

The effect of recombinant human interleukin-5 on eosinophil accumulation and degranulation in human nasal mucosa

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Recombinant human interleukin-5 (rhIL-5) was administered repeatedly onto the nasal mucosa of individuals with Japanese cedar pollinosis outside the pollen season. The numbers of eosinophils and epithelial cells and the amount of eosinophil cationic protein (ECP), secretory IgA (S-IgA), and IgA in the nasal lavage fluid increased significantly after the application of rhIL-5. Responsiveness to histamine was also enhanced after the application. When S-IgA was administered onto the nasal mucosa after application of rhIL-5, the amount of ECP in the nasal lavage fluid was significantly more increased. The above findings together with the facts that IL-5 promotes production of IgA, that IgA receptors are present on eosinophils, and that rhIL-5 does not increase release of ECP from eosinophils isolated from the peripheral blood suggest that a series of possible reactions consisting of (1) IL-5-induced production of IgA from the immune-mediating cells, (2) binding of secretory components released from either serous glandular cells or epithelial cells of the nasal mucosa with IgA, (3) release of ECP from eosinophils induced by S-IgA and/or IgA, (4) epithelial damage to the nasal mucosa, and (5) development of nasal hyperreactivity to histamine. (J ALLERGY CLIN IMMUNOL 1992;90:160-8.)

Key words: Interleukin-5, eosinophil, nasal mucosa, secretory IgA

Infiltration, activation, and degranulation of eosinophils in the nasal mucosa play an important role in the onset of allergic symptoms of the nose, particularly in the late-phase reaction associated with swelling and hyperreactivity of the nasal mucosa.^{1, 2} The factors contributing to migration of eosinophils include PAF, eosinophil chemotactic factor of anaphylaxis, and C5a. Of these factors, PAF has the additional effect of activating eosinophils.^{3, 4} However, studies on the factors that cause degranulation of eosinophils are few.

In recent years, cytokines derived from lymphocytes have been attracting increasing attention for their wide variety of physiologic activities. Among the cy-

Abbreviations used

rhIL-5:	Recombinant human interleukin-5
IL:	Interleukin
PB:	Peripheral blood
PAF:	Platelet-activating factor
ECP:	Eosinophil cationic protein
BSA:	Bovine serum albumin
PBS:	Phosphate-buffered solution
NAR:	Nasal airway resistance
S-IgA:	Secretory IgA

tokines, IL-3, IL-5, and granulocyte-macrophage-colony-stimulating factor are of interest, since these cytokines exert their effect on eosinophils as a common target. Using eosinophils isolated from normal human PB, we have been investigating the degranulation effect of mainly IL-5 in vitro, since it has a selective effect on eosinophils. However, we were not able to obtain clear evidence that IL-5 exerts a direct effect on eosinophils as long as we used ECP as a marker.⁵ In vitro systems have limitations for studying the possibility that IL-5 has an indirect effect by acting

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Received for publication Sept. 10, 1991.

Revised March 19, 1992.

Accepted for publication March 20, 1992.

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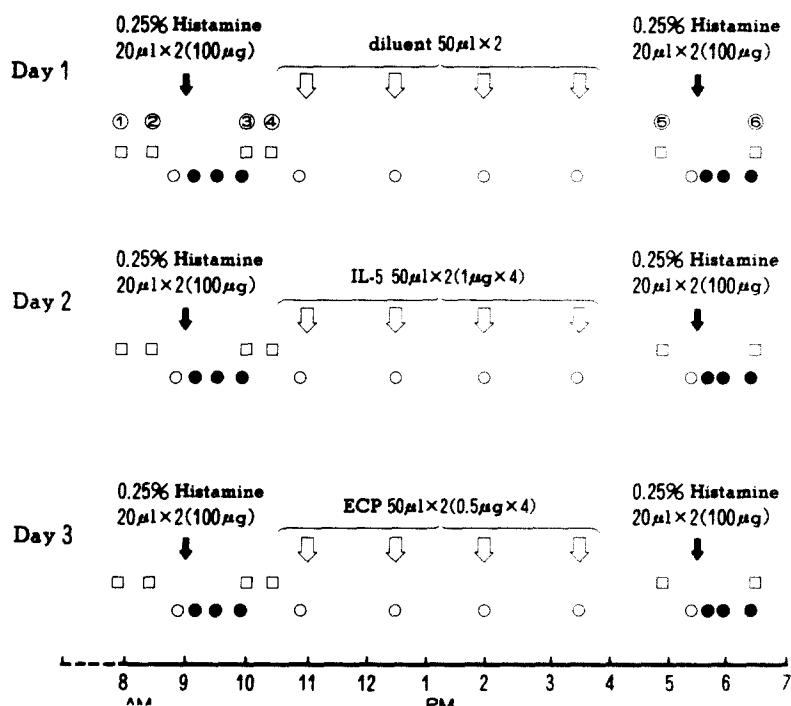


FIG. 1. Time schedule for experiment I. Nasal lavage (□), determination of NAR (○), determination of sneeze, nasal discharge, and NAR (●).

on other cells, causing release of physiologically active substances that subsequently affect eosinophils.

We have clarified various allergic responses occurring locally in the nasal mucosa by analyzing nasal lavage fluid.^{1, 6, 7} In the present study, we determined the effect of rhIL-5 on eosinophil degranulation in human nasal mucosa *in vivo*. At the same time, we investigated changes in histamine reactivity in nasal mucosa attributable to degranulation of eosinophils. The effect of S-IgA on ECP release from eosinophils was also studied.

SUBJECTS AND METHODS

Subjects and study design

Our subjects included 17 patients (aged 19 to 46 years; mean, 24.3 years) with Japanese cedar pollinosis for whom sensitization by Japanese cedar pollens alone had been confirmed by skin tests and RAST. Before the study, informed consent was obtained after adequately explaining the objective and procedures of the present study.

Changes induced by rhIL-5 in eosinophil count, amount of ECP and immunoglobulins in nasal lavage fluid, and in nasal responsiveness to histamine

The study was performed under constant conditions at a temperature of 22° C and in accordance with the schedule depicted in Fig. 1 between early July and late October when

Japanese cedar pollens are absent from the atmosphere. Eight patients participated in this study, which was conducted on 3 different days, at intervals of a minimum of 3 days and a maximum of 7 days between test days.

Day 1. After a rest period of 30 minutes, nasal lavage, Fig. 1, 1 and 2, was performed twice with a 20-minute interval. Each subject was requested to blow his/her nose after nasal lavage for measurement of NAR. The initial histamine challenge was then performed by intranasal administration of 20 µl of 0.25% histamine in physiologic saline drops bilaterally onto the inferior nasal mucosa, a total of 100 µg of histamine. The subject was then asked to blow his/her nose 10, 30, and 60 minutes later, and the weight of the nasal discharge and NAR were determined.

The frequency of sneezing was also recorded. After two nasal lavages with a 20-minute interval, Fig. 1, 3 and 4, 50 µl of 0.1% BSA in PBS was administered by drops (total 100 µl) onto the inferior nasal mucosa bilaterally. This procedure was repeated four times at 90-minute intervals, followed by nasal lavages, Fig. 1, 5. As in the first instance, histamine challenge was induced with 20 µl of 0.25% histamine in physiologic saline, and 10, 30, and 60 minutes later, the frequency of sneezing, weight of nasal discharge, and NAR were determined, and nasal lavage was repeated, Fig. 1, 6.

Day 2. As in day 1, after two nasal lavages, a histamine challenge was performed. After two more nasal lavages, 50 µl of 10 µg/ml of rhIL-5 in PBS containing 0.1% BSA was administered dropwise bilaterally on the inferior nasal mucosa. Administration of rhIL-5 was repeated for a total

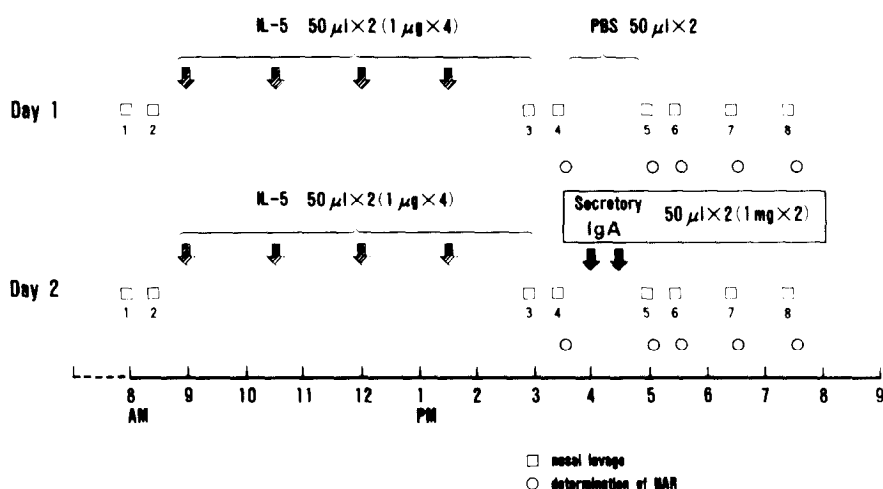


FIG. 2. Time schedule for experiment II. Nasal lavage (□), determination of NAR (○).

of four times at 90 minute intervals (total, 4 μ g of rhIL-5). As on day 1, lavage of the nasal cavity and a histamine challenge followed by nasal lavage were performed.

Day 3. Instead of PBS containing 0.1% BSA administered on day 1 and repeated administrations of rhIL-5 on day 2, 50 μ l of PBS containing 5 μ g/ml of ECP was bilaterally administered (100 μ l in total; 0.5 μ g of ECP). The total volume of ECP administered was 2.0 μ g.

Release of ECP from eosinophils induced by S-IgA

Nine subjects participated in this test on 2 different days. The minimum interval between day 1 and day 2 was 3 days and the maximum was 7 days. The test protocol is illustrated in Fig. 2.

Day 1. After two repeated nasal lavages, Fig. 2, 1 and 2, 4 μ g of rhIL-5 was administered dropwise for a total of four times at 90-minute intervals, followed by two nasal lavages, as depicted in Fig. 2, 3 and 4. This procedure was followed by applying drops of 100 μ l of PBS twice at 10-minute intervals. At 30, 60, 120, and 180 minutes, nasal lavage was performed after each application, Fig. 2, 5, 6, 7, and 8. NAR was determined immediately after each lavage.

Day 2. Two nasal lavages were followed by dropwise administration of rhIL-5, and after another two consecutive nasal lavages, 100 μ l of PBS containing 10 mg/ml of S-IgA was administered dropwise twice at 10-minute intervals. As on day 1, nasal lavage and determinations of NAR were repeated.

Reagents used in the study and the method of processing nasal lavage fluids and determination of ECP and immunoglobulin

rhIL-5 was obtained from Genzyme Corp. (Boston, Mass.). S-IgA was obtained from Organon Teknica (West Chester, Pa.). ECP was kindly supplied by Dr. Christer Peterson (Pharmacia Diagnostics AB, Uppsala, Sweden). NAR was determined by the posterior method with MPR

2100 (Nihon Kodan Co. Ltd., Tokyo, Japan) to obtain a mean of eight measurements. Percent change in NAR (Δ NAR) was expressed by the following equation:

$$\Delta\text{NAR} = \frac{\text{NAR after challenge} - \text{NAR before challenge}}{\text{NAR before challenge}} \times 100$$

The method for lavaging the nasal cavity was described previously.^{1,6} Briefly, each subject was requested to bend his/her head forward. Forty milliliters (20 ml into each nostril) of normal saline at 37° C was instilled into the nostrils through an inserted nozzle connected with a nasal wash pump. After the washing, each subject blew his/her nose into a cylinder coated with Siliconize (Fuji Systems, Tokyo, Japan). The recovery rate was 88.4% $\pm$ 4.0%. After centrifugation of the nasal lavage fluids at 350 g at 4° C for 10 minutes, the pellet and supernatant were removed separately. Eosinophils were counted in a cell-counting plate after staining cells in the lavage solution with Hinkelman's staining, whereas epithelial cells were counted after staining with Türk's stain.

ECP contained in the supernatant was determined by RIA with ECP RIA kit (Pharmacia Diagnostics AB, Uppsala, Sweden). This assay is essentially the same as that described by Venge et al.⁸ and adapted for the nasal procedure. The precision of RIA for ECP was examined with various concentrations of standard samples. Coefficients of variation in intra-assay and interassay were 1.2% to 4.2% and 4.7% to 10.9%, respectively. The amount of IgA, IgG, and IgM was determined by latex agglutination photometric immunoassay.⁹ S-IgA was determined by enzyme immunoassay¹⁰ with S-IgA EIA kit (MBL, Nagoya, Japan). This assay was performed exactly as specified by the manufacturer.

Statistical assessment

The results were expressed as mean $\pm$ SE. Statistical significance was determined by the Student's paired *t* test, with *p* < 0.05 considered statistically significant.

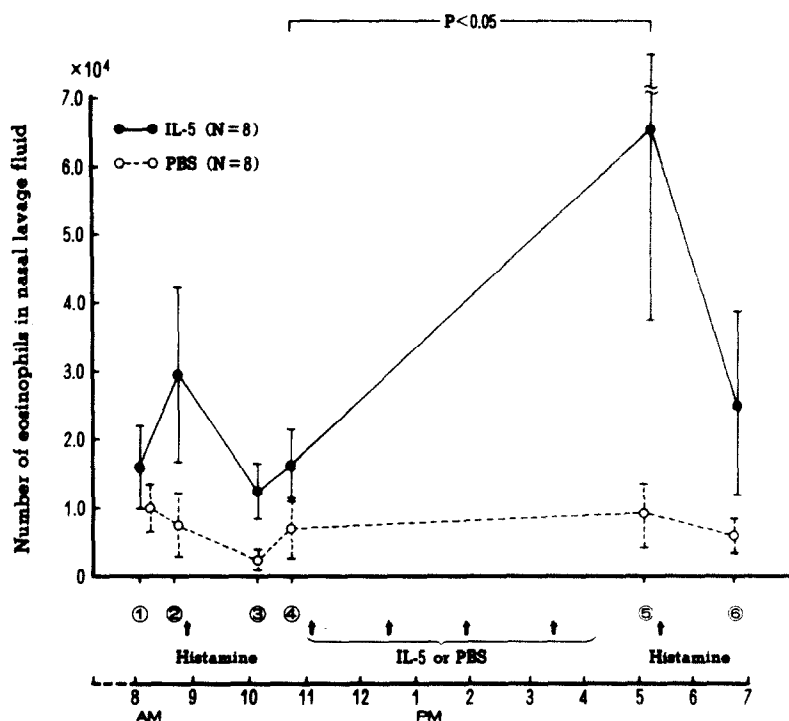


FIG. 3. Changes in number of eosinophils in nasal lavage fluids after application of rhIL-5 and histamine. Significant difference between before and after application of rhIL-5 was observed, $p < 0.05$. Increase in eosinophils after rhIL-5 also significant compared with that of control challenge. Differences in early lavages between rhIL-5-treated and saline-treated subjects were not significant; results expressed as mean $\pm$ SE.

RESULTS

Changes in eosinophil count, amount of ECP, and epithelial cell count in nasal lavage fluids after nasal application of rhIL-5

As illustrated by the dotted line in Fig. 3, there was no significant change in the number of eosinophils in the nasal lavage fluid after the histamine challenge and nasal application of 0.1% BSA containing PBS, followed by a second histamine challenge. Eosinophil counts, amounts of ECP, and epithelial cell counts in the nasal lavage fluid before and after the application of rhIL-5 solution are illustrated in Figs. 3 and 4. The eosinophil count in the total nasal lavage fluid recovered before the nasal application of rhIL-5 was $1.59 \pm 0.59 \times 10^4$ cells; after the application, this increased significantly to $6.54 \pm 2.43 \times 10^4$ cells ($p < 0.02$). The amount of ECP likewise increased significantly from 85.3 ± 27.8 ng to 192.9 ± 53.6 ng after the application ($p < 0.02$), whereas the number of epithelial cells increased significantly from $2.72 \pm 0.50 \times 10^4$ cells before to $6.67 \pm 1.23 \times 10^4$ cells after the application of rhIL-5 ($p < 0.05$). In contrast, after the application of 0.1% BSA in PBS serving as control, the eosinophil count increased slightly but not significantly from $0.61 \pm 0.42 \times 10^4$ cells to $0.91 \pm 0.41 \times 10^4$ cells, the amount of

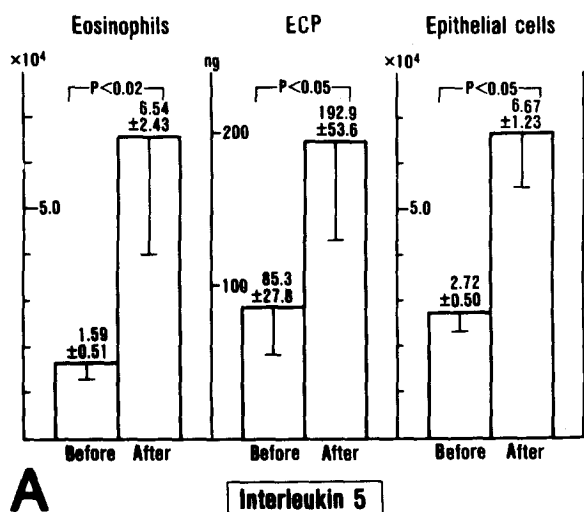
ECP from 60.9 ± 20.6 ng to 84.3 ± 24.3 ng, and epithelial cells from $2.75 \pm 0.62 \times 10^4$ cells to $3.80 \pm 0.90 \times 10^4$ cells.

Changes in immunoglobulin in nasal lavage fluid after nasal application of rhIL-5

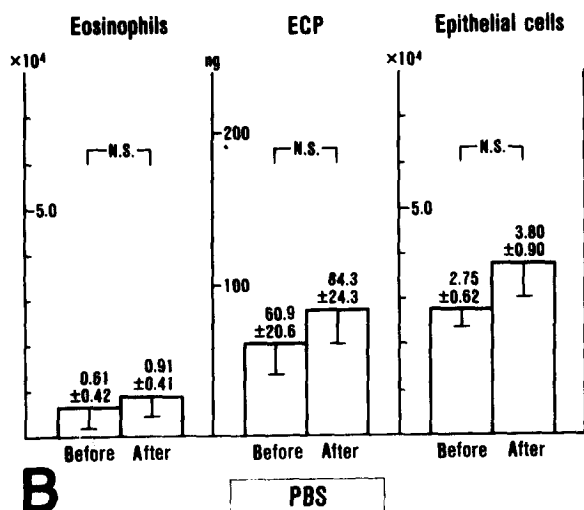
Changes in the amounts of S-IgA, IgA, IgG, and IgM in the nasal lavage fluid after nasal application of rhIL-5 and 0.1% BSA containing PBS are illustrated in Fig. 5. S-IgA in the nasal lavage fluid increased significantly from 322.6 ± 60.7 μ g to 1245.0 ± 462.7 μ g after application of rhIL-5 ($p < 0.05$). Likewise, IgA increased from 270.8 ± 58.8 μ g to 838.9 ± 278.0 μ g ($p < 0.05$), and IgG and IgM from 128.2 ± 40.9 μ g to 203.9 ± 66.6 μ g ($p < 0.1$) and 33.7 ± 19.6 μ g to 116.2 ± 90 μ g ($p < 0.05$), respectively. In contrast, after the application of PBS, there were no significant changes in S-IgA, IgA, IgG, and IgM.

Nasal application of rhIL-5 did not induce sneezing.

The Δ NAR at 30 minutes and 90 minutes after the first of four repeated applications was $3.9\% \pm 8.5\%$ and $10.9\% \pm 12.2\%$, respectively, and at 90 minutes after the fourth application, it was $13.3\% \pm 24.9\%$. These increases in NAR were not significant compared with the prechallenge level.

**A**

Interleukin 5

**B**

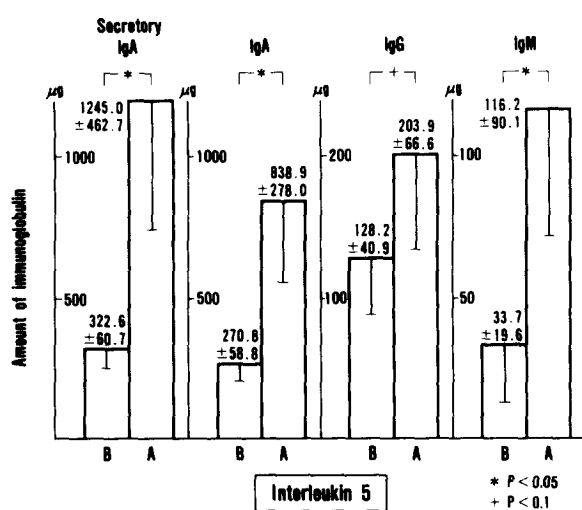
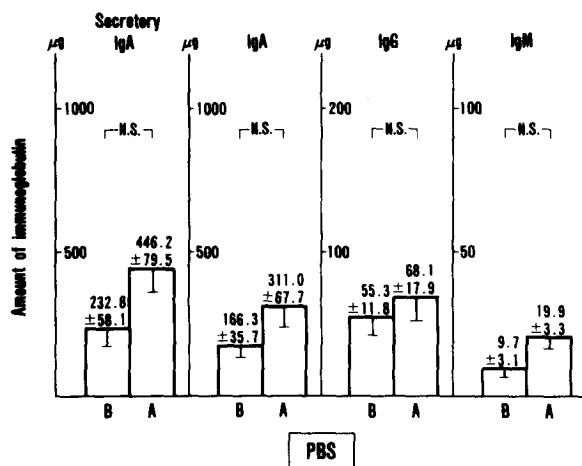
PBS

FIG. 4. Number of eosinophils, amount of ECP, and number of epithelial cells in nasal lavage fluids before and after application of **A**, rhIL-5 and **B**, PBS. Results expressed as mean $\pm$ SE.

Changes in histamine nasal reactivity after application of rhIL-5 and ECP

Changes in histamine nasal reactivity after application of rhIL-5 and ECP are presented in Table I and Fig. 6. The frequency of sneezing and Δ NAR induced within 60 minutes by 20 μ l of 0.25% histamine (in each nostril) were not significantly affected by the application of rhIL-5 or 0.1% BSA containing PBS.

In contrast, the weight of the nasal discharge demonstrated a tendency toward increase from 1.86 $\pm$ 0.34 gm before the application of rhIL-5 to 2.39 $\pm$ 0.39 gm after the application ($p < 0.1$), whereas no significant change was observed after the

* $P < 0.05$
+ $P < 0.1$ 

PBS

FIG. 5. Amount of S-IgA, IgA, IgG, and IgM in the nasal lavage fluids before and after applications of rhIL-5. Results expressed as mean $\pm$ SE.

application of 0.1% BSA containing PBS, that is, from 1.83 $\pm$ 0.36 gm to 1.73 $\pm$ 0.41 gm.

Before application of ECP, the weight of the nasal discharge induced by histamine within 60 minutes was 1.62 $\pm$ 0.28 gm. It increased to 1.91 $\pm$ 0.27 gm after the application ($p < 0.1$; Fig. 6).

The changes in the frequency of sneezing and Δ NAR before and after the application of ECP were not significant (data not presented).

Changes in NAR and ECP in nasal lavage fluid after the application of S-IgA

The amount of ECP in the nasal lavage fluid before the application of S-IgA (and after the application of rhIL-5) was 142.0 $\pm$ 26.6 ng. However, it had increased significantly to 196.1 $\pm$ 31.3 ng 30 minutes after the application of S-IgA (Fig. 7). At 60 minutes

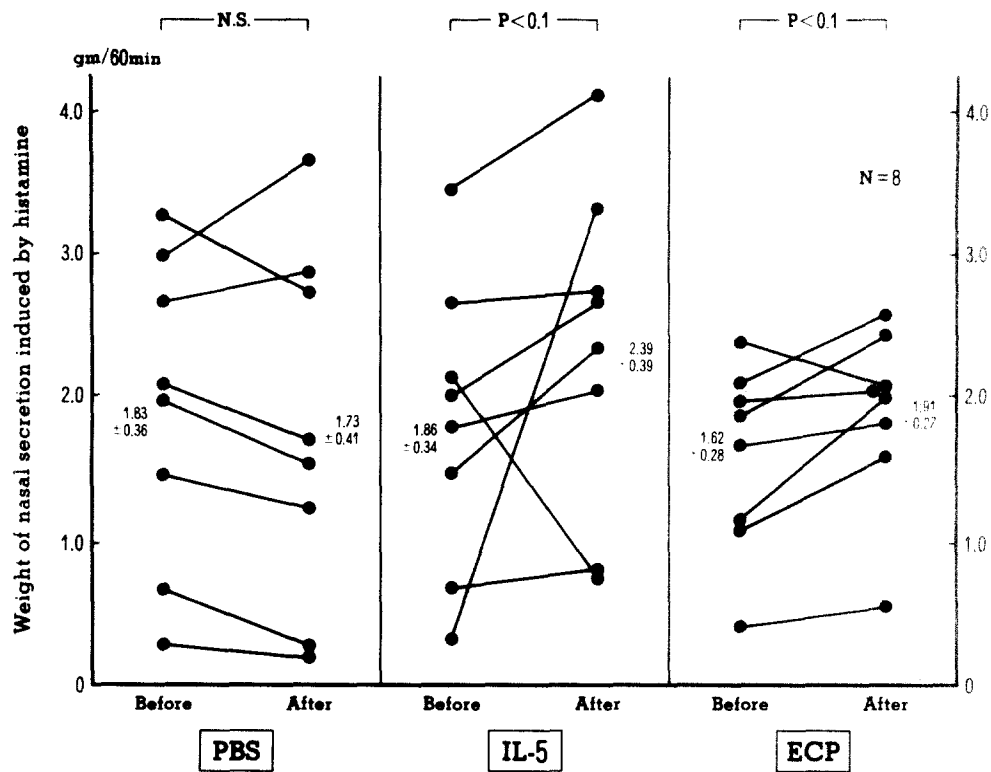


FIG. 6. Changes in weight of nasal discharge induced by histamine after application of rhIL-5 and ECP. Results expressed as mean $\pm$ SE.

TABLE I. Changes in histamine-induced nasal responses after the application of rhIL-5

Responses	IL-5		Control	
	Before	After	Before	After
Sneezing (frequency)	5.8 $\pm$ 1.4	5.9 $\pm$ 0.6	5.6 $\pm$ 1.4	5.3 $\pm$ 0.9
Nasal secretion (gm)	1.86 $\pm$ 0.34	2.39 $\pm$ 0.39*	1.83 $\pm$ 0.36	1.73 $\pm$ 0.41
Δ NAR (%)	35.2 $\pm$ 20.2	13.1 $\pm$ 14.4	27.4 $\pm$ 12.2	25.5 $\pm$ 10.1

Results are expressed as mean $\pm$ SE.

* $P < 0.1$.

after the application, the level had decreased to 123.3 ± 25.7 ng or to the level before the application. By 120 minutes, the amount began to increase gradually to 159.6 ± 32.3 ng and at 180 minutes, it was 179.7 ± 41.7 ng. In contrast, when PBS was applied instead of S-IgA, the amount of ECP before the application was 148.9 ± 19.4 ng, but at 30, 60, 120, and 180 minutes after the application, there was no significant change in the level (143.0 ± 22.1 , 148.8 ± 21.0 , 137.1 ± 24.0 , and 142.0 ± 20.1 ng, respectively).

At 30, 60, 120, and 180 minutes after the application of S-IgA, Δ NAR was $7.2\% \pm 4.8\%$,

$1.6\% \pm 5.1\%$, $5.9\% \pm 5.4\%$, and $7.5\% \pm 6.1\%$, respectively, without demonstrating any significant change.

DISCUSSION

We reported previously that after a single antigen challenge, nasal lavage fluid demonstrated delayed increases in EG2-positive activated eosinophils and the amount of ECP, together with enhancement of nasal histamine reactivity.^{1,2}

It is easy to visualize a development series consisting of (1) degranulation of the activated eosinophils, (2) release of specific granules, such as ECP,

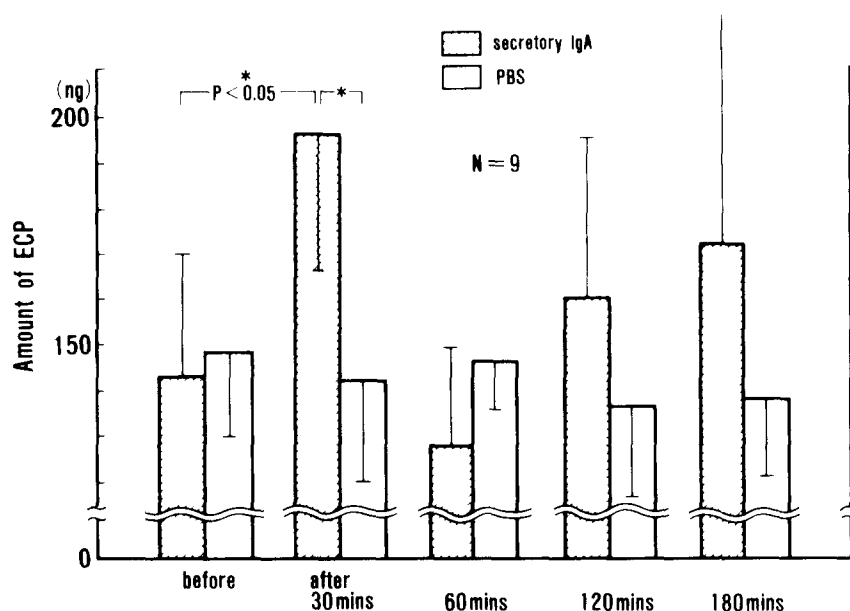


FIG. 7. Changes in amount of ECP in nasal lavage fluids after application of S-IgA or PBS. Results expressed as mean $\pm$ SE.

major basic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, (3) damage to the nasal mucosal epithelium, (4) exposure of sensory nerves present within or immediately beneath the epithelium, and (5) enhanced reactivity of the nasal mucosa as was suggested for the tracheal epithelium by Gleich et al.¹¹ There are, nevertheless, a few problems that remain to be resolved: (1) specifying the factors involved in degranulation of eosinophils and cells producing such factors, (2) whether such specific granules cause epithelial damage in vivo, (3) if these factors induce hyperreactivity of the nasal mucosa in vivo, (4) and confirmation that epithelial damage is essential for the development of nasal hyperreactivity.

IL-5 is known to have a selective and wide-ranging effect on mature eosinophils,¹² such as involvement in their differentiation and proliferation. We have tried to determine whether IL-5 causes degranulation of eosinophils.

The results of in vitro studies with eosinophils separated from PB indicate that the effect of rhIL-5 on eosinophil degranulation is minimum.

By in vitro experiments, it is difficult to study the indirect effect of IL-5 on eosinophil degranulation, such as the responses mediated secondarily by other inflammatory cells or immune-mediated cells, or by chemical mediators and immunoglobulin released from these cells. To investigate the ability of IL-5 to stimulate the migration of eosinophils and to determine the variations in hyperreactivity that can only

be assessed in vivo, we analyzed nasal lavage fluid before and after the application of rhIL-5 in human subjects and at the same time observed changes in histamine reactivity.

The ability of IL-5 to cause migration of eosinophils has been studied only in vitro. However, the results have varied among investigators according to the differences in IL-5 used and in experimental conditions. Wang et al.¹³ reported that IL-5 is a potent and selective chemoattractant for human eosinophils. Yamaguchi et al.¹⁴ confirmed the migration of eosinophils stimulated by murine IL-5. More recently, Gulbenkian et al.¹⁵ reported that eosinophil infiltration in the ovalbumin-sensitized lungs of guinea pigs is inhibited dose dependently by anti-IL-5 antibody. In contrast, Numao et al.¹⁶ demonstrated that eosinophil migration induced by PAF increased when the cells were pre-treated with rhIL-5; however, rhIL-5 itself did not induce any migratory activity.

To investigate the ability of rhIL-5 to cause migration of eosinophils in the nasal mucosa in vivo, we compared the eosinophil counts in the nasal lavage fluids before and after nasal application of rhIL-5.

rhIL-5 caused significant increases in eosinophil counts by a factor of 4.11 ($p < 0.05$). In contrast, there was no appreciable increase in eosinophils after the applications of PBS containing 0.1% BSA serving as control. We also analyzed the nasal mucosa histologically and observed infiltration of eosinophils within and immediately beneath the epithelial layer

of the nasal mucosa, with accumulation of eosinophils in the nasal vasculature (data not presented), which suggests that rhIL-5 at a high concentration can mobilize eosinophils from the vasculature to induce localized nasal infiltration of eosinophils.

Regarding the degranulation effect of rhIL-5 on eosinophils, at least for ECP, this may be ruled out by the results of *in vitro* studies with human PB. If we take into account also the results of studies with eosinophil-derived neurotoxin as a marker, rhIL-5 itself is unlikely to be a definite degranulation factor.¹⁷

In the present study, ECP increased significantly in the nasal lavage fluid after the nasal application of rhIL-5. There were simultaneous significant increases in S-IgA, IgA, and IgM. According to Abu-Ghazaleh et al.,¹⁸ S-IgA, IgA, and IgG bound to Sepharose beads (particularly S-IgA) have a strong degranulation effect on eosinophils. As illustrated in Fig. 7, when S-IgA is applied to the nasal mucosa after mobilization of eosinophils by rhIL-5, the level of ECP in the nasal lavage fluid increases significantly within 30 minutes. This is clearly not due to the prolonged effect of rhIL-5, because the amount of ECP remains essentially the same after application of PBS. The fact that IL-5 induces production of IgA^{19, 20} and that eosinophils have IgA receptors²¹ suggest the involvement of a series of reactions consisting of (1) IL-5 causing IgA production in the immune-mediating cells, (2) binding of IgA with the secretory component released from cells of the serous gland or epithelial cells within the nasal mucosa, and (3) degranulation of eosinophils mediated by S-IgA and IgA.

Epithelial cell counts in the nasal lavage fluid increased significantly after repeated application of rhIL-5 or ECP. In addition, there was a tendency toward an increase in the histamine-induced nasal discharge. Among the parameters to evaluate the nasal response to histamine, including the frequency of sneeze, the weight of nasal discharge, and Δ NAR, the weight of nasal discharge is most sensitive to indicate the changes of nasal reactivity under ordinary circumstances. Because of the limited number of subjects we studied, the increases in nasal histamine reactivity did not achieve conventional levels of statistical significance.

These facts may be the evidence supporting a series of phenomena involving degranulation of eosinophils as mediated by S-IgA or IgA, release of granular proteins, such as ECP, epithelial damage, and the development of nasal histamine reactivity. Nagata and Konno²² demonstrated a lowering of the histamine threshold of the nasal mucosa after epithelial damage caused by scraping the epithelial layer of the nasal mucosa lightly with a curette. It may not be appro-

priate to discuss the effect of physical injury versus the chemical effect of specific granular proteins derived from eosinophils. However, it appears that the epithelial injury is closely related to the development of nasal reactivity.

Repeated application of S-IgA or rhIL-5 did not cause any apparent changes in NAR. It is possible that degranulation of eosinophils mediated by S-IgA and IgA is limited to the release of granular proteins and does not involve the release of leukotriene C₄ and PAF that has been demonstrated to cause elevation of NAR.²³ We are determining the level of PAF in nasal lavage fluid after nasal applications of S-IgA or rhIL-5. We intend to reveal the results, together with results of histamine and tryptase levels, which are being performed to determine their effects on basophilic cells. In the present study, we applied rhIL-5 and ECP to the nasal mucosa to determine the effect of these physiologically active substances on the nasal mucosa. However, it is necessary to determine whether it is the physiologic doses, how deep they reach the nasal mucosa, and what might be the metabolism of applied doses. Until recently, microassay of human IL-5 has not been possible, but we succeeded in measuring IL-5 in the nasal lavage fluid by using an ELISA system developed by Fukuda et al.²⁴ In the near future it will be possible to determine changes in the level of IL-5 in nasal lavage fluid after antigen challenge and to investigate the metabolism of IL-5 in the nasal discharge.

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