

Distinct eosinophil cytokine expression patterns in skin diseases – the possible existence of functionally different eosinophil subpopulations

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Abstract

Background: The function of eosinophils has been attributed to host defense, immunomodulation, and fibrosis. Although eosinophils are found among infiltrating cells in a broad spectrum of skin diseases, their pathogenic role remains uncertain. This study aimed to analyze the cytokine expression by eosinophils in different skin diseases.

Methods: Skin specimens from different skin diseases [allergic/reactive, infectious, autoimmune, and tumors/lymphomas (LY)] were stained by antibodies directed to eosinophil cationic protein, cytokines [tumor necrosis factor (TNF)- α , interleukin (IL)-5, IL-6, IL-10, IL-11, IL-13, IL-17, IL-25, IL-33, interferon- γ , transforming growth factor (TGF)- β , and thymic stromal lymphopoietin], eotaxins (CCL11, CCL24, and CCL26), metalloproteinase (MMP)-9 as well as extracellular matrix proteins (tenascin-C and procollagen-3) and then analyzed by laser scanning microscopy.

Results: The number of eosinophils varied considerably in and between disease groups and did not correlate with the numbers of accompanying inflammatory cells. The expression of IL-5, IL-6, IL-11, TGF- β , CCL24, and MMP-9 by eosinophils significantly differed between disease groups. Eosinophils in tumors/LY predominantly expressed IL-6, TGF- β , and CCL24, but not IL-11. On the other hand, in autoimmune diseases, eosinophils largely contributed to MMP-9 production. IL-5-generating eosinophils were particularly obvious in allergic and infectious diseases.

Conclusion: In skin diseases, eosinophil expresses a broad spectrum of cytokines. The different cytokine expression patterns suggest distinct functional roles of eosinophils in these diseases that might be related to host defense, immunomodulation, fibrosis, and/or tumor development.

In a broad spectrum of skin diseases, eosinophils are found among infiltrating cells (1, 2). However, the pathogenic role of eosinophils in the skin has not been defined yet. The functions of eosinophils have been related to the protection against helminth parasites (3), immunopathology (3, 4), remodeling processes, and immunoregulation. The latter is thought to be mediated by the production and release of a large number of cytokines that modulate the activation and function of other leukocytes, such as dendritic cells, macrophages, lymphocytes, mast cells, and neutrophils, and of resident tissue cells, including epithelial cells, endothelial cells, and fibroblasts (5). By releasing eosinophil extracellular traps (EETs) consisting of

mitochondrial DNA in association with granule proteins, eosinophils also participate in antibacterial defense (6). Recently, we demonstrated EETs in distinct skin diseases (7).

Skin eosinophilia is a characteristic feature of allergic diseases or parasitic infestations, but is also observed in autoimmune diseases as well as in association with hematologic diseases and tumors. In this study, we investigated the cytokine expression by eosinophils to obtain hints about their potential functional role in different skin diseases. In addition, the expression of cytokines responsible for eosinophil recruitment and activation as well as the accompanying inflammatory cells was determined.

Material and methods

Skin specimens from biopsies taken for routine diagnostics were obtained from the archives of the histology laboratory of the Department of Dermatology. The study was approved by an institutional board and the ethics committee of the Canton Bern. Representative specimens (numbers >1 are given) revealing tissue eosinophilia were selected from allergic/reactive diseases [atopic dermatitis (AD, $n = 5$), positive atopy patch test reaction (APT), allergic contact dermatitis (ACD), drug hypersensitivity (DHS, $n = 3$), urticaria (URT, $n = 3$), prurigo nodularis (PN), eosinophil pustular folliculitis (EPF), hypereosinophilic syndromes (HES, $n = 2$), eosinophilic cellulitis (Wells syndrome, WS, $n = 3$), infectious diseases [larva migrans (LM), ectoparasitosis (ECTOP, $n = 3$)], autoimmune diseases [bullous pemphigoid (BP, $n = 5$), pemphigus foliaceus (PF), dermatitis herpetiformis (DH, $n = 2$), connective tissue diseases, dermatomyositis (DM), morphea (MOR, $n = 2$), polyarteritis nodosa (PAN), and eosinophilic fasciitis (EF)], Wegener's granulomatosis (WG, $n = 2$), lymphomas [LY, cutaneous T-cell lymphoma (CTCL, $n = 4$), B-cell lymphoma, pseudolymphoma (PLY, $n = 2$), Langerhans cell histiocytosis (LCH, $n = 2$), cutaneous mastocytosis, angiolymphoid hyperplasia with eosinophilia (ALHE), and solid tumors (TU, $n = 4$), keratoacanthoma, sweat gland carcinoma, benign fibrous histiocytoma, and melanoma metastasis].

Tissue sections of 6 μm were stained with hematoxylin and eosin (H&E) and analyzed using light microscopy to determine eosinophil distribution in the dermis and/or epidermis, shape, extracellular granule depositions, signs of tissue destruction, and fibrosis. To investigate inflammatory cell, cytokine, and mediator expression, immunofluorescence staining using antibodies directed to eosinophil cationic protein (ECP; Pharmacia Diagnostics AB, Uppsala, Sweden), CD4, CD8, CD21 (all AbD Serotec, Oxford, UK), CD3, CD1a, CD57 (all Dako, Glostrup, Denmark), CD11c, NKT (both BD Biosciences, Franklin Lakes, NJ, USA), CD68 (Zymotomed Systems, Berlin, Germany), tumor necrosis factor (TNF- α ; Novus Biologicals, Littleton, CO, USA), interleukin (IL)-5, IL-6, IL-13, IL-17, thymic stromal lymphopoietin (TSLP), metalloproteinase (MMP)-9 (all Santa Cruz Biotechnology, Santa Cruz, CA, USA), IL-10, IL-11, transforming growth factor (TGF)- β , eotaxin-1 (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26) (all R&D Systems, Minneapolis, MN, USA), IL-25 (LifeSpan Biosciences, Seattle, WA, USA), IL-33 (Enzo Life Science, Lausen, Switzerland), and appropriate secondary antibodies labeled with Alexa fluor 488 (Invitrogen Molecular Probes, Paisley, UK) or Cy 3 (Jackson ImmunoResearch Ltd, Suffolk, UK) was performed. Mouse monoclonal IgG1 and rabbit polyclonal IgG control antibodies (both from Dako) and normal goat IgG (R&D Systems) served as negative controls. Extracellular matrix proteins were stained using antibodies to procollagen-3 (Chemicon, Temecula, CA, USA) and tenascin-C (Monosan, Am Uden, Netherlands).

Using confocal microscopy, positive cells were counted in ten consecutive fields (each 0.01 mm^2) in representative areas

of each specimen. Eosinophils were detected by their typical morphology (majority of the experiments) or by double staining with an antibody to ECP (in case of IL-10 expression analysis). The extent of extracellular matrix protein deposits was semiquantitatively analyzed (deposition: 0, none; 1, along dermal–epidermal junction; 2, in papillary dermis; and 3, in dermis). Immunofluorescence staining was evaluated by at least two independent, blinded investigators (D.S., N.R., S.S., M.L., S.H., and R.W.).

Statistical analysis

Mean values ($\pm$ SEM) are given for disease groups. To compare groups, one-way ANOVA was applied, and to describe the correlation between two variables, the Pearson's coefficient was determined. P values < 0.05 were considered statistically significant.

Results

Eosinophil distribution

The number and distribution of eosinophils assessed by immunofluorescence staining varied depending on the skin disease (Fig. 1A). Eosinophils that could easily be detected following H&E stainings mostly appeared round shaped (Fig. 1B). The infiltrates of AD, BP, DHS, and some tumors contained oval- and flat-shaped eosinophils in addition (Fig. 1C). In WS and HES, eosinophils were scattered throughout the dermis (Fig. 1D). Otherwise, eosinophils were part of the inflammatory infiltrate located perivascularly, intralesionally, or perilesionally (Fig. 1E,F). In BP, eosinophils lined up at the basement membrane and were also present in the blisters (Fig. 1G). Eosinophilic spongiosis of the epidermis was seen in PF (Fig. 1H). In WG, an eosinophil infiltration and destruction of blood vessels were seen (Fig. 1J). The accompanying infiltrate assessed by immunofluorescence staining varied considerably between diseases (Fig. 1K). A correlation between eosinophil numbers and the presence or number of other inflammatory cell types was not observed.

Eosinophils as potential regulators of inflammatory responses

To investigate the potential role in modulating skin inflammation, the cytokine expression by eosinophils was examined. We investigated TNF- α , IL-5, IL-6, IL-10, IL-11, IL-13, IL-25, interferon (IFN)- γ , TGF- β , and eotaxins CCL-11, CCL-24, and CCL-26 (Fig. 2). Eosinophils expressing the pro-inflammatory cytokine TNF- α were observed in acute inflammation such as DHS, LM, EPF, as well as in CTCL. By the production of IL-5, IL-13, and IL-25, eosinophils may augment Th2-immune responses. Highest numbers of IL-5-expressing eosinophils were observed in the group of allergic/reactive diseases compared with the other disease groups ($P = 0.034$). IL-13-expressing eosinophils were observed in allergic/reactive diseases, such as AD and APT, and autoimmune diseases. In ALHE, approximately 60% of the eosinophils were positive for IL-13, whereas no IL-13+

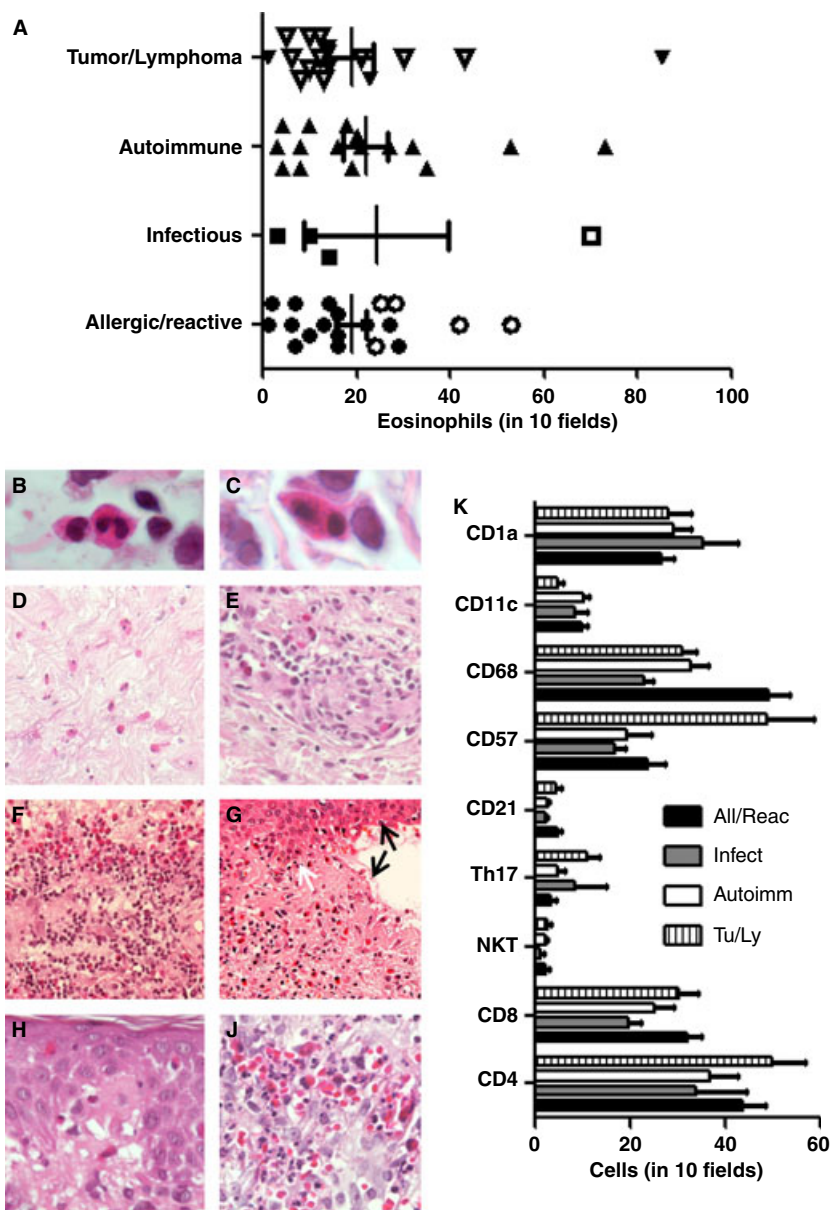


Figure 1 Eosinophil infiltration of the skin. (A) Numbers of eosinophils and means $\pm$ SE in disease groups. Diseases with particular high eosinophil numbers are indicated: Wells syndrome (WS), hypereosinophilic syndromes (open circle), larva migrans (LM) (open rectangle), pemphigus foliaceus (PF), eosinophil pustular folliculitis (open triangle), and solid tumors (closed triangle). (B) Round eosinophil. (C) Flattened eosinophil. (D) Interstitial eosinophils in WS. (E)

Eosinophils in the dermal perivascular infiltrate of atopy patch test reaction. (F) Dense eosinophil infiltrate in LM. (G) Eosinophil lining at dermo-epidermal junction (white arrow) and in the blister (black arrow) of bullous pemphigoid. (H) Eosinophil spongiosis in PF. (J) Eosinophilic vasculitis in Wegener's granulomatosis. Magnifications: B, C $\times 1000$; D–J $\times 400$. (K) Characterization of accompanying infiltrate in disease groups.

eosinophils were found in other tumors and infectious diseases (Table 1). Small numbers of IL-25-expressing eosinophils were detected in allergic/reactive and autoimmune diseases. In LCH, approximately 30% of the eosinophils expressed IL-25 (Table 1).

It has been shown that eosinophils can be an important source of TGF- β (8). In tumors/LY, the number of TGF- β -

expressing eosinophils was significantly higher compared with allergic/reactive diseases ($P < 0.005$). Because TGF- β is involved in the differentiation of Th17 cells, we correlated the number of TGF- β ⁺ eosinophils and Th17 cells. Despite the fact that in all specimens containing IL-17⁺ lymphocytes, TGF- β ⁺ eosinophils were also present, a correlation between the numbers of both cell types was not found. IL-10,

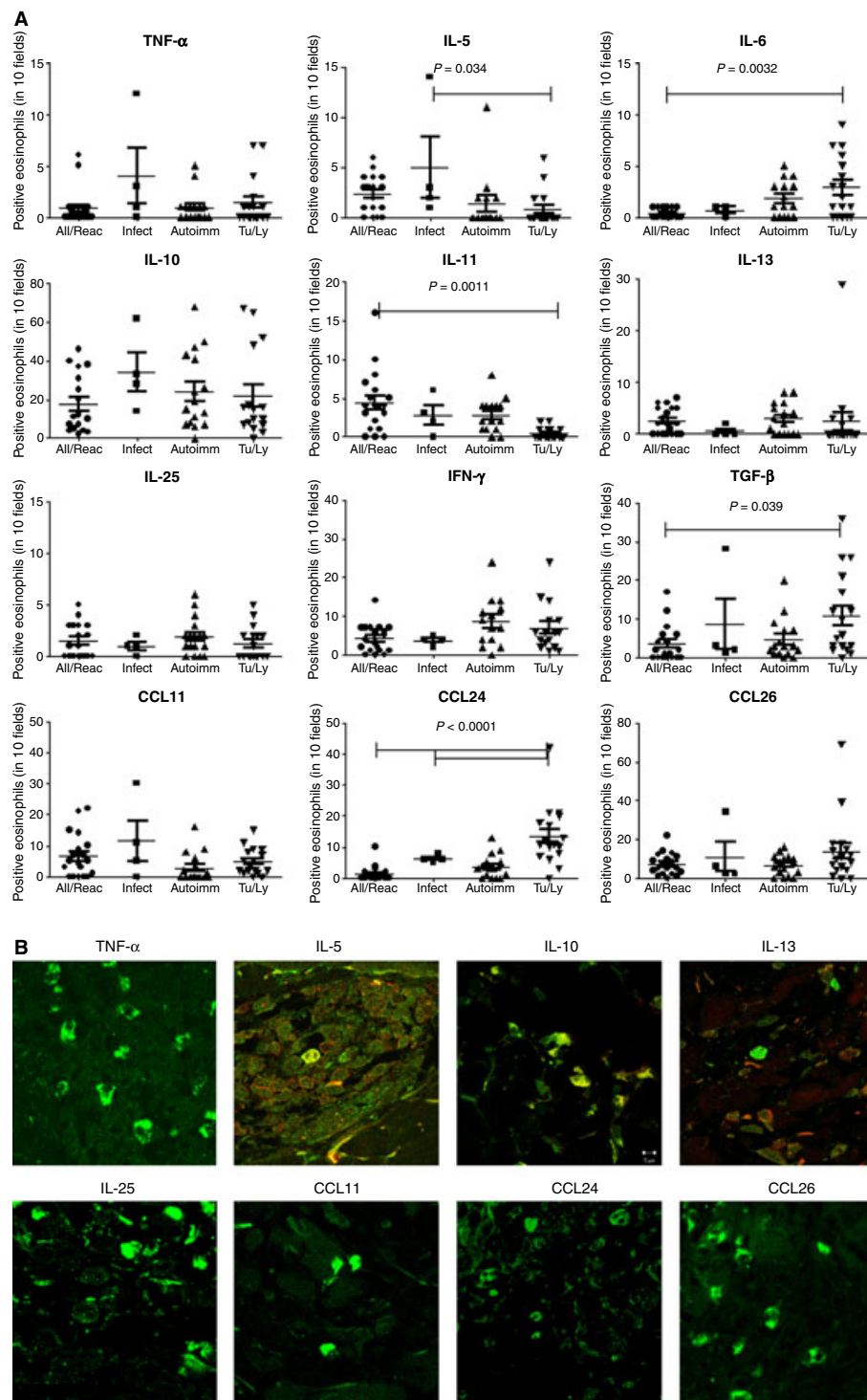


Figure 2 Cytokine expression by eosinophils. (A) Numbers and means $\pm$ SE of cytokine-expressing eosinophils in disease groups are given. Bars indicate significant differences between groups. (B) Representative images of eosinophils expressing tumor necrosis factor- α in cutaneous T-cell lymphoma, IL-5 together with CD4+ cells (red) in allergic contact dermatitis, IL-10 in atopy patch test

reaction (APT), IL-13 together with CD4+ cells (red) in atopic dermatitis (AD), IL-25 in APT, and eotaxins CCL-11 in AD, CCL-24 in dermatitis herpetiformis, CCL-26 in solid tumors. Cytokines/chemokines were stained green, and positive eosinophils were evaluated morphologically or by double staining with an anti-eosinophil cationic protein antibody (red). Magnification $\times 1000$.

Table 1 Patterns of cytokine expression by eosinophils in skin diseases

Disease group	Disease	TNF- α	IFN- γ	IL-5	IL-13	IL-25	IL-10	TGF- β	MMP-9	IL-6	IL-11	Eotaxins		
												CCL11	CCL24	CCL26
Allergic/reactive	AD, APT			+	+	+	++	+			+	++		+
	DHS	+					++	+			+			
	WS, HES						++					+		+
Infectious	ECTOP			+			++	+			+	+	+	
	LM	+		+			++	+				+		+
Autoimmune	BP		+		+		++	+	+					+
	DH		++		+	+	++	+	+	+	+	+		+
	EPF	+	++				++				+			
	MOR							+			+			
Lymphoma/tumor	TU						++			+		+	+	+
	CTCL	+	+				++	+		+		+	+	++
	ALHE		+		++		++	++					+	
	PLY		++				++							
	LCH		+			+	++			++				

Proportions of eosinophils expressing a particular cytokine to numbers of skin-infiltrating eosinophils are given (+, 20–50%; ++, >50%). AD, atopic dermatitis; ALHE, angiolymphoid hyperplasia with eosinophilia; APT, atopy patch test reaction; BP, bullous pemphigoid; CTCL, cutaneous T-cell lymphoma; DH, dermatitis herpetiformis; DHS, drug hypersensitivity; ECTOP, ectoparasitosis; EPF, eosinophil pustular folliculitis; HES, hypereosinophilic syndromes; INF, interferon; LCH, Langerhans cell histiocytosis; LM, larva migrans; MMP, metalloproteinase; MOR, morphea; PLY, pseudolymphoma; TGF, transforming growth factor; TNF, tumor necrosis factor; TU, solid tumors; WS, Wells syndrome.

which is constitutively produced by peripheral blood eosinophils (9), was expressed by the majority of skin-infiltrating eosinophils independent of the underlying disease (Fig. 2, Table 1).

In all skin diseases investigated, eosinophils expressed at least one of the eotaxins (Fig. 2, Table 1). In tumors/LY, significantly more eosinophils expressed CCL-24 compared with allergic/reactive and autoimmune diseases ($P < 0.0001$). Highest absolute numbers of eosinophils expressing CCL-11 were observed in AD, WS, and HES, while CCL-26 expression was highest in tumors/LY.

Eosinophils as potential regulators of fibrosis

Because there is evidence that eosinophils contribute to tissue remodeling and fibrosis (10, 11), we compared the number of eosinophils expressing CCL11, TGF- β , IL-6, IL-11, IL-13 or MMP-9 with the expression of procollagen-3 and tenascin-C in the skin (Fig. 3). Highest numbers of eosinophils expressing MMP-9 were observed in autoimmune diseases, while in allergic/reactive and infectious diseases, eosinophils expressing MMP-9+ could hardly be detected ($P = 0.042$). The numbers of eosinophils expressing IL-6 or IL-11 were minim (Fig. 2). The presence of IL-6+ eosinophils was restricted to tumors/LY ($P = 0.0032$). IL-11+ eosinophils were detected in allergic/reactive, infectious, and autoimmune diseases, but not in tumors/LY ($P = 0.011$). Although the number of eosinophils in MOR was low, approximately 30% of the eosinophils expressed IL-11.

Extensive tenascin-C deposition throughout the dermis was seen in PN, EPF, and PAN, whereas in APT, URT,

LCH, and EF, no tenascin-C deposits could be identified (Table 2). The extent of tenascin-C deposition correlated with the number of TGF- β + eosinophils ($P = 0.0257$) (Fig. 3B), whereas no correlation was found for IL-6, IL-11, IL-13, CCL11, and MMP-9 eosinophils. When the relationship between the numbers of tenascin-C-expressing cells in the dermis and eosinophils expressing profibrotic cytokines or MMP-9 was analyzed, a correlation between CCL11+ ($P = 0.028$) and MMP-9+ eosinophils ($P = 0.024$) was noted (Fig. 3C). However, a correlation between numbers of procollagen-3+ cells and eosinophils expressing IL-6, IL-11, IL-13, CCL11, TGF- β , or MMP-9 was not found.

Cytokines and chemokines regulating eosinophil recruitment to the skin

Eotaxins responsible for the recruitment and activation of eosinophils were expressed in all diseases examined. The numbers of eosinophils in the skin correlated with the numbers of cells expressing CCL11 ($P = 0.0047$), CCL24 ($P = 0.026$), and CCL26 ($P = 0.0006$) (Fig. 4A). Because IFN- γ (12) and IL-13 (13) were shown to play a role in eosinophilic inflammation, the number of tissue eosinophils was related to those of cells expressing IFN- γ and IL-13, revealing a correlation between eosinophils and IFN- γ -expressing cells ($P = 0.0084$) (Fig. 4A). The eosinophil-specific cytokine IL-5 was present in all diseases investigated independently whether there was a more Th1 (IFN- γ)- or Th2 (IL-13)-biased underlying immune reaction. However, the numbers of IL-5-expressing cells did not

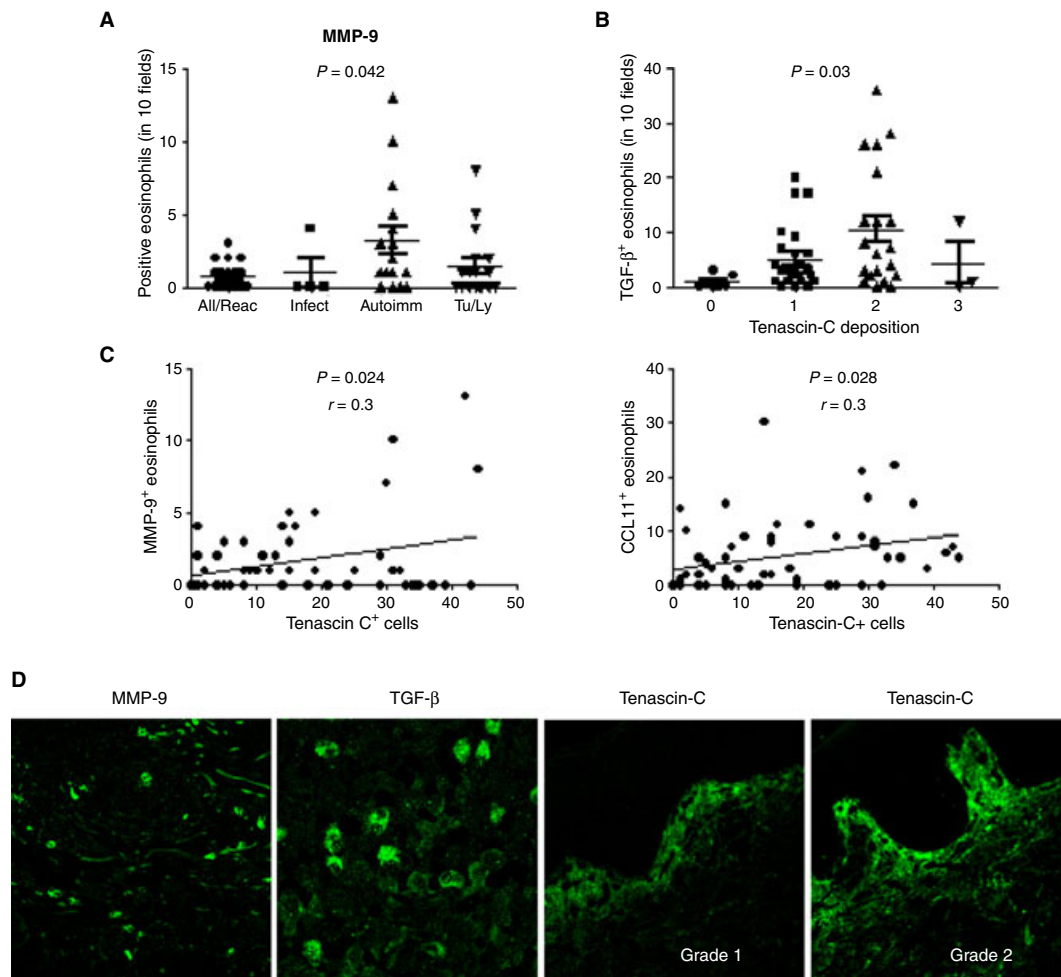


Figure 3 Expression of profibrotic cytokines and mediators by eosinophils. (A) Numbers and means $\pm$ SE of eosinophils producing metalloproteinase (MMP)-9 in disease groups. (B) Numbers of transforming growth factor (TGF)- β producing eosinophils in correlation with tenascin-C depositions (0, no; 1, along dermal–epidermal junction; 2, in papillary dermis; and 3, in dermis). (C) Correlation

between eosinophils expressing MMP-9 or CCL-11 and tenascin-C+ cells in the dermis. (D) Immunofluorescence staining of eosinophils expressing MMP-9 in larva migrans and TGF- β in cutaneous T-cell lymphoma ($\times 1000$) and of tenascin-C depositions at dermal–epidermal junction in bullous pemphigoid (grade 1) and in the papillary dermis in drug hypersensitivity (grade 2) ($\times 400$).

Table 2 Tenascin-C deposition in the skin

Deposition of tenascin	Grading	Examples of eosinophilic skin diseases	Predominant profibrotic cytokines
No tenascin deposits	0	URT, APT, LCH, EF	
Along dermal–epidermal junction	1	ACD, LM, WS, BP, PF, DH, CM	CCL-11, TGF- β , IL-13
Papillary dermis	2	AD, DHS, HES, ECTOP, MOR, CTCL, TU, ALHE	Eotaxin-1, TGF- β , IL-6, IL-11, IL-13
Dermis	3	PN, EPF, PAN	IL-11, TGF- β

ACD, allergic contact dermatitis; AD, atopic dermatitis; ALHE, angiolymphoid hyperplasia with eosinophilia; APT, atopy patch test reaction; BP, bullous pemphigoid; CM, cutaneous mastocytosis; CTCL, cutaneous T-cell lymphoma; DH, dermatitis herpetiformis; DHS, drug hypersensitivity; ECTOP, ectoparasitosis; EF, eosinophilic fasciitis; EPF, eosinophil pustular folliculitis; HES, hypereosinophilic syndromes; LCH, Langerhans cell histiocytosis; LM, larva migrans; MOR, morphea; PAN, polyarteritis nodosa; PF, pemphigus foliaceus; PN, prurigo nodularis; TGF, transforming growth factor; TU, solid tumors; URT, urticaria; WS, Wells syndrome.

correlate with the numbers of eosinophils in the skin (data not shown).

Because TSLP, IL-25, and IL-33 may directly affect eosinophils (14–16), we investigated their expression in the epidermis (Fig. 4). TSLP-positive keratinocytes were observed in the upper parts of the epidermis in AD and APT and, in addition, in the biopsies from ACD, URT, HES, and autoimmune bullous diseases. In most specimens, IL-25+ keratinocytes scattered throughout the epidermis were observed. A more intense staining in the upper part of the epidermis was seen in specimens of DH, EPF, DM, LCH, and PLY. IL-33+ cells were observed throughout the epidermis in ECTOP, WS, HES, BP, CTCL, and TU. In other diseases, e.g., AD, MOR, WG, a patchy distribution of IL-33+ cells in the epidermis was seen.

Specific patterns of cytokine expression by eosinophils

To characterize potential pathogenic functions, we analyzed the dominating cytokine expression by eosinophils in different skin diseases. By rating eosinophil cytokine expression, different patterns can be noticed (Table 1). In AD but also in autoimmune bullous diseases, eosinophils express Th2 cytokines IL-5, IL-13, and IL-25. In ALHE, striking high numbers of eosinophils expressing IL-13 and TGF- β were detected. Eosinophils expressing IL-11 predominated in allergic/reactive, infectious, and autoimmune diseases, but not in

tumors/LY. CCL26 expression was prominent in CTCL. IL-6-expressing eosinophils were detected most notably in LY/tumors.

Next, we analyzed whether certain cytokines were predominantly expressed by eosinophils (Table 3). In most skin diseases, IL-10 and TGF- β were mainly expressed by eosinophils. In LM, eosinophils represented the main proportion of cells expressing TNF- α , TGF- β , CCL11, CCL26, and MMP-9. In addition, eosinophils were the main source of TNF- α in EPF. In LY/tumors, all eotaxins were expressed mainly by eosinophils.

Discussion

Tissue-infiltrating eosinophils are a common feature in many skin diseases; still, their functional role needs to be elucidated. In this study, we observed that eosinophils usually expressed more than one cytokine. Moreover, the examination of the cytokine expression by eosinophils revealed distinct cytokine patterns, suggesting different functional roles of tissue-infiltrating eosinophils in skin diseases. In allergic/reactive skin diseases, in particular in AD, eosinophils were found to be an important source of IL-5, IL-13, IL-25, and CCL26 and thus may contribute to the Th2-biased immune reaction and eosinophil inflammation. Prolonged eosinophil survival was found to represent a key mechanism causing tissue eosinophilia in allergic diseases (17). By releasing IL-5,

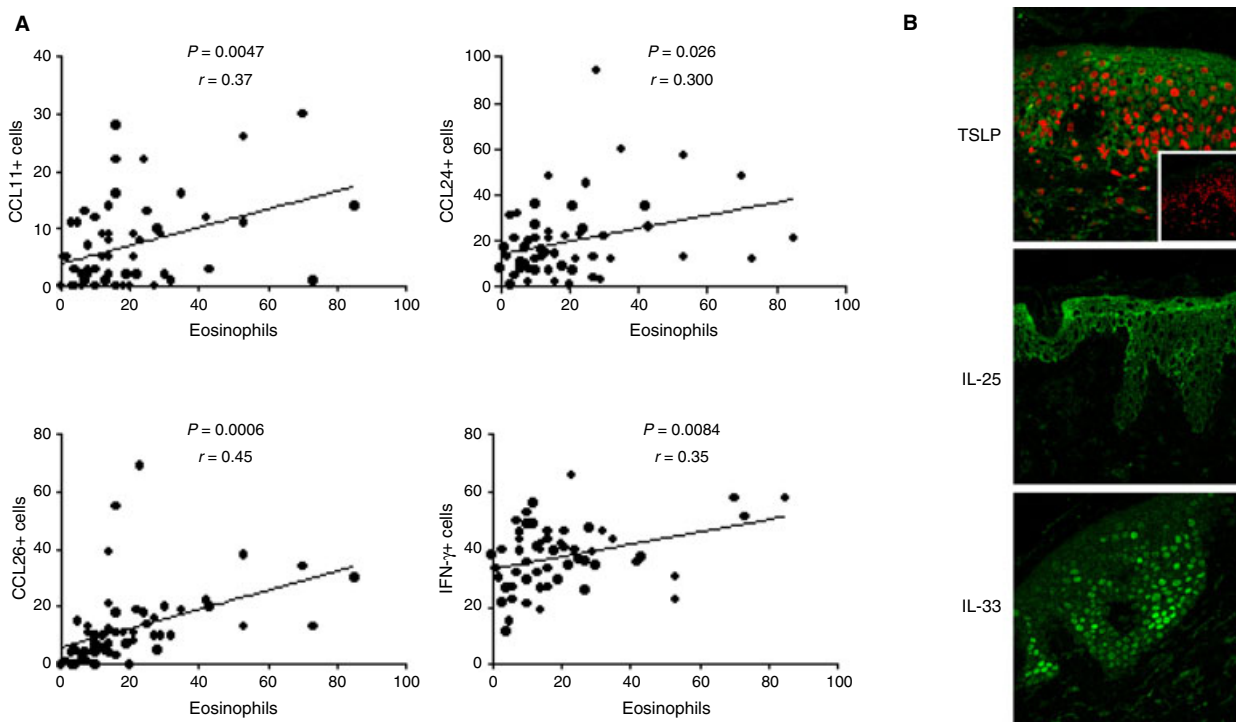


Figure 4 Cytokines mediating eosinophil recruitment. (A) Numbers of infiltrating eosinophils correlated with other inflammatory cells expressing eotaxins CCL-11, CCL-24, CCL-26, or interferon- γ . (B)

Epithelial expression of thymic stromal lymphopoietin in bullous pemphigoid, not in normal skin (insert), IL-25 in atopy patch test reaction, and IL-33 in morphea.

Table 3 Eosinophils as main cytokine-expressing cells in skin diseases

Disease group	Disease	TNF- α	IL-5	IL-13	IL-25	IL-10	TGF- β	MMP-9	Eotaxins		
									CCL-11	CCL-24	CCL-26
Allergic/reactive	AD, APT			+		+			++		
	DHS	++					++				
	WS, HES					+			+		
Infectious	ECTOP					+	+		++	+	+
	LM	+++	++			+++	+++	+++	+++		+++
Autoimmune	BP					+	++	+			++
	DH					++	++	+	++	+	+
	EPF	+++		+		+					
Lymphoma/tumor	TU					+	++		++	++	++
	CTCL					+	++	+	+++	++	+++
	ALHE			++		+	++	+		++	++
	PLY						+			++	++
	LCH				+	+	+			++	

Indicated are those cytokines, which were preferentially expressed by eosinophils. (The particular cytokine was expressed by eosinophils in +, 20–50%; ++, 51–90%; and +++, >90%).

AD, atopic dermatitis; ALHE, angiolymphoid hyperplasia with eosinophilia; APT, atopy patch test reaction; BP, bullous pemphigoid; CTCL, cutaneous T-cell lymphoma; DH, dermatitis herpetiformis; DHS, drug hypersensitivity; ECTOP, ectoparasitosis; EPF, eosinophil pustular folliculitis; HES, hypereosinophilic syndromes; LCH, Langerhans cell histiocytosis; LM, larva migrans; MMP, metalloproteinase; PLY, pseudolymphoma; TGF, transforming growth factor; TNF, tumor necrosis factor; TU, solid tumors; WS, Wells syndrome.

eosinophils were shown to prevent apoptosis in an autocrine fashion (18). Eosinophils were reported to produce and release functional IL-13, a cytokine that is highly expressed in lesional skin of AD (19, 20). IL-25 produced by eosinophils and basophils was shown to enhance the function of memory Th2 cells (21). CCL26 was reported to attract eosinophils, Th2 cells, and basophils via CCR3 receptor, whereas it inhibits the influx of Th1 cells by binding to CCR1 and CCR5 receptors (22, 23).

Furthermore, by expressing TGF- β , IL-11, IL-13, and CCL11, eosinophils may stimulate the production of extracellular matrix proteins and induce remodeling in allergic/reactive skin diseases. Skin-infiltrating TGF- β + eosinophils in association with tenascin-C- and procollagen-expressing cells were observed after intradermal allergen challenge (10). In chronic AD lesions, a significant correlation between IL-11 expression and collagen deposition as well as eosinophil numbers has been reported (24). IL-11 production by eosinophils was suggested to be mediated by an autocrine action of TGF- β (25). Eosinophils as well as TGF- β - and IL-13-stimulated fibroblasts express extracellular matrix proteins (10). CCL11 was shown to induce collagen synthesis and activate lung fibroblasts assuming a direct effect on remodeling (11). The reduction in eosinophils upon anti-IL-5 antibody therapy was associated with a decreased extracellular matrix protein deposition, indicating that eosinophils were actively involved in tissue fibrosis (26, 27).

In the autoimmune bullous skin diseases, eosinophils were demonstrated to express IFN- γ as well as IL-13 and IL-25. Consequently, eosinophils might contribute to the dual Th1–Th2 cytokine profile found in BP and DH (28, 29). According to our observations, TSLP, which has been related exclusively to allergic diseases so far (30), might be

involved in Th2 responses in autoimmune bullous diseases as well. By releasing MMP-9, eosinophils might directly be involved causing tissue damage (31). On the other hand, we found a correlation between the numbers of tenascin-C+ cells and MMP9+ eosinophils, suggesting that eosinophils stimulate extracellular matrix production in skin diseases. In earlier work, MMP-9 has been associated with tissue fibrosis (32).

In infectious skin diseases, remarkable TNF- α expression by eosinophils has been noted in LM pointing to a role of eosinophils in antimicrobial defense (33). Similar to allergic diseases (34), an autocrine stimulation of eosinophils by IL-5 may also occur in infectious diseases. Eosinophils were reported to be directly involved in helminth and bacterial killing (3, 6). On the other hand, eosinophils and their toxic granule proteins have also been accused to cause tissue damage (3). Moreover, eosinophils may initiate tissue repair by producing TGF- β , IL-11, and CCL11.

The high expression of the eotaxins CCL24 and IL-6 by eosinophils was a striking finding in skin tumors/LY. Interestingly, CCL24 and IL-6 were shown to activate fibroblasts (11, 35, 36). Thus, eosinophils may contribute to tumor fibrosis. Eotaxin expression in CTCL (37), Hodgkin LY (38), and TU (39) has been reported, indicating that malignant cells may attract eosinophils. So far, it is not clear whether eosinophils are protective or promote tumor growth. CTCLs were reported to express functional eotaxin receptor CCR3 (37, 40). Thus, eosinophils producing eotaxins might enhance the recruitment and retention of T cells in the skin and promote tumor progression. IL-6 was shown to stimulate malignant cell proliferation and survival in Hodgkin LY (41). Taking into account the dual functions of TGF- β , eosinophils expressing TGF- β might exert either tumor-suppressive or

tumor-promoting function (42). Moreover, the observation that highest numbers of eosinophils expressing IL-6 and TGF- β in association with Th17+ lymphocytes were observed in tumor/LY points to the possibility that eosinophils might be involved in Th17 cell differentiation influencing tumor development (43).

Taken together, our study implicates that eosinophils play different functional roles in skin diseases according to their cytokine expression. Moreover, it supports the view that different eosinophil subpopulations may exist under *in vivo* conditions in one individual/patient (44). Although the number of investigated specimens per disease or disease group was limited and the groups were heterogeneous, the results seem to indicate disease-specific patterns. Eosinophils may contribute to host defense, immunomodulation, fibrosis, and/or tumor development. Furthermore, the expression of IL-10

and/or TGF- β in all skin diseases suggests a major role of eosinophils in immunoregulatory and anti-inflammatory processes.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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