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Attenuated neutrophil axis in atopic dermatitis compared to psoriasis reflects T_H17 pathway differences between these diseases

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To the Editor

Atopic dermatitis (AD) and psoriasis are the most common inflammatory skin diseases. While both are T-cell-associated and share common features such as epidermal hyperplasia and abundant infiltrates, these diseases have different immune and barrier phenotypes.¹ AD is T_H2/T_H22-polarized with an attenuated T_H17 axis and is associated with an impaired barrier, low levels of antimicrobial peptides (AMPs), and increased susceptibility to skin infections with fungi, viruses, and particularly, bacteria (ie, *Staphylococcus aureus*). In contrast, psoriasis is T_H1/T_H17-driven and does not have this increased infection rate.¹ The T_H17 cytokine IL-17 is a key inducer of AMPs and neutrophil chemoattractants.² This cytokine was found to be attenuated in AD compared with psoriasis and paralleled by highly decreased AMP levels, possibly explaining this increased susceptibility to pathogens. Importantly, a direct correlation between bacterial infections and disease exacerbation has been well established in AD.^{3,4}

While psoriasis is established as a disease rich in neutrophils that contribute to cutaneous inflammation,⁵ their role in AD immunity and inflammation is poorly understood. Recently, Choy et al⁶ concluded on the basis of a transcriptome comparison of AD and psoriasis that genes encoding for neutrophil chemoattractants were comparably elevated in both diseases. Limited immunohistochemistry (hematoxylin and eosin, elastase, lipocalin-2 [LCN2]) in their study revealed neutrophilic infiltrates in lesional AD (AL) skin, though

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levels appear up to 2-fold lower than in lesional psoriasis (PL) skin per their quantification. However, as delineated in their patients' clinical characteristics, a majority demonstrated crusts and excoriations; superimposed infection was not thoroughly ruled out. In comparison, prior observations by our group suggested a relative paucity of neutrophils in AD versus psoriasis (unpublished data). Thus, we set to perform a direct comparison of neutrophils and neutrophil-related molecules in these diseases.

By using gene-array analysis of neutrophil-related genes, we compared nonlesional and lesional AD (ANL and AL; $n = 11$ and 9 , respectively) and psoriasis (PNL and PL; $n = 15$ each, respectively) skin with normal skin ($n = 9$). Neutrophil infiltrates were compared by neutrophil elastase (NE) immunohistochemistry on nonlesional, acute/acute-on-chronic lesional, and chronic lesional AD (ANL, ALS, and CLS respectively; $n = 15$ each) and PL and PNL skin ($n = 19$ each). Additional staining identified LCN2-positive neutrophils and differentiated epidermal keratinocyte expression levels of this potent AMP between the diseases ($n = 9$ ANL, 11 ALS, 9 CLS, 10 PNL, and 12 PL samples). All patients with AD and psoriasis exhibited moderate-to-severe disease as measured by Scoring of AD (mean, 55 ± 17) and Psoriasis Area Severity Index (12) (for details, see the Methods section in this article's Online Repository at www.jacionline.org).

Comparative gene-array analysis of neutrophil-signature genes revealed significantly decreased expression in AL skin compared with PL skin (but not compared with normalskin). For example, this difference was demonstrated in gene transcripts of IL-17A (1.9-fold higher in PL, false-discovery rate [FDR] = .001) and neutrophil-inducing molecules such as defensin-2/hBD2/DEFB4A (129-fold), LCN2 (76-fold), IL-8 (22-fold), CC family chemokine ligand 20 (8.9-fold), and CXC family cytokine ligand 1/CXCL1 (6.5-fold, FDR < 0.01) (FDR < 0.001 unless specified; Fig 1).

Using NE immunostaining, marked increases in neutrophils were observed in PL (mean, 98 cells/mm) compared with ALS (mean, 23/mm; $P = .007$), CLS (mean, 19/mm; $P = .005$), ANL (mean, 2/mm; $P = .0004$), and PNL skin (mean, 5/mm; $P = .0002$) (Fig 2, A-f-j-C; see Table E1 in this article's Online Repository at www.jacionline.org). In addition, characteristic psoriatic epidermal neutrophil clusters (Munro microabscesses) were seen only in PL tissues (Fig 2, A-j, red). In AD, only ALS samples demonstrated a notable increase in neutrophils compared with ANL skin ($P = .059$) (Fig 2, C). Staining for LCN2, a potent AMP expressed by keratinocytes² and neutrophils, also supported these findings, demonstrating abundant LCN2-positive neutrophils in PL compared with PNL, ALS, and CLS skin ($P = 4 \times 10^{-25}$, .002, and .0004, respectively) and greater keratinocyte expression in psoriasis (Fig 2, A-k-o).

Of note, 3 AD specimens demonstrated unusually elevated dermal neutrophil infiltrates (Fig 2, C, blue). On closer examination of these outliers, a supraepidermal serum crust was present on hematoxylin and eosin that was absent in other AD samples (Fig 2, B-p, red). This crust contained localized NE- and LCN2-positive neutrophil collections, with dermal infiltrates located directly below (Fig 2, B-q-r) and clusters suggestive of bacteria at higher magnification (Fig 2, B-q, inset). We hypothesized that these patients were secondarily infected, resulting in characteristic crust formation. To confirm infection, we used a green fluorescent protein-tagged binding domain of a bacteriophage lysin specific for *S aureus* on AD tissues with and without large neutrophil infiltrates ($n = 3$ each; see Methods in Online Repository). Dermal bacterial clusters, noted only in samples with large neutrophil infiltrates (Fig 2, B-s, 100 $\times$), fluoresced when exposed to this *S aureus*-specific construct (Fig 2, B-t). Interestingly, an uptrend in gene expression of some IL-17-modulated neutrophil chemoattractants (ie, CXCL1 and CXC family cytokine ligand 2/CXCL2) was noted in infected patients compared with other samples (not shown). Limiting mean calculations to

uninfected AD samples resulted in marked decreases for ALS and CLS skin (means, from 23 and 19 cells/mm to 10 and 7/mm respectively; Fig 2, C, red to green), further enhancing significance compared with PL.

There are several explanations for the differences between our data and findings reported by Choy et al⁶: (1) Despite identifying crusts in 9 of 12 patients with AD and clearly depicting sample tissue similar to our infected patients' in Fig E1, *B* of their paper,⁶ infection was not ruled out by staining or culture; (2) While the published findings focused on smaller regions of tissue consisting of 5 high-power fields at $\times 400$, our histological samples were analyzed in a larger field of 1 mm at $\times 10$, inclusive of the epidermis and dermis; (3) Alternatively, because disease severity measures were not included for psoriasis patients, inclusion of patients with mild psoriasis could skew neutrophil activity to levels comparable to AD.

Overall, we found that neutrophils and their associated molecules are significantly higher in psoriasis than in AD. The differences are particularly striking between uninfected AD lesions (with low neutrophil counts) and psoriasis lesions harboring large neutrophil numbers. In contrast, both diseases exhibit comparably significant CD3⁺ T-cell and CD11c⁺ dendritic-cell infiltrates.⁷ Our data associate higher neutrophil levels in AD with *S aureus* infection and *Staphylococcal* coagulase-induced supraepidermal crust formation. These results suggest a much smaller contribution of the neutrophil axis to AD pathogenesis compared with psoriasis, paralleling similar differences observed in the T_H17/IL-17 pathway.² Interestingly, recent findings suggest that *S aureus* is capable of T_H17/IL-17 induction.^{8,9} Together with our data, this potentially implicates an increasing role for the T_H17/IL-17 axis and its downstream neutrophil-associated molecules in cases of *S aureus* infection in AD, with possible therapeutic implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG 1.

Heatmap of the gene-array analysis of key neutrophil-related genes in normal, lesional, and nonlesional AD and psoriasis skin. Both AL and PL skin demonstrate elevated expression compared with normal skin. However, levels in lesional AD skin were markedly lower than in PL skin as demonstrated by significant fold-change ($\log_2$) differences between groups (Table based on lesional expression values; *FDR < 0.05, **FDR < 0.01, ***FDR < 0.001). *Red*, Upregulated; *blue*, downregulated.

FIG 2.

Immunohistochemistry of ANL, ALS, and CLS and PNL and PL skin. **A-a-e**, Representative hematoxylin and eosins (*H&Es*). **A-f-j**, NE and **A-k-o**, LCN2 immunostainings demonstrate significantly increased neutrophils in PL compared with ALS and CLS skin ($\times 10$). **B-p**, H&E of ALS skin with elevated neutrophils illustrates a supraepidermal serum crust (*arrow*), with **B-q-r**, NE and LCN2 staining within the crust and dermis below ($\times 4$). Higher magnification ($\times 40$) shows a bacterial cluster (inset). **B-s**, A specimen with increased neutrophils reveals dermal clusters ($\times 100$, inset), confirmed as **B-t**, *Staphylococcus aureus* with a GFP-labeled bacteriophage lysin binding domain against *S. aureus*. **C**, NE counts reveal significantly higher infiltrates in PL than in PNL, ALS, and

CLS skin ($P < .01$) (*blue*, infected outliers; *red*, averages with outliers; *green*, averages excluding outliers; *bars*, median). *GFP*, Green-fluorescent protein; *LM*, light microscopy. *Scale bars* 5 100 $\mu\text{m}/\times 10$, 500 $\mu\text{m}/\times 4$, 50 $\mu\text{m}/\times 40$.