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Involvement of Eosinophils in the Early-Phase Allergic Reaction in a Guinea Pig Rhinitis Model

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Key Words

Eosinophils · Allergic rhinitis · Early-phase allergic reaction

Abstract

Background: Eosinophils are found in the nasal lavage fluid (NLF) and nasal biopsies of patients with allergic rhinitis after a nasal antigen challenge, and associated not only with a late-phase allergic reaction (LPR) but also an early phase allergic reaction (EPR). Numerous studies have been carried out to clarify the participation of eosinophils in LPR or airway hyperresponsiveness. However, there has been no published report describing in detail the role of eosinophils during EPR. To better understand the involvement of eosinophils in EPR, we studied the effects of repeated antigen challenges on nasal airway responsiveness and eosinophilic inflammation in EPR using a guinea pig rhinitis model. ***Methods:*** Nasal airway responsiveness was measured as the nasal airway resistance (NAR) after nasal antigen provocation. Eosinophilic inflammation during EPR was assessed by nasal lavage and histopathological examination using two groups of animals: those in group 1 were subjected to a sensitization pretreatment only, and those in group 2 were subjected to a pretreatment of sensitization followed by repeated nasal challenges. ***Results:*** Repeated antigen challenges induced nasal hyperresponsiveness as indicated by a decrease in the antigen provocation dose and a significant increase in NAR. Furthermore, significant increases in eosinophil counts, eosinophil peroxidase (EPO) activity and protein content in NLF during EPR were observed following antigen provocation in group 2. There were significant correlations between the levels of these parameters, and albumin was the most prevalent of the proteins in NLF. Histopathological examination showed that the degree of eosinophil infiltration into the lamina propria of the nasal mucosa of the animals in group 2 was significantly and apparently higher than in group 1. Particularly, epithelial disruption and mucosal edema were significantly elevated after antigen provocation in group 2. ***Conclusions:*** These results suggest that chronic eosinophil accumulation is induced by repeated antigen challenges in the nasal tissue, and that once antigen provocation occurs, eosinophils in the tissue are activated and responsible for the amplification of EPR such as vascular permeability and mucosal edema.

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Introduction

Allergic rhinitis is a hypersensitivity response to specific allergens in sensitized patients that is mediated by IgE antibodies. Sensitized patients with allergic rhinitis have IgE antibodies for specific antigens that bind to high-affinity receptors on mast cells and basophils and to low-affinity receptors on other cells, such as monocytes, eosinophils and platelets. Upon reexposure to the specific allergens, cross-linking of adjacent IgE molecules occurs, followed by mast cell degranulation, releasing a variety of chemical mediators. This immediate allergic response to an antigen, known as the early-phase allergic reaction (EPR), occurs in more than 90% of patients with a history of allergic rhinitis and a positive skin test [1]. A late-phase allergic reaction (LPR) also occurs in approximately 50% of allergic patients, usually approximately 4–12 h following antigen exposure [2], and is characterized by the infiltration of inflammatory cells into the nasal mucosa [3]. Eosinophils play a central role in the mucosal inflammation, although other various inflammatory cells and mediators have been implicated in the recruitment and activation. Gundel et al. [4] demonstrated the association between an increase in airway eosinophils and an increase in airway responsiveness in a repeated antigen inhalation model and suggested that eosinophils were involved in the pathogenesis of a hyperresponsive airway in asthma. Furthermore, eosinophils in the airway mucosa have been considered to induce LPR and airway hyperresponsiveness [5–8]. In this respect, numerous studies have been carried out to clarify the participation of eosinophils in LPR and hyperresponsiveness using animal models [4, 7, 9].

Eosinophils are also found to be present in the nasal lavage fluid (NLF) and nasal biopsies of patients with rhinitis after a nasal allergen challenge [10, 11]. Although this was commonly observed in association with EPR [12, 13], no published report has described in detail the role of eosinophils during EPR. To better understand the involvement of eosinophils in EPR, we studied the effects of repeated antigen challenges on nasal airway responsiveness and eosinophilic inflammation during EPR using a guinea pig rhinitis model.

Materials and Methods

Animals

Male Hartley guinea pigs weighing 250–300 g were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in laboratory animal rooms at about 23°C with a humidity of about 55% and supplied with a standard pellet diet and tap water ad libitum. The experiments were performed in accordance with the guidelines for animal experimentation, which was approved by the Japanese Association for Laboratory Animal Science in 1987.

Agents

Ovalbumin (OVA, grade V, Sigma, St. Louis, Mo., USA) and Eosinostain® (Torii Pharmaceutical Co., Ltd., Tokyo, Japan) were used. The other reagents used were of the highest grade commercially available.

Study Design

The guinea pigs were subjected to the following treatment: (a) sensitization by intraperitoneal injection of OVA (10 μg/head) and aluminium hydroxide gel (alum, 10 mg/head) once a week for 2 weeks (group 1); (b) sensitization with OVA as in group 1, and 1 week after the final sensitization, the animals were challenged by applying 50 μl of antigen solution (1,000 μg OVA/ml in saline) into each nostril and then the antigen challenge was continuously performed once a week for 4 weeks (group 2). One week after these treatments, the animals in each group were provoked with the antigen to study the nasal antigen response. To determine various parameters related to the nasal lavage fluid and histopathological parameters, the animals in both groups were subdivided into a further two groups, and one provoked with saline and the other with antigen. The normal group of animals, which was not subjected to any treatment of sensitization and challenge, was also provoked with saline to clarify the base levels of various parameters related to the NLF and histopathological parameters.

Nasal Antigen Response

To determine the time course of antigen reactivity of the animals in groups 1 and 2, 20 μl of saline or antigen solution (1,000 μg OVA/ml in saline) was applied into each nasal cavity (each group consists of 10 animals). The specific airway resistance (sRaw) was measured before and 15 min, 1, 3, 5, 7 and 24 h after antigen provocation using a two-chambered, double-flow plethysmograph system, Pulmos-I (MIPS, Osaka, Japan), according to the method of Pennock et al. [14]. sRaw was considered as indicative of the nasal airway resistance (NAR) [15]. In the study to determine the antigen threshold in groups 1 and 2, 20 μl of saline and increasing doses of antigen (1, 10, 100 and 1,000 μg OVA/ml in saline) were applied into each nasal cavity (each group consists of 5 animals). Fifteen minutes after the antigen provocation, sRaw was measured.

Nasal Lavage

7–8 animals in groups 1 and 2 were provoked by applying 50 μl of saline or antigen solution (1,000 μg OVA/ml) into each nostril and those in the normal group were provoked with saline. Five minutes after the provocation, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) sufficient to induce anesthesia, and 15 min after the saline or antigen treatment, they were sacrificed by exsanguination. A cannula was inserted into the choana via the tracheostoma for nasal lavage. Nasal cavities were washed with 10 ml of saline and the NLF was collected. The NLF was centrifuged at 120 *g* for 10 min at 4°C. The resultant supernatants were used for measurement of eosinophil peroxidase (EPO) activity and protein content assays. The cell pellets were resuspended in saline solution containing heat-inactivated fetal calf serum and aliquots of the cell suspension were smeared on glass slides using a

Cytospin®-3 cell preparation system (Shandon Inc., Pittsburgh, Pa., USA). Cells were then stained with Diff-Quik solution (International Reagent, Kobe, Japan) for counting the total leukocytes, eosinophils and neutrophils in NLF per milliliter under a light microscope.

Measurement of EPO activity and Protein Content in the NLF

After centrifugation of NLF as described above, EPO activity and protein content in the supernatant were assayed. EPO activity was assayed according to the method of Schneider and Issekutz [16]. The protein content of NLF per milliliter was measured by the DC protein assay (Bio-Rad Laboratories, Tokyo, Japan).

SDS-PAGE and Immunoblotting

The amount of albumin, which is a marker of nasal vascular permeability, was determined in NLF by SDS-PAGE and immunoblotting as follows. After the centrifugation of NLF as described above, the supernatants were pooled and the proteins in each supernatant and guinea pig albumin (Sigma, St. Louis, Mo., USA) were separated by SDS-PAGE according to the method of Laemmli [17] and visualized using Coomassie Brilliant Blue R-250. Immunoblotting was performed to detect albumin in NLF as follows. Proteins separated by SDS-PAGE as described above were transferred electrophoretically to a nitrocellulose membrane using the Tefco transfer blotting system (Tefco, Tokyo, Japan). After blocking the membrane with PBS containing 5% skimmed milk, it was incubated with the same buffer containing the anti-guinea pig albumin IgG (Inter-Cell Technologies Inc., USA). After washing with PBS containing 0.05% Tween 20, the membrane was incubated with anti-rabbit IgG antiserum labeled with horseradish peroxidase in PBS. The membrane was washed with PBS containing 0.05% Tween 20 and then developed with Konica Immunostaining HRP-1000 (Konica, Tokyo, Japan).

Histopathological Study

After nasal lavage had been performed as described above, the nasal cavity was gently flushed with 10% buffered formalin solution. The head was fixed with 10% buffered formalin solution for at least 7 days and then decalcified with formic acid for at least 10 days. In accordance with the methods of Young [18], blocks of the transverse section of the nasal cavity at the second palatal ridge (level 3) were cut and embedded in paraffin blocks. Tissue sections (4 μm) were placed on microscope slides, deparaffinized and stained with Eosinostain® (Torii Pharmaceutical Co., Ltd., Tokyo, Japan) or hematoxylin and eosin. The degrees of epithelial disruption, mucosal edema and eosinophil infiltration into the epithelium and lamina propria of the entire nasal mucosa were evaluated by light microscopy. The scoring system used to classify the severity of these symptoms is as follows: 0 = no symptoms; 1 = slight epithelial disruption, slight edema or slight eosinophil infiltration; 2 = moderate epithelial disruption, moderate edema or moderate eosinophil infiltration, and 3 = severe epithelial disruption, severe edema or severe eosinophil infiltration.

Statistical Analysis

Data were presented as the means or medians ± SEM. Statistical analyses were performed using the paired or unpaired Student t test, Mann-Whitney U-test and Fisher's r to z transformation. A probability value (p) of less than 0.05 was considered to be statistically significant.

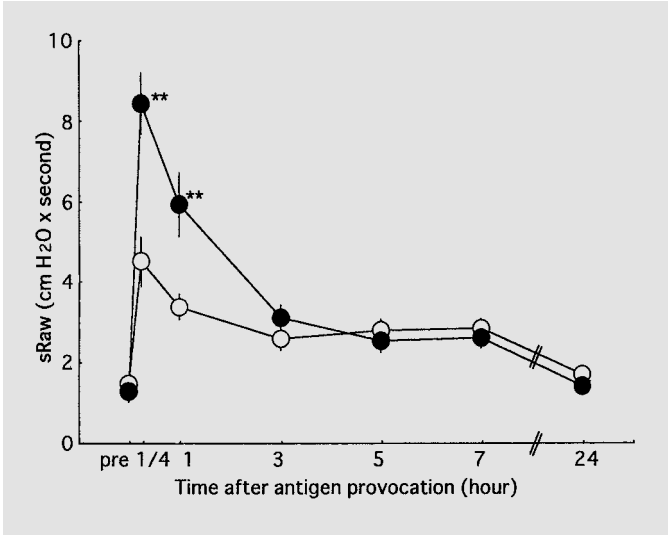

Fig. 1. Time-course of changes in NAR after antigen provocation. Each of the 10 animals in a group was nasally provoked with 1,000 μg/ml of antigen a week after the final sensitization (○: group 1) and a week after the fifth antigen challenge (●: group 2). sRaw was measured before, and 15 min, 1, 3, 5, 7 and 24 h after antigen provocation. ** $p < 0.01$ vs. group 1 (unpaired Student t test). Values are presented as means ± SEM.

Results

Nasal Antigen Response

To investigate the effect of repeated antigen challenges on the time course of allergic reaction, 1,000 μg/ml of antigen solution was applied to the animals in groups 1 and 2. In both groups, the maximal level of sRaw was attained at 15 min, after which time it gradually decreased and returned to the baseline level after approximately 24 h (fig. 1). sRaw of group 2 was significantly higher than that of group 1 at 15 min and 1 h ($p = 0.0009$ and 0.0077, respectively). Since a marked increase in sRaw was observed in group 2, the antigen provocation threshold was determined next (fig. 2). Application of 100 and 1,000 μg/ml of antigen solution resulted in a significant increase in NAR in group 2 ($p = 0.0457$ and 0.0152, respectively). On the other hand, only 1,000 μg/ml of antigen induced significant elevation in NAR in group 1 ($p = 0.0266$). At 100 and 1,000 μg/ml of antigen challenge, sRaw of group 2 was significantly higher than that of group 1 ($p = 0.0240$ and 0.0355, respectively). No significant difference in baseline (saline application) sRaws was observed between both groups.

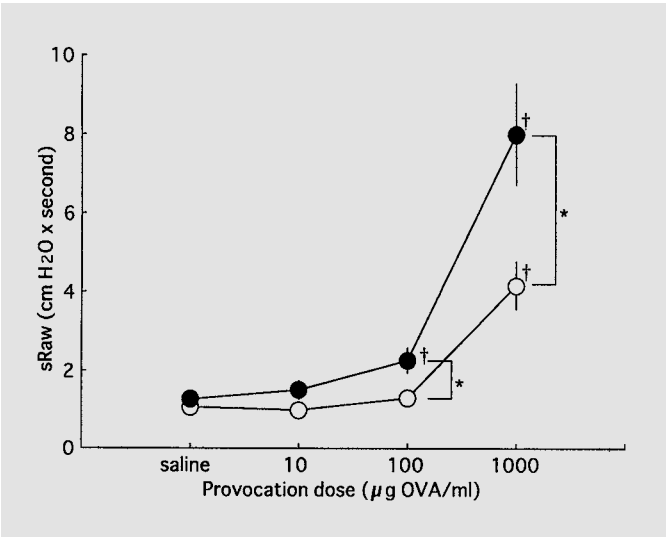

Fig. 2. Threshold antigen dose in nasal response. Each of the 5 animals in a group was nasally provoked with various concentrations of antigen a week after the final sensitization (○: group 1) and a week after the fifth antigen challenge (●: group 2). sRaw was measured 15 min after each provocation. * $p < 0.05$ vs. group 1 (unpaired Student t test). † $p < 0.05$ vs. saline application (paired Student t test). Values are presented as means ± SEM.

Effects of Repeated Antigen Challenges on the Number of Eosinophils, EPO Activity and Protein Content

NLF was obtained under five conditions to evaluate the involvement of inflammatory cells in EPR. Eosinophil counts (table 1), EPO activity and protein content (table 2) of NLF obtained from the groups with saline provocation instead of antigen were not different from those of the normal group. These NLF parameters were significantly different from each other after antigen provocation. Particularly, these parameters were significantly elevated after antigen provocation in group 2 (eosinophil counts, $p = 0.0003$; EPO activity, $p = 0.0005$, and protein content, $p = 0.0084$). In group 1, eosinophil counts were slightly but significantly elevated after antigen provocation ($p = 0.0037$). All these parameters of group 2 were significantly higher than those of group 1 after antigen provocation (eosinophil counts, $p = 0.0008$; EPO activity, $p = 0.0041$, and protein content, $p = 0.0123$). To determine the possible role of eosinophils in nasal inflammation, we calculated the correlation efficiencies between these NLF parameters obtained from all animals in both groups, which are shown in figure 3. There is a significant positive correlation between the level of EPO activity and the number of eosinophils ($p < 0.0001$, $r = 0.627$; fig. 3A).

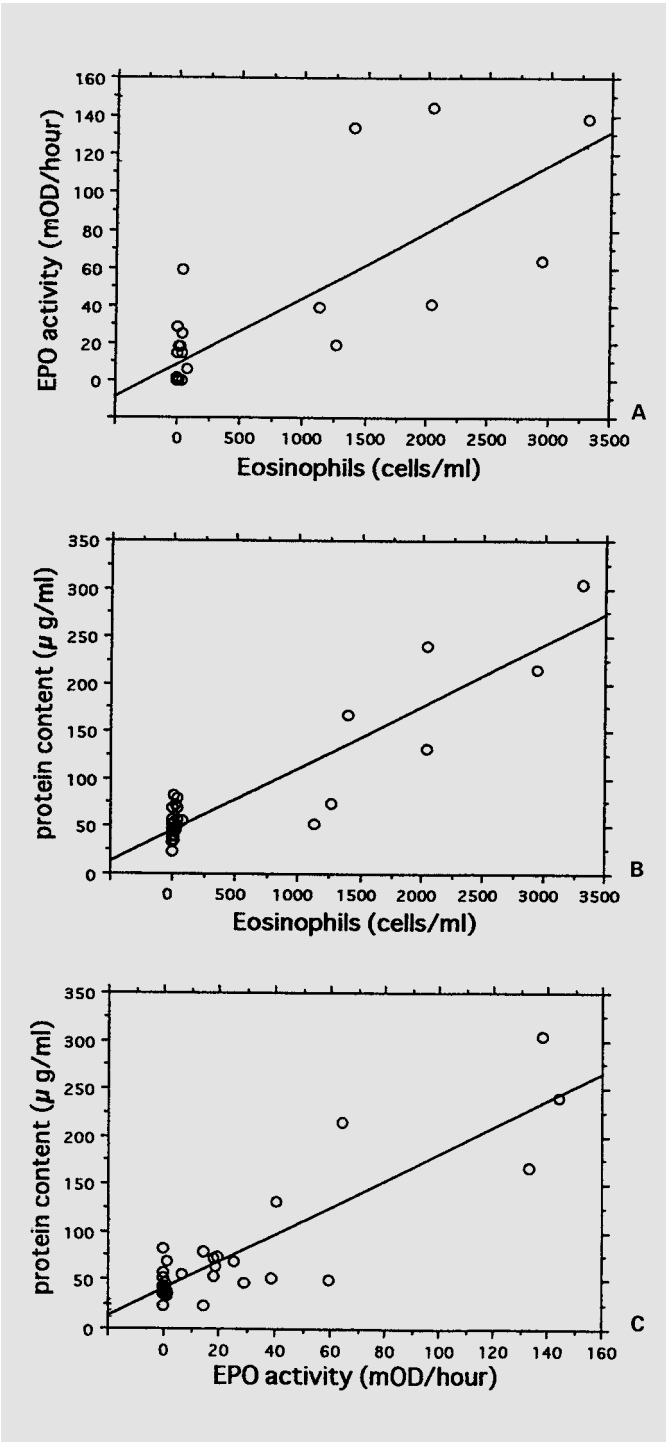

Fig. 3. Relationship between the number of eosinophils, EPO activity and protein content in NLF. Correlations between the number of eosinophils and EPO activity (**A**), the number of eosinophils and protein content (**B**), and EPO activity and protein content (**C**) in NLF obtained from 31 animals in groups were evaluated. Positive correlations were obtained in **A** ($r = 0.627$, $p < 0.0001$), **B** ($r = 0.915$, $p < 0.0001$) and **C** ($r = 0.727$, $p < 0.0001$) by Fisher's r to z transformation.

Table 1. Cellular compositions in the nasal lavage fluid

Group	Pretreatment	Provocation	Total leukocytes cells/ml	Eosinophils cells/ml	Neutrophils cells/ml
Normal	no sensitization	saline	46 ± 13.9	11 ± 4.8	4 ± 2.9
Group 1	sensitization	saline	10 ± 2.9	4 ± 1.5	5 ± 2.8
Group 1	sensitization	antigen	35 ± 8.8*	32 ± 8.5**	3 ± 1.1
Group 2	sensitization + repeated challenges	saline	21 ± 7.6	10 ± 11.6	11 ± 6.5
Group 2	sensitization + repeated challenges	antigen	1,792 ± 372.1**,††	1,772 ± 370.8**,††	13 ± 3.4†

Fifteen minutes after saline or antigen application to each of the 7–8 animals in a group, NLF was obtained. NLF was also obtained from nonsensitized animals (normal). Values are presented as means ± SEM.

* $p < 0.05$ and ** $p < 0.01$ vs. each group with saline provocation (unpaired Student t test).

† $p < 0.05$ and †† $p < 0.01$ vs. group 1 (unpaired Student t test).

Table 2. EPO activity and protein content of NLF

Group	Pretreatment	Provocation	EPO activity mOD/h	Protein content μg/ml
Normal	no sensitization	saline	18.9 ± 5.11	3 ± 9.7
Group 1	sensitization	saline	7.9 ± 3.95	42 ± 4.9
Group 1	sensitization	antigen	12.0 ± 3.66	58 ± 5.7
Group 2	sensitization + repeated challenges	saline	0.3 ± 0.16	51 ± 6.1
Group 2	sensitization + repeated challenges	antigen	79.8 ± 17.87**,††	155 ± 32.2**,†

Fifteen minutes after saline or antigen application to each of the 7–8 animals in a group, NLF was obtained. NLF was also obtained from nonsensitized animals (normal). Values are presented as means ± SEM.

** $p < 0.01$ vs. each group with saline provocation (unpaired Student t test).

† $p < 0.05$ and †† $p < 0.01$ vs. group 1 (unpaired Student t test).

Similar tendencies are also observed between the protein content and the number of eosinophils ($p < 0.0001$, $r = 0.915$; fig. 3B) and between the protein content and EPO activity ($p < 0.0001$, $r = 0.727$; fig. 3C). To determine whether or not albumin, which is a marker of vascular permeability, was increased in NLF after antigen provocation, each NLF sample was analyzed by SDS-PAGE (fig. 4A). Lane 4 in figure 4A contains a single protein band corresponding to the position of the guinea pig albumin, marked by an arrowhead. Comparing lane 4 with the other lanes, the protein was found to be increased and the most prevalent of the proteins, namely, in the NLF of group 2 with antigen provocation. It was confirmed that the protein band was albumin by immunoblot analysis (fig. 4B).

Changes in Eosinophil Infiltration into the Nasal Mucosa

Nasal tissues were obtained under five conditions to investigate the inflammatory conditions of the nasal mucosa. The degrees of epithelial disruption, mucosal edema and eosinophil infiltration into the epithelium and the lamina propria of the nasal mucosa obtained from group 1 with saline provocation instead of antigen were almost the same as those of the normal group. The degrees of epithelial disruption, mucosal edema and eosinophil infiltration into the epithelium of the animals in group 2 with saline provocation were slight and not significantly different from those of group 1 with saline provocation (table 3). However, eosinophil infiltration into the lamina propria of those in group 2 with saline provocation was

significantly and apparently elevated compared with that of group 1 with saline provocation (p = 0.0016). These histopathological parameters were significantly different from each other after antigen provocation. Particularly, the degrees of epithelial disruption, mucosal edema and eosinophil infiltration into the epithelium were significantly elevated after antigen provocation in the case of group 2 (p = 0.0054, 0.0034 and 0.0084, respectively). In the case of group 1, only the degree of mucosal edema was significantly elevated after antigen provocation (p = 0.0026). The degrees of all these parameters for group 2 were significantly higher than those of group 1 with antigen provocation (epithelial disruption, p = 0.0074; mucosal edema, p = 0.0284; and eosinophil infiltration into the epithelium and the lamina propria, p = 0.0045 and 0.0045). Typical photomicrographs of the nasal mucosa exhibiting these inflammatory conditions are shown in figure 5. A significant amount of eosinophils can be observed in the lamina propria of the animals in group 2 that received repeated antigen challenges.

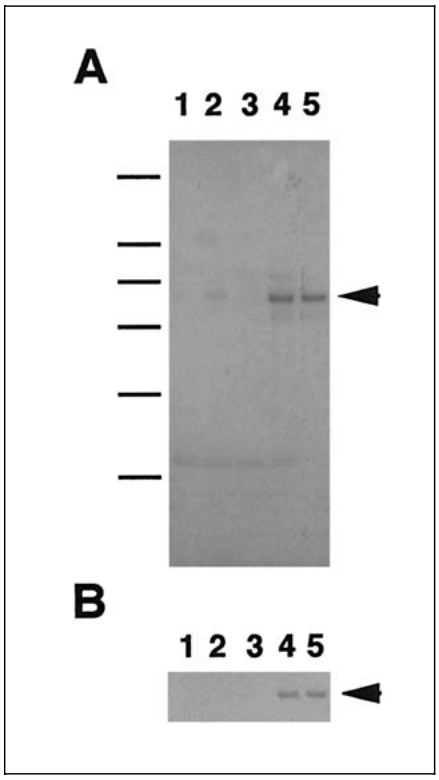

Fig. 4. SDS-PAGE and immunoblot analysis of albumin in NLF. 5 μl of a NLF supernatant from each group was electrophoresed in a 4–20% linear gradient gel and stained with CBB (**A**) or blotted with the antialbumin IgG after transfer to a nitrocellulose membrane (**B**). Lanes: 1, group 1 with saline provocation; 2, group 1 with antigen provocation; 3, group 2 with saline provocation; 4, group 2 with antigen provocation; and 5, purified guinea pig albumin, 0.5 μg. The bars at the left of the gel indicate the positions of marker proteins of 200, 97, 66, 55, 31 and 14 kD from top to bottom. The arrowhead shows the position of guinea pig albumin.

Discussion

In the present study, we used OVA-sensitized guinea pigs as a model for human nasal allergy and studied the effects of repeated antigen challenges on EPR. The sensitized animals were challenged once a week for 5 weeks. Under these experimental conditions, it was observed that the antigen provocation dose for the animals in group 2, which were subjected to the pretreatment of sensitization followed by repeated antigen challenges, was

Table 3. Effects of repeated antigen challenges on the nasal tissue based on histopathological observations

Group	Pretreatment	Provocation	Epithelial disruption	Mucosal edema	Eosinophil infiltration	
					epithelium	lamina propria
Normal	no sensitization	saline	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Group 1	sensitization	saline	0.0 ± 0.09	0.0 ± 0.09	0.0 ± 0.08	0.0 ± 0.08
Group 1	sensitization	antigen	0.0 ± 0.14	1.0 ± 0.21**	0.0 ± 0.07	0.0 ± 0.07
Group 2	sensitization + repeated challenges	saline	0.0 ± 0.13	0.5 ± 0.11	0.5 ± 0.13	1.0 ± 0.21††
Group 2	sensitization + repeated challenges	antigen	2.0 ± 0.45**,††	2.0 ± 0.20**,†	2.0 ± 0.25**,††	2.0 ± 0.20††

Fifteen minutes after saline or antigen application to each of the 7–8 animals in a group, various parameters regarding the nasal mucosa were histopathologically evaluated. Values are presented as medians ± SEM.

** $p < 0.01$ vs. each group with saline provocation (Mann-Whitney U test).

† $p < 0.05$ and †† $p < 0.01$ vs. group 1 (Mann-Whitney U test).

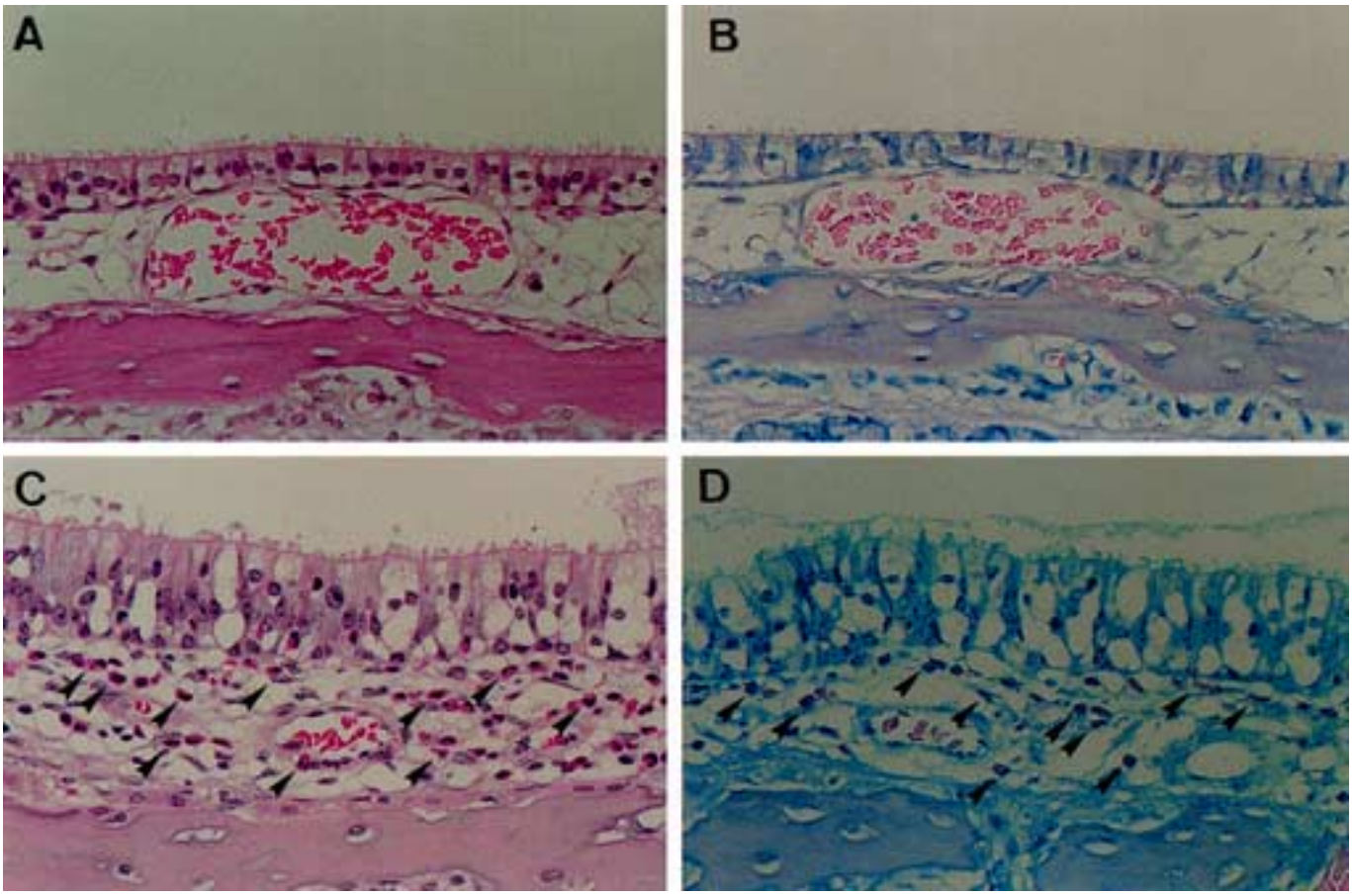

Fig. 5. Representative photomicrographs of the nasal mucosa. The nasal tissue specimens obtained from groups 1 (**A**, **B**) and 2 (**C**, **D**) of the animals subjected to saline provocation were stained with hematoxylin and eosin (**A**, **C**) or Eosinostain® (**B**, **D**). The arrowheads indicate the eosinophils. Original magnification ×100.

significantly lower than that of the animals in group 1, which were subjected to the sensitization pretreatment only. Moreover, the level of sRaw in group 2 was significantly higher than that in group 1. In the preliminary experiment, we applied 50 μl of dye solution into each nostril as a nasal drip and examined the distribution of dye in the upper and lower respiratory tracts. We found that almost all of the dye was distributed in the nasal cavity, while none was found in the larynx and lower respiratory tract. Thus, the observed changes in sRaw are thought to result from the stimulation of the upper respiratory tract, particularly the nasal cavity. Furthermore, these aforementioned data suggest that antigen-specific hypersensitivity can be acquired in animals when subjected to repeated antigen challenges.

It was reported that activated eosinophils release toxic proteins such as eosinophil cationic protein and EPO, leading to tissue damage of the host [19], and are therefore known to cause airway hyperreactivity to specific antigen or nonspecific stimuli including histamine in patients with allergic asthma and rhinitis. Eosinophils also release lipid mediators such as leukotrienes and platelet-activating factor [20, 21], causing nasal congestion by increasing vascular permeability and dilating capacitance vessels in allergic rhinitis [22, 23]. To clarify the cause of hypersensitivity, NLF was obtained from both groups, and the eosinophil count, EPO activity and protein content of NLF were measured. Although marked changes were not observed in group 2 treated with saline provocation, all these parameter levels were significantly elevated after antigen provocation. In group 1, no marked changes were observed even after antigen provocation. A positive correlation was observed between the eosinophil count, EPO activity and protein content in these animals. It was con-

firmed in this study that albumin, which is a marker of vascular permeability, was the most prevalent of the proteins in NLF after antigen provocation, and histopathological examinations revealed that nasal edema in group 2 was significantly severer than that in group 1. From these data, it is suggested that the activation of eosinophils was responsible for the increase in vascular permeability, and the elevation of sRaw might be mainly due to the complication of mucosal congestion and increase in mucosal vascular permeability followed by mucosal edema. Furthermore, epithelial disruption and eosinophil infiltration into the epithelium of the nasal mucosa were observed after antigen provocation in group 2. Taking these into consideration, it is suggested that the activation of eosinophils was involved in the hypersensitive reaction acquired by the animals in group 2 subjected to repeated antigen challenges.

Allergen-specific nasal challenge is a valid and reliable tool for studying the pathophysiological mechanisms involved in allergic inflammation. Nasal challenge induces an immediate clinical response in allergic subjects and the concomitant appearance of inflammatory cell infiltration. The mucosal inflammation may persist up to 48–72 h following allergen exposure. If the subject is rechallenged within this period, the response is more pronounced: the so-called priming effect [24]. Pipkorn et al. [25] reported that the priming effect, in contrast to the immediate response, was significantly reduced by prednisone pretreatment, suggesting that cell-mediated inflammation may be involved in the priming effect. Furthermore, it was reported that numerous eosinophils were observed in the bronchoalveolar lavage fluid and NLF during the late allergic response. In the present study, no significant increase in the eosinophil count of NLF was observed in group 2 subjected to saline provocation, which received the final antigen challenge 7 days before nasal lavage. Histopathological examination was then carried out to clarify the existence of nasal mucosal inflammation in these animals. The eosinophil infiltration into the lamina propria was significantly and apparently elevated in the animals in group 2, compared with those in group 1, which were not subjected to any challenges. On the other hand, epithelial disruption, mucosal edema and eosinophil infiltration into the epithelium were not observed in group 2 not subjected to antigen provocation. These observations suggest that chronic eosinophil accumulation was induced by repeated antigen challenges. Recently, it was reported that resolution of the eosinophilic inflammation was likely the consequence of eosinophil apoptosis [26] and delayed apoptosis was important for specific accumulation of this cell type in chronic allergic responses [27]. In group 2 not subjected to antigen provocation, which received the final antigen challenge 7 days before nasal lavage, chronic eosinophil accumulation was observed without changes in various inflammation parameters, such as baseline sRaw, eosinophil counts, EPO activity and protein content of NLF, and epithelial disruption and mucosal edema, as determined by histopathological examination. In this study, the mechanisms underlying chronic eosinophil accumulation were not identified. However, data suggest that chronic eosinophil accumulation is induced by repeated antigen challenges in the nasal tissue, and that once the animals are subjected to an antigen provocation, eosinophils in the tissue are activated and responsible for the amplification of EPR such as mucosal congestion, vascular permeability and mucosal edema. Furthermore, the priming effect of repeated antigen challenges might be the result of activation of eosinophils observed in the nasal mucosa in our experimental model.

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