

Review Article

Mechanisms of eosinophilic inflammation in the mucosa of the nasal cavity and paranasal sinus

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ABSTRACT

More than one mechanism contributes to persistent increases in eosinophils in the nasal cavity and paranasal sinus. The levels of eotaxin in lavage samples obtained after antigen challenge showed strong correlation with lavage levels of eosinophil counts and eosinophil protein-X. In the eosinophil endothelial transmigration (TEM) assay using nasal microvascular endothelial cells, eotaxin showed the most potent effect among various eosinophil chemoattractants. In addition, treatment of eosinophils with anti-CCR3 monoclonal antibody significantly blocked eosinophil TEM induced by homogenate of nasal mucosa. These results indicate that eotaxin has an important role in eosinophil-dependent inflammation in nasal mucosa and suggest that blocking eotaxin or CCR-3 may be useful for new therapeutic tools of allergic rhinitis. Several mechanisms for eosinophilic inflammation in paranasal sinus may be proposed: a series of reaction systems may be involved, such as swollen mucosa caused by type I allergic reactions in the nasal cavity, closure of natural orifices, a hypoxic state in the maxillary sinus, accelerated production of eotaxin and RANTES from fibroblasts and eosinophilic infiltration. These reaction systems would include tumor necrosis factor- α and interferon- γ , as well as Th2 cytokines, including interleukin-4 and -13, so that this phenomenon hardly occurs in infective rhinitis.

Key words: allergic rhinitis, eosinophil, eotaxin.

INTRODUCTION

The pathogenesis of allergic rhinitis is based on interactions of allergen with membrane-bound allergen-specific IgE on the surface of mediator cells (i.e. basophils and mast cells) leading to the release of allergic mediators (both preformed and newly synthesized), including histamine, leukotrienes and eosinophil-derived mediators, such as eosinophil cationic protein (ECP). The inflammation is characterized by the recruitment of inflammatory leukocytes, predominantly eosinophils, and their subsequent migration from the vessels into the tissue. T helper 2 (Th2) lymphocytes and epithelial and endothelial cells play major roles in this cascade by secreting cytokines and, yet, the mechanism responsible for their selective accumulation is not fully understood. In the present paper, we review some of the mechanisms leading to eosinophilic inflammation in the nasal mucosa. We also address the relationship of allergic rhinitis with sinusitis and consider the mechanism of eosinophil accumulation in the paranasal cavity.

MECHANISMS OF EOSINOPHILIC INFLAMMATION IN THE MUCOSA OF THE NASAL CAVITY: THE CENTRAL ROLE OF EOTAXIN

Among the various chemical mediators released from eosinophils, platelet-activating factor (PAF) and leukotrienes dilate the capacitance vessels and induce leakage of plasma components from post-capillary veins, resulting in swelling of the nasal mucosa.^{1–3} Furthermore, PAF promotes the histamine-induced swelling of the nasal mucosa, while leukotriene (LT) D₄ and ECP augment the histamine-mediated secretion of nasal discharge.^{1,2} Thus,

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eosinophils play an important role in the development of the swelling and hyperreactivity of the nasal mucosa from non-specific stimulation.

More than one mechanism contributes to persistent increases of eosinophils in the nasal cavity and paranasal sinus (Fig. 1). First, there is the possibility of enhanced bone marrow production and activation or prolonged survival of mature eosinophils. With regard to this, the role of interleukin (IL)-5 should be most important. Interleukin-3 and granulocyte-macrophage colony stimulating factor (GM-CSF) also contribute to the bone marrow events. Recent reports have demonstrated that eotaxin (CCL11) stimulates the production and differentiation of myeloid hematopoietic progenitors in the bone marrow and that eotaxin induces a rapid release of eosinophils and their progenitors from the bone marrow.^{4,5}

Another mechanism is the process of increased infiltration of eosinophils. The infiltration of eosinophils initially

depends on an influx from systemic blood into local tissue. Chemokines, such as eotaxin, regulated upon activation, normal T expressed and secreted (RANTES), monocyte chemoattractant protein (MCP)-3, MCP-4 and macrophage inflammatory protein 1, and inflammatory cytokines, such as IL-3, IL-5 and GM-CSF, regulate this influx. Among those, the eosinophil chemoattractant eotaxin seems to be most important in local eosinophil recruitment in the nasal mucosa.⁶ In subjects with allergic rhinitis, allergen challenge leads to parallel increases in eosinophil counts, levels of eosinophil protein-X (EPX) and eotaxin concentrations in nasal lavage fluid. The levels of eotaxin in lavage samples show strong correlation with lavage levels of eosinophil counts and EPX. In addition, although to a lesser extent, the levels of IL-16 and IL-5 in lavage samples are significantly correlated with lavage levels of eosinophil counts and EPX. No significant correlation is observed between PAF and eosinophil counts or EPX or between LTB₄ and eosinophil

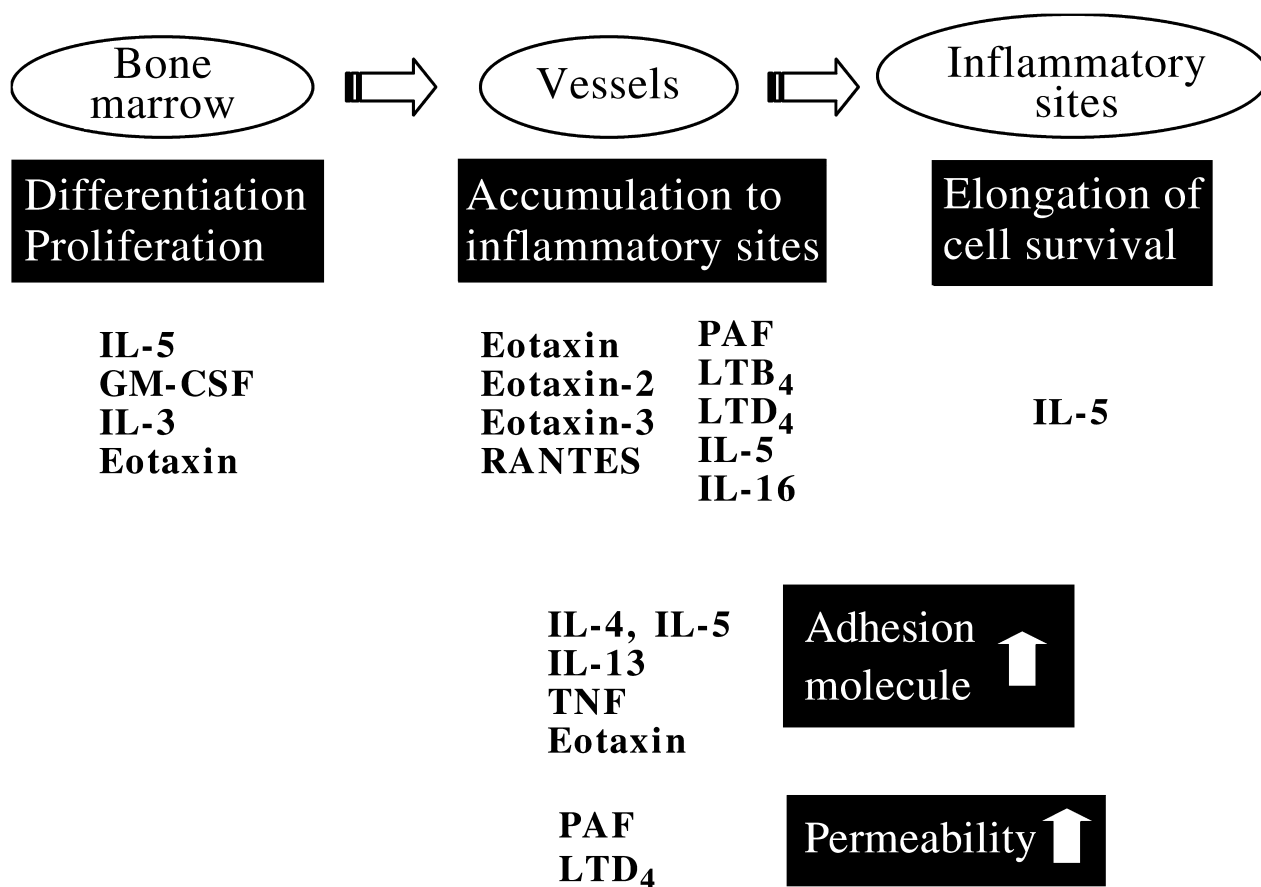


Fig. 1 The possible mechanism of eosinophilic inflammation in the nasal cavity and paranasal sinus. IL, interleukin; GN-CSF, granulocyte-macrophage colony stimulating factor; PAF, platelet-activating factor; LTB₄, LTD₄, leukotriene B₄ and D₄, respectively; RANTES, regulated upon activation, normal T expressed and secreted; TNF, tumor necrosis factor.

counts or EPX. In our experiments of a eosinophil transendothelial migration (TEM) assay using the human nasal mucosal microvascular endothelial cells (HMMEC), eotaxin, MCP-4 and RANTES induced eosinophil transendothelial migration across HMMEC in a concentration-dependent manner. The rank order of potency was eotaxin > MCP-4, RANTES > IL-5 > PAF > LTB₄, IL-16. The maximal responses induced by eotaxin significantly exceeded those produced by RANTES, MCP-4 or other eosinophil chemoattractants.⁶ In addition, when eosinophils were pretreated with anti-CCR3 monoclonal antibody (mAb) or anti-IL-5 receptor α mAb, eosinophil TEM induced by the homogenate of nasal mucosa was reduced in a dose-dependent manner. At optimal concentrations, CCR3 mAb and IL-5R α mAb inhibited eosinophil TEM by 35.2 ± 9.2 and $12.4 \pm 8.1\%$, respectively. In contrast, the PAF receptor antagonist WEB 2086 and the LTB₄ receptor antagonist Y-24180, at optimal or higher concentrations, only weakly inhibited eosinophil TEM.⁶

CCR3 is now known to be among the most promiscuous of chemokine receptors, signaling in response to upwards of a dozen different ligands.⁷ We cannot completely exclude the possibility that the other CCR3-activating chemokines could be more important than eotaxin itself. However, when eotaxin in nasal extracts was neutralized by excessive anti-eotaxin mAb, eosinophil TEM induced by the homogenate of the nasal mucosa was reduced by $27.2 \pm 4.4\%$, which is similar to the percentage inhibition seen following treatment with anti-CCR-3 mAb. In contrast, anti-RANTES mAb, anti-MCP-3 mAb and anti-MCP-4 mAb inhibited eosinophil TEM at minimum levels.⁶ It is well known that, unlike most other chemokines, which are themselves promiscuous, eotaxin shows great fidelity, signaling exclusively via CCR3. On the basis of these findings, it is highly possible that eotaxin is the most important eosinophil chemoattractant of the CCR3-activating chemokines.

The inflammatory process also requires the expression of cell adhesion molecules. It is well known that IL-4, tumor necrosis factor (TNF)- α and interferon (IFN)- γ , alone or in combination, increase adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1.⁸⁻¹¹ Interleukin-13 increases VCAM-1 expression in HMMEC, the adhesiveness of endothelial cells to eosinophils and the transendothelial migration. In contrast, IL-13 decreases the adhesiveness of eosinophils to HMMEC and, as a result, accelerates eosinophil infiltration.¹² These

effects are more potent than those of IL-4. Considering that the amount of IL-13 in nasal mucosa is higher than that of IL-4, IL-13, as well as IL-4, may be important in eosinophilic inflammation in the nasal mucosa. Eotaxin also upregulates the expression of ICAM-1 and VCAM-1 on HMMEC, but not human umbilical vein endothelial cells (HUVEC).¹³ It is likely that IL-5 works not only as an eosinophil selective chemoattractant, but also as an enhancing factor of adhesion molecules in nasal mucosa in subjects with nasal allergy. When the enhancement of adhesion molecule gene expression by IL-5 was studied by quantitative polymerase chain reaction (PCR), IL-5 induced ICAM-1 gene expression in the nasal mucosa of patients with nasal allergy but not in patients with non-allergic rhinitis.¹⁴

In nasal mucosa, eosinophils degranulate and release various kind of mediators. CCR3 ligands may also have a role in this process. It has been shown that calcium influx is induced by MCP-1, MCP-3, MCP-4, RANTES, eotaxin, IL-8 and stromal cell-derived factor 1 α , which are chemokines that bind several chemokine receptors. However, degranulation is induced only by CCR3 ligands, including MCP-3, MCP-4, RANTES and eotaxin.¹⁵ Eotaxin induces a rapid concentration-dependent activation of extracellular signal-regulated kinase (ERK) 2 and p38 in eosinophils and activation of these mitogen-activated protein kinases (MAPK) is essential for eotaxin-stimulated degranulation.¹⁶

MODULATION OF EOSINOPHIL CHEMOTAXIS BY CELL-TO-CELL INTERACTIONS

Throughout the process of migration of eosinophils from blood vessels into the nasal cavity through the interposed layers of the nasal mucosa, the eosinophils repeatedly adhere to and detach from endothelial and epithelial cells and the intercellular matrix. It is suggested that eosinophil functions are regulated by surface adhesion molecules during this migration, as evidenced by the prolonged survival rates of eosinophils and enhanced production of LTC₄ after the adhesion of the eosinophils to fibronectin.¹⁷ Conversely, clarification of the messages conferred from the eosinophils to endothelial and epithelial cells (HNEC) are also of great interest. We have shown that a certain established system seems to induce the signal transfer from eosinophils to HNEC to inhibit RANTES production, thus decreasing further eosinophil infiltration.¹⁸ In particular, RANTES production in nasal epithelial cells, induced by TNF- α and IFN- γ , is

decreased by the presence of eosinophils. The degree of the decrease is a function of the number of eosinophils involved. In this respect, eosinophil adhesion to HNEC is crucial because, after pretreatment of eosinophils with anti-CD18 or coculture with HNEC in transwell culture inserts, eosinophils did not inhibit RANTES production.

CCR3 ANTAGONISTS MAY BE A USEFUL THERAPEUTIC TOOL FOR ALLERGIC RHINITIS

These data mentioned above raise the possibility that anti-CCR3 antibody or CCR3 antagonists may attractively and innovatively regulate eosinophilic inflammation in the nasal mucosa. Very recently, four different small molecule antagonists of human CCR3 have been created and reported: UCB-35625 (J113863), SB-297006, SB-328437 and Compound X.^{19–21} All four compounds were identified by high-throughput screening of existing chemical libraries and are effective antagonists of CCR3 *in vitro*, with IC₅₀ values in the low nanomolar range. These CCR3 antagonists possess a basic nitrogen within their structure that may interact with a conserved glutamate counterion in the seventh transmembrane helix of CCR3, as has been concluded for the action of the CCR2 and CCR5 antagonists.^{22,23} These CCR3 antagonists may be a useful therapeutic tool for allergic disorders in the near future.

RELATIONSHIP BETWEEN ALLERGIC RHINITIS AND SINUSITIS

When sinusitis is classified according to its causal factors, it is bipolarized with infectious sinusitis and allergic sinusitis. Although these two pathologic conditions coexist in many cases, allergic factors are conceivably increasing more than infectious ones and the so-called allergic sinusitis is becoming more prevalent in general. Although worldwide criteria for allergic sinusitis could not be found, it is considered generally defined in Japan as to: (i) having nasal allergy; (ii) showing a shadow on X-ray in the paranasal sinus; and (iii) having no infections.

The mechanism by which allergic reactions trigger sinusitis has yet to be clarified in many aspects. Karlsson *et al.*²⁴ proposed the following mechanisms; (i) the allergen directly reaches the sinus cavity and causes the same kind of allergic inflammation; (ii) antigens are absorbed through the skin or digestive tract and hematogenously reach the paranasal sinuses; (iii) the ostium of the paranasal sinuses is narrowed or closed by an allergic

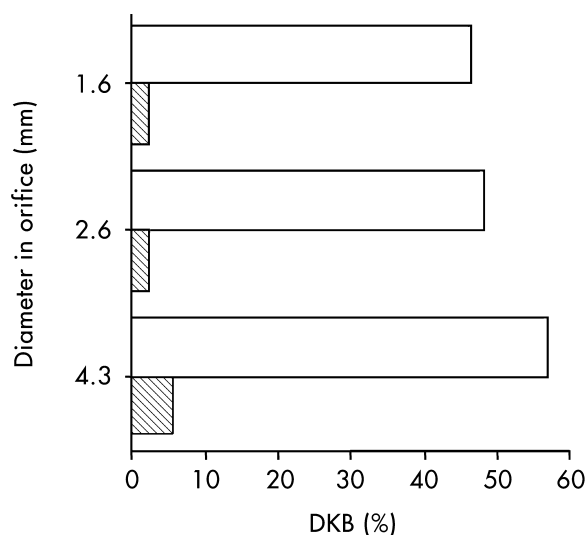


Fig. 2 The relationship between the inner diameter in a natural orifice model and dibekacin sulfate (DKB) concentrations in the nasal cavity model. Particle diameter: 0.5–0.7 μm (□); > 5 μm (▨).

reaction that occurs in the nasal sinuses; and (iv) paranasal sinuses are affected by nervous reactions. Of these mechanisms, the first two are defined as allergic sinusitis in a strict sense. Whether a primary allergic reaction occurs in the sinuses in association with naturally occurring allergic rhinitis remains to be clarified. Pelikan *et al.* performed nasal provocation challenges in patients with allergic rhinitis and found a large number who demonstrated mucosal edema or opacification of the sinuses on radiographic examination.²⁵ These responses were accompanied by increased pressure in the maxillary sinuses, acute headache and sometimes otalgia. However, these findings occurred as a result of provocative challenge and not naturally occurring rhinitis. Slavin *et al.*²⁶ attempted to determine whether allergic rhinitis results in sinus inflammation that can be detected by computed tomography (CT) scan. However, Slavin *et al.* found that adult patients with symptomatic allergic rhinitis demonstrated infrequent and minimal changes in the sinuses on limited coronal CT during natural seasonal exposure.²⁶ In addition, when the nose was challenged with supra-physiologic doses of radiolabeled ragweed pollen, no radioactivity was detected in the region of the ipsilateral paranasal sinus posterior nasopharynx and oropharynx, whereas considerable radioactivity was noted in the nasal airway. Liu *et al.* found tissue-specific IgE antibodies to house dust mite in the nasal mucosa of dust-sensitive

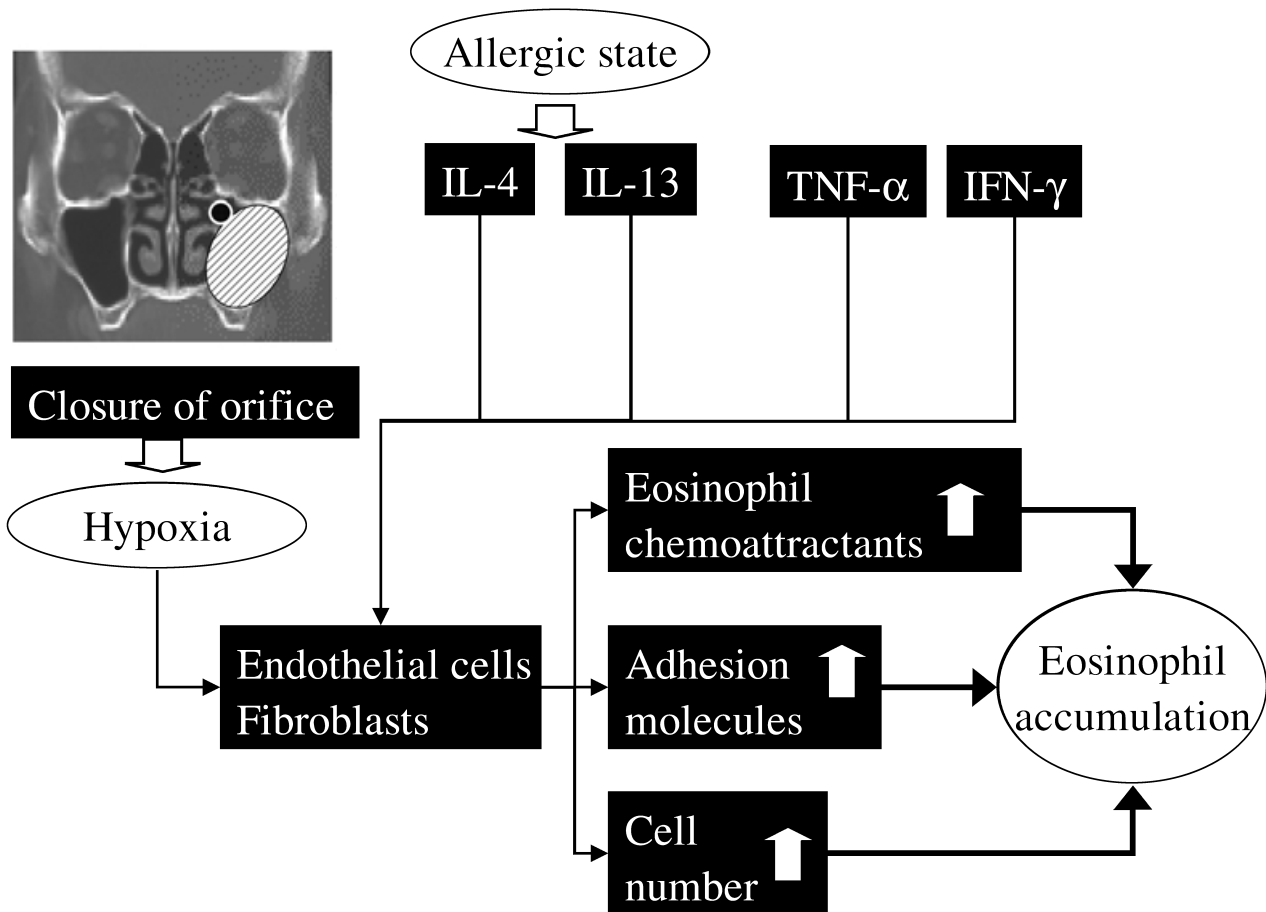


Fig. 3 The possible mechanism for eosinophil accumulation in hypoxic conditions. IL, interleukin; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ .

patients, but levels in the sinus mucosa were similar to those seen in non-atopic patients with rhinitis.²⁷

Investigations of the antibiotic dibekacin sulfate (DKB) sprayed into the paranasal sinus with a jet-type nebulizer in a nasal cavity model prepared to approximate aeration resistance and cross-section area demonstrated that DKB particles with a diameter larger than 5 μm or more hardly entered the maxillary cavity. When the inner diameter in a natural orifice model is 1.6–2.6 μm , the passing frequency is only 2% (Fig. 2). Under non-pressurized conditions, DKB concentrations are less than the detection limit when the inner diameter is less than 2.6 μm . From these findings, it is hardly possible that pollen, with a diameter of the larger particles of at least 20–30 μm , directly enters the maxillary cavity. Fragmented components in house dust or mites may enter the maxillary

cavity. Soluble components of the antigen may also enter the maxillary cavity through the natural orifice. However, this is considered difficult in the case of normal villous function. In addition, after the allergic reaction occurs near the orifice, it will make it even more difficult for further entrance of the allergen.

Moreover, if the antigen infiltrates the maxillary cavity directly through the natural orifice to cause type I allergic reactions in the mucous membrane of the maxillary cavity, it is considered that mast cells or histamine levels will increase in the mucous membrane or in the retention fluid of the maxillary cavity. However, as far as we know, there have been no reports comparing these levels between allergic rhinitis patients and normal healthy subjects. In contrast, Yamada *et al.* studied infiltrated cells in the mucosae of maxillary sinus in which X-ray revealed an

abnormal shadow in patients with a nasal allergy and in those with chronic sinusitis.²⁸ Those authors found that neither the number of formalin-sensitive nor -resistant mast cells are significantly different between the two groups.

Several mechanisms for eosinophilic inflammation may be proposed: a series of reaction systems may be involved, such as swollen mucosa caused by type I allergic reactions in the nasal cavity, closure of natural orifices, a hypoxic state in the maxillary sinus, accelerated production of eotaxin and RANTES from fibroblasts and eosinophilic infiltration (Fig. 3). These reaction systems would include TNF- α and IFN- γ , as well as Th2 cytokines, including IL-4 and IL-13, so that this phenomenon hardly occurs in infective rhinitis. In addition, it is well known that hypoxia rapidly activates endothelial cells and fibroblasts to release inflammatory mediators and growth factors. These inflammatory mediators are able to recruit and promote the adherence of inflammatory cells to the endothelium, where they become activated. In addition, longer periods of hypoxia increase the expression of specific genes, such as those encoding certain cytokines as well as platelet-derived growth factor and vascular endothelial growth factor. The transcriptional induction of these genes is mediated through the activation of several transcription factors, the most important being hypoxia-inducible factor-1. Recently, Pinsky *et al.*²⁹ demonstrated that hypoxia alone induces a calcium-dependent exocytosis of the Weibel–Palade bodies. These organelles, typical of endothelial cells, store von Willebrand factor (vWF). This exocytosis leads to the release of vWF³⁰ and to the overexpression of P-selectin, which then sustains eosinophil and neutrophil binding.

Allergic fungal sinusitis (AFS) is a distinct form of non-invasive fungal sinusitis that has recently been delineated as a distinct clinicopathologic entity. The diagnosis of AFS and its pathophysiology is controversial and problematic. Recently, Ponikau *et al.* showed a new aspect of sinusitis.³¹ They demonstrated that fungal cultures of nasal secretions were positive in 202 of 210 consecutive chronic rhinosinusitis (CRS) patients (96%) and that eosinophilic mucin was found in 97 of 101 consecutive surgical cases of CRS (96%). However, they also observed that only 42% of patients had a detectable type I hypersensitivity by skin test and only 30% had an elevated fungus-specific IgE level by radioallergosorbent test. Thus, more than half their patients showed no evidence of increased IgE levels to fungi. It is obvious that exposure alone to these fungi appears to be insufficient

to initiate clinical manifestations of the disease (nasal polyps, expansile mucocoele formation, allergic fungal mucin etc.). At the present time, it is likely that initiation of the inflammatory cascade leading to AFS is a multifactorial event, requiring the simultaneous occurrence of such things as IgE-mediated sensitivity (atopy), specific T cell human leukocyte antigen receptor expression, exposure to specific fungi and aberration of local mucosal defense mechanisms.

Since 1983, a form of sinusitis histologically similar to AFS except for the absence of fungal hyphae has also been noted. The designation eosinophilic mucin rhinosinusitis (EMRS) is proposed for this condition. Its relationship to AFS is also controversial and problematic. Polyp occurrence was almost 100% in the AFS and EMRS groups.²⁹ However, 55% of AFS patients had bilateral disease, in contrast with 100% of EMRS patients with bilateral disease ($P < 0.0001$). Forty-one percent of patients with AFS were asthmatic compared with 93% of patients with EMRS ($P < 0.0001$). It is postulated that AFS is an allergic response to fungi in predisposed individuals, whereas EMRS occurs because of a systemic dysregulation of immunologic controls. Because EMRS is a systemic disease, unilateral disease is not seen. In contrast, AFS, an allergic response to fungi, may occur unilaterally or bilaterally depending on the antigenic stimulation.

Although the reason is not clear, in Japan the numbers of patients with AFS and EMRS are relatively small compared with the number of patients with infectious sinusitis. The future awaits an exploration of the pathophysiologic basis of these differences.

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