

## Airway inflammation in nasal polyposis: immunopathological aspects of relation to asthma

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### Summary

**Background** Nasal polyposis (NP) is a chronic inflammatory disorder of the upper respiratory tract, which is often coexist with asthma. However, the pathogenesis of especially in patients with NP is still a matter of debate.

**Objective** To better understand the immunopathologic mechanism involved in this relationship, we investigated the inflammatory cell profiles in bronchial and nasal tissues of patients with NP alone and with concomitant asthma.

**Methods** Seventeen patients with NP (six male, 11 female, age range: 19–63, mean age:  $38.29 \pm 13.27$  years) were selected for the study. Subjects were divided into two groups based on the presence of asthma or bronchial hyper-responsiveness (BHR). NP without BHR (Group 1) ( $n = 8$ ), NP and asthma or BHR (Group 2) ( $n = 9$ ). All patients underwent atopy evaluation including detailed history, skin prick test (SPT), total and specific IgE determination in sera. None of the subjects had taken inhaled, nasal or oral corticosteroids for at least 1 month before the study. Respiratory symptoms of asthmatic patients were controlled with only short acting  $\beta_2$ -agonist inhaler drugs as needed. NP tissue, nasal and bronchial mucosa biopsies were taken from all patients using fiberoptic endoscopy. CD3, CD8, CD16, CD68, AA1 (mast cell tryptase), human leucocyte antigen-DR (HLA-DR) and eosinophil peroxidase (EPO) expressing cells in specimens were determined by immunohistochemical methods. Positively staining inflammatory cell types were counted. Subepithelial lamina propria and periglandular areas were separately evaluated.

**Results** No significant difference was found in polyp tissue, nasal and bronchial CD3<sup>+</sup>, CD8<sup>+</sup>, CD16<sup>+</sup>, CD68<sup>+</sup>, AA1<sup>+</sup>, HLA-DR<sup>+</sup> and EPO<sup>+</sup> positive cells between groups. There were significantly higher numbers of CD8<sup>+</sup>, CD16<sup>+</sup>, HLA-DR<sup>+</sup>, EPO<sup>+</sup> cells in the polyp tissue and nasal mucosa vs. the bronchial mucosa in all groups ( $P < 0.05$ ). However, CD8<sup>+</sup> cells were significantly increased in the polyp tissue and bronchial mucosa of patients with NP alone when compared with the patients with both asthma and NP ( $P < 0.05$ ). CD3<sup>+</sup>, CD68<sup>+</sup> and CD16<sup>+</sup> cell counts were tended to be higher within the nasal polyp tissue of patients with isolated NP compared with counts within nasal and bronchial mucosa of patients with NP and asthma. Also, patients with isolated NP showed more HLA-DR<sup>+</sup> cells in the nasal polyp tissue and nasal mucosa than those of patients with NP and asthma. Immunoreactivity for EPO<sup>+</sup> eosinophils within the nasal and bronchial mucosa was more prominent in patients with NP and asthma compared with patients with NP alone. The number of EPO<sup>+</sup> eosinophils within the polyp tissue, nasal and bronchial mucosa was higher in the skin prick test negative (SPT – ve) group than the SPT positive (SPT +ve) ones.

**Conclusions** Our results demonstrate that infiltration of inflammatory cells in the nasal and the lower airways do not remarkably differ between patients with NP alone who has no evidence of BHR and asthmatic patients with NP. However, patients with SPT – ve NP reveal more intense eosinophilic inflammation in the entire respiratory mucosa.

**Keywords** asthma, bronchial hyper-responsiveness, eosinophil, immunohistopathology, inflammation, nasal polyp

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### Introduction

Nasal polyps (NPs) are arised from the paranasal sinus mucosa, which prolapses into the nasal cavity and cause persistent nasal obstruction. They are often coexist with asthma, rhinitis and aspirin intolerance. Like asthma, NP

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have been ascribed to infection and allergen exposure. Among the various aetiologic factors, the role of atopy has been controversial [1]. Histologically, NP are characterized by an edematous stroma with a dense eosinophilia underneath a thickened basement membrane. The ciliated epithelium is commonly damaged. The inflammatory cell infiltrate generally includes eosinophils, T-helper lymphocytes, plasma cells and mast cells. These chronic inflammatory changes are similar to those observed in the bronchial mucosa in asthma, and a feature of polyp tissue whether or not allergy is present. The accumulation of activated eosinophils is considered to be the primary hallmark of asthma and NP. In studies involving large series of patients with NP, asthma was found in 30% to 70% [2–4]. Non-specific bronchial hyper-responsiveness (BHR) and/or asthma occur more frequently in patients with NP than in the general population. Several authors have also reported evidence of BHR in patients with NP even in those with no clinically asthma, especially non-atopics [5–7]. Lamblin et al. [8] found that patients with NP without BHR did not have eosinophilic bronchial inflammation compared with patients with NP having asthma or asymptomatic BHR. On the other hand, subjects with rhinitis who had no history of asthma or BHR have exhibited inflammatory changes in bronchial mucosa [9, 10]. There are some evidences supporting that the presence of non-specific BHR in NP can be a risk factor for the development of obvious asthma [11].

The often coexpression of these disorders suggest that they probably share common pathophysiology affecting the upper and lower airways. NP is a chronic inflammatory disease of the nasal mucosa; however, the mechanisms playing role in polyp pathogenesis remain largely unclear. The possibility that a similar inflammatory process could be involved in the NP that is associated with asthma or not, has not yet fully understood.

We designed our study to further investigate concomitantly the upper and lower airway inflammation in patients with NP alone and associated with asthma or BHR. In this study; first we obtained both nasal mucosa and polyp tissue, and also bronchial mucosa specimens from subjects with NP without BHR and having asthma or BHR for immunohistochemical analyses. The primary aim of this study was to compare bronchial and nasal inflammation between the NP alone and NP with asthma or BHR groups. In the second part of study, we evaluated whether or not this inflammation is correlated with clinical conditions such as skin test positivity and extent of polyposis.

## Materials and methods

### Patient selection

Seventeen patients (six male and 11 female) consecutively referred to the Ankara University School of Medicine, Departments of Allergy and ENT were included in this prospective study. The mean age was  $38.29 \pm 13.27$  years (age range, 19–63 years). All patients had NP and radiographic evidence of sinusitis on a recently taken sinus computed tomographic (CT) scan. Nine of 17 patients were associated with asthma. Eight patients with isolated NP who had no

history of lower airway symptoms exhibited negative bronchial response to methacholine inhalation challenge (methacholine PC<sub>20</sub> value of greater than 8 mg/mL). Later, they were divided into either an SPT +ve or a SPT –ve NPs with or without asthma on the basis of the results of skin test reactivity.

### Study protocol

This study was approved by the Ankara University School of Medicine Ethical Committee and informed consent was obtained from all patients. On the first visit, subject characteristics were documented by a detailed questionnaire on upper and lower respiratory symptoms, history of atopy and asthma, medications for NP and/or asthma. Although we cannot rule out local IgE production in the NP tissue, SPTs were applied, serum total and specific IgE levels were measured for atopy screening. Pulmonary function tests and airway responses to inhaled methacholine were assessed by using standardized procedures. On the second visit, all patients underwent a functional endoscopic sinus surgery (FESS) to obtain biopsies from nasal mucosa and polyp tissue. On the third visit (the day after), a fiberoptic bronchoscopy procedure was performed to the all patients for bronchial biopsy. Exclusion criteria were respiratory tract infection in the previous 8 weeks, and the treatment with inhaled, oral and intranasal corticosteroids or an leukotriene (LT) modifier within the last 4 weeks before the study. None of the subjects smoked or used medication that could influence the results of this study, with the exception of antihistamine and salbutamol as needed.

### Definitions

Asthma was diagnosed according to the criteria as described by the Global Initiative for Asthma [12]. The asthmatic patients exhibited a history of episodic dyspnea and wheezing, a documented (recently or in the past) reversible airway obstruction ( $12 \geq \%$  increase in forced expiratory volume in 1 s (FEV<sub>1</sub>) from baseline after inhalation of salbutamol, or hyper-responsiveness to methacholine (PC<sub>20</sub> of less than 8 mg/mL) in bronchial challenge test. Methacholine was administered according to a standardized tidal breathing method [13]. NPs were identified in all patients by the following criteria; nasal symptoms (obstruction, anosmia, sneezing, rhinorrhea, itching) visualization of polyps by anterior rhinoscopic examination and nasal endoscopy. Coronal sinus CT scanning was performed all patients. Scans were analysed for evidence of mucosa thickening in the sinuses, osteomeatal complexes, and nasal cavities. A CT-scan score was assigned, as previously described by Lund and Mackay [14]. This system grades each paranasal sinus (maxillary, frontal, sphenoid, anterior, and posterior ethmoids) as follows; 0 = no abnormality, 1 = partial opacification, and 2 = total opacification. Ostiomeatal complex is scored as 0 = not obstructed or 2 = obstructed. The total score possible ranges from 0 to 24. SPTs were performed with a common panel of inhalant allergens including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, molds *Cladosporium* and *Alternaria*, cat and dog animal danders, cockroach, grass, tree and weed pollens (Stallergenes,

Pasteur, France). Negative and positive controls were histamine (10 mg/mL) and phenolated glycerol-saline, respectively. Reactions were measured after 15 min. A mean wheal diameter larger than 3 mm was considered positive. Atopy was defined as the presence of history and positive SPT response to at least one inhalant allergen. Serum total and specific IgE concentrations were measured by uniCAP system (Pharmacia Diagnostics, Uppsala, Sweden). The normal limit of total IgE was 100 kU/L. Specific IgE was accepted positive if it was higher than 3.5 kU/L.

#### *Pulmonary function tests*

All measurements were made with the patient in the sitting position. FEV<sub>1</sub>, forced vital capacity (FVC), and maximal midexpiratory flow (FEF<sub>25-75</sub>) were obtained from flow-volume curves with a spirometer (Sensormedics Vmax series 2130 spirometer, Sensor Medics BV, Bilthoven, The Netherlands). All data were expressed in absolute values and in percent of predicted normal values.

#### *Fiberoptic bronchoscopy*

The bronchoscopy procedure was performed at the morning. All bronchial biopsy specimens were taken by the same pulmonary physician (N.N.). After local anesthesia of the throat, larynx, and bronchi was achieved with 2% xylocaine spray, a flexible bronchoscope (Olympus CV-230 Evis video system center, and Olympus BF type I-T200 Evis Bronchovideoscope) was introduced into the bronchial tree via the nasal route. Mucosal biopsy specimens were taken from segmental bronchus of the right middle lobe through biopsy forceps (FB-19CR). Vital signs and oximetry readings were monitored throughout the procedure, and the patient had an intravenous infusion to provide venous access. All patients with asthma had stable and FEV<sub>1</sub>  $\geq$  70% predicted in the bronchoscopy day.

#### *Nasal surgical procedure*

All patients underwent FESS under local anesthesia (lidocaine 4%) and sedation. Hopkins telescopes (0° and 30°, 4 mm) were used. The same ENT specialist (Y.A.) operated on all cases. Nasal mucosa biopsy specimens were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge with a forceps or microdebrider (Wizard-Xomed Co.). Nasal tissues were obtained from subjects undergoing polypectomy. All freshly obtained tissue and biopsy specimens were fixed in 10% formalin for histopathologic examination and immunohistochemistry.

#### *Immunohistochemistry and staining*

The nasal polypectomy and nasal, bronchial mucosa biopsy specimens were fixed with 10% buffered formaline for 24–48 h in room temperature and embedded in paraffin wax. Four to 6  $\mu$ m sections per biopsy specimen were cut and taken on slides coated with poly-L-lysine (Sigma, Sigma-Aldrich Chemie, Germany) for immunohistochemical stainings, except one which was stained with hematoxylin and eosin. Immunohistochemistry were performed with anti-CD3 (DAKO Ltd, Dako Cytomation, Denmark) for

T lymphocytes, anti-CD8 (DAKO Ltd) for suppressor/cytotoxic T lymphocytes, anti-CD16 (DAKO Ltd) for natural killer cells, anti-CD68 (DAKO Ltd) for alveolar macrophages, anti-HLA-DR (DAKO Ltd) for antigen presenting cells, anti-eosinophil peroxidase (EPO/Neogene) for identifying eosinophils and anti-mast cell tryptase (AA1/DAKO Ltd) monoclonal antibodies for mast cells by using streptavidin-biotin-peroxidase method. The antibodies were incubated for 1 h in room temperature. Antigen retrieval (for anti-CD8 and anti-CD16) with microwave (4X min/750 W) and treatment with trypsin (for anti-CD3, anti-CD68, AA1 and EPO) were performed before monoclonal incubation. The sections were washed with phosphate-buffered saline (PBS) between the each staining steps except protein blocking and monoclonal antibody incubation. Amino-ethyl-carbazole was used as chromogen and Mayer's Haematoxylen was used for counterstaining. Lymph node for anti-CD3, anti-CD8, anti-CD16, anti-CD68, anti-HLA-DR; skin for AA1 and nasal polyp tissues were used as positive controls, the control sections were incubated with PBS instead of monoclonal antibody. The cytoplasmic reddish-brown staining was evaluated as positive.

The sections of nasal polyp, nasal mucosa and bronchial mucosa were evaluated for each monoclonal antibody in a blinded fashion by using an Olympus BX40 microscope equipped with an eyepiece graticule. The positively stained cells were counted in total 20 graticule area at  $\times$  400 magnification and the mean positive cells per 1 graticule area (625  $\mu$ m) were calculated for each biopsy under the basement membrane. The positive cells in the lamina propria area under the surface epithelium and periphery of glands were counted separately.

#### *Statistics*

Differences between groups for each histopathological region were analysed with repeated measures analysis of variance (ANOVA). Duncan test was used to compare intra-group differences. Correlation coefficient was calculated to assess the relationship among the duration of NP and CT-scan scores. All of the results are expressed as mean  $\pm$  SEM. A *P*-value of less than 0.05 was considered statistically significant.

## **Results**

#### *Clinical data*

We studied 17 patients with NP. Characteristics of patients are shown in Table 1. Two groups were formed according to the presence of asthma or BHR. Group 1: NP without BHR (*n* = 8), Group 2: NP and asthma (*n* = 9). Whether or not having asthma, all patients with NP were also grouped based on the skin test positivity. Group 3: SPT +ve NPs (*n* = 8) and Group 4: SPT –ve NPs (*n* = 9). SPT +ve and SPT –ve NPs included equal number of asthmatic patients (*n* = 4 and *n* = 5, respectively). Among them, only two patients were documented aspirin-sensitive asthma. The 4 groups were not different in regard to age and history of NP. History of NP was  $7.8 \pm 2.7$  years. Patients associated with asthma and SPT –ve polyposis tended to have longer history of NP. The mean duration of asthma was 4.1 years. There was a

**Table 1.** Characteristics of patients

|                      | NP without BHR<br>(Group 1) | NP with asthma<br>or BHR (Group 2) |
|----------------------|-----------------------------|------------------------------------|
| Patients (n)         | 8                           | 9                                  |
| Age (year) (mean)    | 38.5 ± 9.8                  | 38.1 ± 16.3                        |
| Gender (male/female) | 3/5                         | 3/6                                |
| Atopy*               | 4                           | 4                                  |
| Total IgE (kU/L)     | 186.7 ± 83.7                | 279.1 ± 76.4                       |
| History of NP (year) | 7.3 ± 5.5                   | 8.6 ± 7.7                          |
| Polypectomy number   | 1                           | 6                                  |
| CT-scan score        | 11.7 ± 1.9                  | 15.5 ± 3.1**                       |

Data are expressed as mean ± SEM.

\*Positive skin prick test response to at least one inhalant antigen.  
\*\* $P < 0.01$ . NP, nasal polyps; BHR, bronchial hyper-responsiveness; CT, computed tomography.

significant correlation between duration of asthma and NP ( $r = 0.71$ ,  $P < 0.01$ ). The serum total IgE level (mean ± SEM) was  $245.5 \pm 106$  kU/L (range 18–790 kU/L). Although total IgE levels did not significantly differ between groups, it tended to be higher in the SPT +ve NPs than the SPT –ve NPs ( $327 \pm 104$  vs.  $172 \pm 49$ , respectively). In addition, NP and asthma group had higher total IgE values when compared with isolated NP group ( $296.3 \pm 78.0$  vs.  $186.9 \pm 83.7$ , respectively). In the whole group of 17 patients, the mean CT-scan score was  $13.5 \pm 1.1$ . When we compared the CT-scan scores in the groups, patients with SPT –ve polyps and asthma had significantly increased CT-scan score ( $P < 0.05$ ). In addition, NP with asthma group showed higher CT-scan score than the isolated NP group ( $P < 0.01$ ). CT-scan scores of all patients was positively correlated with their duration of NP ( $r = 0.56$ ,  $P < 0.05$ ).

### Immunohistochemistry

#### T lymphocyte infiltration

**CD3<sup>+</sup> cells** There was no significant difference in CD3<sup>+</sup> cells in bronchial and nasal mucosa and polyp tissue among the four groups. However, CD3<sup>+</sup> T cells of SPT –ve subjects tended to be lower in the polyp tissue than the SPT +ve group ( $7.9 \pm 1.5$  and  $14.4 \pm 3.4$ , respectively). Although not significant, CD3<sup>+</sup> cell number in periglandular area of polyp tissue was higher in isolated NP group than polyp and asthma group ( $16.3 \pm 3.1$  and  $6.7 \pm 1.0$ , respectively).

**CD8<sup>+</sup> cell counts** Isolated NP group showed increased density of CD8<sup>+</sup> cells in the lamina propria of bronchial mucosa when compared with the NP and asthma group ( $16 \pm 5.3$  and  $6.4 \pm 2.0$ , respectively). Significantly increased CD8<sup>+</sup> cells were also observed in the polyp tissue of isolated nasal polyp group than the polyp and asthma group ( $30.7 \pm 5.66$  and  $18.5 \pm 3.73$ , respectively) ( $P < 0.05$ ) (Fig. 1a). Subjects with SPT +ve polyps had higher CD8<sup>+</sup> cells in the bronchial mucosa than that of the SPT –ve group ( $15.6 \pm 5.09$  and  $6.7 \pm 2.43$ , respectively) (not significant). However, SPT –ve polyp group had significantly less CD8<sup>+</sup> cells in the bronchial mucosa ( $6.7 \pm 2.43$ ) compared with polyp tissue and nasal mucosa ( $17.2 \pm 4.57$  and  $25.4 \pm 1.77$ , respectively) ( $P < 0.05$ ) (Fig. 1b).

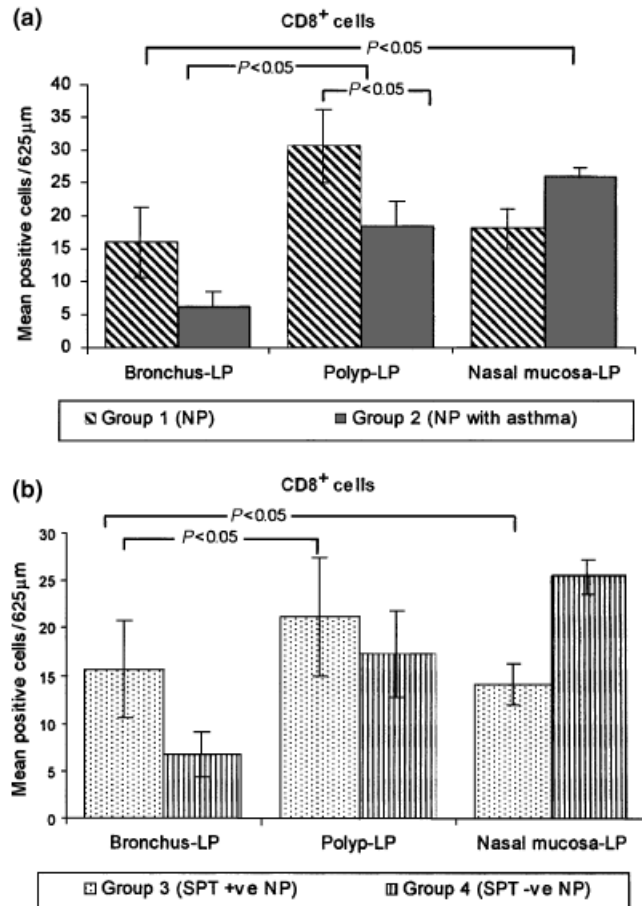


**Fig. 1.** CD8<sup>+</sup> cell counts in the bronchial mucosa, polyp tissue and nasal mucosa lamina propria (LP) (a) from patients with isolated nasal polyp (NP) and NP with asthma, (b) in skin prick test (SPT) +ve and SPT –ve NP patients. Results are expressed as the number of positive cells per 625 μm. Data represent mean ± SEM. Statistically significant differences are shown.

**HLA-DR<sup>+</sup> cells** In all patient subgroups, HLA-DR<sup>+</sup> cells were significantly more dense in the polyp tissue and nasal mucosa compared with bronchial mucosa ( $P < 0.05$ ) (Fig. 2a). Patients with isolated NP had more HLA-DR<sup>+</sup> cells in polyp tissue and nasal mucosa than in the NP with asthma group (polyp tissue:  $3.44 \pm 1.70$  vs.  $2.48 \pm 1.0$ , nasal mucosa:  $4.50 \pm 2.3$  vs.  $2.93 \pm 1.0$ , respectively). Whereas bronchial mucosa did not differ between groups ( $1.10 \pm 0.66$  vs.  $1.08 \pm 0.42$ , respectively).

**CD68<sup>+</sup> cells** There was no significant difference between groups in the numbers of CD68<sup>+</sup> cells in nasal and bronchial mucosa and polyp tissue. Patients with NP alone had higher numbers of CD68<sup>+</sup> macrophages in the periglandular area both polyp tissue and bronchial mucosa as compared with patients associated with asthma (polyp tissue:  $6.5 \pm 2.55$  vs.  $3.6 \pm 0.47$ , bronchial mucosa:  $3.3 \pm 0.85$  vs.  $1.7 \pm 0.04$ , respectively), but the difference did not reach statistical significance (Fig. 2b). Although not significant, SPT +ve polyp group displayed more CD68<sup>+</sup> cells in the polyp tissue and bronchial mucosa than the SPT –ve group (polyp tissue:  $6.5 \pm 2.62$  vs.  $4.1 \pm 1.1$ , bronchial mucosa:  $5.0 \pm 0.0$  vs.  $1.62 \pm 0.03$ , respectively).

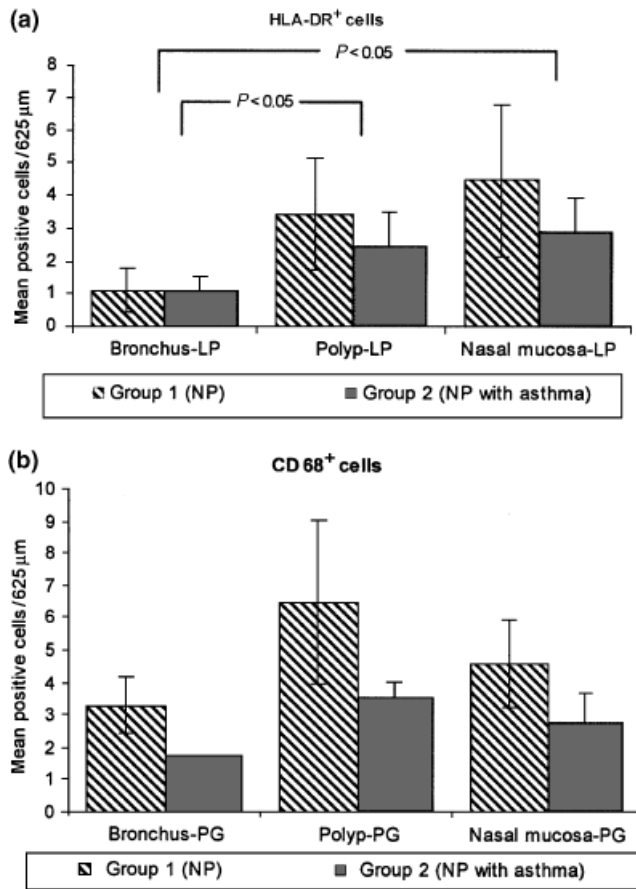


**Fig. 2.** (a) Distribution of HLA-DR<sup>+</sup> cells in lamina propria (LP) and (b) CD68<sup>+</sup> cells in periglacial area of bronchial mucosa, polyp tissue and nasal mucosa from patients with nasal polyposis (NP) and NP with asthma.

**CD16<sup>+</sup> cells** Neither subgroup differed from each other in terms of CD16<sup>+</sup> cells. In comparison with bronchial mucosa, CD16<sup>+</sup> cell numbers of nasal mucosa and polyp tissue were significantly higher in all groups ( $P < 0.05$ ). Patients with isolated NP had a tendency to have more CD16<sup>+</sup> cells in the polyp tissue than in the polyp and asthma group ( $6.4 \pm 1.63$  vs.  $4.0 \pm 2.0$ ) (not significant) (Fig. 3a). SPT +ve NP group showed fewer CD16<sup>+</sup> cells in the lamina propria of polyp tissue ( $3.3 \pm 1.24$  vs.  $6.72 \pm 2.11$ ) (Fig. 3b).

**Eosinophil** The density of EPO<sup>+</sup> eosinophils was significantly increased in the nasal mucosa and polyp tissue than in the bronchial mucosa of all groups ( $P < 0.05$ ). In comparison with isolated NP group, there was an abundant EPO<sup>+</sup> cells in the nasal mucosa of patients with NP and asthma ( $35.11 \pm 14.27$  vs.  $16.75 \pm 6.01$ ) (not significant) (Fig. 4a). This indicated that activated eosinophils are involved in the nasal polyp generation. Although not significant, SPT –ve NP group exhibited higher numbers of EPO<sup>+</sup> eosinophils in the polyp tissue ( $44.06 \pm 13.18$  vs.  $16.34 \pm 4.86$ ) (Fig. 4b).

**Mast cell** All subjects had tryptase-containing mast cells (AA1), which did not differ significantly among the subgroups in terms of polyp tissue, nasal and bronchial mucosa. The number of mast cells was similar in the groups (data were not shown).

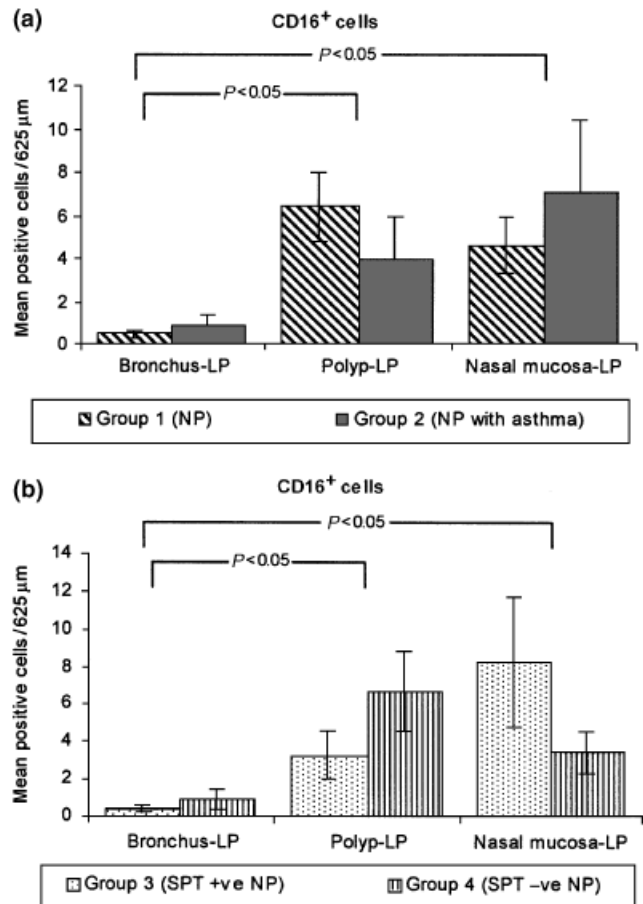


**Fig. 3.** CD16<sup>+</sup> cell counts in the bronchial mucosa, polyp tissue and nasal mucosa lamina propria from patients with (a) isolated nasal polyposis (NP) and NP with asthma, (b) in skin prick test (SPT) +ve and SPT –ve NP patients.

## Discussion

This is the one report to describe a relationship between nasal and bronchial inflammation of patients with NP alone and with concomitant asthma. In the present study; in NP with or without asthma or/BHR, inflammation of the nasal tissues and bronchial mucosa represented similar distribution of inflammatory cells including eosinophils, T lymphocytes, macrophages and mast cells. Our findings suggest the presence of lower airway inflammation, which it does not lead to symptoms or BHR, in patients with NP alone.

Although it has long been known that NP is commonly associated with asthma, the pathogenetic mechanisms underlying this relationship are not yet clear. Histological studies have shown that majority of the polyps are characterized by infiltration of the mucosa with eosinophils, T lymphocytes and mast cells, epithelial damage and expression of Th2-type cytokines similar to that documented in asthma [15–17].

In our study, patients having isolated NP showed increased CD8<sup>+</sup> cells in both bronchial mucosa and polyp tissue when compared with polyp with asthma group. The predominance of CD8<sup>+</sup> cells in polyp tissue is in agreement with previous observations of some authors [18–20]. In fact, the major difference of our study from others was to include asthmatic patients with NP not only NP patients.

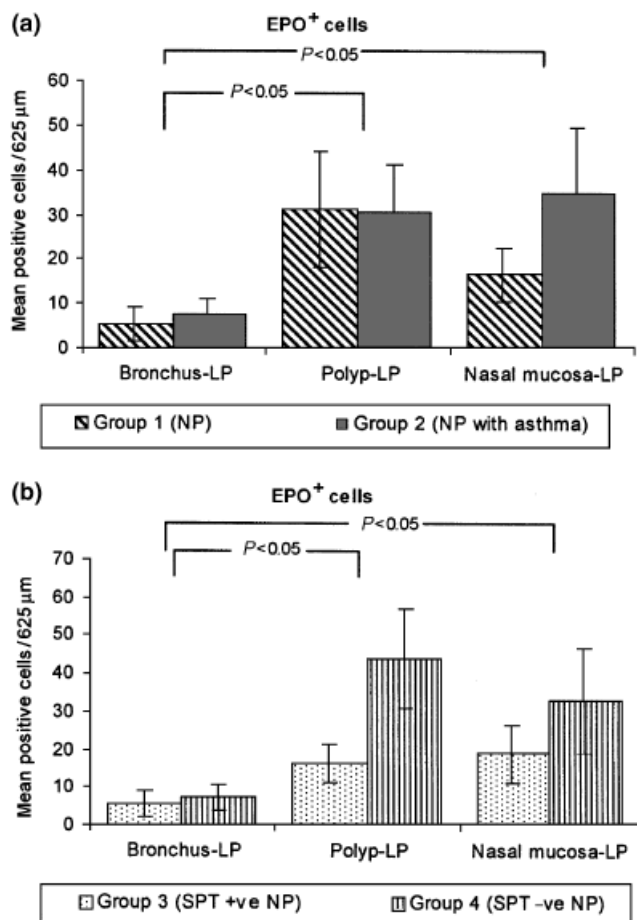


**Fig. 4.** Comparison of density of eosinophil peroxidase (EPO<sup>+</sup>) eosinophils in the bronchial mucosa, polyp tissue and nasal mucosa lamina propria from patients with (a) isolated nasal polyp (NP) and NP with asthma, (b) in skin prick test (SPT)+ve and SPT -ve NP patients.

Despite it is well known that CD4<sup>+</sup> T cells play a central role in the mucosal inflammation in both asthma and NPs, the mechanisms involved in the generation of CD8<sup>+</sup> suppressor cells are poorly understood. We were not able to detect CD4<sup>+</sup> T cells in the specimens because of some technical reasons. But, our finding could suggest that higher numbers of CD8<sup>+</sup> cells in the bronchial mucosa might have a suppressive effect on the induction of local inflammatory response against development of asthma or BHR in isolated NP. Also, it is possible that increased CD8<sup>+</sup> suppressor cell activity in the nasal mucosa and polyp infiltrate could protect to the host from harmful effect of local immune reaction. We observed that the numbers of CD8<sup>+</sup> T cells were lower in the bronchial mucosa than in the nasal mucosa and polyp tissue of patients with SPT -ve NP. Whereas CD8<sup>+</sup> T cells were found to increased in the bronchial mucosa of patients with SPT +ve NP. Recently, it is thought that although, CD4<sup>+</sup> T cells particularly, initiate and sustain allergic type immune response, regulatory suppressor CD8<sup>+</sup> T cells modulate CD4-driven inflammatory responses with down-regulatory activities [21]. Here, greater number of HLA-DR<sup>+</sup> cells were observed in nasal mucosa and polyp tissue than in the bronchial mucosa of all NP patients with or without asthma in this study. Our findings are consistent with those of

Stoop et al. [18] demonstrating an abundant presence of HLA-DR<sup>+</sup> cells in the epithelium and nasal mucosa of patients with NP in whom some of them had asthma. The similar data was shown with the appearance of HLA-DR<sup>+</sup> cells in the lamina propria of NP in patients with allergic or non-allergic NP by Liu et al. [22]. These authors also detected more CD8<sup>+</sup> suppressor/cytotoxic cells in the polyp tissue. In contrast, bronchial mucosa have not been evaluated in these studies. It should be kept in mind that HLA-DR<sup>+</sup> cells act as antigen-presenting cells in the nasal mucosa and HLA-DR positivity is a marker of cell activation. It is well known that nasal mucosa as an area of chronic antigenic stimulation is continuously more exposed to noxious agents and inhalant antigens than the bronchial mucosa. In the upper airways, raised expression of HLA-DR may thus an attempt to control the inflammatory reaction by inducing immune responses in nasal polyp.

In this study, CD16<sup>+</sup> natural killer (NK) cells were more abundant in the nasal mucosa and polyp tissue than in the bronchial mucosa of all patients. As interesting, patients having NP without asthma had increased numbers of CD16<sup>+</sup> NK cells in the polyp tissue when compared with patients with asthma. These cells contribute to the first natural defense mechanism in the upper respiratory tract because of their cytotoxic/suppressor properties in the immune response [23]. Therefore, high numbers of CD16<sup>+</sup> and CD8<sup>+</sup> cells in the upper airways may indicate a potential role to prevent from downloading inflammation to the lower airways in NP patients not yet developed asthma or BHR.

The number of tryptase-containing mast cells were similar in all biopsy specimens of both nose and bronchi of patients with NP alone and with concomitant asthma. Our data partly support the results of a previous study, in which AA1<sup>+</sup> mast cells did not differ in all polyp specimens among allergic, non-allergic and aspirine sensitive patients [24]. According to these data, we can assume that mast cells could participate in nasal polyp generation whether NP is associated with asthma and allergy or not.

In line with our observations, a large number of histologic studies have shown that tissue eosinophilia is a prominent feature of most NPs [16, 25–27]. Park et al. [24] noted that activated eosinophils was abundant in NPs irrespective of whether it was allergic or non-allergic, but they tended to be higher in aspirine-sensitive asthmatics. In our study, the predominance of activated eosinophils in the polyp tissue of patients with SPT -ve polyposis suggest that the other possible mechanisms not be driven by T lymphocytes may account for the selective eosinophil accumulation into the tissues. It has long been recognized that eosinophils are major effector cells in asthmatic inflammation, a disease that is frequently coexist with NP [28]. An important aspect of our study was to examine both nasal and bronchial biopsy specimens of the same patients. In addition, more eosinophils were found to exist in the nasal mucosa of NP patients with asthma in the present study. It is likely that being of asthmatic may induce more eosinophil infiltration into the nose. Or alternatively, the presence of extensive eosinophilic inflammation in the upper airways might be expected to enhance the epithelial damage as observed in NP patients having asthma.

Several investigators have provided convincing evidence that the cellular characteristics of NP were closely related to those seen in the asthma [8, 29–31]. In the study of Lamblin

et al., they reported that NP patients without BHR did not have eosinophilic lower airway inflammation. They also found equal amounts of mast cells, macrophages, and lymphocytes in the bronchial mucosa among groups. But, nasal inflammation of patients with NP was not examined in their study [8]. An important observation of the present study was that accumulation of activated eosinophils was more pronounced in the upper airways of patients with NP and asthma than in the NP alone. Moreover, total IgE levels tended to be higher in patients with NP and asthma. Thus, this finding provide further evidence the prior studies suggesting that the intensity of sinonasal inflammation as reflected by eosinophils might have contributing effect for the bronchial involvement. In our study, the presence of high numbers of eosinophils in polyp tissue from patients with and without asthma also suggests that it is the polyp tissue itself that is responsible for driving the increased eosinophils.

In the other aspect of this study, we evaluated the possible relations of some factors. As expected, there was a significant positive correlation between the extent of sinonasal disease and duration of NP. Here, patients with a more advanced NP appeared to be associated with asthma showing high scores on sinus CT scans and longer duration of NP. In accordance with this, duration of NP correlated with history of asthma. Extensive sinus disease have been shown in 24% of patients having severe asthma and its relation to sputum eosinophilia in a study reported by Brinke et al. [32].

In summary; as determined by immunocytochemistry, nasal and bronchial tissues differed significantly in terms of the number of activated (EPO<sup>+</sup>) eosinophils, CD8<sup>+</sup>, CD16<sup>+</sup> and HLA-DR<sup>+</sup> cells. In our all patients, NPs and nasal mucosa contained more EPO<sup>+</sup> eosinophils, CD8<sup>+</sup>, CD16<sup>+</sup> and HLA-DR<sup>+</sup> cells as compared with the bronchial mucosa. Because only two patients with NPs had aspirin intolerant asthma, we could not comment on whether they have any differences in cell numbers with other subjects.

In addition, this study indicates that NP without BHR and with concomitant asthma or BHR represent very similar immunohistopathologic features revealing marked eosinophilic inflammation in the nose of patients with asthma. Moreover, bronchial inflammation exists in patients with NP even in not having asthma or BHR, but it seems to be far less extensive than in asthma. These findings along with the often clinical overlap suggest that NP and asthma are linked by common inflammation of entire airway mucosa, in particular eosinophils, propagation from the nose down to the bronchi.

However, polyp tissue of NP patients not having asymptomatic BHR or asthma has more cytotoxic/suppressor cells such as CD16<sup>+</sup> and CD8<sup>+</sup> cells. The imbalance of these cells might be crucial factor for the development of later BHR and asthma in patients with NP alone. It needs to further studies to elucidate mechanisms by which inflammatory response in the NP increases the risk of asthma even in the absence of BHR.

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