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ORIGINAL PAPER

Epidermal expression of eotaxins and thymic stromal lymphopoietin in eosinophil rich dermatoses

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Abstract

Eosinophils are seen in a number of dermatologic conditions. While the extent of their function in these diseases remains to be fully elucidated, pathogenic activity in bullous pemphigoid suggests a more significant role than previously thought. Several dermatoses have a fairly characteristic histologic morphology of eosinophil infiltration. We hypothesized that epidermal expression of eotaxins and TSLP would differ by disease, perhaps explaining the different histologic morphologies. We performed a retrospective study of eosinophil rich dermatoses to perform immunohistochemistry. We collected 49 specimens composed of bullous pemphigoid ($n=15$), atopic dermatitis ($n=12$), drug rash ($n=8$), arthropod assault ($n=5$), and non-bullous pemphigoid eosinophilic spongiosis ($n=5$). We used lichen planus ($n=4$) as a control for lymphocyte-mediated inflammation. TSLP was diffusely expressed in all epidermal samples, whereas eotaxins demonstrated a weaker staining. Eotaxins and TSLP demonstrated a gradient between basal and spinous keratinocytes. The correlation between overall basal keratinocyte and spinous keratinocyte staining of eotaxins and TSLP with the number of eosinophils demonstrated a significant correlation between eotaxin-1 ($R=0.404$, $P=0.004$), eotaxin-2 ($R=0.576$, $P<0.001$), and eotaxin-3 ($R=0.512$, $P<0.001$), but not TSLP ($R=0.164$, $P=0.251$). These remained significant after correcting for multiple comparisons. While we were unable to detect significant differences in epidermal expression of eotaxins and TSLP in various eosinophil rich dermatoses, we identified a significant correlation of spinous keratinocyte eotaxin staining with tissue eosinophilia. Our identification of a correlation of spinous keratinocyte eotaxin staining with tissue eosinophilia may provide insight into local eosinophil chemotaxis.

Keywords CCL11 · CCL26 · CCL24 · Thymic stromal lymphopoietin · Bullous pemphigoid · Atopic dermatitis

Introduction

Eosinophils are seen in a number of dermatologic diseases including bullous pemphigoid (BP), arthropod bites, drug reactions, and incontinentia pigmenti [1]. Their function as potent immune effector cells in innate and adaptive immune responses is mediated through activation and degranulation of their cytoplasmic granules [2]. Release of immunomodulatory mediators from eosinophils promotes inflammation in the local microenvironment, provides recruitment signals for other effector cells as well as mediates local tissue destruction and fibrosis [2, 3].

Despite the presence of eosinophils in several conditions, the morphology of their infiltration is intriguing. While BP manifests with an eosinophil-rich inflammatory infiltrate with eosinophils lined against the basement membrane zone (BMZ) as well as percolating into the epidermis via eosinophilic spongiosis, atopic dermatitis is characterized by a striking absence of eosinophils on histology due to eosinophil cytolysis [4, 5]. Given the prevalence and function of eotaxins and TSLP in BP [6–13] as well as atopic dermatitis [5, 14–18], we hypothesized that eotaxins and TSLP expression would have a differential expression in the various eosinophil-driven diseases, perhaps explaining the different histologic morphologies.

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Eosinophil recruitment and activation are guided by the eotaxin family, comprised of three chemokines; eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26). Collectively, all bind and modulate eosinophil activity through interaction with the C–C chemokine receptor (CCR) 3, which is universally expressed on peripheral eosinophils and upregulated in activated states [1, 10, 19–21]. However, this chemokine family has little homology and plays differing roles in a potentially sequential manner to hone and stimulate eosinophils [22]. Stimulation by Th2 cytokines (IL-4, IL-13, TNF-α) results in upregulation of the eotaxin family [1, 22]. Likewise, we have demonstrated that eosinophil degranulation protein, eosinophil cationic protein, and eosinophil-derived neurotoxin induce keratinocyte expression of eotaxin-1, resulting in a positive feedback loop [23]. Each eotaxin functions as a potent eosinophil chemoattractant; however, there appears to be a temporal sequence to their expression and activity [22, 24].

Thymic stromal lymphopoietin (TSLP), an upregulated epithelial cytokine in AD and BP, induces a pro-inflammatory Th2 cytokine response, while modulating eosinophil survival through activation of the p38 mitogen-associated protein kinase (MAPK) and NF-κB signaling pathways [25]. Acting in conjunction with eotaxins, TSLP precisely regulates expression of cell surface adhesion molecules to facilitate recruitment and transmigration of eosinophils into inflammatory sites. TSLP-driven eosinophil cytokine/chemokine expression can result in local amplification and perpetuate the local inflammatory response [5, 26]. TSLP can additionally directly induce pruritus [13, 27].

To better understand the lesional expression of eotaxins and TSLP among these diseases, we performed a retrospective study of eosinophil rich dermatoses to using immunohistochemistry.

Materials and methods

Reagents

Anti-human eotaxin 1 monoclonal, eotaxin-2 polyclonal, eotaxin-3 polyclonal, and TSLP polyclonal antibodies were purchased from Thermo Fisher (Waltham MA).

Specimen selection

Upon receiving independent review board exemption from the University of California Irvine, the dermatopathology database was queried to identify samples consistent with bullous pemphigoid, atopic dermatitis, drug rash, arthropod assault, and eosinophilic spongiosis. Normal overlying skin from lipoma excisions was additionally used as negative control for stains. Eosinophils were used for positive control of eotaxin expression. Cases of bullous pemphigoid were confirmed by results of direct immunofluorescence, indirect immunofluorescence, and/or ELISA for BP180 and BP230. Lichen planus was used as a control for epidermal expression background in lymphocytic inflammation. Based on a primary outcome measure comparing bullous pemphigoid to atopic dermatitis, we determined a sample size of 15 for bullous pemphigoid and 12 samples with atopic dermatitis assuming an α of 0.05 and a 1-β of 0.80 assuming a 0.6 effect size. Criteria for specimen selection are shown in Table 1.

Immunohistochemistry

Five sections from each block were cut to a thickness of 4 microns and heated for 1 h at 60 °C. Sections were then inserted into Ventana Bench Mark Ultra (automated IHC platform). Deparaffinization, dehydration, antigen retrieval, cell conditioning, as well as pH, temperature control, and incubation were completed automatically within the platform.

Table 1 Inclusion criteria for specimens used in retrospective analysis

Disease	Features
Bullous pemphigoid	Clinical suspicion + subepidermal blister with eosinophils along basement membrane zone, eosinophilic spongiosis, confirmatory direct or indirect immunofluorescence and/or ELISA
Atopic dermatitis	Clinical history of atopic dermatitis, minimal eosinophils within the inflammatory infiltrate
Drug rash	Clinical suspicion + spongiosis with presence of eosinophils within the infiltrate, ± interface changes
Arthropod assault	Clinical suspicion + deep and superficial inflammatory infiltrate with eosinophils
Non-BP eosinophilic spongiosis	Eosinophilic spongiosis with bullous pemphigoid ruled out by direct or indirect immunofluorescence and/or ELISA
Lichen planus	Clinical suspicion + band-like lymphocytic infiltrate with an absence of eosinophils or parakeratosis

Specimen grading

Following acquisition of high-quality digital photomicrographs, images were reviewed by two investigators independently and rated qualitatively on a scale of 0–3 based on assessing three high-powered fields of epidermis. Intraepidermal leukocytes were not included in the grading, nor were intraepithelial vesiculations. In light of the presence of intraepidermal vesiculation, bullae, or interface changes, computerized quantitative scoring which would potentially include infiltrating immune cells could not be performed [28]. Basal keratinocytes and spinous keratinocytes were scored separately. A basal to spinous keratinocyte gradient was mathematically calculated by subtracting the spinous score from basal. Three random high power fields were selected to assess superficial dermal/epidermal eosinophil counts. Eosinophil counts were cut off at 16 for specimens with greater than 15 eosinophils.

Statistical analysis

Mean basal keratinocyte and spinous keratinocyte IHC grading was recorded. Basal–spinous gradient was calculating by subtracting the mean spinous score from the mean basal score for each chemokine or cytokine. A Kruskal–Wallis H test was used to compare each chemokine or cytokine's mean basal, spinous, or gradient between each disease entity. Comparisons between two independent groups were made using a Mann–Whitney rank sum test. To correlate epidermal expression pattern with eosinophil numbers, we used a Spearman's rank correlation. To account for multiple comparisons between each cytokine and chemokine, we used a Bonferroni correction to account for four different groups (eotaxin 1–3, and TSLP). All tests were two-sided with significance defined as $P < 0.0125$. Statistics were performed using SPSS 25 (IBM Corporation. Armonk, NY).

Results

We collected 49 specimens composed of bullous pemphigoid ($n = 15$), atopic dermatitis ($n = 12$), drug rash ($n = 8$), arthropod assault ($n = 5$), eosinophilic spongiosis ($n = 5$), and lichen planus ($n = 4$). Basal and spinous keratinocyte staining did not significantly differ between each disease entity for all eotaxins and TSLP. Mean basal and spinous keratinocyte scorings are shown in Fig. 1. Individual comparisons of BP with atopic dermatitis were also not significant.

Morphologically, TSLP was diffusely expressed in all epidermal samples, whereas eotaxin-2 demonstrated weak staining. Eotaxins and TSLP demonstrated a gradient, whereby expression was more intense in basal keratinocytes as compared to spinous keratinocytes as shown in Fig. 2. The extent of this gradient did not, however, differ between diseases.

Next, we sought to determine whether epidermal expression of eotaxins and TSLP correlated with dermal and epidermal eosinophils. Mean eosinophil counts per disease are shown in Fig. 3. Spearman's

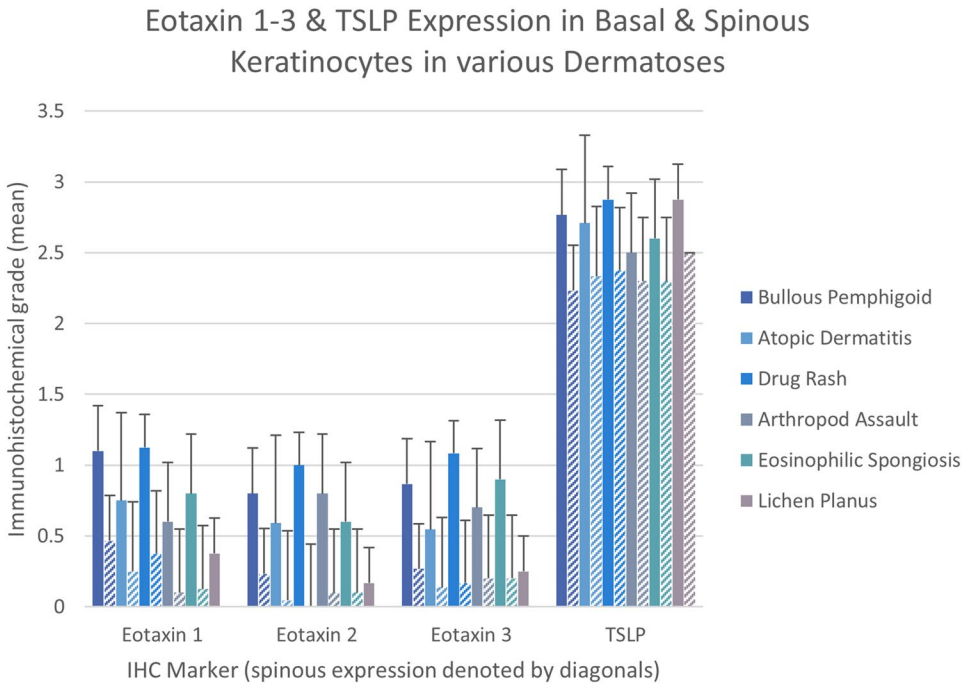

Fig. 1 Mean grading of epidermal expression of eotaxin-1, eotaxin-2, eotaxin-3, and thymic stromal lymphopoietin

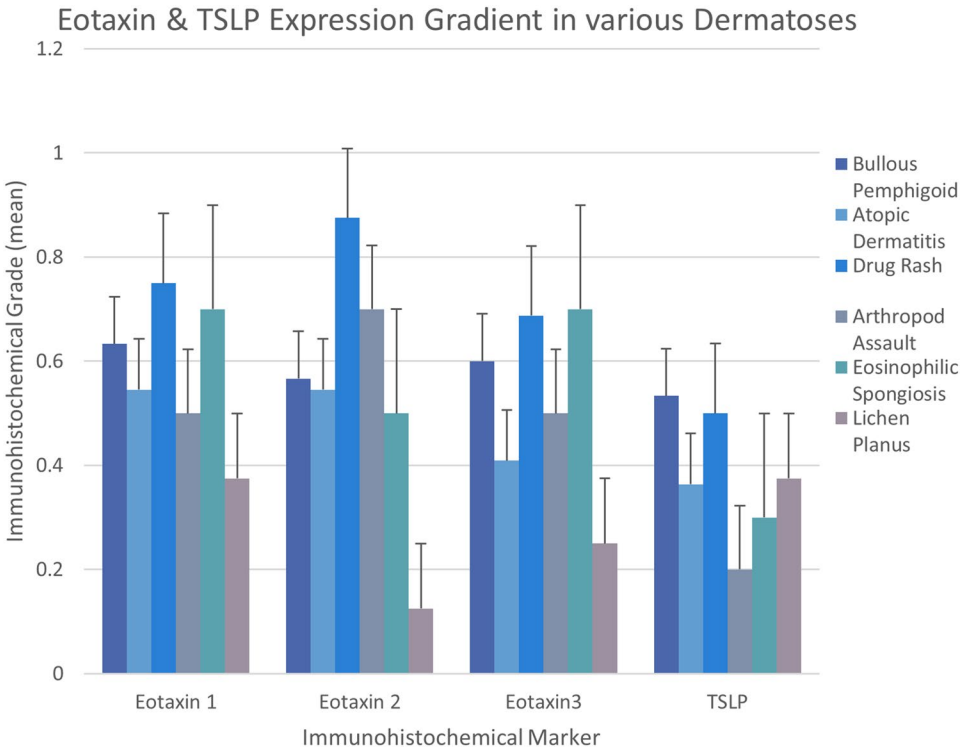

Fig. 2 Mean gradient of spinous keratinocyte and basal keratinocyte expression of eotaxin-1, eotaxin-2, eotaxin-3, and thymic stromal lymphopoietin

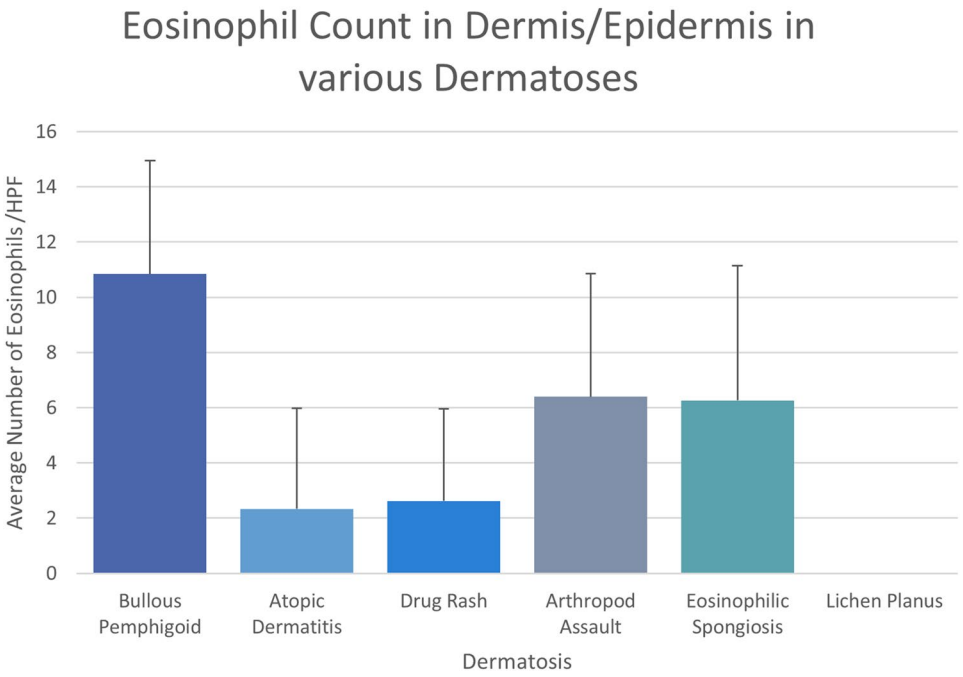

Fig. 3 Mean number of eosinophils in each condition, as measured by averaging the number seen in the three higher power fields. *AD* atopic dermatitis, *BP* bullous pemphigoid, *ES* non-bullous pemphigoid eosinophilic spongiosis cases, *LP* lichen planus

rank correlation between overall basal keratinocyte and spinous keratinocyte expression of eotaxins and TSLP demonstrated a significant correlation between eotaxin-1 ($R = 0.404$, $P = 0.004$), eotaxin-2 ($R = 0.576$, $P < 0.001$), and eotaxin-3 ($R = 0.512$, $P < 0.001$), but not TSLP ($R = 0.164$, $P = 0.251$). These remained significant after correcting for multiple comparisons. Subgroup analysis of these correlations only remained significant for spinous keratinocytes. We additionally explored whether cytokine/chemokine gradient between the basal

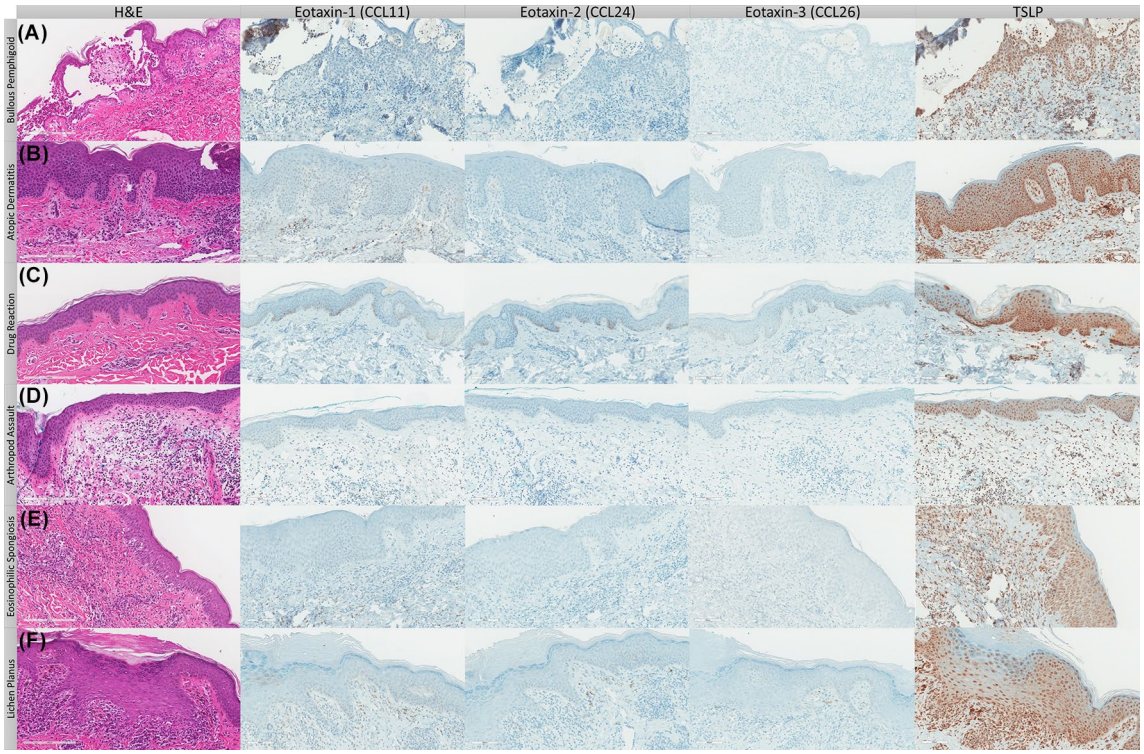

Fig. 4 Characteristic specimens used for studying eotaxin-1, eotaxin-2, eotaxin-3, and thymic stromal lymphopoietin (TSLP) expression in **a** bullous pemphigoid, **b** atopic dermatitis, **c** drug reaction, **d** arthropod assault, **e** non-bullous pemphigoid eosinophil spongiosis, **f** lichen planus

keratinocyte and spinous keratinocytes is associated with tissue eosinophilia. Of these, only TSLP had a weak correlation ($R = 0.283$), which when corrected for multiple comparisons, no longer maintained statistical significance ($P = 0.044$) (Fig. 4).

Discussion

Eosinophils are present in several dermatologic diseases and are likely to play an important role in their pathogenesis. In BP, they are known to be pathogenic [2]. While we were unable to detect significant differences in epidermal expression of eotaxins and TSLP in various eosinophil rich dermatoses, we identified a significant correlation of spinous keratinocyte eotaxin staining with tissue eosinophilia. While basal keratinocytes demonstrate a more robust staining, they failed to correlate with eosinophilia. We hypothesize this may be due to chronicity of pro-eosinophilic inflammation resulting in more differentiated keratinocyte still strongly expressing pro-inflammatory chemokines.

Given the pathology of BP, whereby autoantibodies bind to the NC16a domain of collagen XVII in the basal keratinocyte eventually resulting in subepidermal blistering, this is particularly unexpected and intriguing. These findings suggest a more important interplay of basal keratinocytes and spinous keratinocyte than may have previously been thought.

Our study is limited by its retrospective nature, resulting in use of paraffin-embedded samples. Immunofluorescence would potentially allow more quantitative scoring. Likewise, our study was underpowered to compare all the eosinophil rich dermatoses but was rather based on two-way comparisons between BP and atopic dermatitis.

Our identification of a correlation of spinous keratinocyte eotaxin expression with tissue eosinophilia may provide insight into chemotaxis at the dermal/epidermal level. Further single-cell sequencing studies may be helpful to determine the expression profile of these clearly distinct populations of keratinocytes. We were unable to find an association between basal or spinous eotaxin and TSLP expression with disease subtype.

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Compliance with ethical standards

Conflict of interest The authors report that they have no potential conflict of interest.

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