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Electron microscopical studies of the cell population in nasal secretions*

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SUMMARY

The purpose of this study is to identify the cell types and ultrastructural changes of the cells in nasal secretions, and to understand the pathology of allergic and infectious rhinitis. Nasal secretions from 20 patients with allergic rhinitis and 15 patients with infectious rhinitis have been observed by transmission electron microscopy. The cell population of the allergic group consists of (in order of predominance): epithelial cells, eosinophils, neutrophils, lymphocytes, basophilic cells (basophil leukocytes and mast cells), and macrophages. In the infectious group the population contains: neutrophils, epithelial cells, macrophages, and lymphocytes. Marked degranulation has been observed in the granules of eosinophils in allergic nasal secretions together with granule fusion, vacuolation, and signs of phagocytosis. Increased numbers of basophil leukocytes and mast cells are also a feature of the allergic nasal secretion. Degranulation of neutrophils is markedly increased in the infectious group as compared to the allergic group. Clustered epithelial cells are observed in the allergic group more often than in the infectious group. Four types of lymphocytes with different morphological features are observed in both groups, i.e. small lymphocyte, T-lymphocyte-like cells, large granular lymphocyte-like cells, and plasma cells. The results of the present study show special ultrastructural characteristics in the cell population of allergic nasal secretions, i.e., an increase in the number of degranulated eosinophils and basophilic cells, clustered epithelial cells, and large granular lymphocytes, while an increase in degranulated neutrophils and macrophages with marked phagocytosis are characteristic for infectious nasal secretions.

Key words: ultrastructural cytology, nasal secretion, allergic rhinitis, infectious rhinitis

INTRODUCTION

As early as 1922, Eyerman demonstrated the correlation between allergic rhinitis and eosinophilia in nasal mucus. Since then, eosinophils in nasal secretions have been utilized as an aid in the diagnosis of nasal allergy for many years. The cytology of nasal secretions - especially the eosinophils - has been extensively studied and is well documented (Malmberg and Holopainen, 1979; Mullarkey et al., 1980; Whelan, 1980; Bogaerts and Clement, 1981), but most of these studies have been done by light microscopy. Due to the limited resolution of the light microscope, it is not easy to differentiate between all cell types. Furthermore, changes in the fine structure of the cells indicating functional changes in the cells, cannot be observed lightmicroscopically. Although reports have been published on the ultrastructure of cells in nasal secretions, most of them were limited to only some features of the eosinophils or basophils (Okuda et al., 1978; Masuyama et al., 1988). The purpose of this study is to elucidate the characteristics of the cell population in the nasal secretion in the differentiatial diagnosis of allergic and infectious rhinitis, and for understanding the pathology of both diseases by means of electron microscopy.

MATERIAL AND METHODS

Patients

The *allergic group* consisted of 11 male patients and nine female patients with a mean age of 23.8 years (range: 5-55 years). They were identified according to the following criteria: (1) clinical symptoms such as nasal itching, sneezing, hypersecretion, and nasal blockage at the time of collection of nasal secretion; (2) positive skin tests and nasal challenge tests to one or several antigens (most of the patients in this group were positive to house dust mite and Japanese cedar pollen); (3) elevated specif-

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ic IgE antibody levels in the serum; and (4) absence of associated airway infections, both chronic or acute, for at least two weeks prior to the study.

The *infectious group* consisted of eight male and seven female patients with a mean age of 25.1 years (range: 6–57 years) suffering from chronic rhinosinusitis with perennial clinical symptoms such as nasal obstruction and purulent or mucopurulent nasal discharge for more than one year. Examination of the nasal cavity showed congestion of the inferior turbinate with purulent discharge in the middle or inferior nasal meatus. X-rays of the paranasal sinuses (occipito-frontal and Waters' views) showed increased density in the ethmoidal and/or the maxillary sinuses.

Collection of specimen

Nasal secretions were collected by aspiration directly from the nasal cavity (about 0.3–0.5 ml per patient) and fixed in 10 ml of 3% glutaraldehyde in phosphate-buffered saline (PBS; pH 7.4) at a temperature of 4°C for 24 h. The specimen were then centrifuged at 400*g* for 10 min. The pellet was washed in 0.1 M PBS three times for 10 min, post-fixed in 1% osmium tetroxide for 2 h, followed by two rinses in 0.1 M PBS, dehydrated in a graded series of increasing concentrations of ethanol and then embedded in epoxy resin. Ultrathin sections (90 nm) were cut using an ultramicrotome and taken from three different layers of each specimen; next, they were contrast-stained with uranyl acetate and lead citrate and observed in a Hitachi H-800 electron microscope. All the cells in each field were observed and photographed at a magnification of × 6,000.

Identification of cell types

The *leukocytes* in nasal secretions (including eosinophils, basophil leukocytes and neutrophils) in general reveal condensation of chromatin in the margins of the nucleus.

Eosinophils have many typical granules with a homogeneous matrix and 1-3 electron-dense cores in the centre of the granules, and a lobulated nucleus (Kelly, 1984; Junqueira et al., 1986; see Figure 1).

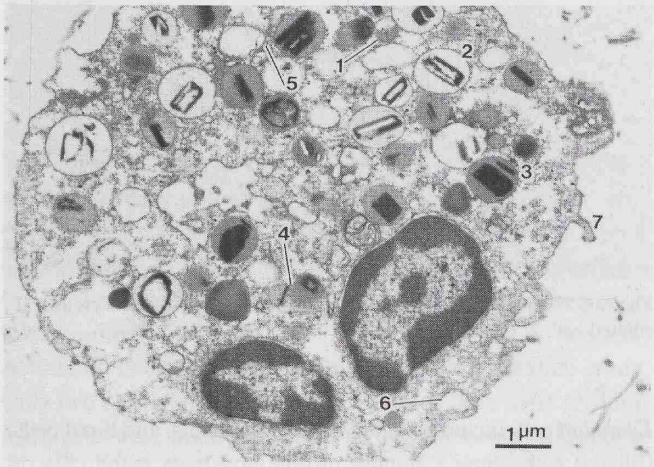

Figure 1. Eosinophil from an allergic nasal secretion (1: partial degranulation of matrix; 2: total degranulation of matrix; 3: no degranulation; 4: granule fusion; 5: vacuolization in granule; 6: pinocytotic vesicle; 7: microvillus-like process).

Basophilic cells (blood basophil leukocytes and mast cells) appear round or oval in shape, and the nucleus demonstrates 1–4 segmentations. The specific granules in the cytoplasm have a homogeneous finely-granular matrix, but differ in size from cell to cell. They are divided into two types of cells (Okuda et al., 1978): one is similar to blood basophil leukocytes (Figure 2A). and the other to mucosal-type mast cells (Figure 2B).

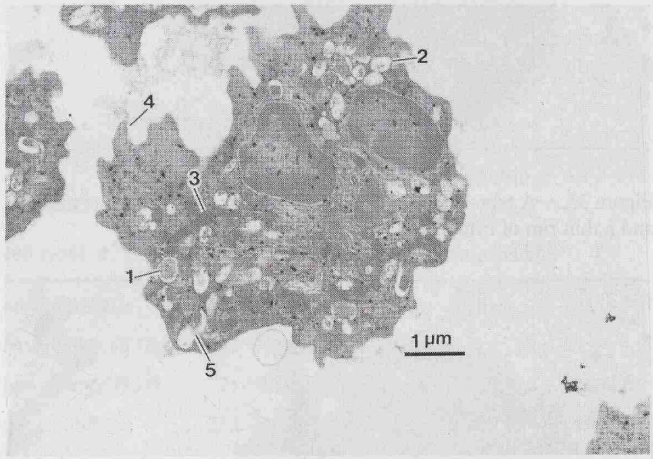

Figure 2A. A basophil leukocyte from a nasal secretion showing many granules with a homogeneous finely-granular matrix (1: partial degranulation; 2: total degranulation; 3: no degranulation; 4: microvillus-like process; 5: granule fusion).

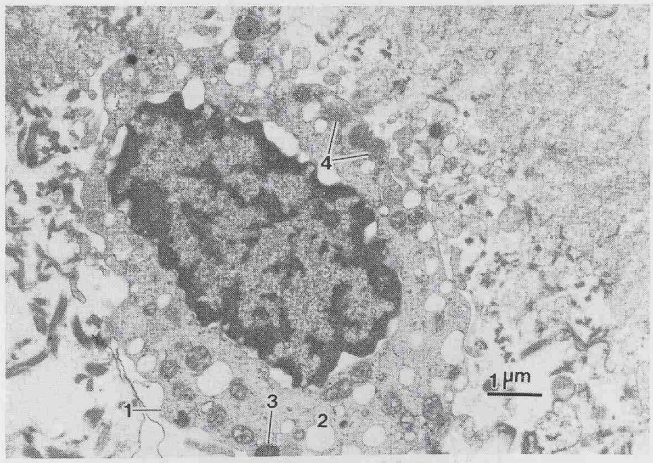

Figure 2B. A mucosal-type mast cell (1: partial degranulation; 2: total degranulation; 3: no degranulation; 4: fusion of granule with adjacent granule and granule with cellular membrane).

Lymphocytes have a large nucleus/cytoplasm ratio. With respect to their size, the shape of nucleus and the cytoplasmic organelles, lymphocytes in nasal secretions can be divided into four types. Type I has a relatively large round nucleus and scanty organelles in the cytoplasm (Figure 3A); type II has an irregular nucleus and is rich in cytoplasmic organelles (Figure 3B); type III has several granules of different sizes and many organelles in the cytoplasm, and its ultrastructural composition is similar to that of natural killer cells and large granular lymphocytes (Prasthofer, 1987; Figure 3C); type IV is characterized by an abundance of rough endoplasmic reticulum cisterns and several mitochondria, similar to plasma cells (Figure 3D).

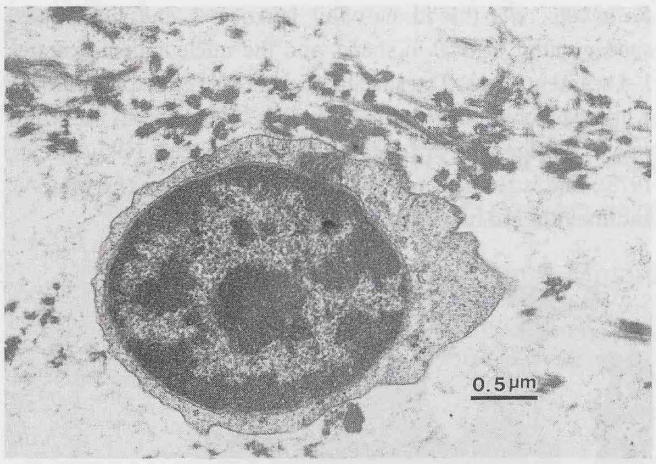

Figure 3A. A type-I lymphocyte with a round, relatively large nucleus and a thin rim of cytoplasm.

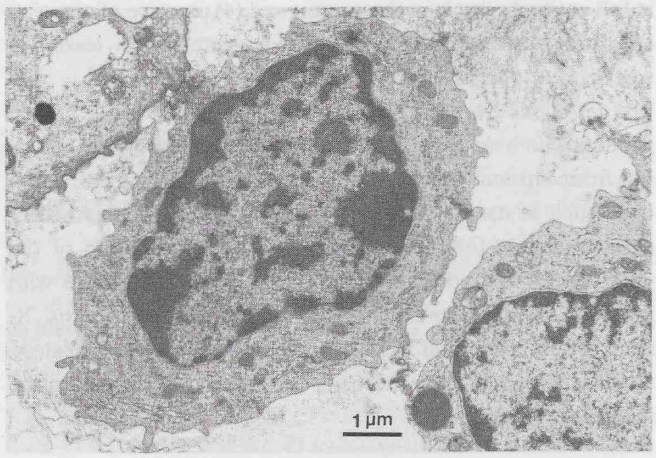

Figure 3B. A type-II lymphocyte with an irregularly-shaped nucleus and several cytoplasmic organelles.

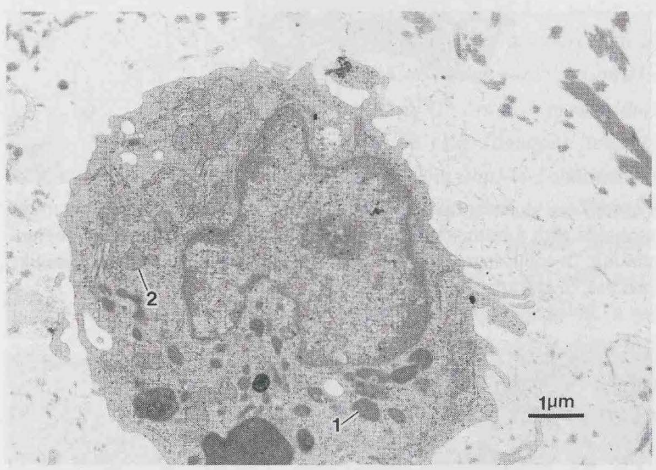

Figure 3C. A type-III lymphocyte with several specific granules in different sizes and abundant cytoplasmic organelles (1: specific granule; 2: mitochondria).

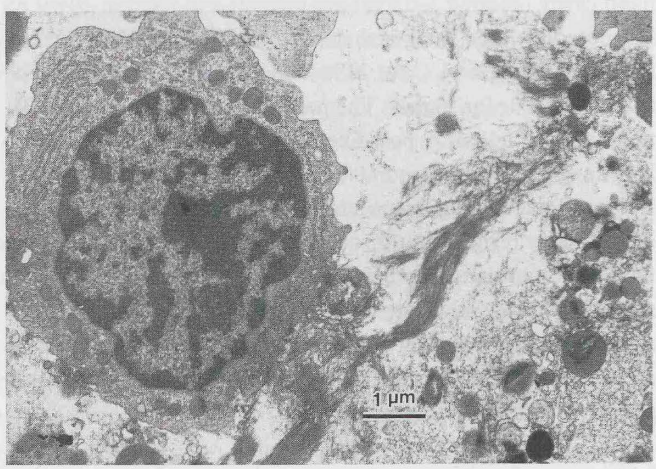

Figure 3D. A type-IV lymphocyte rich in rough endoplasmic reticulum and several mitochondria.

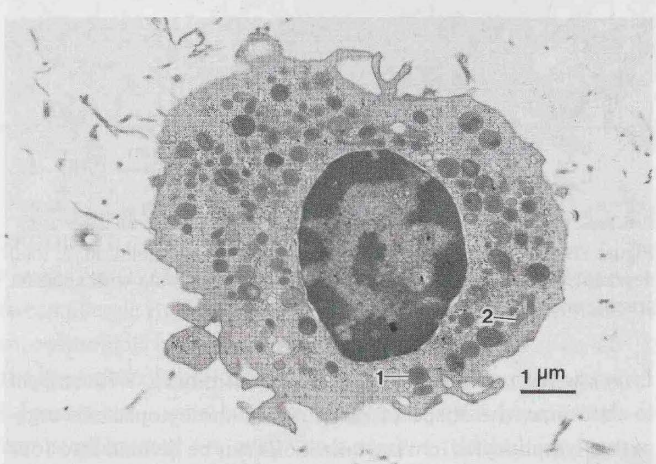

Figure 4. Neutrophil from an allergic nasal secretion (1: azurophilic granule; 2: specific granule).

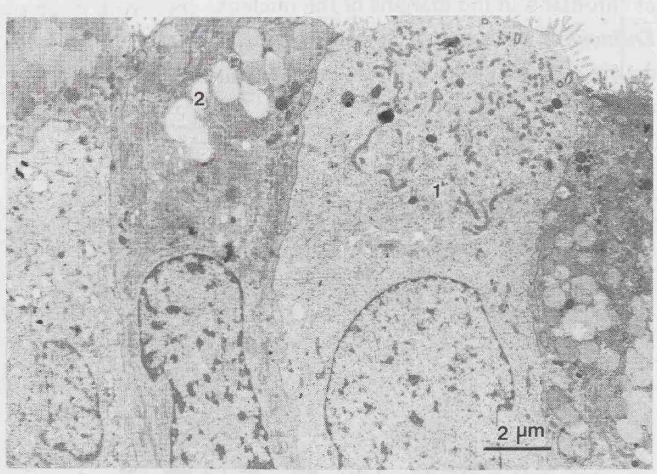

Figure 5. Clustered epithelial cells from an allergic nasal secretion (1: ciliated cell; 2: goblet cell).

Neutrophils have a segmented nucleus with 1–5 indentations, and are characterized by the presence of two types of cytoplasmic granules, i.e. azurophilic and specific granules (Kelly, 1984). They show many phagosomes of irregular and different sizes (Junqueira et al., 1986; Figure 4).

Epithelial cells include ciliated cells, goblet cells, and basal cells. Ciliated cells show a slender profile, and have many cilia on their surface. Goblet cells are filled with large secretory granules. Clumps of epithelial cells are referred to as clustered epithelial cells (Figure 5).

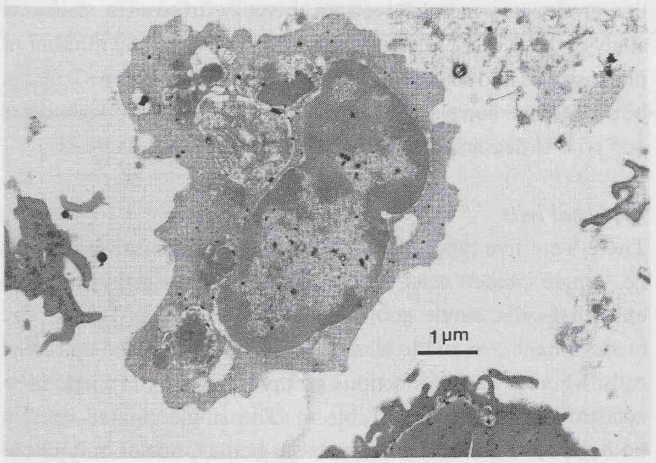

Figure 6. Macrophage with several phagosomes from an allergic nasal secretion.

Macrophages usually have non-lobulated or kidney-shaped nuclei, in the cytoplasm many phagosomes and lysosomes are present, but only few azurophilic granules (Junqueira et al., 1986; Figure 6).

Observations of micrographs

We measured the maximum diameter of each cell, as well as the transverse diameter from the centre of the maximum diameter perpendicularly. Furthermore, the number of nuclear lobes, microvillus-like processes on the cell surface, phagosomes (including pinocytotic vesicles), and mitochondria in the cytoplasm were counted.

The degranulated granules of eosinophils were divided into three types: (1) Type I or partial degranulation; (2) Type II or complete degranulation; and (3) no degranulation (Type III; Figure 1). We counted degranulation separately in the matrix and the core, because they did not always degranulate simultaneously. Degranulation of the basophilic cells was classified in a similar way as the eosinophils.

Statistical methods

The data were analysed using the Student's t test, the Chi-square test, and Pearson's correlation analysis.

RESULTS

We examined a total of 1,407 cells in the allergic group. In order of predominance, the cells observed were: epithelial cells (n=654; 46.5%), eosinophils (n=344; 24.5%), and neutrophils (n=233; 16.6%). Basophilic cells (n=57; 4.0%), macrophages (n=30; 2.1%) and lymphocytes (n=71; 5.0%) were observed in lower numbers (Table 1).

A total of 1,000 cells were examined in the infectious group. Only two eosinophils (0.2%) were observed. The most predominant cells were neutrophils (n=786; 78.6%), being followed by epithelial cells (n=109; 10.9%), which were significantly less than those in the allergic group. Lymphocytes (n=15; 1.5%) were much less, but macrophages (n=71; 7.1%) were significantly more than those in the allergic group.

The ratio of each cell type to the total number of cells varied greatly from case to case: eosinophils (0–78.1%), basophilic cells (0–-33.1%), lymphocytes (0–23.4%), neutrophils (0–72.5%), epithelial cells (2.0–56.5%), and macrophages (0–8.9%) in the allergic group. In the infectious group, these numbers were: eosinophils (0–1.7%), lymphocytes (0–7.9%), neutrophils (26.3–100%), epithelial cells (0-31.8%), and macrophages (0–22.9%).

Table 1. The number of each cell type in nasal secretions.

	allergic group		infectious group		
cell types	*n*	% (mean±SD)	*n*	% (mean±SD)	P
eosinophil (Eo)	344	24.5±21.8	2	0.20	
basophilic cell (Ba)	57	4.0±7.5			
lymphocyte (L)	71	5.0±6.3	15	1.5±2.4	<0.0001
neutrophil (N)	233	16.6±21.4	786	78.6±20.4	<0.0001
epithelial cell (Ep)	654	46.5±19.9	109	10.9±20.7	<0.0001
macrophage (Mo)	30	2.1±2.9	71	7.1±7.5	<0.0001
unidentified	18	1.3±2.2	17	1.7±3.2	
total number	1,407		1,000		

Table 2. The fine structures of eosinophils in nasal secretions.

		allergic group		infectious group
		mean	SD	mean
max. diameter (µm)		9.11	1.68	7.77
transverse diameter		6.24	1.20	6.86
nuclear segmentation (cells)	1	168		1
	2	92		
	3	3		
	0	81*		
no. of total granules		15.34	9.00	35.5
matrix degranulation:	I**	0.67	1.11	0
	II	1.07	1.85	0
	III	15.37	8.80	35.5
core degranulation:	I	1.29	1.37	0
	II	1.01	1.66	0
	III	5.69	3.90	0
vesiculation of granules		0.49	0.35	0
microvillus-like processes		2.95	3.07	10.00
phagosomes		2.36	2.13	3.50
fusion of granules		0.96	1.44	0
lipid droplets		0.50	0.90	0.50
mitochondria		1.51	1.77	0

(*): There were 81 eosinophils in which the nuclei were not visible due to inadequate sectioning angles.

(**): Degranulation I: partial degranulation; degranulation II: total degranulation; degranulation III: no degranulation.

Eosinophils

The mean maximum and transverse diameters of eosinophils were 9.11 and 6.24 μm, respectively, in the allergic group. There were a few lipid droplets (0.5±0.9/cell), pinocytotic vesicles, and 15 granules per cell profile. The values of partial and complete degranulation in the matrix were 4.3% and 6.9%, respectively, comparable to those in the core (8.3% and 6.5%, respectively). Granule fusion (0.9±1.4/cell), vacuolation of granules (0.4±0.3/cell), mitochondria (1.5±1.7/cell), and a few ribosomes were also observed (Table 2).

Neutrophils

In the allergic group, 16.5% of the total cells examined were neutrophils. Their average maximum diameter was 7.17 μm. A few phagosomes were dispersed throughout the cytoplasm. There were two types of granules in the cytoplasm. One was large, round-shaped with a homogeneous matrix (azurophilic granule), whereas the other was either round, dumbbell-shaped or claviform and was smaller in size (specific granule). Many microvillus-like processes (6.9±4.4/cell) were observed on the cell surface (Table 3).

Table 3. The fine structures of neutrophils in nasal secretions.

	allergic group		infectious group		
	mean	SD	mean	SD	P
max. diameter	7.17	0.57	9.03	0.82	<0.0005
transverse diameter	5.90	0.49	6.48	0.56	<0.05
phagosomes	0.85	0.99	2.54	1.33	<0.01
total granules	25.05	6.36	17.08	4.21	<0.001
granule I*	8.85	4.01	6.37	3.33	>0.05
granule II	16.45	8.18	10.57	4.86	<0.0001
degranulation I	0.65	1.14	1.28	1.14	<0.001
degranulation II	0.45	0.76	1.03	0.96	<0.01
microvillus-like processes	6.90	4.42	3.24	2.86	<0.01

Correlation analysis: Phagosome and microvillus-like processes: r=-0.49, p <0.005; phagosome and degranulation II: r=0.59, p <0.0005. (*) Granule I: azurophilic granules: Granule II: special granules.

In the infectious group, the neutrophils were also predominating (78.6%) and were present in significantly higher numbers than in the allergic group. The shape of the cells was the same as that in the allergic group. About 38.3% (295 cells of the total neutrophils) showed different degrees of degeneration, but could still be identified as neutrophils, on basis of the two types of cytoplasmic granules. The size of the neutrophils in the infectious group was significantly larger than in the allergic group. The phagosomes (2.5±1.3/cell) in the cytoplasm contained bacteria, electron-dense material, and electron-lucent vacuoles. The numbers were significantly higher in the infectious group than in the allergic group (p <0.01). The degree of degranulation was significantly higher than in the allergic group. Microvillus-like processes were observed on the cell surface. On statistical analysis there was a negative correlation between the number of phagosomes and microvillus-like processes (r=-0.49; p <0.0005), but a positive correlation between the number of phagosomes and type-II degranulation (r=0.59, p <0.0005; see Table 3).

Epithelial cells

There were five types of epithelial cells in the nasal secretions, i.e., single ciliated cells, metaplastic squamous cells, clustered epithelial cells, single goblet cells, and single basal cells. The predominant cells in the allergic group were clustered epithelial cells, whereas in the infectious group they were the single metaplastic squamous cells (Table 4). The single ciliated cells in both groups showed kinocilia, basal bodies, nuclei, mitochondria, and Golgi complexes. Vacuoles were also seen in the cytoplasm, the number varying from cell to cell. Metaplastic squamous cells showed slender profiles. Their cytoplasmic organelles were not obvious, due to degenerative processes. Clustered epithelial cells consisted of three types of cells: ciliated cell, goblet cell, and basal cell. These cells were the main epithelial cells observed in the allergic group, and they were more numerous than in the infectious group. There was no statistical correlation between the number of eosinophils and epithelial cells in nasal secretion.

Table 4. Types of epithelial cells in nasal secretions.

	allergic group		infectious group		
	n	%	n	%	P
single ciliated cells	56	8.56	32	29.36	<0.0001
single goblet cells	8	1.22	0		
single basal cells	15	2.29	8	7.34	<0.05
metaplastic squamous cells	189	28.90	53	48.62	<0.05
clustered epithelial cells*	386	59.02	26	23.85	<0.001
total	654		109		

* Clustered epithelial cells in the allergic group include 212 ciliated cells, i.e. 84 basal cells and 90 goblet cells; and in the infectious group 18 ciliated cells and 8 basal cells.

Lymphocytes

Four types of lymphocytes (71 cells; 5.0%) were observed mostly in the allergic group. Type I showed a relatively large nucleus with diffuse heterochromatin; very few mitochondria (mean: 0.8/cell) and no granules were observed (Figure 3A). Type-II lymphocytes showed an irregular nucleus, the heterochromatin of which was not so dense as that of the type-I cells. Mitochondria (mean: 13.8/cell) were observed very often (Figure 3B). Type-III cells showed a nucleus positioned eccentrically, which was kidney-shaped or irregular in shape; it was composed of condensed chromatin distributed along its margins. Several electron-dense granules (21.3 per cell profile) were observed in the cytoplasm (0.1–0.6 μm in diameter), similar to

Table 5. The fine structures of lymphocytes in nasal secretions.

type	I		II		III		IV	
group	allergy	infect.	allergy	infect.	allergy	infect.	allergy	infect.
n	18	8	30	2	51	5	6	0
%	17.1	53.3	28.6	13.3	48.6	33.3	5.7	
C.M.D.	5.19	5.28	8.48	7.96	9.33	8.98	9.00	
SD	0.82	0.91	2.32	3.13	0.72	1.34	0.94	
C.T.D.	3.73	4.10	6.81	6.31	6.45	6.56	6.67	
SD	1.04	1.21	2.04	1.95	1.02	1.21	1.05	
N.M.D.	3.72	3.64	5.94	6.12	6.10	6.21	6.25	
SD	0.56	0.81	1.62	1.96	2.31	2.14	1.32	
N.T.D.	3.11	3.15	3.90	4.01	3.48	3.64	4.59	
SD	0.41	0.64	0.64	0.89	0.49	1.03	0.86	
G.n.	0	0	0	0	21.33	10.56	8.50	
SD					5.34	3.28	1.31	
Mv	3.17	4.24	8.10	10.0	13.3	12.5	5.00	
SD	2.89	1.56	2.92	3.46	3.80	4.12	2.48	
Mt	0.83	1.04	13.8	12.5	18.3	15.3	11.5	
SD	1.42	1.50	3.42	4.03	3.49	3.54	3.14	
R.N.C.	0.72	0.70	0.69	0.68	0.65	0.68	0.69	
SD	0.16	0.13	0.16	0.14	0.15	0.13	0.12	

Allergy: allergic group; Infect: infectious group; C.M.D.: cell max. diameter; C.T.D.: cell transverse diameter; N.M.D.: nucleus max. diameter; N.T.D.: nucleus transverse diameter; G.n.: granule number; Mv: microvillus-like processes; Mt: mitochondria; R.N.C.: ratio of nucleus/cell. The figures express the means. Standard deviation (SD) is mentioned separately.

large granular lymphocytes. They consisted of a homogeneous electron-dense matrix and a thin electron-lucent rim separating the matrix of the granule from its limiting membrane. The cell surface showed numerous microvillus-like processes (mean: 13.3/cell). Golgi complexes, rough endoplasmic reticulum, and free ribosomes were often observed. There were several mitochondria (mean: 18.3/cell). Multivesicular bodies, microtubules, and microfilaments were sometimes observed (Figure 3C). Type-IV-like plasma cells were characterized by an abundant rough endoplasmic reticulum, arranged in a parallel fashion around the nucleus. There were moderate numbers of mitochondria (mean: 15.3/cell). No specific granules were observed (Figure 3D; Table 5).

Basophilic cells

Fifty-seven basophilic cells were observed in the allergic group, but not in the infectious group. The basophilic cells were divided into two types according to critera used in our laboratory (Okuda et al., 1978). Forty-one type-I cells were blood basophilic leukocytes, and 16 type-II cells were similar to nasal-mucosal-type mast cells. There were more degranulated specific granules in type-I cells than in type-II cells ($p < 0.05$). The average maximum cell diameter was 7.8±0.8 μm in type-I cells and 9.5±1.0 μm in type-II cells ($p < 0.05$). There were 5-7 microvillus-like processes on the cell surface of both two types. Granule fusion was observed very often. Both types of cell had a few phagosomes (Table 6).

Table 6. The fine structures of basophilic cells in the allergic nasal secretions.

	type I (n=41)		type II (n=16)		
	mean	SD	mean	SD	P
max. cell diameter	7.83	0.85	9.57	1.03	<0.05
transverse cell diameter	6.13	0.55	7.16	0.82	>0.05
total number of granules	35.95	12.35	32.13	13.14	<0.05
degranulation I*	13.80	3.60	10.34	5.35	<0.05
degranulation II	6.33	5.14	7.62	6.14	>0.05
degranulation III	18.57	9.52	15.32	10.14	<0.05
microvillus-like processes	7.00	4.64	5.67	4.69	>0.05
granule fusion	4.76	2.23	5.54	3.03	>0.05
mitochondria	2.33	1.82	3.12	2.04	>0.05
phagosomes	4.19	2.27	5.69	3.13	>0.05

(*): Degranulation I: partial degranulation; degranulation II: total degranulation; degranulation III: no degranulation.

Macrophages

The macrophages were present in higher numbers in the infectious group (n=71; 7.1%) than in the allergic group (n=30, 2.1%; $p < 0.0001$). Their size was larger in the infectious group than in the allergic group ($p < 0.05$). The contents of the phagosomes in

Table 7. The fine structures of macrophages in nasal secretions.

	allergic group		infectious group		
	mean	SD	mean	SD	P
max. cell diameter	8.57	0.64	9.69	0.81	<0.05
transverse cell diameter	5.71	0.86	5.86	0.92	>0.05
microvillus-like processes	6.45	3.28	3.08	1.57	<0.01
phagosomes	3.29	1.66	7.13	3.12	<0.01
mitochondria	1.38	0.96	1.29	0.66	>0.05

the macrophages was the same as those in the neutrophils; i.e., the electron-lucent and electron-dense types were observed in the allergic group, while bacteria-containing phagosomes also were observed in the infectious group. The number of phagosomes was significantly higher in the infectious group than in the allergic group (Table 7).

DISCUSSION

The results of the present study indicate that there is an increased number of eosinophils in allergic, but not in infectious nasal secretions, and this supports the earlier finding that eosinophilia of nasal secretions is a very useful parameter in the diagnosis of allergic rhinitis and in differentiating it from non-allergic rhinitis (Murry, 1970; Bhandari and Baldwa, 1976; Malmberg, 1979; Henderson and Chi, 1985; Lans, 1989; Rivasi and Bergamini, 1988). The eosinophils were characterized by marked degranulation of different types together with fusion of the cytoplasmic membrane with granule or granules with adjacent granules, as Okuda et al. (1981) already stated in their study of the allergic nasal mucosa.

Many epithelial cells were observed in the secretion of patients with both diseases. The question arose if these cells appeared as a result of desquamation of the dead epithelial cells or were due to the mechanical stimulation induced by suction during collecting the specimen. It has been stated that in asthma eosinophil cationic proteins released from the increased number of eosinophils in the mucosa of lower airway may damage and destroy the epithelium, resulting in exfoliation (Frigas et al., 1980, 1986).

On the other hand, contradictory results have been obtained in allergic rhinitis. In histological and nasal smear studies, the lining of the nasal epithelium are usually well-preserved (Masuyama et al., 1988; Okuda et al., 1991), and mucociliary transport is normal in allergic rhinitis. In addition, there is no positive correlation of the number of increased eosinophils with the severity of symptoms, nasal provocation reaction, and non-specific nasal hyperreactivity (Ishikawa et al., 1991; Okuda et al., 1993). However, *in vitro* challenge studies have demonstrated that ciliary beating of nasal ciliated cells is inhibited after application of eosinophil cationic proteins (Liu, 1988; Hisamatsu et al., 1990). In the late-phase response after allergen challenge, eosinophils are increased in allergic rhinitis as well as in asthma (Pelikan and Pselika-Filipesk, 1989; Okuda et al., 1993). Contrary to the concept that the epithelial cells are destroyed by eosinophils, we have found in the present study many clustered epithelial cells without any signs of cellular degeneration, suggesting that suction used for collection of specimen caused mechanical epithelial desquamation. In addition, the number of eosinophils does not correlate with the number of epithelial cells in nasal secretions.

There is also a large number of neutrophils in both allergic and infectious nasal secretions. The main role of neutrophils in an infection is to remove the foreign bodies, including bacteria, by phagocytosis as observed in the infectious group, while in allergic group the main contents of phagosomes in neutrophils were electron-lucent or homogeneously electron-dense substances that surely were not bacteria. Daems and Oort (1962) have suggested that neutrophils are able to phagocytize antigen-antibody complexes *in vivo* in a similar way as they take up bacteria. Since there are antigen-antibody complexes in the allergic nasal mucosa, one of the possible functions of neutrophils in the secretion of allergic rhinitis is to discard with these complexes.

There is little known about lymphocytes in nasal secretions since they reside mainly in the nasal mucosa. However, we have observed a small number of lymphocytes in the nasal secretion. Type-I lymphocytes are smaller in size, scanty in cytoplasmic organelles, and are the same as small lymphocytes (Kelly, 1984). Type-II cells show irregularly shaped nuclei, a few mitochondria, many microvilli, and are significantly larger in size than the type-I cells; as such they are similar to T cells. Ultrastructurally, type-III cells are similar to the large granular lymphocytes which are characterized by specific endoplasmic granules (Prasthofer et al., 1987; Kaneda and Wake, 1985), and they possess relatively abundant cytoplasm, many mitochondria, and rough endoplasmic reticulum. However, to confirm that type-II cells are T cells and type-III cells are large granular lymphocytes, staining of surface markers is required. Type-IV cells have abundant rough endoplasmic reticulum, which is arranged around the nucleus in a parallel fashion, similar to that in plasma cells. They might reside in the epithelium and appear in the nasal secretion due to exfoliation of epithelial cells.

Appearance of basophilic cells is a special feature of allergic nasal secretions (Okuda et al., 1978). Both basophil leukocytes and mucosal-type mast cells play an important role in triggering the allergic reaction upon contact with the allergen.

Macrophages are important for antigen presentation to T cells by phagocytosis and processing of the antigen. They are large and have more phagosomes in the infectious group than in the allergic group, suggesting increased activity in infection.

The results of the present study showed difference in cell population in nasal secretions when compared with those of nasal epithelium (Okuda et al., 1991). In the nasal epithelium of allergic patients, the predominant migrating cells are lymphocytes, followed by (in order of predominance) eosinophils, mast cells and basophils, granular leukocytes, and neutrophils, whereas in nasal secretions the predominant migrating cells are eosinophils, followdby neutrophils, lymphocytes, basophils, and macrophages. On the basis of these differences, it seems to be true that mast cells and lymphocytes play their own role in the

allergic reaction within the nasal epithelium. On the other hand, neutrophils and eosinophils are more numerous in nasal secretions than in the epithelium. What is the special role of eosinophils and neutrophils in nasal secretions? Do they have a special function in nasal secretion? Do they migrate into the nasal secretion as their final fate, without any specific role? It is easy to speculate that eosinophils and neutrophils deliver phagocytized antigens and antigen-antibody complexes into the nasal secretion; macrophages take up and process antigen, and present antigenic information to lymphocytes; large granular lymphocytes remove dead epithelial cells; basophils and mast cells release chemotactic factors upon contact with allergens to remove the allergen particles. However, we need more precise experimental evidence to support these speculations.

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