98%±0.12 vs AD 1.45%±0.26; $CD8^+IL\text{-}22^+$ Psor 0.44%±0.13 vs AD 0.73%±0.27 (Figure 2B).

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 using the same FACS gating strategy as in peripheral blood. We confirmed that the frequency of IFNγ-producing Th1 T-cells was significantly increased in psoriasis compared

to AD (Psor 13.90%±1.31, AD 8.27%±1.47, $p<0.01$), while the frequency of IL-4-producing Th2 T cells was significantly higher in AD compared to psoriasis (Psor 1.53%±0.30, AD 5.04% ±0.95, $p<0.005$) (Figure 3A). We also observed a lower frequency of IL-17-producing Th17 T-cells in AD lesions compared to psoriasis (Psor 7.82%±1.03, AD 4.23%±0.75, $p<0.05$). However, despite the relative Th17 deficiency in AD compared to psoriasis, the mean percentages of IL-22-producing $CD4^+$ T-cells were similar in both diseases (Psor 8.01%±0.82, AD 10.86%±3.22) (Figure 3A). 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^+ Th17 cells, with only a small proportion of cells co-synthesizing both IL-17 and IL-22 (Figure 3A, FACS plots 3rd panel from top). While only a small proportion of IL-4^+ Th2 T-cells were shown to produce IL-22 in both diseases, the frequency of IL-22-producing Th2 T-cells in AD was significantly higher than in psoriasis (Psor 0.12%±0.02, AD 0.72%±0.24, $p<0.005$) (Figure 3A FACS plots 4th panel from top).

$CD8^+$ T-cells comprised approximately 20% of total T-cells in both AD and psoriasis skin biopsies (supplementary Fig. 1). In psoriasis, majority of the $CD8^+$ T cells were IFNγ–producing Tc1 cells (Psor 41.62%±2.90) (Figure 3B). Tc1 T-cells were also found in AD, although at a significantly lower frequency than in psoriasis (AD 28.86%±3.37, $p<0.01$). 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 to psoriasis (Psor 3.50%±0.42, AD 20.56%±6.56, $p<0.05$) (Figure 3B) and majority of these cells did not co-synthesize IL-17 or IL-4 (Figure 3B, 3rd and 4th FACS plots from the top).

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

To further characterize 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, then co-synthesis of IFNγ, IL-4 and IL-17 was determined within these IL-22^+ T-cells (Figure 4A to 4C).

In psoriasis, IL-22 production within $CD4^+$ T cell subsets was observed in Th1 (10.43%±1.3) and Th17 (39.17%±3.4) cells. However, the largest subset of IL-22 producing $CD4^+$ T cells (49.90±3.01%) did not co-secrete IFNγ, IL-4 or IL-17 (Figure 4B).

In AD lesions, IL-22 production was noted within Th1 (6.96%±1.64), Th2 (4.90%±1.17) and Th17 (17.31%±4.00) cell subsets. However, the overwhelming majority of IL-22 producing $CD4^+$ T cells in AD lesions (70.83%±4.54) were neither Th1, Th2 nor Th17 cells, and may represent a unique subset of IL-22 producing T helper cells (Figure 4B).

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

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 (Figure 5A). 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 ($r=0.78$, $p<0.05$) (Fig. 5B). While a similar trend was noted for $CD4^+$IL-22^+ T cells, it did not reach statistical significance ($r=0.39$, $p<0.22$) (Fig. 5C). No correlation was observed 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 as compared to psoriasis. Our findings expand current knowledge on the presence of Th17 T-cells and shed new light on a unique subset of IL-22 producing T-cells in blood (Figure 2) and skin (Figures 3 and 4) compartments of chronic AD patients.

We found a significantly decreased frequency of $CD4^+$ Th17 T-cells in AD lesional skin compared to psoriasis, whereas comparable numbers of these cells were obtained in peripheral blood. However, no significant difference was observed in IL-17 producing $CD8^+$ T-cells (Tc17) in skin lesions of AD and psoriasis. We previously reported that, compared to psoriasis, there was little expression of IL-17 and IL-23 mRNAs in AD lesions.[13] In the present study, we found a 2-fold difference in Th17 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 Th17 T-cell frequencies could potentially mean that although Th17 T-cells exist in AD lesions, in contrast to psoriasis, they are not activated. Another possibility is that increased Th2 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 Th2 cytokines on IL-17-induced antimicrobial protein secretion.[17]

Ultimately, several factors might collectively contribute to the reduction of Th17/IL-17 in AD skin: 1) Reduced recruitment of Th17 T-cells to AD skin due to either a lack of Th17 attracting chemokines (e.g. CCL20 that is expressed at lower levels in AD skin), or a lack of Th17-polarizing DC;[18] 2) Failure to activate existing Th17 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 proteins (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 histological 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 Th17 T-cells, a significantly increased expression of IL-22 mRNA and up-regulated production of IL-22 from $CD4^+$ and $CD8^+$ T-cells was observed in lesional AD skin, as compared to 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, hypogranulosis and suppression of terminal differentiation.[8] *In vivo* over-expression of IL-22 induces marked hyperplasia and inflammation in murine skin, bearing similarities to both psoriatic and AD skin.[9] Moreover, this cytokine down-modulates 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 co-synthesize IFNγ, IL-4, or IL-17. While the $CD4^+IL\text{-}22^+$ population is evident in both diseases, a significant proportion of IL-22 production is still derived from Th17 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 Th1, Th2, and Th17 T-cells that co-produce 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 mice[5] and humans.[3, 17] Our data establishes 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 pro-inflammatory chemokines by "T17" T-cells, and keratinocyte proliferation and modulation of terminal differentiation by "T22" T-cells (Figure 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 IL-22 producing memory T-cell populations differentiate from naïve T-cells, as opposed to the independent functional modulation of these cytokines within a common T-cell population.

Of particular interest is the $CD8^+IL\text{-}22^+$ T-cell population that although highly increased in AD skin, it was negligible in psoriasis, precluding further analysis. Interestingly, we found a strong correlation between the frequency of $CD8^+IL\text{-}22^+$/"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 "Th22" and "Tc22" T-cells contribute to the increased IL-22 expression in chronic AD skin (Figure 6). Our results support a Th2/T22 immune polarization in chronic AD, compared to the deviation toward a Th1/Th17 phenotype in psoriasis (Figure 6). The increased IL-22/"T22" signal despite a relative deficiency of Th17/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 $IL\text{-}22^+$ Th17 cells in AD.

Further elucidation of the role of these novel "T22" T-cells in both chronic and acute AD, as well as the interplay between these cells and the Th17/IL-23 pathway to produce the AD phenotype, are warranted. Our data, supported by the clinical correlation with disease severity potentially suggest targeting "T22" 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 Th17/IL-23 pathway, these investigations become even more critical.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

AD	Atopic dermatitis
AMP	Antimicrobial peptide
DC	Dendritic cell
FACS	Fluorescence-activated cell sorting
IFNγ	Interferon-gamma
IL	Interleukin
IHC	Immunohistochemistry
mRNA	Messenger RNA
Norm	Normal skin
IRB	Institutional review board
PBMC	Peripheral blood mononuclear cells
PMA	Phorbol myristate acetate
Psor	Psoriasis
Th	CD4 T helper cell
Tc	CD8 T cell

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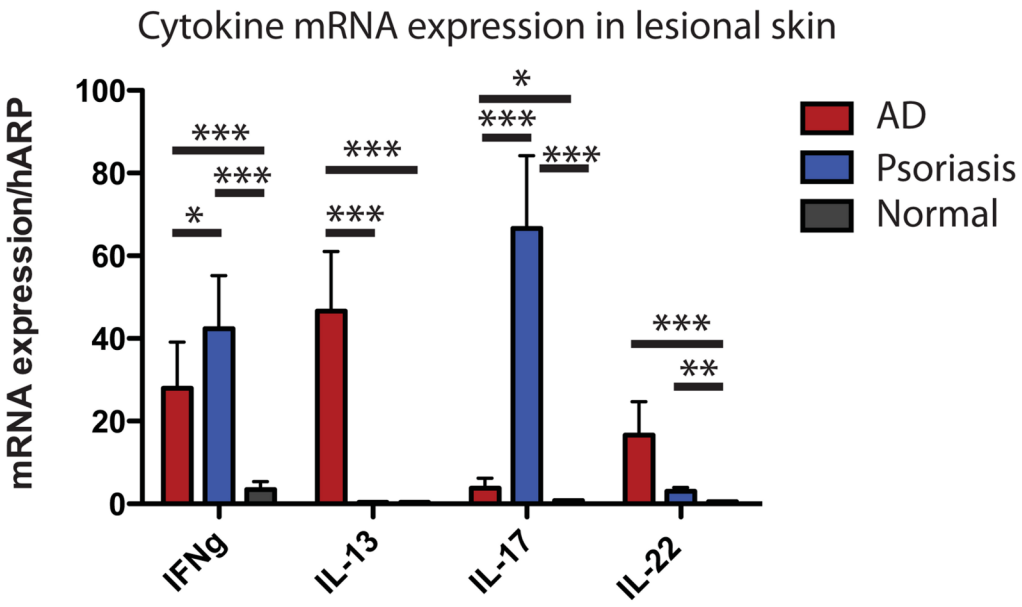

Figure 1. The cytokine microenvironment in atopic dermatitis (AD) and psoriasis skin lesions

Quantitative RT-PCR analysis of cytokine mRNA expression normalized to hARP in skin biopsies of lesional AD, psoriasis, and normal skin. Th1/Th17(IFNγ/IL-17) polarization was observed in psoriasis, Th2(IL-13) polarization in AD, with upregulation of IL-22 expression in both diseases. *p<0.05, **p<0.01, ***p<0.005.

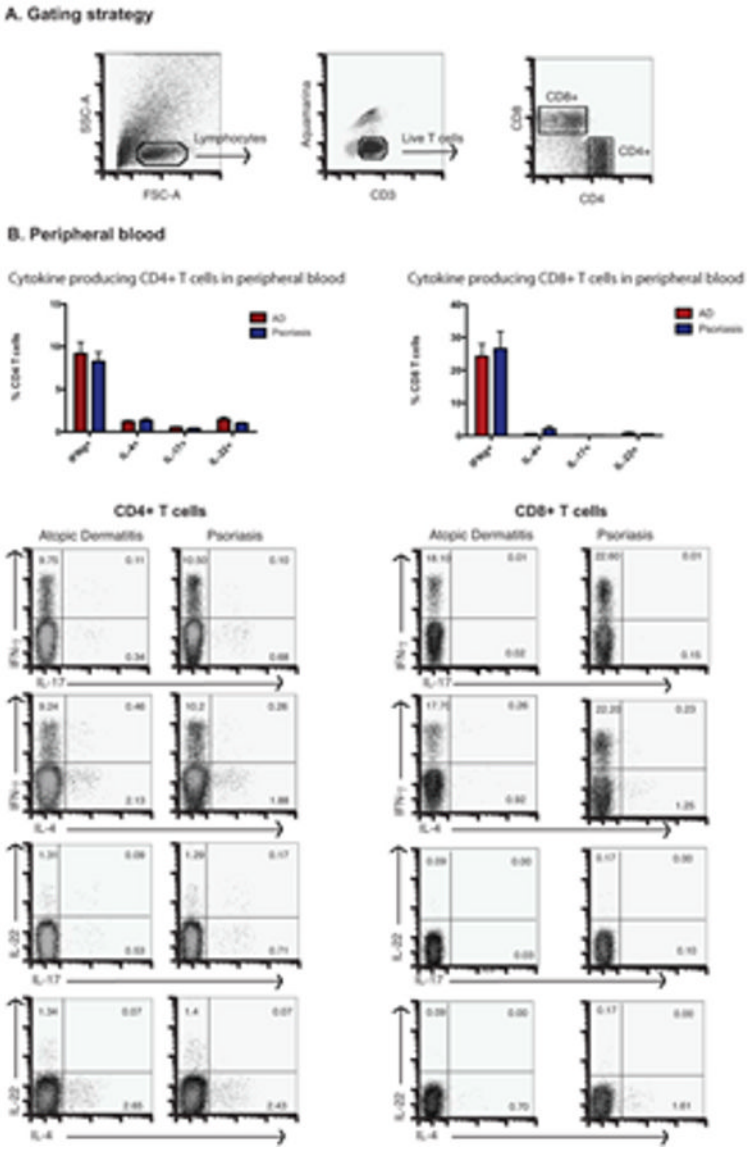

Figure 2. Circulating CD3$^+$ T-cell profiles are similar in atopic dermatitis (AD) and psoriasis

(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.

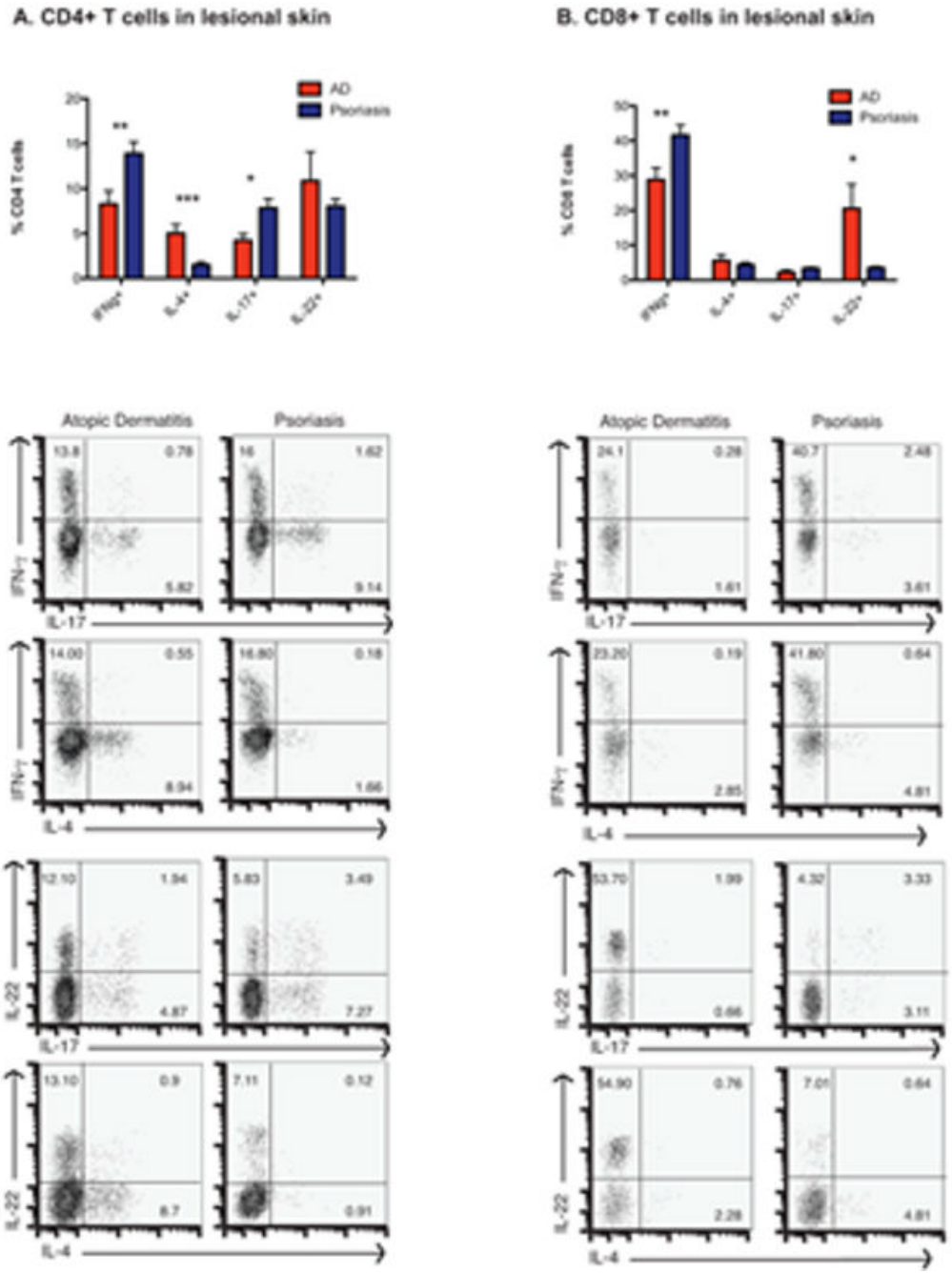

Figure 3. Atopic dermatitis (AD) and psoriasis lesions have distinct T-cell profiles

(A) Graph of $CD4^+$ T-cell subsets in AD and psoriasis lesions showing Th1/Th17(IFNγ/IL-17) polarization in psoriasis, Th2(IL-4) in AD, and similar $IL\text{-}22^+CD4^+$ frequencies in both. Representative FACS plots follow. (B) Graph of skin $CD8^+$T-cell subsets showing Tc1 (IFNγ) polarity in psoriasis and $IL\text{-}22^+CD8^+$ polarity in AD. Representative FACS plots follow. *p<0.05, **p<0.01, ***p<0.005.

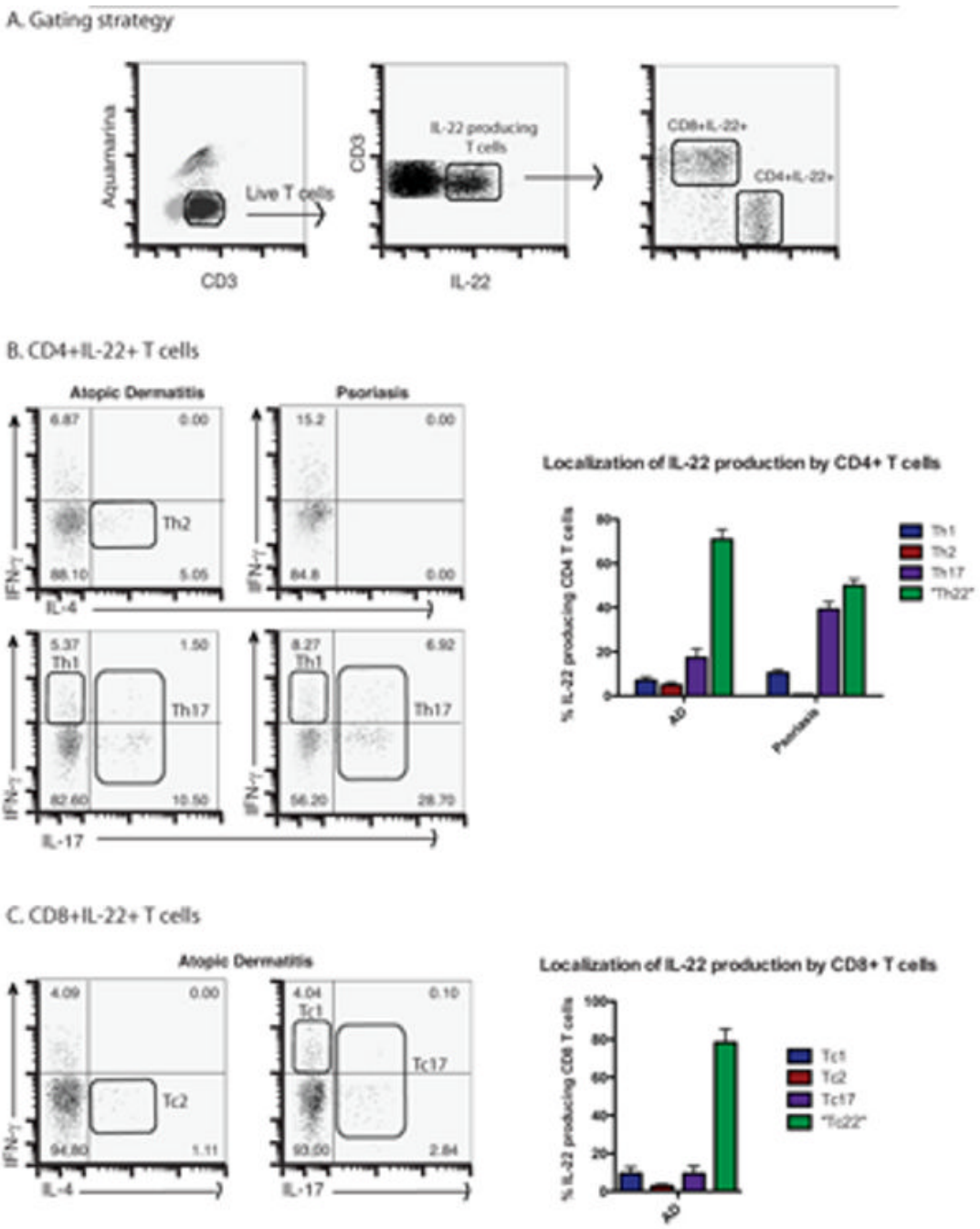

Figure 4. Unique IL-22-producing CD4$^+$ and CD8$^+$ subsets are abundant in AD lesions

(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 Th1, Th2 or Th17 cells. Psoriasis IL-22 is produced by Th1, Th17 and "Th22" cells. Representative FACS plots shown. (C) Majority of IL-22-producing CD8$^+$T-cells in AD are "Tc22" cells.

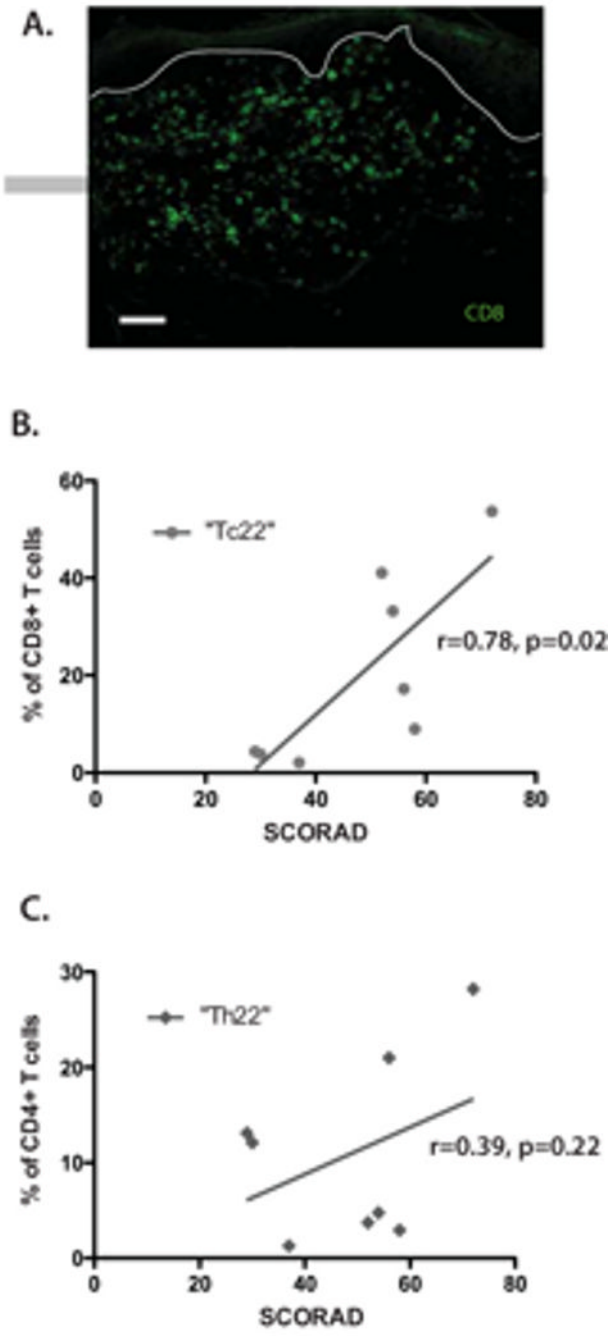

Figure 5. IL-22$^+$CD8$^+$ T-cell frequency correlates with disease severity

(A) Immunofluorescence showing abundant CD8$^+$T-cells in AD dermis. Bar: 100μm. (B) Linear regression showing positive correlation between IL-22-producing CD8$^+$T-cells ("Tc22") and AD clinical severity (SCORAD) ($r=0.78$, $p<0.05$). (C) Linear regression of IL-22-producing CD4$^+$T-cells ("Th22") showing positive trend with disease severity but not statistically significant.

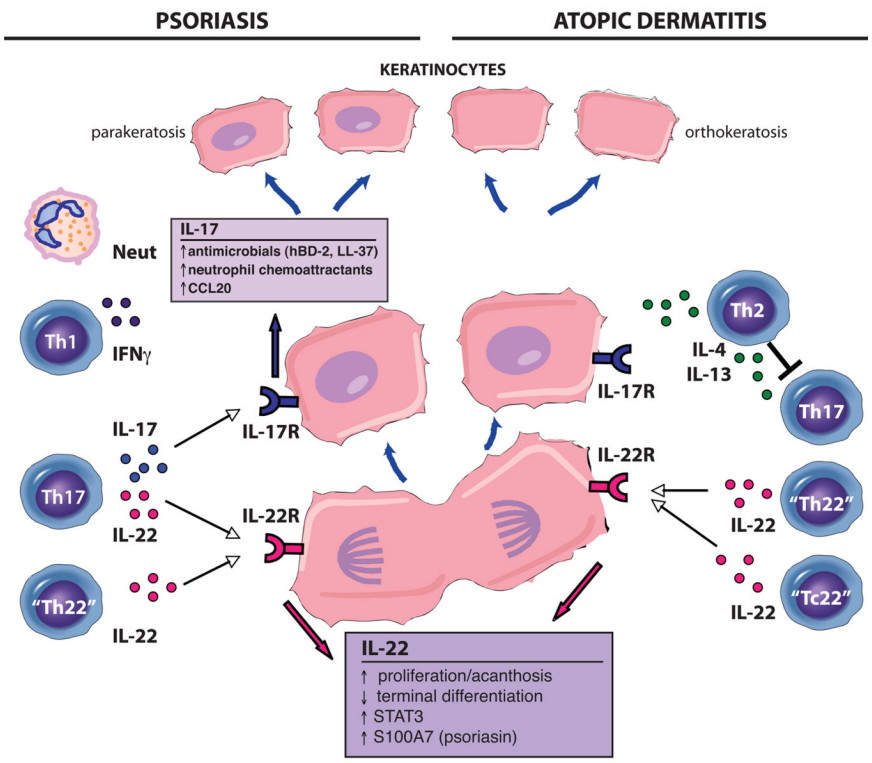

Figure 6. Model of major T-cells and their cytokines in chronic skin lesions of psoriasis vs. atopic dermatitis (AD)

In psoriasis, Th1 cells produce IFNg, Th17 cells produce both IL-17 and IL-22 cytokines, and "Th22" cells produce IL-22 but not IL-17. Upregulated IL-17 axis in psoriasis results in increased production of anti-microbial proteins, neutrophil chemoattractants and CCL20 chemokine, while upregulated IL-22 causes epidermal acanthosis. In AD, the Th2 cytokines IL-4 and IL-13 potentially inhibit Th17 T-cell activity resulting in reduced IL-17 effects. Both "Th22" and "Tc22" T-cells in AD are responsible for significantly increased IL-22 levels. The upregulated 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.

Table I

Profiles of AD and Psoriasis patients

AD	Age	IgE	Eos	SCORAD	Atopic Background	Psoriasis	Age	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

Abbreviations: AD-Atopic dermatitis; IgE-Immunoglobulin E; Eos – Eosinophil count; SCORAD-Scoring Atopic Dermatitis Index; PASI – Psoriasis Area and Severity Index