

Immunohistochemical characterization of cellular infiltrate in nasal polyp from aspirin-sensitive asthmatic patients

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Background: The immunopathologic mechanism of nasal polyp in aspirin-sensitive asthma remains to be further defined.

Objective: To characterize the features of the inflammatory cellular infiltrate in the nasal polyp tissue from aspirin-sensitive asthmatic patients.

Methods: We have taken nasal polyp tissue during nasal polyp resection from 13 aspirin-sensitive asthma, 6 allergic, and 12 non-allergic subjects. Immunohistochemistry was employed to stain and enumerate the individual inflammatory cell types using monoclonal antibodies against tryptase (AA1) to identify mast cells, against secreted forms of eosinophil cationic protein (EG2), to identify activated eosinophils, against neutrophil elastase (NE) for neutrophils and against T cell surface markers (CD3) to identify total T cells.

Results: There were no significant differences in AA1 + cells among three groups ($P > .05$). EG2 + cells tended to be higher in ASA-sensitive asthmatic patients than in allergic and non-allergic subjects, but no statistical significance was observed. NE+ cells were found in most subjects of the three groups and their numbers were significantly higher in allergic subjects than in aspirin-sensitive asthma ($P < .05$). Some patients had CD3+ cells with no statistical significance among the three groups. Significant correlation was found in numbers between NE+ cell and AA1+ cell ($r = .44$, $P = .01$), and between NE+ cell and EG2+ cell ($r = .40$, $P = .02$).

Conclusion: These findings suggested that major effector cells such as mast cells and eosinophils might be placed in the center of the inflammatory response of nasal polyps, regardless of their association with aspirin sensitivity.

Ann Allergy Asthma Immunol 1998;81:219-224.

INTRODUCTION

Patients with bronchial asthma and sensitivity to aspirin and/or other non-steroidal anti-inflammatory drugs have a high prevalence of accompanying symptoms of rhinosinusitis including rhinorrhea, nasal congestion, anosmia, loss of taste, and recurrent severe nasal polyposis.^{1,2} The pathogenetic mechanism of aspirin-induced asthma still remains to be further studied. Upon chal-

lenge with aspirin, these patients increased cysteinyl leukotriene release as detected in urine³ and in nasal lavage,^{4,5} which does not occur in aspirin-tolerant subjects. Leukotrienes are known to be potent bronchoconstrictors, which stimulate bronchial mucus secretion, increase vascular permeability, and induce chemotaxis to inflammatory cells.⁶ Immunohistochemical study of the bronchial mucosa from both ASA-sensitive and non-ASA-sensitive asthmatic subjects showed an increased inflammatory cell infiltration which include eosinophils, mast cells, and lymphocytes.⁷ The pathogenetic mechanism for development of the nasal polyp associated with aspirin sensitivity remains unclear. There have

been few studies dealing with immunohistochemical analysis of nasal polyp tissue with a panel of monoclonal antibodies to inflammatory cells. Histologic examination showed that nasal mucosa from aspirin-sensitive asthmatic patients did not seem to differ from those removed from ASA-tolerant patients.⁸ The present study was designed to understand further the underlying immunopathologic mechanism for the development of nasal polyps in ASA-sensitive asthmatic subjects by using an immunohistochemical method with monoclonal antibodies to four major inflammatory cells.

MATERIALS AND METHOD

Subjects

Nasal polyps were obtained from 13 subjects with ASA-sensitive asthma, aged between 29 and 62 years (mean 45.8 years) who had complained of rhinorrhea and nasal obstruction as well as asthmatic symptoms at Allergic Clinic of Ajou University Hospital, Suwon, Korea. To confirm aspirin sensitivity, lysine-aspirin (L-ASA) bronchoprovocation test was performed and they showed nasal and asthmatic symptoms with a significant fall of FEV₁ after the inhalation of L-ASA. As controls, six allergic subjects under the polypectomy, aged 28 to 50 (mean 38.1 years), who were sensitive to house dust mite were chosen. They fulfilled the following criteria as $\geq 3+$ A/H ratio to house dust mite on skin prick test, and $\geq 2+$ d2-RAST. Sixteen non-allergic subjects under the polypectomy, aged between 28 and 55 years (mean 40.5 years), who showed all negative responses on skin prick

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Received for publication September 15, 1997.

Accepted for publication June 3, 1998.

test with common inhalant allergens were enrolled. None of the control patients had aspirin intolerance. The clinical characterization of the three study groups are listed in Table 1. There were no significant differences in age and duration of nasal symptoms before the polypectomy. None of all the study

subjects had used topical steroids for at least 4 weeks prior to obtaining the polyp. The subjects having any evidence of bacterial or viral infections during the nasal polypectomy were excluded in this study. Immediately after the surgical removal, the polyps were washed with normal saline to remove

stagnant mucus. The middle portion of the polyp was cut off, and a paraffin-embedded tissue was prepared immediately. All the subjects gave their written informed consents as required by Ajou University Hospital, Suwon, Korea.

Staining Procedures

A tissue section 4 μm thick was stained with each monoclonal antibodies to inflammatory cells. The antibodies were applied in optimal dilutions with Tris-buffered saline (TBS) which was determined by prior titration. After deparaffinization and hydration, sections were incubated with 10% normal goat serum to block non-specific binding. Subsequently, they were incubated overnight with primary antibodies in either 1:200 AA1 (Dako, Denmark), 1:200 EG2 (Pharmacia, Sweden), 1:20 CD3 (Dako, Denmark) or 1:200 NE (Dako, Denmark) at room temperature. For detection, a streptavidin-peroxidase complex (Dako, Denmark) with deaminobenzidine as chromogen was used, and sections were counter-stained with Mayors' haematoxyline. For negative controls, the primary antibody was omitted.

Evaluation

The sections were evaluated by conventional light microscopy ($\times 100$, $\times 200$, $\times 400$). The numbers of stained cells were counted and presented as cells/ mm^2 of tissue area according to Fokkens et al.⁹ Four areas per one tissue were estimated by superimposing a grid of 100 points (intersection of crosses covering a surface area of 0.5 mm^2) on the section and by using an eyepiece graticulate at 200 magnification. Mean cell counts were derived from the summation of four fields.

Statistical Analysis

The Mann-Whitney U and ANOVA tests were applied using the SPSS version 7.0 (Chicago, USA) to evaluate the statistical differences among the data. A *P* value of .05 or less was regarded as significant.

Table 1. Clinical features of the study subjects

Patient	Sex/ Age	Total Eosinophil Count	Duration of Nasal Symptoms (m)	Aspirin Intolerance	Topical Steroid
I. ASA-intolerant group					
1	F/29	600	38	P	stopped for 4 weeks prior to the study
2	F/30	400	26	P	"
3	F/57	400	28	P	"
4	F/52	300	48	P	"
5	M/36	400	25	P	"
6	F/54	200	36	P	"
7	F/57	400	46	P	"
8	F/55	300	28	P	"
9	F/33	400	34	P	"
10	F/62	600	26	P	"
11	F/32	400	48	P	"
12	M/56	200	50	P	"
13	F/43	400	60	P	"
II. Non-allergic group					
1	F/42	500	24	N	"
2	F/35	400	28	N	"
3	M/28	600	36	N	"
4	F/30	300	43	N	"
5	F/43	400	24	N	"
6	M/30	200	28	N	"
7	F/30	300	38	N	"
8	M/34	400	34	N	"
9	F/55	600	52	N	"
10	F/48	400	60	N	"
11	F/50	900	48	N	"
12	M/45	300	36	N	"
13	F/46	200	44	N	"
14	F/42	400	22	N	"
15	F/38	300	28	N	"
16	F/52	400	38	N	"
III. Allergic group					
1	M/28	600	28	N	"
2	M/35	400	36	N	"
3	F/32	800	34	N	"
4	F/45	400	26	N	"
5	F/50	600	32	N	"
6	M/38	300	48	N	"

F = female, M = male, m = month, P = positive (intolerant) and N = negative (tolerant).

RESULTS

Eosinophil

In all the subjects, there was an abundant EG2+ cells in both stroma and epithelium (Fig 1A), which did not differ significantly among the three groups ($P=.16$) (Fig 2). The EG2+ cell of subjects with ASA-sensitive asthma and allergic subjects tended to be higher than non-allergic controls, however, with no statistical significance ($P=.22$). This indicated that activated eosinophils are involved in the nasal polyp generation in ASA-sensitive asthma.

Mast Cell

All subjects had tryptase-containing mast cells (AA1) in both epithelium and stroma (Fig 1B), which did not differ significantly among the three groups as shown in Fig 2 ($P=.54$). There was a tendency for AA1+ cells to be higher in the subjects with ASA-sensitive asthma and allergic subjects than in non-allergic controls, but no statistical significance was observed ($P=.28$, $P=.08$, respectively). No statistical significance was noted between the subjects with ASA-sensitive asthma and allergic subjects ($P=.51$).

Neutrophil

Elastase-containing neutrophils were found in most subjects of three individual groups, and their numbers were significantly higher in allergic subjects than in aspirin-sensitive asthma as shown in Fig 2 ($P=.02$). Comparing the allergic and non-allergic subjects, NE+ cells were significantly higher in allergic subjects than in non-allergic controls ($P=.01$).

Lymphocytes

The number of lymphocytes varied considerably among biopsy specimens; the number of CD3+ T lymphocytes/mm² section ranged between 0 and 106/mm². There was no significant difference in CD3+ cells among the three groups, although CD3+ T cells of allergic subjects tended to be higher than in non-allergic controls or ASA-sensi-

tive asthmatic subjects ($P=.62$, $P=.13$, respectively).

Relationship Between Inflammatory Cells

Figure 3 shows correlation between NE+ and AA1+ cells (3A) or EG2+ cells (3B). Significant correlation was noted in the numbers between NE+ and AA1+ cells ($r=.44$, $P=.01$) or EG2+ cells ($r=.40$, $P=.02$).

DISCUSSION

Inflammatory processes within the mucosa of the upper respiratory tract is believed to play an important role in development of nasal polyp. The inflammatory cellular infiltrate in nasal polyps has been shown to consist of eosinophils, lymphocytes, plasma cells, and mast cells¹⁰⁻¹² to an extent which is similar to that observed in the

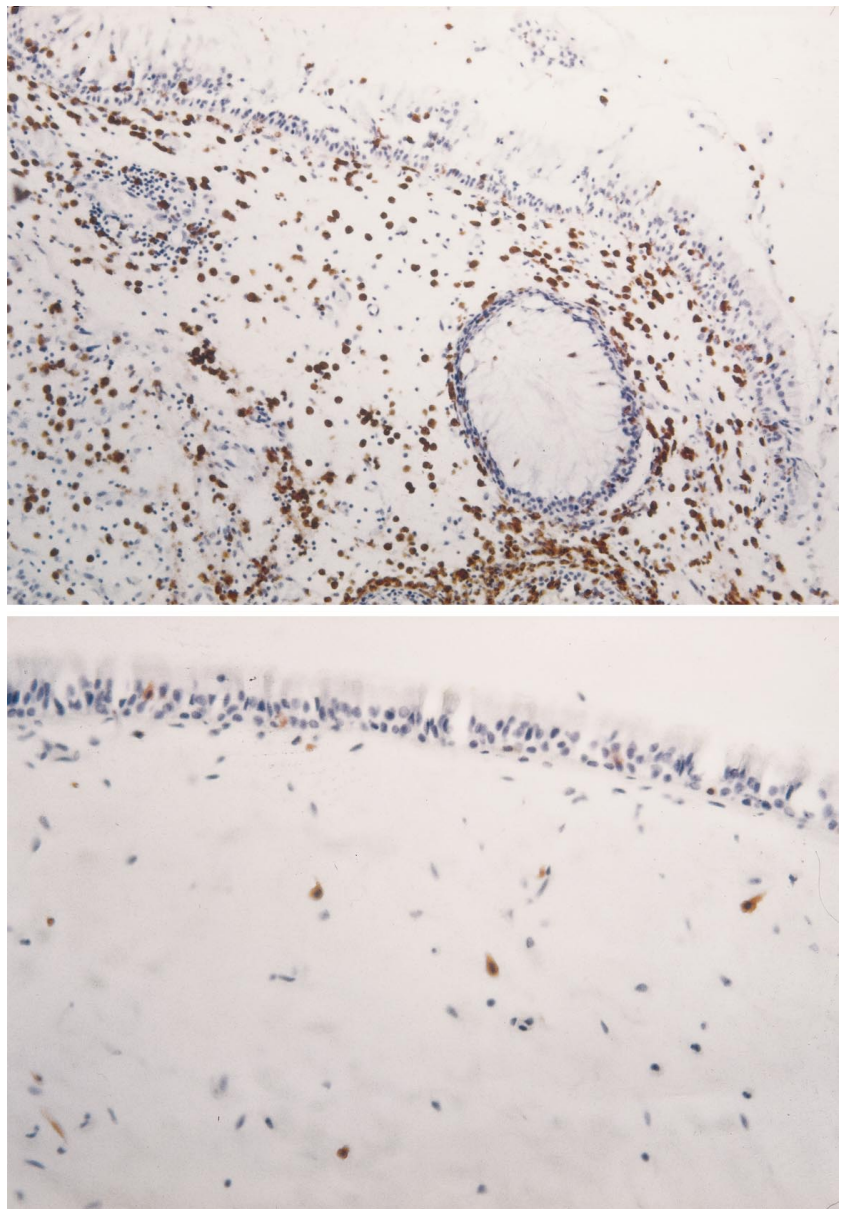


Figure 1. Infiltrated cells of nasal polyp tissue section of aspirin-sensitive asthma, positively stained with antibodies to secreted form of eosinophil cationic protein (upper panel, $\times 100$) and mast cell tryptase (lower panel, $\times 200$).

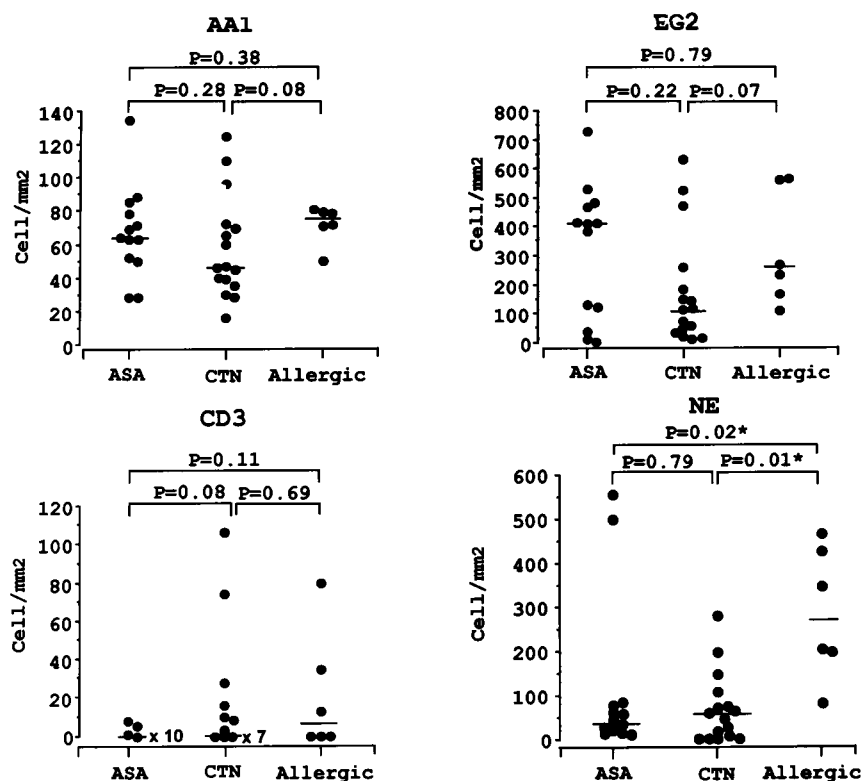


Figure 2. Comparison of inflammatory cell counts expressing AA1 (mast cell), EG2 (activated eosinophil), CD3 (pan T cells), and NE (neutrophil) in nasal polyp tissues from ASA-sensitive asthma, allergic, and non-allergic controls. Expressed as the number of positive cells per mm². Median values are represented by horizontal bars.

bronchial mucosa of asthmatics. This suggests that the inflammatory mechanisms of the two diseases may be similar.¹³ Nasal polyps are commonly found in patients with chronic respiratory disorders, especially in ASA-sensitive asthma. When compared with allergic and non-allergic subjects, the information on the immunologic processes occurring in the nasal polyp of ASA-sensitive asthma is rather limited. Immunohistochemical studies employing monoclonal antibodies to immunologically relevant cells can be helpful to understand these aspects. The present study was undertaken to detect any differences in inflammatory cellular populations and their extent in nasal polyps that were derived from allergic and non-allergic subjects, and those from patients with ASA-sensitive asthma.

Studies of epithelial toxicity exerted by arginine-rich, cationic proteins of

eosinophils, and bronchial biopsy and lavage studies from asthmatics suggested eosinophils as prime effector cells in asthma irrespective of the presence of atopy.¹⁴ Although the mechanism of eosinophilia is poorly understood, their presence in nasal polyp tissue implicated them in the pathogenesis of this disease as well.¹⁵ In addition to releasing many inflammatory and pro-inflammatory mediators that are either vaso-active and/or bronchoactive or toxic to the epithelium, eosinophil is an important source of cytokines, particularly IL-3,¹⁶ GM-CSF,¹⁶ and IL-5.¹⁷ A recent study¹⁸ showed more EG2+ cells in biopsy specimens of both nasal polyps and unaffected mucosa of the middle and inferior turbinate than in biopsies of the nasal mucosa of healthy control subjects. Further, more EG2+ cells were noted in patients with nasal polyps who had chronic obstructive pul-

monary disease or asthma than in those without.

Higher eosinophil cationic protein and eosinophil counts were noted in nasal lavage fluid from ragweed-sensitive rhinitis patients with polyposis than those without them, suggesting that an excessive response by activated eosinophils may induce more profound inflammatory process of the nasal polyps.¹⁸ In this study, EG2+ cells were abundant in both epithelial and stromal layers of three individual groups, irrespective of whether they had allergic, non-allergic background, or ASA-sensitive asthmatic subjects. EG2+ cell numbers, however, tended to be higher in nasal polyps from ASA-sensitive asthma than in the other two groups, indicating that the activated eosinophils could participate in the generation of nasal polyps in ASA-sensitive asthma. Further investigations with extended sample size of each group will be needed to understand the role of eosinophils in nasal polyp inflammation from aspirin-sensitive asthmatic subjects.

Mast cells are believed to be the key cells in allergic inflammation with an ability to release both chemical mediators and cytokines in response to cross-linkage of surface-bound IgE by allergen.¹⁹ Our study has shown that all polyp specimens had tryptase-containing mast cells in both epithelial and stromal layers. The numbers of AA1+ cell tended to be higher in the ASA-sensitive asthma and allergic subjects than in non-allergic subjects, suggesting that activated mast cells which degranulated and released tryptase could participate in nasal polyp generation in ASA-sensitive asthma similarly in allergic and non-allergic subjects.

There have been several studies on the role of neutrophils in allergic inflammation. Following allergen challenge, one study showed that BAL neutrophil numbers increased relative to baseline in asthmatics but not in normal subjects at 4 hours, whereas by 24 hours this difference had disappeared. High molecular weight neutrophil chemoattractant thought to be released by mast cells was detected

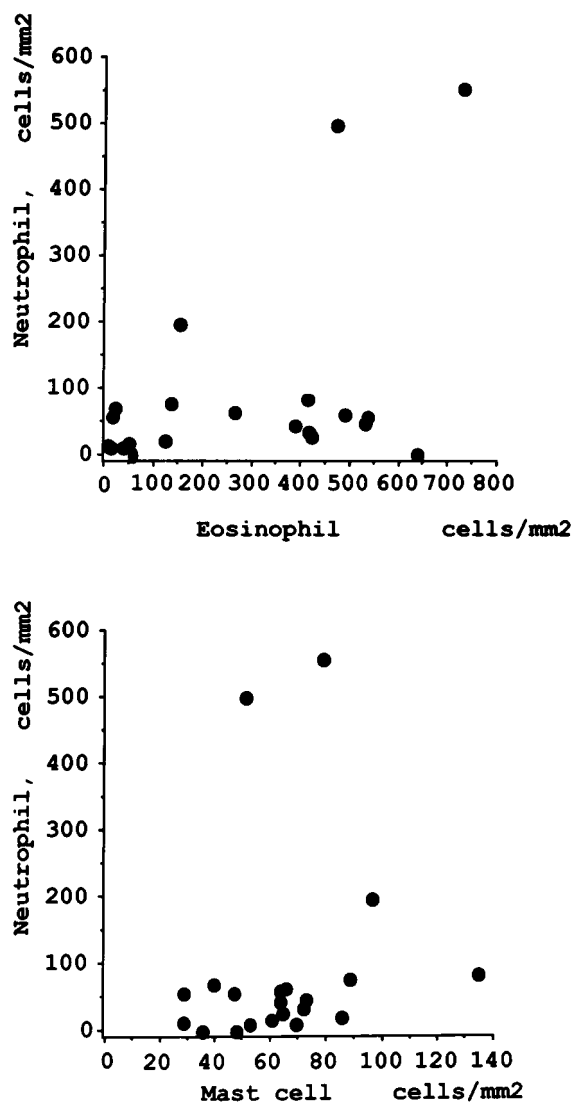


Figure 3. Correlation between NE+ and AA1+ cells (upper panel). Significant correlation was noted between NE+ cell and AA1+ cells ($r=.44$, $P=.01$) or EG2+ cells ($r=.40$, $P=.02$).

following allergen challenge, appearing within minutes and persisting for at least 24 hours.²⁰ In this study, neutrophils were found in most subjects of the three groups. Interestingly, their numbers were significantly higher in allergic subjects than those from ASA-sensitive asthma and non-allergic controls in spite of no definite evidence for associated infections. NE+ cell count was not related to nasal polyp size. Further, significant correlation was found in counts between NE+ and AA1+ cell. These findings may sug-

gest that mast cells might participate in the neutrophil recruitment in inflammation response of nasal polyp and there has been a possible involvement of neutrophil in the nasal polyp inflammation. Further studies will be needed to investigate the role of neutrophil in nasal polyp inflammation.

It was suggested that T cell has a regulatory role in airway inflammation.²¹ Using the cryosection of nasal polyp, a recent study showed an evidence of lymphocytic infiltration in the tissue,²² and found that their subsets

(CD3, CD4, and CD8) were not different between atopic and non-atopic patients. In the present study, although T cell number varied, 25% of ASA-sensitive asthma (three subjects) of ASA-sensitive asthma, 50% of allergic and 11.3% of non-allergic subjects had CD3+ cells in both epithelial and stromal layer of nasal polyp tissue. It should be noted, however, that the median numbers of CD3+ cell were not significantly different among them. These results suggested a possible regulatory role of T cell in inflammatory reaction of nasal polyp tissue, regardless of whether they were derived from ASA-sensitive asthma, allergic, or non-allergic subjects.

In conclusion, this study suggests that major effector cells, eosinophils, and mast cells may be responsible for inflammatory response of nasal polyps, whether the nasal polyp is associated with aspirin-sensitivity or not.

ACKNOWLEDGMENT

The authors are grateful to Dr. Andrew Walls (Southampton University, UK) for providing AA1 antibody.

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