Cytolysis and piecemeal degranulation as distinct modes of activation of airway mucosal eosinophils

Jonas S. Erjefält, PhD,[a,e] Morgan Andersson, MD, PhD,[c] Lennart Greiff, MD, PhD,[c] Magnus Korsgren, MD,[a] Mariuz Gizycki, MSc, MD,[e] Peter K. Jeffery, PhD,[e] and Carl G. A. Persson, PhD[b,d] *Lund, Sweden, and London, United Kingdom*

Background: Cytotoxic eosinophil granule proteins are considered important in the pathogenesis of inflammatory airway diseases, including asthma, rhinitis, and polyposis. However, little is known about the mechanisms involved in the deposition of these tissue-damaging granular products in vivo.
Objective: We sought to determine the occurrence of degranulating eosinophils, those with morphologic evidence of cytolysis with associated clusters of free eosinophil granules (Cfegs), and to identify the frequency of apoptotic eosinophils in inflamed upper airway tissue.
Methods: Eosinophil-rich nasal polyps were processed for transmission electron microscopy and for light microscopic evaluation of whole-mount preparations subjected to deep tissue staining for eosinophil peroxidase.
Results: The mean proportion of eosinophil subtypes were intact and resting (6.8%), intact but degranulating (83%), cytolytic or Cfegs (9.9%), and apoptotic (0.0%). All degranulating eosinophils exhibited piecemeal degranulation. The occurrence of Cfegs was confirmed in nonsectioned whole-mount preparations. Depending on the appearance of their core and matrix, the specific granules were divided into four subtypes, and a degranulation index (altered per total granules) was calculated for each eosinophil. Cytolytic eosinophils had a much lower degranulation index than intact eosinophils present in the same tissue ($P < .001$).
Conclusions: These data indicate that eosinophil cytolysis is present in human airway mucosa, that its occurrence is not an artifact of the means of tissue handling, and that cytolysis of eosinophils may occur without prior extensive degranulation. We suggest that eosinophil cytolysis is a major activation mechanism, which occurs along with, but is distinct from, other types of degranulation. (J Allergy Clin Immunol 1998;102:286-94.)

Key words: *Allergy, nasal polyps, eosinophils, cytolysis, apoptosis, degranulation, eosinophil peroxidase, electron microscopy*

Abbreviations used
Cfegs: Clusters of free eosinophil granules
EPO: Eosinophil peroxidase
PBS: Phosphate-buffered saline
PMD: Piecemeal degranulation

The activation of eosinophils and release of eosinophil granule mediators in the airway mucosa is considered an essential process in the pathogenesis of asthma, allergic rhinitis, and nasal polyposis.[1-4] There is also ample evidence for the presence of granule products, such as major basic protein and eosinophil cationic protein, in airway tissues and secretions or exudations.[2,5-7] However, despite research focused on the airway eosinophil, little is as yet established regarding several basic features of degranulation of eosinophils in inflamed airway tissues.

On the basis of ultrastructural evidence, it is possible to discern at least two degranulation events in eosinophils: piecemeal degranulation (PMD), whereby the granular content is released from intracellular granules leaving more or less empty granules in the intact cell,[8,9] and eosinophil cytolysis, whereby the cell membrane ruptures, causing the release of clusters of free eosinophil granules (Cfegs).[10] There is general agreement as to the occurrence of PMD, but the ultrastructural patterns of granules during this event as it proceeds in the airway mucosa remain largely unexplored.

Eosinophil cytolysis was recently suggested as a potentially important eosinophil activation mechanism.[10] In support of this, cytolysis of eosinophils and Cfegs could be promptly induced in vivo by common topical challenges.[11,12] Furthermore, studies involving cell types other than eosinophils have shown that cytolysis can be a highly organized process[13,14] that may be no less *programmed* than apoptotic cell death, which is now receiving much attention in eosinophil research.[15-17] Outside the tissue, eosinophils are susceptible to apoptotic cell death as shown by demonstrations of apoptotic eosinophils in airway lumenal samples (bronchioalveolar lavage fluids and sputum)[18,19] and in cell cultures.[20,21] However, the occurrence and frequency of eosinophil apoptosis (particularly in relation to eosinophil cytolysis) within airway tissues has remained unknown.

In a century's literature on eosinophilic diseases, one can find numerous illustrations of eosinophil cytolysis and Cfegs,[10] and the occurrence of Cfegs in bronchial

From [a]the Departments of Physiology and Neuroscience, [b]Clinical Pharmacology, and [c]Otolaryngology, University Hospital, Lund; [d]Preclinical Research, Astra Draco, Lund; and [e]Lung Pathology Unit, Asthma and Allergy Research Group, National Heart and Lung Institute, Imperial College School of Medicine, London.

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Reprint requests: Jonas S. Erjefält, PhD, Department of Physiology and Neuroscience, E-Blocket, University Hospital 221 85, Lund, Sweden.

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biopsy specimens of asthmatic subjects has already been illustrated and quantified in electron microscopic studies.[22] Workers who currently report on lysed eosinophils may frequently regard this event as a result of intense PMD of eosinophils, inferring that high cytoplasmatic levels of toxic granule products have led to the cytolysis.[23-26] Another factor that may have prevented recognition of cytolysis is the possibility that mechanical artifacts (e.g., inflicted during the taking of biopsy specimens) have contributed to findings of Cfegs in various tissues.[10] Furthermore, the lack of data on the molecular regulation of this phenomenon may have contributed to the fact that eosinophil cytolysis and Cfegs have been almost completely ignored in current discussions of eosinophil activation in inflammation and allergic conditions.[10]

In this study we have used nasal polyps to examine morphologic characteristics of human airway eosinophils. PMD has been described previously and would appear to be a frequent phenomenon in the polyps,[27,28] whereas eosinophil cytolysis and Cfegs have not been reported as yet. Our aim was to determine the occurrence of eosinophil cytolysis and Cfegs in relation to states of activation and apoptosis of eosinophils in this tissue and to shed light on the possibility that eosinophil cytolysis is a primary mechanism distinct from other forms of degranulation. It was further considered that we should take account of potential artifacts and avoid their contribution. To this end we chose to use nasal polyps because they are eosinophil-rich and readily available in large, intact pieces from which whole-mount preparations could be made as a complement to ultrastructural analysis.

METHODS

Subjects and tissue sampling

Eight patients with nasal polyposis were recruited. Nasal polyps were excised by intranasal snare polypectomy with topical anaesthesia (2% tetracaine and 0.01% epinephrine), gently cut into blocks, and immediately placed in fixative (1% glutaraldehyde and 3% formaldehyde in a phosphate-buffered saline [PBS] buffer) overnight at 4° C. Polyps with tissue eosinophilia (five of the eight patients) were selected for further detailed electron microscopic analysis (Table I). The atopic patients experienced symptoms (e.g., itching and sneezing) when exposed to the relevant allergen. However, none of these patients were exposed to their allergens and were symptom free at the time of the polypectomies.

Transmission electron microscopy

From each patient, two blocks ($\approx 3 \times 3 \times 5$ mm, representing two separate regions) were rinsed in buffer, postfixed in 1% osmium tetroxide for 1 hour, dehydrated in graded acetone solutions, and embedded in Polybed 812 (Poly Science). Plastic sections (1 μm thick) were cut on an ultratome (Ultracut E, Leica, Germany), stained with toluidine blue, and examined in a light microscope (Axioscop, Zeiss, Germany). Areas with intact surface epithelium were selected for further electron microscopic analysis. Ultrathin sections (90 nm) were cut and placed on 200-mesh, thin-bar copper grids and stained with uranyl acetate and lead citrate.[29] The specimens were examined by using a Hitachi transmission electron microscope (H-7000; Hitachi, Japan).

TABLE I. Patients from which polyps were selected for detailed transmission electron microscopic analysis

Patient	Atopy	Steroid treatment*
1	—	—
2	—	—
3	Timothy, mugwort	—
4	Timothy, mugwort, cat	—
5	—	—

*Defined as treatment with glucocorticosteroids within 4 weeks before polypectomy.

Eosinophil peroxidase staining in whole-mount preparations

To detect scattered subepithelial Cfegs in tissues subjected to a minimum of mechanical stress, mucosal whole-mount preparations were stained for cyanide-resistant eosinophil peroxidase (EPO).[11] Immediately after surgical excision, polyps were gently cut into blocks (~50 mm^2 of the mucosal surface and 3 to 4 mm deep), rinsed with saline, and placed in PBS supplemented with 4% formaldehyde overnight at 4° C. The surface of the airway epithelium was visualized by histochemical staining for tissue-nonspecific alkaline phosphatase.[30] The samples were rinsed in TRIS-buffer and one drop of incubation solution (TRIS-buffer [pH 9.0] containing 0.1% Naphtol AS-BI phosphate [Sigma] and azo-dye TR-salt [Sigma]) was placed on the mucosal surface for 7 minutes. The specimens were then rinsed in buffer and incubated for 10 minutes in PBS buffer containing 3.3 diaminobenzidine tetrahydrochloride (75 mg/100 ml [Sigma]), H_2O_2 (100 μl/100 ml), and NaCN (50 mg/100 ml). After rinsing in PBS, the samples were mounted in Kaisers medium and examined with a Zeiss light microscope. An intact epithelial surface was identified by a characteristic blue mosaic-like pattern. Subepithelial EPO-stained eosinophil granules were examined by altering the focal plane to focus down to a depth of 0 to 100 μm below the epithelial basement membrane.

Quantification

Inflammatory cells. Subepithelial inflammatory cells in ultrathin sections (>150 per sample) were counted to a depth of 0 to 250 μm below the epithelial basement membrane. Mast cells, eosinophils, neutrophils, plasma cells, and macrophages were identified by their characteristic ultrastrucural morphology.[29] Cell counts were expressed as total numbers per 0.1 mm^2 subepithelial tissue. Areas of tissue occupied by vessels and submucosal glands were excluded from the evaluation. The subepithelial area assessed in each sample was calculated from low-power electron micrograph by using computer-assisted image analysis (NIH Image 1.33; National Institutes of Health, Bethesda, Md.) on a Macintosh computer (Cupertino, Calif.)

Ultrastructural morphology of eosinophils. Eosinophils (43 to 120 per patient, mean = 64) and Cfegs were recorded on electron micrographs at 5000X magnification and divided into subgroups defined in the following categories. Resting eosinophils was defined as no ultrastructural signs of activation (i.e., all specific granules had an electron-dense core surrounded by an intact matrix). Degranulating eosinophils was defined as intact cells displaying characteristic changes of specific granules[9] (i.e., presence of granules type II to IV [see below]). Occurrence of partly empty intracellular granules with no signs of granule extrusion was here defined as PMD. Eosinophil cytolysis was defined as the presence of chromatolysis and loss of plasma membrane integrity.[9] Apoptotic eosinophils were defined as the presence of chromatin condensation with preserved

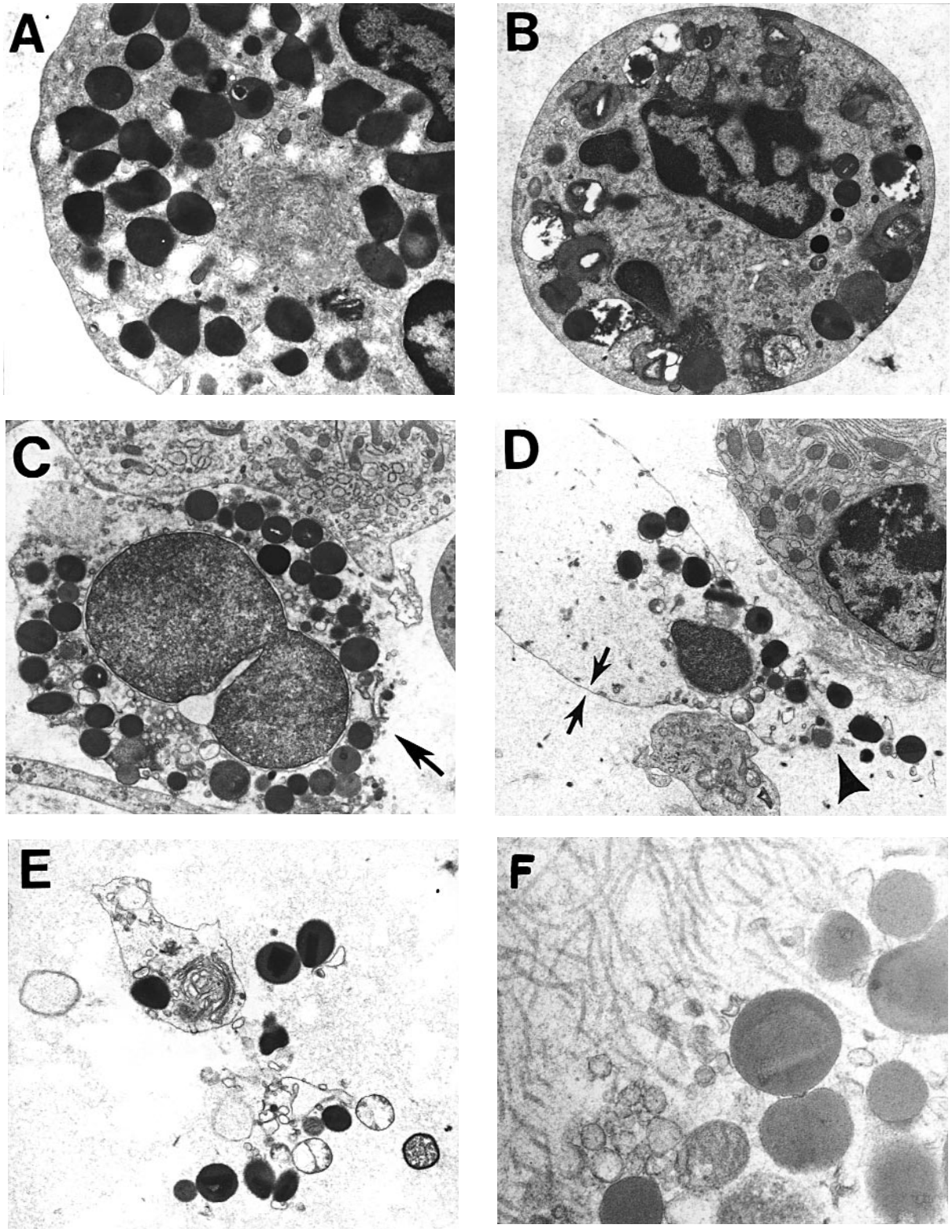

FIG. 1. Transmission electron micrographs demonstrating eosinophil with mostly intact specific granules **(A)**; degranulating (PMD) eosinophil with structural changes in most specific granules **(B)**; eosinophil cytolysis in which plasma membrane is ruptured (*arrow*) and cell nucleus displays signs of chromatolysis **(C)**; late-stage cytolysis in which eosinophil granules are released into extracellular matrix (*arrowhead*), with parts of cell membrane remaining (*arrows*) but virtually all organelles (except for specific granules) absent **(D)**; clusters of free eosinophil granules and scattered cell debris **(E)**; and high-power micrograph demonstrating extracellular eosinophil granules, some of which have retained their granule membrane **(F)**.

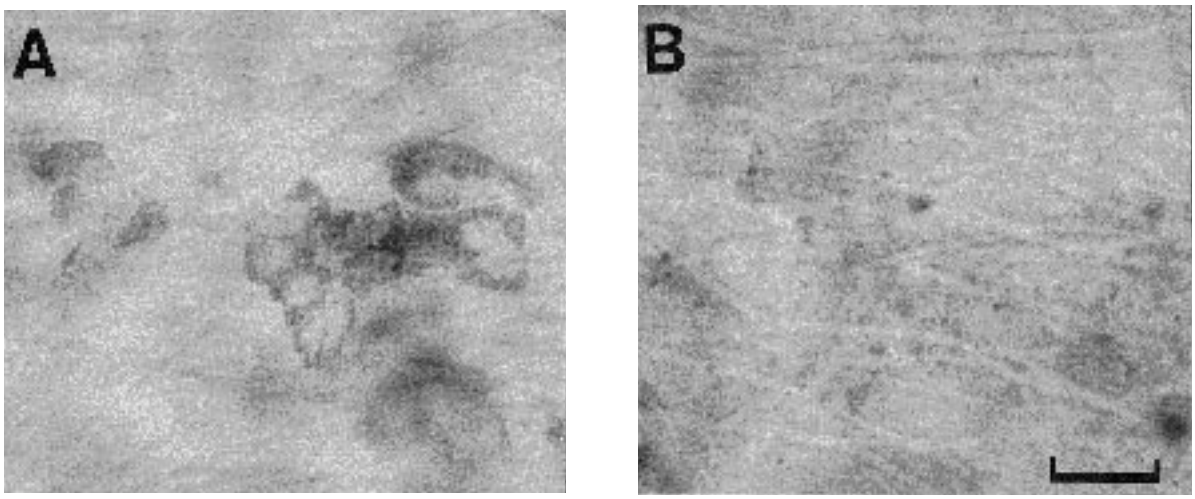

FIG. 2. Light micrographs demonstrating distribution of EPO-stained eosinophil-specific granules in whole-mount preparation of nasal polyp. **A**, Area with only intact eosinophils. Space occupied by cell nuclei can be observed as nonstained islets in cells. **B**, EPO-positive granules lying scattered in subepithelial tissue. Both micrographs represent regions 0 to 20 μm below epithelial basement membrane and were taken from areas covered by intact surface epithelium. Scale bar = 7 μm.

plasma membrane and nondilated organelles.[31,32] Cfegs was defined as the occurrence of clusters (three or more) of identifiable extracellular eosinophil-specific granules.[10,22]

The number of intracellular granules was expressed as total numbers per 50 μm^2 cell cytoplasmic area. To determine the cytoplasmic area, each micrograph was digitized by using an Epson EU-10 scanner (Seiko-Epson Corp, Japan). The total cell area and nuclei area were calculated by tracing the perimeter of the plasma and nucleus membranes with the NIH Image 1.33 image analysis system.

Evaluation of eosinophil degranulation. In each eosinophil the specific granules were divided into four groups: *type I*, intact granules with no signs of degranulation (i.e., intact core and matrix); *type II*, ragged loss of core material but intact matrix; *type III*, intact core or nearly intact core and the granule matrix partly or completely empty (early stages of matrix changes were identified by a characteristic coarsening of the matrix); and *type IV*, nearly complete or complete loss of both core and matrix material. To express the degree of degranulation for each eosinophil, a degranulation index was calculated as follows: DI = 100 × (numbers of activated granules, II to IV/total granules).

Statistical analysis

Differences among groups were examined by Student's *t* test. Data on granule numbers were logarithmically transformed before analysis. Correlations between degranulation indices and total granule numbers were assessed by Spearman's rank correlation test. Statistical differences between groups were assumed for *P* values less than .05. All statistical tests were performed with Microsoft Excel v5.0 and Astute v1.5.

RESULTS

Inflammatory cells

The numbers of inflammatory cells present in the polyp tissues are presented in Table II. All samples were characterized by tissue eosinophilia together with infiltration of plasma cells, macrophages, and lymphomononuclear leukocytes (Table II). The degree of tissue eosinophilia varied among the patients, with a range of 4 to 137 eosinophils per 0.1 mm^2 subepithelial area (Table II).

Eosinophil subtypes

The proportions of eosinophil subtypes in each polyp are shown in Table III. Eosinophil cytolysis and Cfegs comprised approximately 10% of the tissue eosinophils. Both the eosinophil cytolysis and Cfegs were scattered throughout the tissue and were frequently seen close to intact cells (Fig. 1, *C* and *D*), including intact eosinophils. The presence of Cfegs was confirmed by light microscopic examination of EPO-stained whole-mount preparations (Fig. 2). In all patients the majority of the eosinophils were involved in either degranulation of intact cells (Fig. 1, *B*) or eosinophil cytolysis (Table II, Fig. 1, *C* to *E*). All the degranulating but intact eosinophils displayed signs of PMD. No signs of granule extrusion (i.e., exocytosis) were observed. Extracellular eosinophil granules that had retained their granule membrane were frequently observed amidst membrane-free or partly dissolved granules (Fig. 1, *F*). Eosinophils displaying the characteristic features of apoptosis were absent. However, apoptotic cells other than eosinophils were observed.

Eosinophil granule subtypes

Several morphologies of eosinophil-specific granules were noted in eosinophils undergoing PMD. Four distinct granule types were discerned (Fig. 3). The pattern of granule subtypes varied among the patients but was generally consistent within each polyp (Fig. 4). The exception was patient 3, in whom the granule pattern differed among different regions of the polyp (Fig. 4). Large differences in the mean degranulation index were observed among the polyps (Fig. 5) and in patient 3 also between different regions within the same polyp (Fig. 5). A weakly negative but statistically significant correlation was

TABLE II. Inflammatory cells

Patient	Eosinophils	Neutrophils	Mast cells	Macrophages	Plasma cells	Total cells
1a	9.3	7.2	10.2	6.3	27.5	108.0
1b	4.1	5.3	4.3	4.3	36.1	89.5
2a	25.8	0.0	4.3	17.1	36.7	137.9
2b	21.7	0.0	2.9	19.6	37.0	135.3
3a	14.1	0.0	5.4	12.4	54.1	123.3
3b	13.5	0.0	0.5	7.6	14.6	55.6
4a	60.3	1.3	3.8	11.2	42.3	157.6
4b	51.7	0.0	7.1	8.7	31.2	127.4
5a	137.0	2.2	0.9	4.8	46.3	239.7
5b	101.2	0.6	1.8	5.5	69.4	217.8
X ± SEM	43.7 ± 14	1.7 ± 0.8	4.1 ± 0.9	9.8 ± 1.7	39.5 ± 4.7	139.0 ± 17

Cells are expressed as total numbers per 0.1 mm^2 subepithelial tissue.

TABLE III. Proportions of eosinophil subtypes

Patient	Intact	Degranulating and intact	Apoptotic	Cytolytic	Cfegs
1a	0.0	100.0	0.0	0.0	0.0
1b	0.0	100.0	0.0	0.0	0.0
2a	19.2	42.3	0.0	26.9	11.2
2b	14.3	57.1	0.0	17.9	10.7
3a	0.0	96.1	0.0	3.8	0.0
3b	0.0	96.7	0.0	0.0	3.2
4a	10.3	89.6	0.0	0.0	0.0
4b	21.4	78.6	0.0	0.0	0.0
5a	1.6	82.6	0.0	8.2	6.6
5b	0.0	89.6	0.0	6.0	4.5
X ± SEM	6.7 ± 2.8	83.4 ± 6.1	0.0 ± 0.0	6.3 ± 2.9	3.6 ± 1.4

The data are presented as percent of total eosinophil counts.

observed between the degranulation index and the cell content of specific granules (Fig. 6).

Granule changes in cytolytic eosinophils

The eosinophils in Fig. 5, *A* and *B*, representing samples containing both eosinophil cytolysis and PMD (Figs. 4 and 5, Table III), were selected for a detailed analysis of degranulation indices. It was found that the cytolytic eosinophils had a markedly lower degranulation index ($P < .0001$) than neighboring intact eosinophils (Fig. 7, *A*). The number of intact granules was significantly higher ($P < .003$) in the cytolytic than in the intact eosinophils (Fig. 7, *B*).

DISCUSSION

This study provides evidence that eosinophil cytolysis, and the ensuing release of free granules, is a significant fate of human airway eosinophils, which is distinct from other forms of degranulation events such as PMD. In support of this mechanism, we demonstrated that cytolysis was induced in eosinophils exhibiting no or few signs of PMD. This observation, together with demonstrations of Cfegs in nonsectioned whole-mount preparations, ruled out mechanical artifacts as a cause of eosinophil cytolysis. Almost all the intact eosinophils in the polyps displayed granules with signs of PMD. These features allowed classification of granule subtypes and the determination of a novel index of PMD. In contrast to the frequent occurrence of cytolysis and PMD, apoptotic eosinophils were lacking.

The lack of apoptotic eosinophils is in agreement with the infrequent reports of apoptotic eosinophils within human airway tissues in vivo. It is only after maintaining polyp tissue in culture for several days (and with proapoptotic cytokine-blocking drugs present) that tissue eosinophils undergoing apoptosis may become evident.[33]

Several findings suggest that Cfegs are important effectors in eosinophilic diseases. First, the recent observations on subepithelial Cfegs deep within whole-mount preparations of guinea-pig airways,[11,12] well away from sectioning sites, are confirmed herein. The present observation of cytolytic eosinophils lying scattered amidst intact eosinophils and other intact cells indicates further that cytolysis is not confined to any particular area of necrosis that might develop through disease processes or mechanically. Indeed, the distinct pattern of degranulation indices for cytolytic eosinophils suggests that eosinophil cytolysis is a physiologic phenomenon in eosinophilic airway conditions. Cfegs have been repeatedly depicted in histologic illustrations of airway tissue and surface material, particularly in asthma.[10,22,29] These data further suggest that after cytolytic death, the cytoplasm is lost by rupture of the plasma membrane, which creates *ghost cells* (Fig. 1, *D*). Even at this stage, the specific granules, spilt into the surrounding milieu as Cfegs, remain surprisingly intact, indicating that the release of

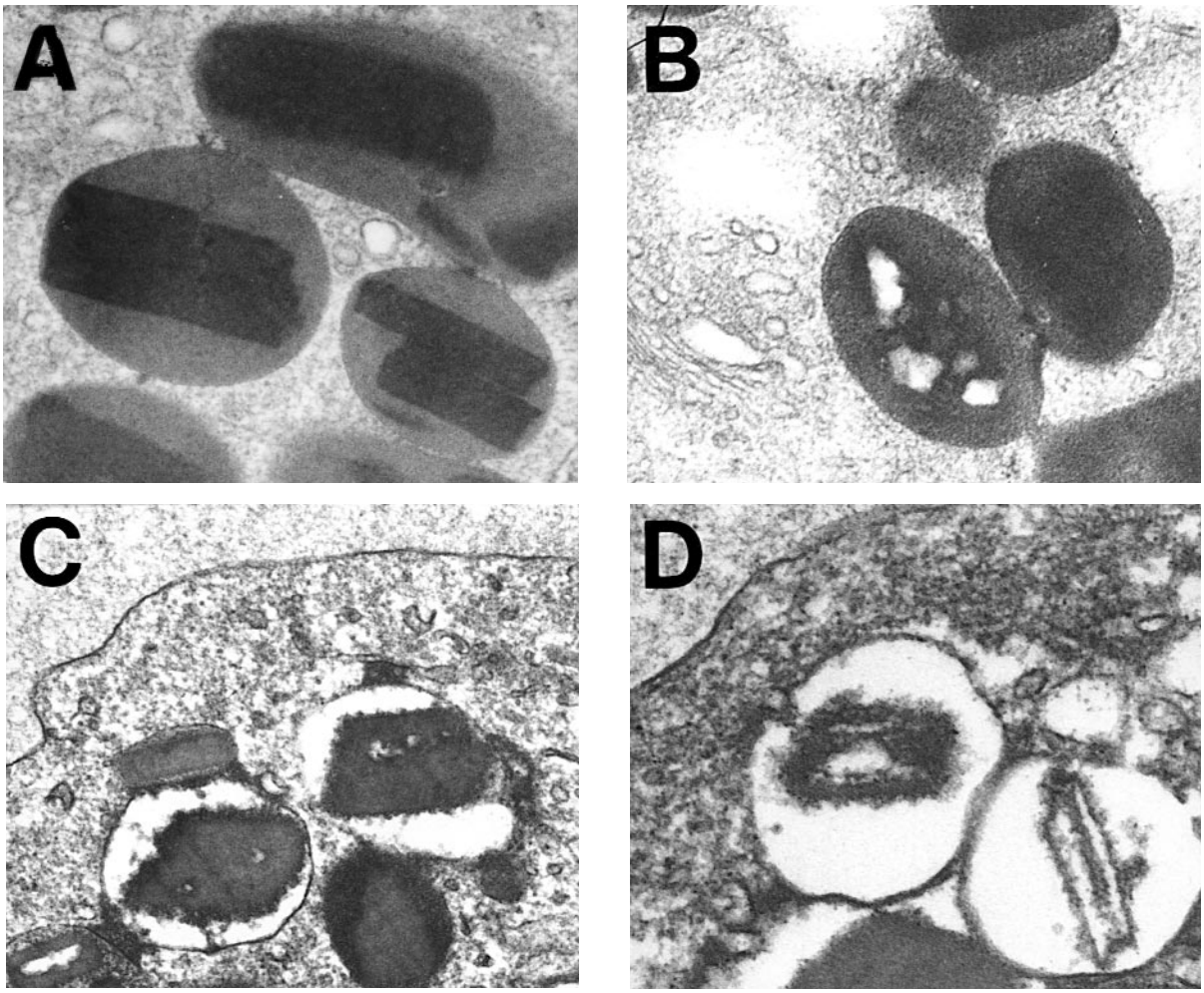

FIG. 3. Transmission electron micrographs demonstrating ultrastructural changes in specific granules during PMD. Different types of granular changes are depicted: **A**, Type I, intact matrix and intact electron-dense cores; **B**, type II, ragged loss of material in core; **C**, type III, loss of granular material but cores largely intact; **D**, type IV, extensive loss of both core and matrix material.

granule proteins at eosinophil cytolysis would take place late in the cytolytic process. Eosinophil granules rarely occurred in phagosomes of macrophages in this study. Hence free but intact granules appear not to be doomed to phagocytosis and would be expected to release gradually all their content into the extracellular matrix, thus contributing to widespread tissue distribution of eosinophil granule proteins. This notion is supported by Filley et al.[5] who examined airways from patients who died from status asthmaticus and reported extensive tissue immunoreactivity for major basic protein in association with areas rich in eosinophilic granular debris. Taken together, the potential leakiness of Cfegs[5,25] and reports indicating that eosinophil cytolysis is common in nasal polyposis (this study), allergic rhinitis,[34] and asthma[5,10,22,29] suggest that cytolysis of eosinophils should be regarded as a biologically important mechanism by which airway eosinophils may have widespread effects. In support of this, it was recently reported that allergen challenge of sensitized guinea pigs induced eosinophil cytolysis, and the release of Cfegs was significantly associated with sites of epithelial damage.[12]

Eosinophil cytolysis, which is induced promptly in vivo by allergen exposure[12] or in vitro by incubation of eosinophils with immunoglobulin-coated dextran particles[24] or calcium ionophores,[35] may differ from the cell necrosis that is commonly viewed as a process of passive degeneration.[36] It is an attractive possibility that eosinophil cytolysis may be regulated by well-controlled intracellular mechanisms and that cytolysis is subject to physiologic and pharmacologic control.[10] Indeed, the existence of procytolytic intracellular pathways have recently been described in cell types other than eosinophils.[13,14,37,38]

Consistent with previous reports,[39,40] the present eosinophil-rich inflammation in nasal polyps proceeds in association with the tissue recruitment of plasma cells, macrophages, and lymphocytes. Our data on PMD agree with the work by Takasaka et al.[27] who reported that the majority of nasal polyp eosinophils exhibit altered granules. In addition, this study has provided new information on the occurrence of morphologic subtypes of the specific granules in diseased human airways. The present

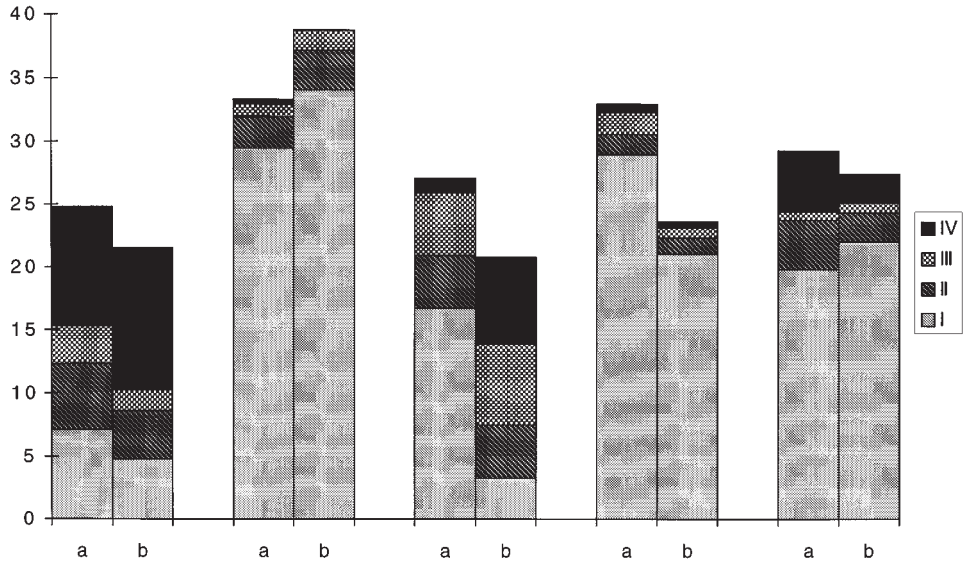

FIG. 4. Proportion of granule subtypes in individual tissue samples. For each granule, subtype data are presented as mean numbers of granules per 50 μm^2 cytoplasm. Grouped bars represent data from two regions (*a* and *b*) within same patient. Patients 1 to 5 are shown from left to right. Approximately 900 granules per sample were analyzed.

spectrum of granule changes is in agreement with previous observations on PMD in cultured eosinophils,[8,9,41,42] but the occurrence of different subtypes of granules in diseased tissues has not been extensively examined in previous work. Interestingly, the pattern of PMD, as based on the present division into granule subtypes, shows little variations within each polyp, suggesting individual polyp homogeneity in this regard. This observation may also strengthen the validity of the degranulation index that we have described in this study as a representative measure of eosinophil activation through PMD. However, to appreciate fully the utility of the ultrastructural signs of PMD, we need to learn more about the changes in the specific granules that reflect aging of the mucosal eosinophils. Because currently available molecular markers of degranulation (e.g., the monoclonal antibody EG2) may fail to distinguish degranulating eosinophils reliably,[43] the present index, together with the determinations of the occurrence of eosinophil cytolysis and Cfegs, could find utility in defining the nature of tissue eosinophilia and its consequences in disease and in models of eosinophilic disease. For example, it has been noted that the ultrastructural signs of eosinophil activation are lacking in the widely used mouse immunomodels of asthma.[44] Ultrastructural indices of eosinophil activation in vivo are also needed for validation and development of new molecular markers of different forms and degrees of eosinophil degranulation, which are central to the interpretation of data, showing similar degrees of eosinophilic infiltration in asthma and exacerbations of chronic bronchitis,[45] yet with distinct pathologies.

This study has demonstrated that cytolysis occurs in eosinophils that, although maintaining a *normal* number of specific granules, differ from the general population of eosinophils by having a low degranulation index. These data support our notion that eosinophil cytolysis and PMD are distinct mechanisms and that cytolysis of eosinophils may be a primary mechanism of degranulation in the human airway mucosa in vivo.[10] Our observations do not exclude the possibility[23-25] that intracellular release of cytotoxic mediator may also induce cytolysis. Eosinophil cytolysis results in widespread release of eosinophil content, which would have an effect on the intact eosinophil numbers per se. This may explain why there are reports of heterogeneity in the numbers of intact eosinophils identified by conventional hematoxylin and eosin staining of airway tissues in cases of fatal asthma.[46,47]

We speculate that PMD and cytolysis represent two functionally different processes by which eosinophils release their granule mediators. PMD provides the possibility of a long-lasting and selective[48] release of granule content. These features are in agreement with the suggested role of eosinophil granular proteins in immunoregulation.[49-51] On the other hand, eosinophil cytolysis, which is expected to yield rapid and complete release of mediators, may be an important host mechanism in parasite defense[52] and in the tissue disturbances that characterize eosinophilic diseases.[53] This latter aspect makes it of interest to explore the biologic and pharmacologic control of eosinophil cytolysis in an attempt to find novel drugs for the treatment of eosinophilic disorders.[10]

In conclusion, this study has examined nasal polyp tissue and demonstrated the occurrence of eosinophil cytolysis and Cfegs. Apoptosis of eosinophils was rarely detected, and classical exocytosis of eosinophil granules was not observed, which is in agreement with studies of other airway eosinophilic conditions. Four readily identifiable morphologic subtypes of the granules revealed dif-

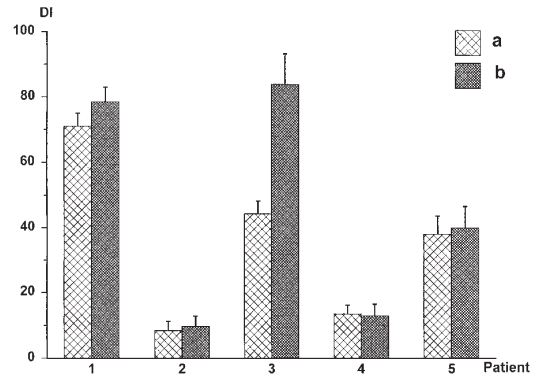

FIG. 5. Variation in mean degranulation index among polyps and regions within same polyp. Data are shown as means ± SEM. *DI*, Degranulation index.

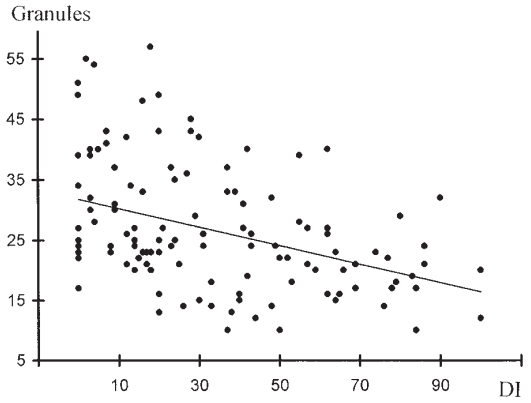

FIG. 6. Correlation between degranulation index and total content of specific granules (expressed as numbers per 50 μm² cytoplasm). *Dots* represent values from eosinophils randomly selected from all five polyps. *DI*, Degranulation index.

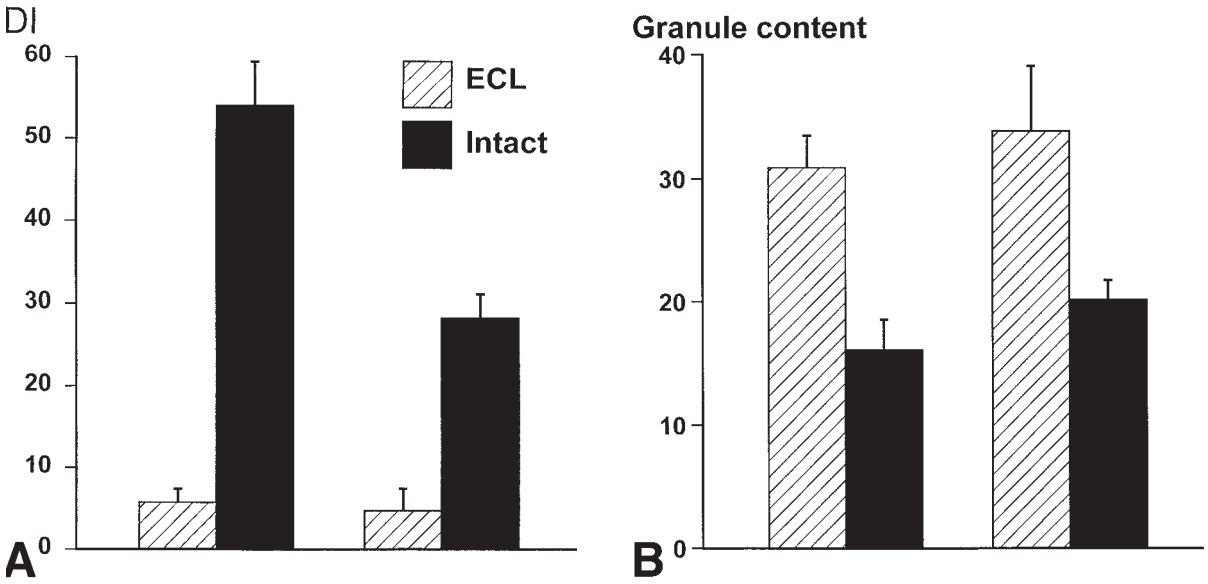

FIG. 7. A, Degranulation index in intact and cytolytic eosinophils in two separate polyp regions (regions a and b are shown as grouped bars to left and right, respectively). **B**, Total numbers of intact granules. Eosinophils with signs of advanced cytolysis (where cell border could not be traced) were excluded from analysis. Data are shown as means ± SEM.

ferent patterns of PMD among the patients; the index of PMD was high in intact but low in cytolytic eosinophils. These findings suggest that eosinophil cytolysis and PMD are distinct mechanisms for release of eosinophil granule products in human airways. Nothing is known as yet about the rate of eosinophil cytolysis or its regulation. However, our data suggest that eosinophil cytolysis is a common and potentially important fate for human airway eosinophils.

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