

Dermal eosinophils in atopic dermatitis undergo cytolytic degeneration

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Background: Immunofluorescent staining for eosinophil granule proteins in lesional skin of patients with atopic dermatitis shows extensive extracellular deposition throughout the upper dermis with relatively few intact eosinophils.

Objective: This study was carried out to determine whether eosinophil granule protein deposition in atopic dermatitis occurs by classical exocytosis, by piecemeal degranulation, or as a result of cytolysis.

Methods: Skin biopsy specimens from 10 patients with atopic dermatitis were examined by electron microscopy.

Results: The biopsy specimens showed varying degrees of dermal eosinophil granule major basic protein deposition by indirect immunofluorescence. Specimens from seven patients showed striking alterations of eosinophils by electron microscopy including intact eosinophils with granule alterations (reversal of core staining and/or core lucency) and with uropod processes. Biopsy specimens from six patients showed evidence of eosinophil degeneration with disruption of nuclear and/or plasma membranes. In four patients' specimens, membrane-bound eosinophil granules were present near degenerating eosinophils or were present in the absence of recognizable eosinophils. Evidence of classical exocytotic degranulation was not observed. Two of the specimens were also examined by immunoelectron microscopy for major basic protein localization. In these, major basic protein appeared to be lost from the granule core and distributed in the eosinophil cytoplasm as granules disintegrated and the cell disrupted.

Conclusion: These findings support the hypothesis that eosinophils undergo cytolysis with release of granule contents and membrane-bound granules; this is likely the usual mechanism of eosinophil granule protein release in atopic dermatitis. (*J Allergy Clin Immunol* 1997;99:683-92.)

Key words: Eosinophil, atopic dermatitis, MBP, cytolytic degeneration, electron microscopy, granule, uropod

Atopic dermatitis is a common chronic inflammatory disease. It is characterized by pruritic, eczematous lesions and affects 10% to 15% of the population.¹ Diag-

Abbreviations used

MBP: Major basic protein
TBS-T: Tris-buffered saline with Tween-20

nostic criteria have been established.^{2,3} Atopic dermatitis occurs early in life, usually in infancy and childhood, and tends to improve with age; however, atopic dermatitis may have a variety of clinical presentations throughout life. Allergic associations are prominent and include markedly elevated IgE levels.³ The pathogenesis of atopic dermatitis remains speculative. Bone marrow transplantation studies have demonstrated transmission of atopic dermatitis from atopic donors⁴ and suggest that the basic defect is carried in bone marrow-derived cells, which infiltrate the skin. Several cells likely play a role in the inflammation, including mast cells, Langerhans cells, lymphocytes, basophils, and eosinophils.^{5,6} The late phase of the IgE-mediated reaction has been proposed as a model of chronic allergic inflammation in atopic dermatitis.⁷ However, as determined by immunohistology, late-phase reactions differ from atopic dermatitis lesions in that the late-phase reaction shows neutrophil elastase deposition and atopic dermatitis does not; both show prominent eosinophil granule protein deposition.^{8,9}

Investigations of eosinophils have shown that they are important effectors of inflammation through release of toxic cationic granule proteins.¹⁰ Peripheral blood eosinophilia is commonly observed in patients with atopic dermatitis, but histologic specimens of atopic dermatitis lesions show a paucity or an absence of eosinophils by hematoxylin-eosin staining.^{11,12} Yet, by immunohistologic staining, eosinophil granule proteins are extensively deposited extracellularly throughout the dermis.^{9,13} Several studies have shown a relationship between the eosinophil and atopic dermatitis disease activity.^{9,14} Some reports have shown that peripheral blood eosinophils are increased in severe disease^{15,16} and that decreased peripheral blood eosinophils accompany therapeutic improvement.¹⁷⁻¹⁹ Other reports have shown a positive correlation between eosinophil granule protein levels in blood and disease activity.* The mechanism of eosinophil degranulation culminating in the deposition of granule proteins in atopic dermatitis skin is

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*References 9, 14, 16, 17, and 19-24.

TABLE I. Eosinophils in atopic dermatitis

Patient No.	Age (yr)	Gender	Serum IgE (U/ml) (Normal, < 367)	Serum MBP (ng/ml) (normal, 87-287) (mean = 185, SD = 46)	Body surface involvement (%)	Tissue MBP extracellular* (normal, 0)	Electron microscopy					Total identifiable eosinophils†
							Normal eosinophils	Eosinophils with abnormal granules	Eosinophils with abnormal granules and uropods	Loss of cytoplasmic and/or nuclear membrane (>75%)	Regions of free eosinophil granules	
1	39	M	23,738	1,264	70	+3	0	10	10	30	10	50
2‡	11	F	76	292	50	0	0	0	0	0	0	0
3	52	M	8,250	778	80	+2	0	2	6	4	2	12
4	30	F	1,917	2,430	20	+3	0	0	0	0	4	0
5‡	10	F	459	398	75	+2	0	1	1	1	0	3
6‡	31	F	27,002	334	45	+2	0	2	6	2	3	10
7‡	9	M	1,257	389	30	+1	0	0	0	0	0	0
8‡	50	M	9,557	300	45	+1	0	1	0	1	0	2
9	41	M	10,281	385	70	+1	0	3	1	2	0	6
10‡	39	M	8,400	337	60	+1	0	0	0	0	0	0
Total							0	19	24	40	19	83

SD, Standard deviation

*Extracellular protein deposition based on intensity and extent of fluorescence in tissue.

†Sum of normal eosinophils, intact eosinophils with granule abnormalities, intact medusa cells, and cells with loss of cytoplasmic and/or nuclear membranes; note that regions of free granules are not included in the total.

‡Patients 2, 5, 6, 7, 8, and 10 in this study were patients 6, 16, 12, 4, 7, and 11 in the study by Ott et al.⁹

unknown. In this study we examined eosinophil degeneration in atopic dermatitis lesions by electron microscopy, and we present evidence that eosinophils undergo cytolytic degeneration in the skin of patients with atopic dermatitis.

METHODS

Ten patients satisfying the diagnostic criteria for atopic dermatitis² were studied. Each patient had chronic recurrent disease for years, with active disease at the time of biopsy. Specimens of lesional skin from the 10 patients with atopic dermatitis were obtained by 5 or 6 mm punch biopsy from chronic lesions, to which no or mild topical glucocorticoids (class 4 or greater²⁵) had been applied, which were not responding to treatment. Approximately 2 mm thick portions of each biopsy specimen were fixed in Trump's fixative²⁶ (1% glutaraldehyde and 4% formaldehyde in 0.1 mol/L phosphate buffer, pH 7.2). After fixation, the tissue was washed for 30 minutes thrice in 0.1 mol/L phosphate buffer, pH 7.2. The tissue was postfixed in 1% osmium tetroxide in phosphate buffer for 1 hour. The tissue was thrice washed again in distilled water for 30 minutes and stained en bloc with 2% uranyl acetate for 30 minutes at 60° C. The tissue was washed three additional times in distilled water, dehydrated in progressive concentrations of ethanol and 100% propylene oxide, and embedded in Spurr's resin.²⁷

Thick sections of tissue were cut, stained with toluidine blue, and examined by bright field microscopy. Thin (700 Å) sections were cut from the dermis where inflammatory cell infiltration was identified on the thick sections, mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with a JEM-1200 Ex II transmission electron microscope (JEOL Ltd., Tokyo, Japan). Representative electron photomicrographs (approximately 600) were taken. Eosinophils were enumerated by morphologic appearance in tissue and included

normal-appearing eosinophils, intact eosinophils with granule abnormalities, eosinophils with uropod processes, eosinophils with loss of cytoplasmic and/or nuclear membrane, and free eosinophil granules.

Tissue sections were also examined after indirect immunogold staining²⁸ for eosinophil granule major basic protein (MBP). Thin sections were mounted on 200 mesh nickel grids, which were floated on saturated sodium metaperiodate for 40 minutes and thrice rinsed with distilled water for 30 minutes. Nonspecific binding sites were blocked by incubating the sections in Tris-buffered saline containing 0.1% Tween-20 (TBS-T), 1% ovalbumin, and 1% normal goat serum. After blocking, the grids were incubated in affinity chromatography-purified rabbit MBP antibody (271 µg/ml) diluted 1:4 in TBS-T for 2 hours at room temperature. Control grids were incubated with normal rabbit IgG and with TBS-T alone. Sections were washed thrice for 30 minutes with TBS-T and incubated in goat anti-rabbit IgG conjugated to 15 nm of colloidal gold (Amersham, Arlington Heights, Ill.) diluted 1:100 in TBS-T for 1 hour at room temperature. The grids were washed thoroughly, at least four times with TBS-T, followed by at least four washes with distilled water. The sections were stained with uranyl acetate and lead citrate and examined with a Philips CM-10 transmission electron microscope (Philips Electronic Instruments, Endhoven, Netherlands).

IgE levels in patients' sera were measured by using the Quanticlone IgE Radioimmunoassay (Kallestad Diagnostics, Chaska, Minn.). Eosinophil granule MBP levels were measured by using a two-site immunoradiometric assay with protein A-purified mouse monoclonal antibodies showing optimal MBP binding, sensitivity and discrimination ranges, and nonspecific binding, as previously described.²⁹ An indirect immunofluorescent staining method was used to detect the presence of MBP in formaldehyde-fixed, paraffin-embedded portions of the skin biopsy specimens as described previous-

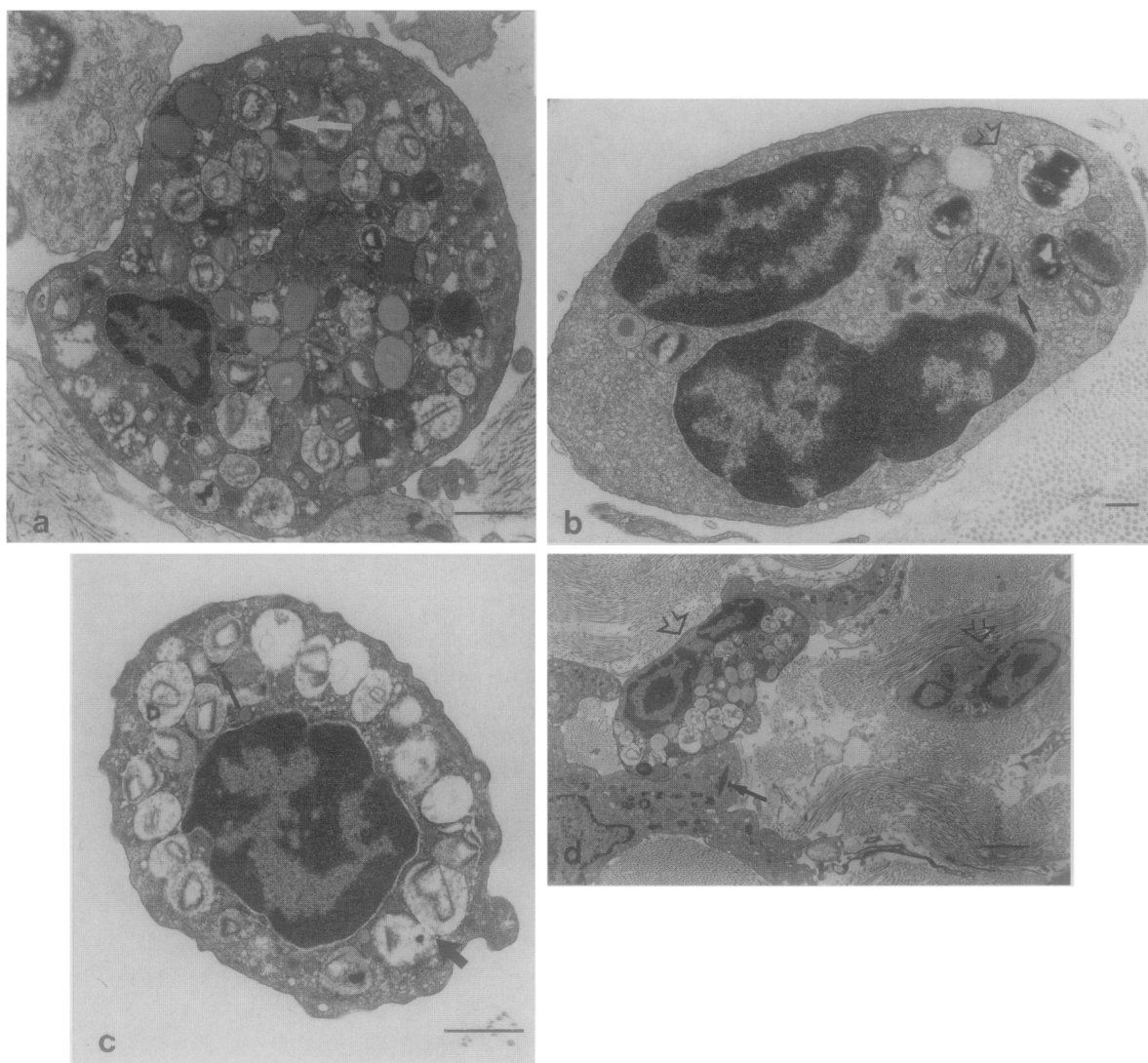


FIG. 1. Electron photomicrographs of abnormal, intact eosinophils in the dermis from lesional skin of patients with atopic dermatitis. **a**, Intact eosinophil with multiple abnormal cytoplasmic granules from patient 1. Granules show vacuolization and matrix lucency. Electron-dense core-like material appearing as "dark clouds" is present about granules (white arrow). Scale bar = 1 μ m. **b**, Mainly intact eosinophil with few abnormal cytoplasmic granules from patient 9. Granule membranes are distinct and mainly intact. Granules show core loss, as well as matrix lucency. "Dark clouds," especially prominent adjacent to one granule, are present (closed arrow). Cytoplasmic vesicles are also present (open arrow). Scale bar = 500 nm. **c**, Mainly intact eosinophil with markedly abnormal cytoplasmic granules from patient 8. Granules are almost empty with remnants of lucent cores and empty matrices. "Dark clouds" are present (thin arrow). Some granules may be fusing (thick arrow). Granule membranes are not distinct. Vesicles are present in the cytoplasm. Scale bar = 1 μ m. **d**, Lower-power view of mainly intact eosinophils (open arrows) in dermis from patient 1. Cytoplasmic granules in the central eosinophil are almost empty, with loss of both core and matrix contents. This eosinophil is directly adjacent to a cell containing a Charcot-Leyden crystal (closed arrow). The other eosinophil in the field is paucigranular; the few identifiable granules are abnormal, with loss of core and vacuolization. Scale bar = 2 μ m.

ly.^{3, 9, 13, 30} The stained sections were evaluated microscopically and assigned a grade from 0 to +4 for extracellular MBP localization on the basis of the intensity and extent of the fluorescent staining throughout the skin biopsy section, an area 6 to 9 mm². Visual analog grades were scored as follows for extracellular deposition of MBP on each tissue section: 0, no extracellular protein deposition; +1, focal areas of extracellular

protein deposition; +2, focal but dense areas of extracellular protein deposition; +3, extensively distributed areas of extracellular protein deposition; and +4, massive confluent areas of extracellular protein deposition.

Various parameters recorded for each patient were compared by Spearman's rank correlation (Stat View II; Abacus Concepts, Inc., Berkeley, Calif.) to analyze relationships. These

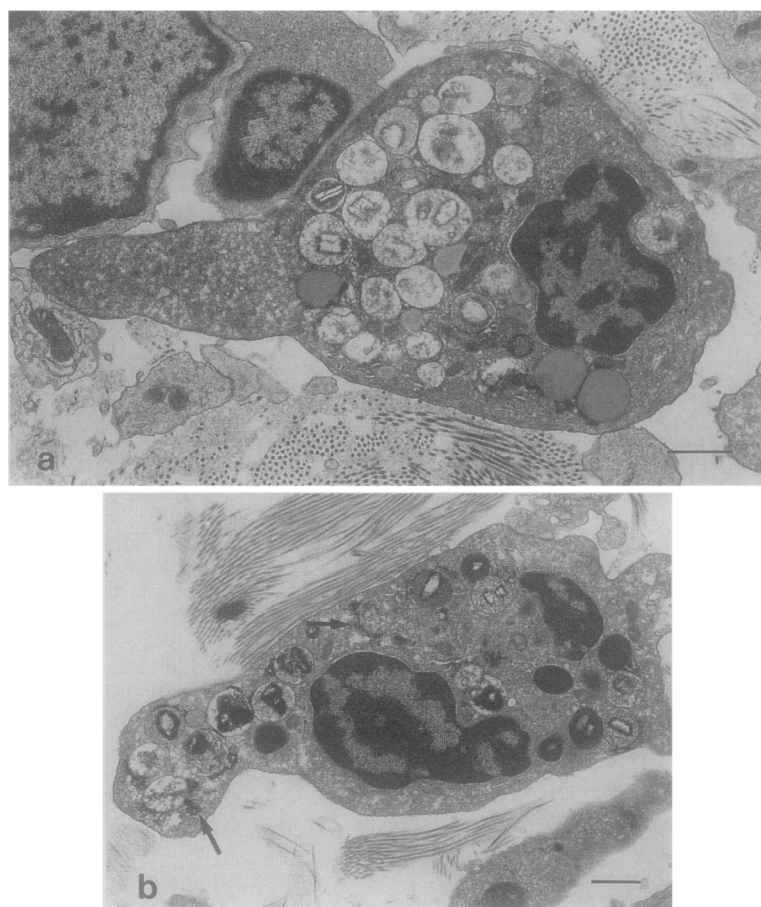


FIG. 2. Electron photomicrographs of eosinophils with uropods. **a**, Intact eosinophil with prominent cytoplasmic projection and abnormal granules from patient 1. Scale bar = 1 μ m. **b**, Intact eosinophil with cytoplasmic projections from patient 3. Cytoplasmic granules are abnormal and extend into the cytoplasmic projections. "Dark clouds" (arrows) are seen outside of granules with core lucency. Scale bar = 1 μ m.

parameters included serum IgE, serum MBP, tissue MBP deposition, total numbers of identifiable eosinophils in each patient's specimen, numbers of intact (albeit abnormal) eosinophils, and numbers of obviously degenerated eosinophils.

RESULTS

A summary of findings from the 10 patients with atopic dermatitis is shown in Table I. Six of the patients were included in another report.⁹ Nine of the patients had elevated IgE levels. All 10 had increased serum MBP levels compared with serum levels from a control group of 50 blood bank donors with no history of smoking, asthma, or allergies; seven of the sera showed slight MBP elevations, and three had marked elevations (patients 1, 3, and 4). Indirect immunofluorescence of the skin biopsy specimens from chronic lesions showed varying degrees of dermal MBP deposition, ranging from none to +3. As determined by electron microscopy, three of the specimens (from patients 2, 7, and 10) did not show eosinophils or eosinophil granules; of these, the specimen from patient 2 had no MBP depo-

sition in tissue, and specimens from patients 7 and 10 showed +1 (minimal, focal) MBP deposition by immunofluorescence. Six specimens (from patients 1, 3, 5, 6, 8, and 9) showed striking abnormalities, which included intact eosinophils with abnormal granules (reversal of core staining and/or core lucency) (Fig. 1). Five of these six specimens showed eosinophils with uropods^{31, 32} (Fig. 2), and all six showed eosinophil disruption with loss of nuclear and/or plasma membranes (Fig. 3). Eosinophil granules in areas around degenerating eosinophils were also observed, as were granules between collagen bundles without recognizable adjacent eosinophils (Fig. 4). The specimen from patient 4 showed only free eosinophil granules in the dermis. Fifty-nine areas from the seven patients' specimens showed either markedly degenerated eosinophils or regions of free granules. Evidence of classical exocytotic degranulation was not observed in 83 eosinophils examined in the specimens from these 10 patients.

Eosinophil granule MBP was localized by immunoelectron microscopy in two patients' specimens (patients

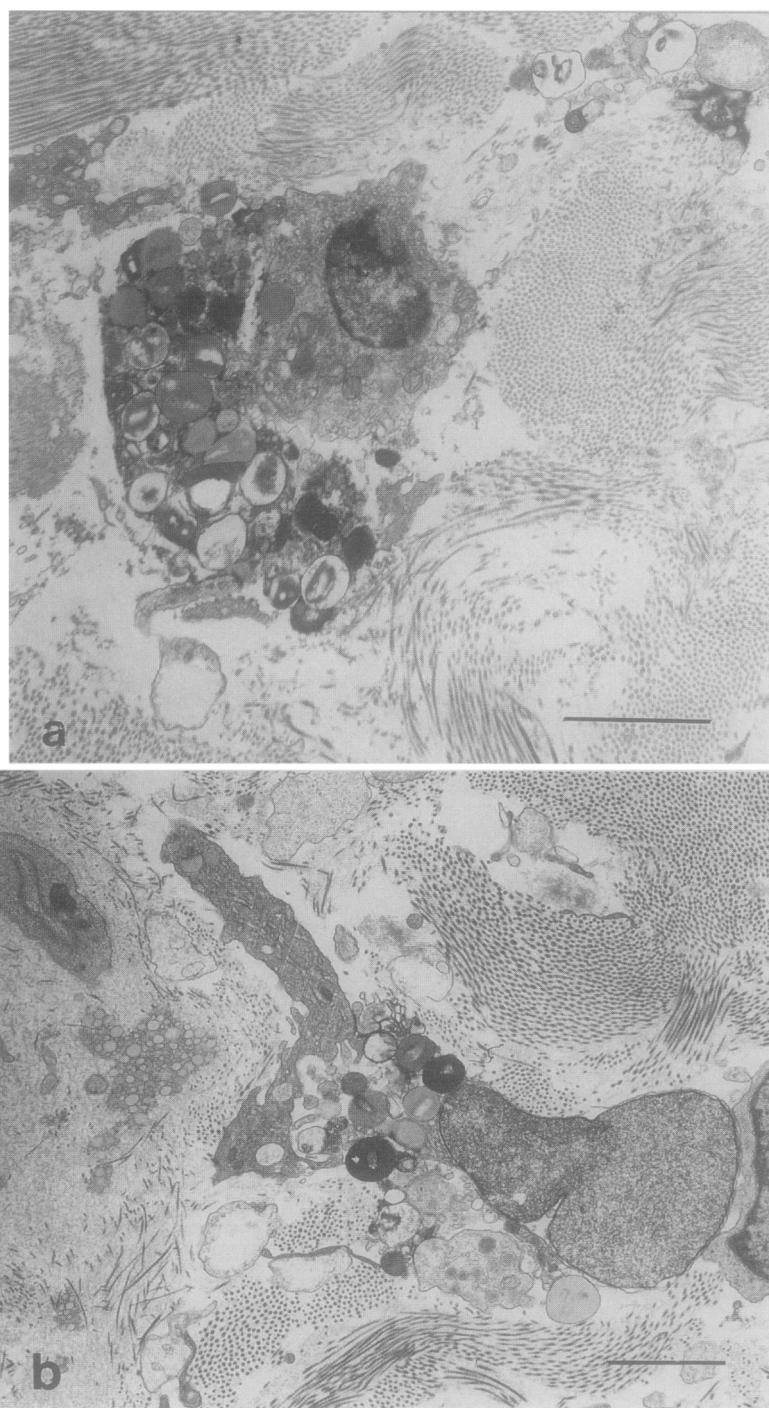


FIG. 3. Electron photomicrographs of degenerating eosinophils with loss of cytoplasmic and/or nuclear membranes. **a**, Eosinophil no longer contained by a cell membrane with markedly abnormal granules showing core lucency, matrix lucency, and vacuolization (from patient 1). Scale bar = 2 μ m. **b**, Degenerated eosinophil with loss of cytoplasmic membrane (from patient 1). Chromatolysis of the nucleus is also apparent. Abnormal eosinophil granules are in contact with the extracellular space. Scale bar = 2 μ m.

1 and 3). Analysis of largely intact eosinophils showed localization of MBP to the granule core, except in areas where the granule core had become indistinct; here, MBP was distributed throughout the granule substance

(Fig. 5, *a*). Other eosinophils had more marked granule abnormalities (Fig. 5, *b*). Lucent areas were present within granule cores, and MBP was distributed on either side of the lucent areas. In one area, granules appeared

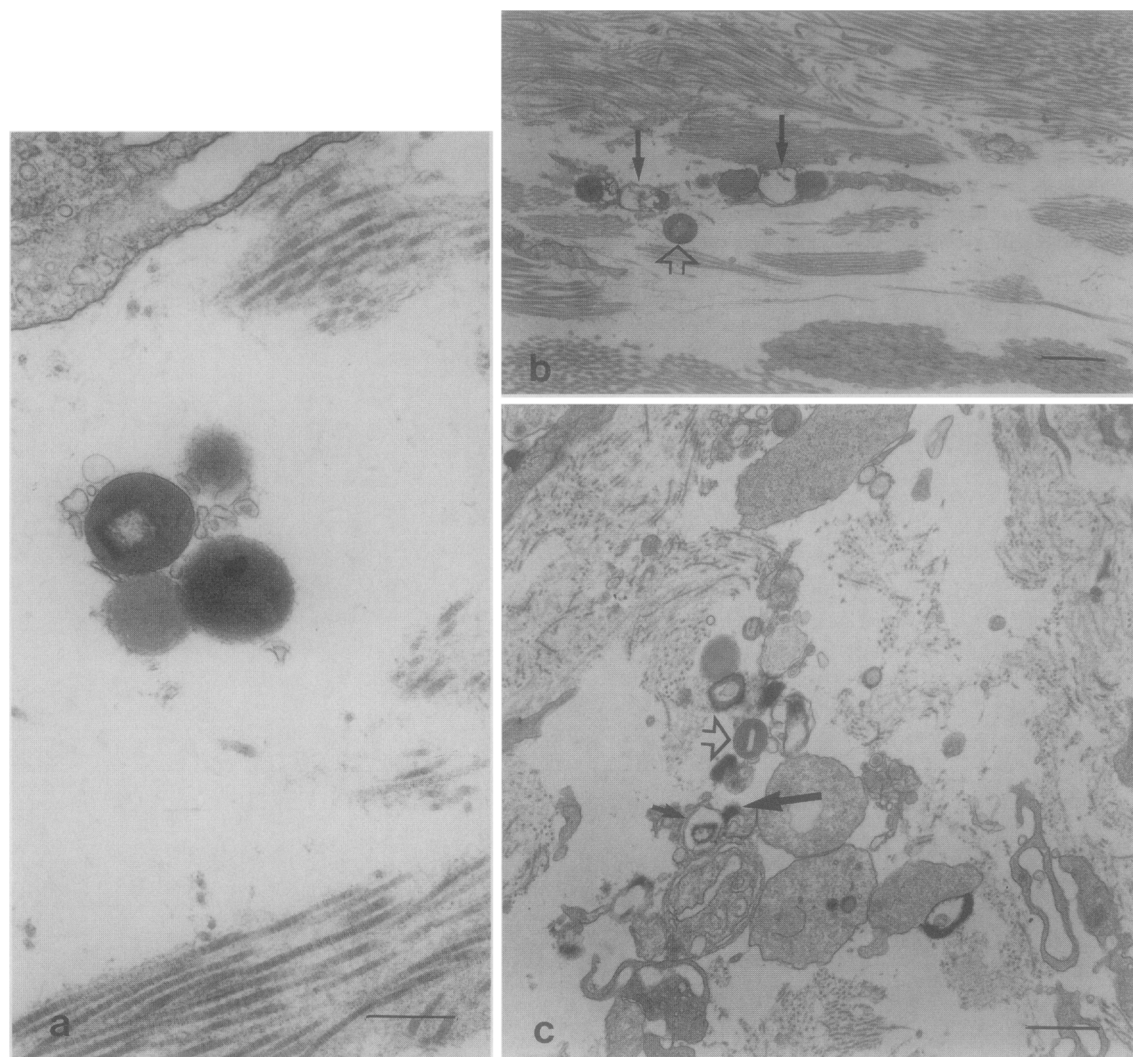


FIG. 4. Electron photomicrographs of free abnormal eosinophil granules without recognizable adjacent eosinophils. **a**, Eosinophil granules outside a cell between collagen bundles (from patient 3). Scale bar = 500 nm. **b**, Free eosinophil granules between collagen bundles (from patient 6). Granules appear to be membrane-bound, and two are largely empty (*closed arrows*). One shows core lucency (*open arrow*). Scale bar = 1 μ m. **c**, Free eosinophil granules in dermis (from patient 1). Granules are membrane-bound and markedly abnormal. One is largely empty with a remaining partially lucent core (*short closed arrow*); another shows prominent core lucency (*open arrow*). "Dark clouds" are present (*long arrow*). Scale bar = 1 μ m.

TABLE II. Spearman's rank correlation coefficient ($n = 10$)

Serum	Tissue	r_s Value	p Value
MBP	MBP deposition by immunofluorescence	0.82	0.0139
IgE	Intact eos by EM	0.66	0.0461
IgE	Degenerated eos by EM	0.65	0.0496

eos, Eosinophils; *EM*, electron microscopy.

Intact eos by EM: normal eosinophils plus eosinophils with granule abnormalities plus eosinophils with abnormal granules and uropods, as observed by electron microscopy.

Degenerated eos by EM: eosinophils with loss of cytoplasmic and/or nuclear membranes, as observed by electron microscopy.

to have fused, forming a large sac. In other areas, gold particles were localized in the apparent absence of intact granules. MBP localization was also observed in eosinophils that had lost plasma membranes and had undergone nuclear chromatolysis (Fig. 5, c). In these, MBP was distributed in a layer outlining the central lucent areas in the granule cores. Control sections incubated with normal rabbit IgG showed sparse and widely scattered immunogold particles without specific localization; controls incubated with buffer alone showed no immunogold particles.

Significant correlations among the parameters compared are summarized in Table II. Serum MBP corre-

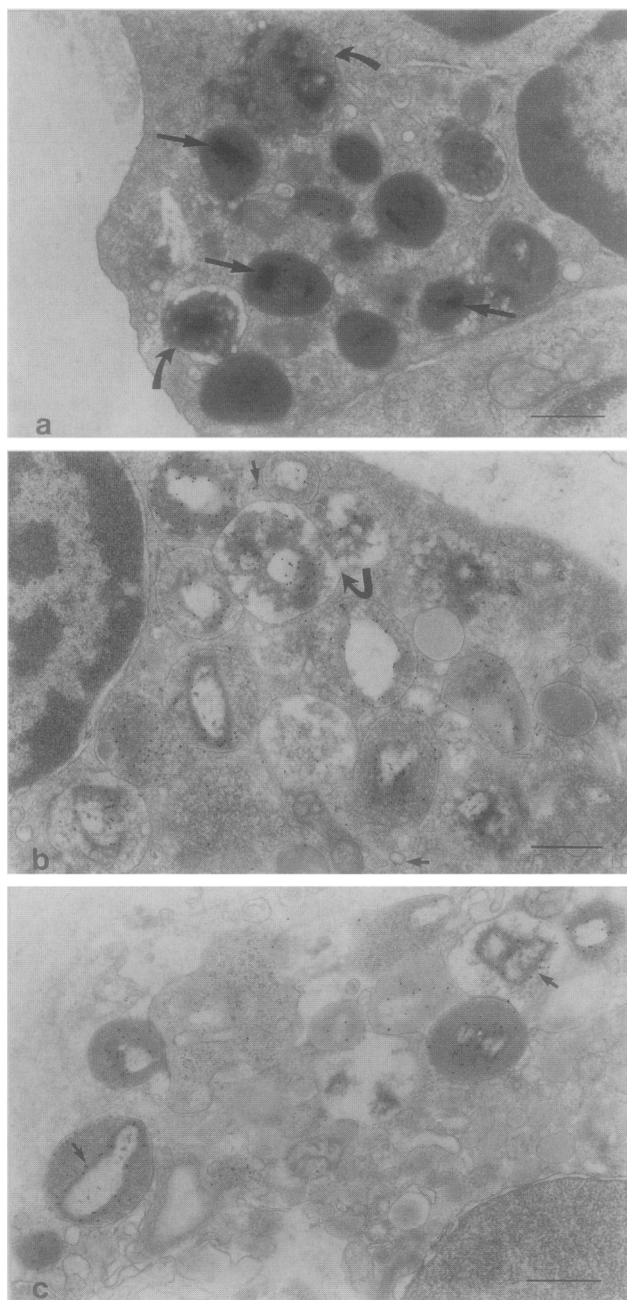


FIG. 5. Localization of MBP by immunoelectron microscopy. **a**, Eosinophils with largely intact granules showing localization of gold particles predominantly to the granule core (*straight arrows*). Several granules (*curved arrows*) show loss of definition of the core, and gold particles are scattered throughout the granule structure. Scale bar = 500 nm. **b**, Intact eosinophil showing more marked granule abnormalities with formation of clear areas within the core region and with deposition of MBP around the core areas. Occasional MBP deposition is seen in the cytoplasm in the absence of membrane-bound structures. In some areas, gold particles are localized on vesicles (*short arrows*). Granules may have fused (*curved arrow*). Scale bar = 500 nm. **c**, Eosinophil granules in a degenerating cell with deposition of MBP around the center of lucent granules (*arrows*). In some granules, the matrix is still intact, whereas in others the granule matrix has become lucent. In this eosinophil, the cell membrane has been lost. Nuclear chromatolysis is present. Scale bar = 500 nm.

lated with tissue MBP deposition by indirect immunofluorescence ($r_s = 0.82$, $p = 0.0139$). Serum IgE levels correlated weakly with both intact eosinophils ($r_s = 0.66$, $p = 0.046$) and degenerated eosinophils ($r_s = 0.65$, $p =$

0.049) in tissue identified by electron microscopy. Although the number of patients studied was small so that conclusive pathogenetic interpretations from this relationship could not be made, the two patients with the highest

IgE levels (patients 1 and 6) had among the greatest numbers of eosinophils identified in their tissues.

DISCUSSION

The histopathologic changes in skin lesions of atopic dermatitis are variable, depending on the lesional stage. Acute lesions show intracellular edema of the epidermis (spongiosis) and dermal inflammatory cell infiltration with lymphocytes, macrophages, and rare eosinophils and neutrophils.^{11, 12} Chronic lesions show epidermal hyperplasia with minimal spongiosis and dermal inflammatory cell infiltration consisting of lymphocytes and monocytes-macrophages.^{11, 12} Although eosinophils are found in both acute and chronic atopic dermatitis lesions, with slightly greater numbers in chronic lesions,³³ they are not a prominent component of the cellular infiltration. However, striking deposition of eosinophil granule proteins has been found in chronic lesions of atopic dermatitis.^{9, 13, 14} Compared with uninvolved skin, both acute and chronic lesions of atopic dermatitis have greater numbers of cells expressing messenger RNA for IL-4 and IL-5 as determined by *in situ* hybridization; chronic lesions compared with acute lesions showed fewer IL-4 mRNA-expressing cells and greater IL-5 mRNA along with increased eosinophil infiltration.³³ Although IL-4 mediates various immunologic effects, including the induction of IgE receptors on Langerhans cells³⁴ and the regulation of IgE synthesis influencing the switch of IgM to IgE production in B cells,³⁵ IL-5 has specific effects on eosinophils. IL-5 induces eosinophilopoiesis,³⁶ eosinophil activation,³⁷ and eosinophil chemotaxis³⁸; it likely accounts for the common presence of eosinophils and products of activated eosinophils in allergic reactions.

The eosinophil contains several cationic granule proteins including MBP, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin, which are released with cell activation.¹⁰ These proteins are potent toxins to various targets including helminths, protozoa, bacteria, and normal mammalian cells. In addition, they stimulate basophil and mast cell mediator release and activate neutrophils.¹⁰ Moreover, the granule proteins potentially cause vascular permeability.³⁹ The oxidative products of eosinophils include superoxide anions, hydroxyl radicals, and singlet oxygen, which also damage cells. The activated eosinophil produces small molecular weight mediators such as platelet activating factor and eicosanoids, including leukotriene C₄, which induce vasodilatation and have other inflammatory activities. Therefore the eosinophil has a variety of products that implicate its participation in mediating disease.

On the basis of the recognition that tissue-damaging eosinophil granule proteins are deposited in chronic atopic dermatitis lesions and that mRNA for an eosinophil-active cytokine, IL-5, is also present, the mode of eosinophil granule protein release in chronic atopic dermatitis was sought. Three mechanisms of eosinophil degranulation in particular were considered: (1) classical granule exocytosis with release of granule contents to

the exterior of the cell through fusion of granule membranes with the cell membrane and extrusion of granule contents, (2) release of granules through cytolytic degeneration of eosinophils with deposition of membrane-bound granules on the exterior of the cell, and (3) piecemeal degranulation. Piecemeal degranulation, a term coined by Dvorak et al.,⁴⁰ has been used to describe the gradual emptying process of granule contents through vesicles from basophils and mast cells. Dvorak et al.,⁴¹ in studies of mature eosinophils induced from umbilical cord blood cells in IL-5-supplemented, phytohemagglutinin-stimulated, T-cell-conditioned media found that eosinophils could undergo extensive piecemeal degranulation characterized by partially empty and completely empty secondary cytoplasmic granule chambers. Small, smooth vesicles were evident within the empty granule chambers, as well as adjacent to them, and eosinophils formed close associations with phagocytic macrophages in the culture system. They further showed that vesicular transport of eosinophil peroxidase from the granule matrix compartment to the cell surface was associated with piecemeal degranulation.⁴² In these culture systems, damaged or dead eosinophils with subsequent release of free membrane-bound granules were not observed.

Ultrastructural analyses of human eosinophils, studied both *in vitro* and *in vivo*, have shown a variety of alterations in specific eosinophil granules. For example, focal and complete losses of specific granule cores in tissue eosinophils have been found in bowel samples from patients with Crohn's disease.⁴³ Matrix alterations, including loss of density and accumulation of vesicles in granule membranes, have also been observed.⁴⁴ Moreover, eosinophil secretion by exocytosis was identified ultrastructurally in intestinal biopsy specimens from patients with intestinal diseases including ulcerative colitis and Crohn's disease.⁴⁵ Although eosinophil degeneration was not observed ultrastructurally in eosinophil cultures stimulated from human cord blood,⁴¹ disrupted eosinophils have been observed ultrastructurally in the syndrome of episodic angioedema associated with eosinophilia⁴⁶ and in the IgE-mediated late-phase reaction,⁸ and eosinophil necrosis was found in skin lesions in the hypereosinophilic syndrome⁴⁷ and bullous pemphigoid.⁴⁸

In the electron microscopic analysis reported here, seven of the 10 lesional atopic dermatitis biopsy specimens contained eosinophils and/or eosinophil granules. No normal-appearing eosinophils were present, and various stages of eosinophil degeneration were observed including intact eosinophils with abnormal granules, intact eosinophils with granule abnormalities and pseudopod-like extensions (uropods), eosinophils with disrupted cell membranes and/or nuclear membranes, and free eosinophil granules in the dermis near or in the absence of degenerating eosinophils. Inspection of approximately 600 electron photomicrographs showed no evidence of classical granule exocytosis. In contrast, evidence for eosinophil cytolysis was striking. Eosinophil

granules lost their integrity, and MBP became distributed in the cytoplasm near degenerating or intact granules and/or dispersed to the outside of the cell. Many eosinophils showed granule lucency and numerous vesicles, some apparently budding from granules, and suggested the occurrence of piecemeal degranulation. However, localization of MBP by immunoelectron microscopy gave only scant support to this mechanism in that relatively few gold particles were localized in vesicles. Thus the evidence from this study supports the occurrence of eosinophil degranulation in association with eosinophil cytolysis and possibly by piecemeal degranulation in atopic dermatitis.

The significance of tissue eosinophils with pseudopod-like processes is unknown. Hanker et al.⁴⁹ described ameboid eosinophils and termed them *medusa cells*. Their concept of the ameboid eosinophil was an eosinophil that temporarily produced cytoplasmic protrusions or pseudopods, which could function in locomotion and feeding through phagocytosis. Medusa cells have been frequently found in contact with erythrocytes, platelets, and leukocytes in specimens from patients with eosinophilia accompanying allergy, parasitosis, or malignancy⁵⁰; and ultrastructural morphology has been described.⁵¹ However, medusa cells usually refer to giant processes as observed on whole mounts of cells. Others have described single, blunt cytoplasmic projections as uropods (or motile structures); and these have been identified in lymphocytes,⁵² basophils,³² neutrophils,⁵³ and eosinophils.³¹ Recent studies demonstrate that uropod formation occurs with chemokine stimulation and is accompanied by translocation and clustering of adhesion molecules to the uropod.^{52, 53} This phenomenon may represent a mechanism for recruitment and adhesive interactions of cells in inflammation.

Our studies showed a progression of eosinophil abnormalities by electron microscopy in atopic dermatitis, ranging from marked granule abnormalities in the cell to complete disruption of eosinophils with free granules in contact with the extracellular space. Interestingly, eosinophils were identified with pseudopod-like projections. No evidence of classical exocytotic degranulation was observed. On the basis of these findings, we conclude that eosinophil disruption with deposition of granule contents occurs in lesional atopic dermatitis skin and that eosinophil disruption is a cytolytic process. The granule protein deposition may be contributed to by piecemeal degranulation. These studies provide further information on the eosinophil's role in the disease.

REFERENCES

- Larsen FS, Holm NV, Henningsen K. Atopic dermatitis: a genetic-epidemiologic study in a population based twin study. *J Am Acad Dermatol* 1986;15:487-94.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;92(suppl)44-7.
- Hanifin JM. Atopic dermatitis. *J Allergy Clin Immunol* 1984;73:211-22.
- Agosti JM, Sprenger JD, Lum LG, Witherspoon RP, Fisher LD, Storb R, et al. Transfer of allergen-specific IgE-mediated hypersensitivity with allogeneic bone marrow transplantation. *N Engl J Med* 1988;319:1623-8.
- Cooper KD. Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994;102:128-37.
- Leung DYM, editor. Atopic dermatitis: from pathogenesis to treatment. Austin (TX): RG Landes Company, 1996.
- Gleich GJ. The late phase of the immunoglobulin E-mediated reaction: a link between anaphylaxis and common allergic disease. *J Allergy Clin Immunol* 1982;70:160-9.
- Leiferman KM, Fujisawa T, Gray BH, Gleich GJ. Extracellular deposition of eosinophil and neutrophil granule proteins in the IgE-mediated cutaneous late phase reaction. *Lab Invest* 1990;62:579-89.
- Ott NL, Gleich GJ, Peterson EA, Fujisawa T, Sur S, Leiferman KM. Assessment of eosinophil and neutrophil participation in atopic dermatitis: comparison with the IgE-mediated late-phase reaction. *J Allergy Clin Immunol* 1994;94:120-8.
- Gleich GJ, Adolphson CR, Leiferman KM. Eosinophils. In: Gallin JI, Goldstein IM, Snyderman R, editors. Inflammation: basic principles and clinical correlates. New York: Raven Press, 1992:663-700.
- Mihm MC Jr, Soter NA, Dvorak HF, Austen KF. The structure of normal skin and the morphology of atopic eczema. *J Invest Dermatol* 1976;67:305-12.
- Soter NA. Morphology of atopic eczema. *Allergy* 1989;44(suppl 9):16-9.
- Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venencie PY, Gleich GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis: comparison with onchocerciasis. *N Engl J Med* 1985;313:282-5.
- Kapp A. The role of eosinophils in the pathogenesis of atopic dermatitis: eosinophil granule proteins as markers of disease activity. *Allergy* 1993;48:1-5.
- Uehara M, Izukura R, Sawai T. Blood eosinophilia in atopic dermatitis. *Clin Exp Dermatol* 1990;15:264-6.
- Tsuda S, Kato K, Miyasato M, Sasai Y. Eosinophil involvement in atopic dermatitis as reflected by elevated serum levels of eosinophil cationic protein. *J Dermatol* 1992;19:208-13.
- Kagi MK, Joller-Jemelka H, Wuthrich B. Correlation of eosinophils, eosinophil cationic protein and soluble interleukin-2 receptor with the clinical activity of atopic dermatitis. *Dermatology* 1992;185:88-92.
- Hanifin JM, Schneider LC, Leung DY, Ellis CN, Jaffe HS, Izu AE, et al. Recombinant interferon gamma therapy for atopic dermatitis. *J Am Acad Dermatol* 1993;28:189-97.
- Walker C, Kagi MK, Ingold P, Braun P, Blaser K, Bruijnzeel-Koomen CA, et al. Atopic dermatitis: correlation of peripheral blood T cell activation, eosinophilia and serum factors with clinical severity. *Clin Exp Allergy* 1993;23:145-53.
- Paganelli R, Fanale-Belasio E, Carmini D, Scala E, Meglio P, Businco L, et al. Serum eosinophil cationic protein in patients with atopic dermatitis. *Int Arch Allergy Appl Immunol* 1991;96:175-8.
- Wuthrich B, Kagi MK, Joller-Jemelka H. Soluble CD14 but not interleukin-6 is a new marker for clinical activity in atopic dermatitis. *Arch Dermatol Res* 1992;284:339-42.
- Czech W, Krutmann J, Schopf E, Kapp A. Serum eosinophil cationic protein (ECP) is a sensitive measure for disease activity in atopic dermatitis. *Br J Dermatol* 1992;126:351-5.
- Krutmann J, Czech W, Diepgen T, Niedner R, Kapp A, Schopf E. High-dose UVAI therapy in the treatment of patients with atopic dermatitis. *J Am Acad Dermatol* 1992;26:225-30.
- Juhlin L, Venge P. Eosinophilic cationic protein (ECP) in skin disorders. *Acta Derm Venereol (Stock)* 1992;71:495-501.
- Stoughton RB. Percutaneous absorption of drugs. *Annu Rev Pharmacol Toxicol* 1989;29:55-69.
- McDowell EM, Trump BF. Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Pathol Lab Med* 1976;100:405-14.

27. Spurr AR. A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastructure Res* 1969;26:31-43.
28. Bendayan M, Zollinger M. Ultrastructural localization of antigenic sites on osmium-fixed tissues applying the protein A-gold technique. *J Histochem Cytochem* 1983;31:101-9.
29. Wagner JM, Bartemes K, Vernof KK, Dunnette S, Offord KP, Checkel JL, et al. Analysis of pregnancy-associated major basic protein levels throughout gestation. *Placenta* 1993;14:671-81.
30. Peters MS, Schroeter AL, Kephart GM, Gleich GJ. Localization of eosinophil granule major basic protein in chronic urticaria. *J Invest Dermatol* 1983;81:39-43.
31. Greenwood B. The motility of blood eosinophils on glass. *Br J Dermatol* 1969;81(suppl 3):36-7.
32. Galli SJ, Dvorak AM, Hammond E, Morgan E, Galli AS, Dvorak HF. Guinea pig basophil morphology in vitro. 1. Ultrastructure of uropod-bearing (motile) basophils and modulation of motile structures by serum and substrate effects. *J Immunol* 1981;126:1066-74.
33. Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870-6.
34. Vercelli D, Jabara HH, Lee B, Woodland N, Geha RS, Leung DYM. Human recombinant interleukin-4 induces FcεR2/CD23 on normal human monocytes. *J Exp Med* 1988;167:1406-16.
35. Gauchat J-F, Lebman DA, Coffman RL, Gascan H, deVries JE. Structure and expression of germline ε transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990;172:463-73.
36. Silberstein DS, Austen KF, Owen WF Jr. Hemopoietins for eosinophils. Glycoprotein hormones that regulate the development of inflammation in eosinophilia-associated diseases. *Hematol Oncol Clin North Am* 1989;3:511-33.
37. Rothenberg ME, Peterson J, Stevens RL, Silberstein DS, McKenzie DT, Austen KF, et al. IL-5 dependent conversion of normodense human eosinophils to the hypodense phenotype use 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody dependent cytotoxicity. *J Immunol* 1989;143:2311-6.
38. Yamaguchi Y, Hayashi Y, Sugama Y, Miura Y, Kasahara T, Kitamura S, et al. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J Exp Med* 1988;167:1737-42.
39. Minniccozi M, Duran WN, Gleich GJ, Egan RW. Eosinophil granule proteins increase microvascular macromolecular transport in the hamster cheek pouch. *J Immunol* 1994;153:2664-70.
40. Dvorak AM, McLeod RS, Onderdonk A, Monahan-Earley RA, Cullen JB, Antonioli DA, et al. Ultrastructural evidence for piecemeal and anaphylactic degranulation of human gut mucosal mast cells in vivo. *Int Arch Allergy Immunol* 1992;99:74-83.
41. Dvorak AM, Furitsu T, Letourneau L, Ishizaka T, Ackerman SJ. Mature eosinophils stimulated to develop in human cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. I. Piecemeal degranulation of specific granules and distribution of Charcot-Leyden crystal protein. *Am J Pathol* 1991;138:69-82.
42. Dvorak AM, Ackerman SJ, Furitsu T, Estrella P, Letourneau L, Ishizaka T. Mature eosinophils stimulated to develop in human-cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. II. Vesicular transport of specific granule matrix peroxidase, a mechanism for effecting piecemeal degranulation. *Am J Pathol* 1992;140:795-807.
43. Dvorak AM. Ultrastructural evidence for release of major basic protein-containing crystalline cores of eosinophil granules in vivo. Cytotoxic potential in Crohn's disease. *J Immunol* 1980;125:460-2.
44. Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, editor. *Blood cell biochemistry*. vol. 2. London: Plenum Publishing, 1991.
45. Dvorak AM, Onderdonk AB, McLeod RS, Monahan-Earley RA, Antonioli DA, Cullen J, et al. Ultrastructural identification of exocytosis of granules from human gut eosinophils in vivo. *Int Arch Allergy Immunol* 1993;102:33-45.
46. Gleich GJ, Schroeter AL, Marcoux JP, O'Connell EJ, Kohler PF. Episodic angioedema associated with eosinophilia. *N Engl J Med* 1984;310:1621-6.
47. Dvorak AM, Weller PF, Monahan-Earley RA, Letourneau L, Ackerman SJ. Ultrastructural localization of Charcot-Leyden crystal protein (lysophospholipase) and peroxidase in macrophages, eosinophils, and extracellular matrix of the skin in the hypereosinophilic syndrome. *Lab Invest* 1990;62:590-607.
48. Dvorak AM, Mihm MC, Osage JE, Kwan TH, Austen KF, Wintroub BU. Bullous pemphigoid, an ultrastructural study of the inflammatory response: eosinophil, basophil and mast cell granule changes in multiple biopsies from one patient. *J Invest Dermatol* 1982;78:91-101.
49. Hanker JS, Chandross RJ, Weatherly NE. Medusa cells: the morphology and cytochemistry of common ameboid variants of eosinophils. *Histochem J* 1980;12:701-15.
50. Hanker JS, Chandross RJ, Solic JJ, Weatherly NF, Laszlo J, Moore JO, et al. Medusa cells: cytostructure and cytochemistry of ameboid eosinophils with pseudopod-like processes. *Histochem J* 1981;13:905-19.
51. Berman EL, Carter HW, Ambrose WW, Hanker JS. Studies of eosinophil medusa cells by electron imaging modes. *Scanning Electron Microsc* 1983(pt 1):311-21.
52. del Pozo MA, Sánchez-Mateos P, Nieto M, Sánchez-Madrid F. Chemokines regulate cellular polarization and adhesion receptor redistribution during lymphocyte interaction with endothelium and extracellular matrix. Involvement of cAMP signaling pathway. *J Cell Biol* 1995;131:495-508.
53. Dore M, Burns AR, Hughes BJ, Entman ML, Smith CW. Chemoattractant-induced changes in surface expression and redistribution of a functional ligand for P-selectin on neutrophils. *Blood* 1996;87:2029-37.