

Molecular mechanisms in allergy and clinical immunology

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Molecular pathology of allergic disease II: Upper airway disease

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Allergic upper airway diseases such as allergic rhinitis and chronic sinusitis are an increasing problem. Although the pathogenesis remains elusive, an individual's genetic predisposition as well as exposure to the allergen are currently considered factors in their development. Clinical symptoms of sneezing, rhinorrhea, and congestion are primarily a consequence of granulocyte release of chemical mediators such as histamine, prostanoids, and leukotrienes as well as the infiltration of inflammatory cells. Observations subsequent to allergen provocation are comparable to natural exposure and as such much of our understanding of allergic responses is derived from this model. A prominence of CD4⁺ T cells and eosinophils, synthesis and release of T_H2 cytokines, and the coordinate expression of chemokines and adhesion molecules are all characteristic of the allergic response observed in rhinitis and sinusitis. Corticosteroids and immunotherapy target these inflammatory processes and have been observed to successfully reduce and shift the predominantly T_H2 environment toward T_H1 cytokine expression. As our understanding of the pathophysiologic features of allergic upper airway disease improves, as well as the relationship between their development and that of lower airway disease, new strategies of diagnosis and treatment will allow for more effective modulation of the allergic process and associated morbidity. (*J Allergy Clin Immunol* 2000;105:211-23.)

Key words: Allergic rhinitis, chronic sinusitis, inflammation, cytokines, chemokines

HISTOPATHOLOGIC FEATURES OF THE NASAL AND SINUS MUCOSA

The nasal mucosa consists of a ciliated pseudostratified columnar epithelium composed of ciliated and non-ciliated columnar epithelial cells, goblet cells, and basal cells.^{1,2} All 4 cell types rest on a basement membrane composed of collagen types I, III, and IV fibrils³; how-

Abbreviations used

CHS/NP:	Chronic hyperplastic sinusitis with nasal polyposis
ECP:	Eosinophil cationic protein
EPO:	Eosinophil peroxidase
ICAM-1:	Intercellular adhesion molecule-1
LFA-1:	Lymphocyte function antigen-1
LT:	Leukotriene
MBP:	Major basic protein
MCP:	Monocyte chemotactic protein
mRNA:	Messenger RNA
NO:	Nitric oxide
PAF:	Platelet-activating factor
STAT-6:	Signal transducer and activator protein-6
TGF- β :	Transforming growth factor- β
VCAM-1:	Vascular cell adhesion molecule-1
VLA-4:	Very late antigen-4

ever, not all cells reach the luminal surface, hence the illusion of a stratified layer. Immediately beneath the basement membrane is an almost cell-free zone composed of fibronectin and collagen types III and V and a submucosal layer consisting of glands, inflammatory and interstitial cells, extracellular matrix, nerves, and blood vessels. Three types of glands can be found within this layer: mucous, seromucous, and serous glands. These glands, along with epithelial goblet cells, synthesize the mucus that overlies the epithelium and provides an antimicrobial function as well as transport for particulate matter, antigens, or bacteria by mucociliary clearance.^{4,5} Serous glands also produce secretory IgA, an important component of mucosal immunity.⁶ The submucosal gland area is increased in patients with perennial allergic rhinitis, representing approximately 25% of the lamina propria, compared with only 15% in individuals with no nasal allergies. This increase is consistent with reports of mucus hypersecretion in allergic airway disease.⁷ Under normal conditions, the cells in the nasal mucosa are primarily lymphocytes, macrophages, and mast cells, which are interspersed by fibroblasts. Nasal mast cells are generally found just beneath the basement membrane as well as within the epithelial layer.⁸⁻¹³

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The paranasal sinuses are 4 paired structures surrounding the nasal cavities: the ethmoid, sphenoid, maxillary, and frontal sinuses. They are lined by an epithelial-submucosal layer contiguous with that of the nose and is as such composed of similar histologic features. The anterior ethmoid and maxillary sinuses both drain into the middle meatus, and airflow is mainly in contact with the middle turbinate and ethmoid complex; as such the ethmoid sinus is under continual environmental insult.¹⁴ The maxillary sinus, however, normally receives only a small amount of its gas from the environment, ensuring relative protection from exogenous injury.¹⁵

INFLAMMATION IN ALLERGIC RHINITIS

Allergic rhinitis can be triggered by perennial or seasonal allergens, the most common of which are house dust, animal dander, mold spores, and pollen. Encounter with the allergen of sensitivity induces sneezing, nasal itch, rhinorrhea, and nasal blockage, resulting from afferent nerve stimulation,¹⁶ glandular hypersecretion,¹⁷ increased vascular permeability,¹⁸ and the infiltration of inflammatory cells.¹⁹

The mast cell plays an essential role in mediating the immediate response to allergen. Although the total number of these cells does not change during the allergy season, a higher proportion are observed just beneath or within the epithelial layer.^{9,20,21} Furthermore, in perennial allergic rhinitis there are elevated numbers of mast cells expressing FcεRI, indicating their increased ability to bind IgE.²² Mast cells store a number pro-inflammatory mediators, including tryptase, histamine, and cytokines such as TNF-α^{23,24} and IL-4.²⁵ On allergen inspiration and cross-linkage of IgE, they become activated, degranulate, and release these stores, as evidenced by the detection of elevated tryptase and histamine levels in nasal lavage fluid of patients with allergic rhinitis.²⁶ Allergen activation also induces mast cell synthesis of membrane-derived mediators such as leukotrienes (LTB₄, LTC₄, LTD₄, LTE₄), prostaglandins (PGD₂), and platelet-activating factor (PAF)²⁷ as well as bradykinin.²⁸ LTC₄/D₄ and PGD₂ are increased within nasal lavage fluid in perennial allergic rhinitis patients compared with healthy control subjects.²⁹ Nitric oxide (NO) is also produced in higher concentrations by the nasal mucosa of untreated allergic rhinitis patients than in healthy individuals.³⁰ It arises from the reaction between L-arginine and NO synthase, which is expressed by endothelial cells (type III), macrophages, neutrophils, mast cells, and fibroblasts (type II) as well as by parasympathetic neurons. There is good evidence that these substances mediate the immediate allergic response and together are considered to induce the characteristic symptoms.³¹⁻³³

ANTIGEN CHALLENGE MODEL OF ALLERGIC RHINITIS

Antigen challenge of the nasal mucosa has been a successful tool for monitoring the allergic response. Importantly,

the clinical response to antigen provocation and the ensuing inflammation are comparable to what is observed in patients after natural allergen exposure. The nasal response to antigen stimulation manifests as an early phase reaction, developing and subsiding within 60 minutes, followed by a delayed or late-phase reaction starting within 3 to 6 hours, peaking at 6 to 8 hours and subsiding 12 to 24 hours after allergen challenge. Dual responses, both an early and late phase, are observed in 40% to 50% of patients.³⁴ A single antigen challenge in patients with allergic rhinitis induces lasting inflammatory alterations in the nose. Indeed, Iliopoulos et al³⁵ have shown that a single antigen challenge can induce an enhancement of clinical symptoms and an increased mediator release on rechallenge.

Kinetic studies with use of antigen challenge demonstrated that sneezing and nasal secretion peaks within the first 2 minutes and parallels with histamine release.^{35,36} Histamine appears to be a crucial mediator of the early response because most of the symptoms can be initiated by histamine challenge.³⁷ The cys-LT and PGD₂ are also released during the early phase³⁸ and are considered to increase vascular permeability and glandular secretion induced by the action of the cys-LTs³⁷ and PGD₂.^{39,40} on sensory nerve fibers.

Clinically, the late-phase response is noted by a recurrence of sneezing, rhinorrhea, and increased nasal air flow resistance. The late response is considered to derive primarily from the early expression of cytokines and chemokines, which lead to the recruitment and infiltration of inflammatory cells. The number of CD4⁺ T cells, eosinophils, and basophils are increased within the nasal mucosa of individuals with allergic rhinitis as well as after antigen challenge.⁴¹⁻⁴⁴

Eosinophils play a particularly important role in the late reaction. These cells are a potent source of LTC₄.⁴⁵ This mediator is elevated in nasal lavage fluid of seasonal allergic rhinitis after allergen challenge⁴⁶ and has been suggested to be of greater importance than histamine for nasal blockage.⁴⁷ Increased levels of eosinophil-derived proteins such as major basic protein (MBP) and eosinophil cationic protein (ECP) are also present within the nasal mucosa and secretions in allergic rhinitis, which have been shown to cause degranulation of other inflammatory cells as well as epithelial cell damage.⁴⁸⁻⁵³ Basophil number is also increased within nasal lavage fluid obtained 24 hours after allergen challenge.⁴² Similar to the mast cell, they bind allergen by IgE/FcεRI and release histamine on activation.⁵⁴ Because the level of histamine, but not tryptase or PGD₂, is increased during the late-phase response, it is attributed to basophil activation rather than to secondary mast cell degranulation.^{42,54,55}

Biopsy tissue obtained from allergic individuals 24 hours after allergen challenge outside the pollen season demonstrates a marked increase in the number of CD4⁺ T lymphocytes.⁴¹ Furthermore, with use of *in situ* hybridization with antisense complementary riboprobes to detect cytokine messenger RNA (mRNA), increased numbers of the T_H2-type cytokines IL-3, IL-4, IL-5, IL-13, and GM-

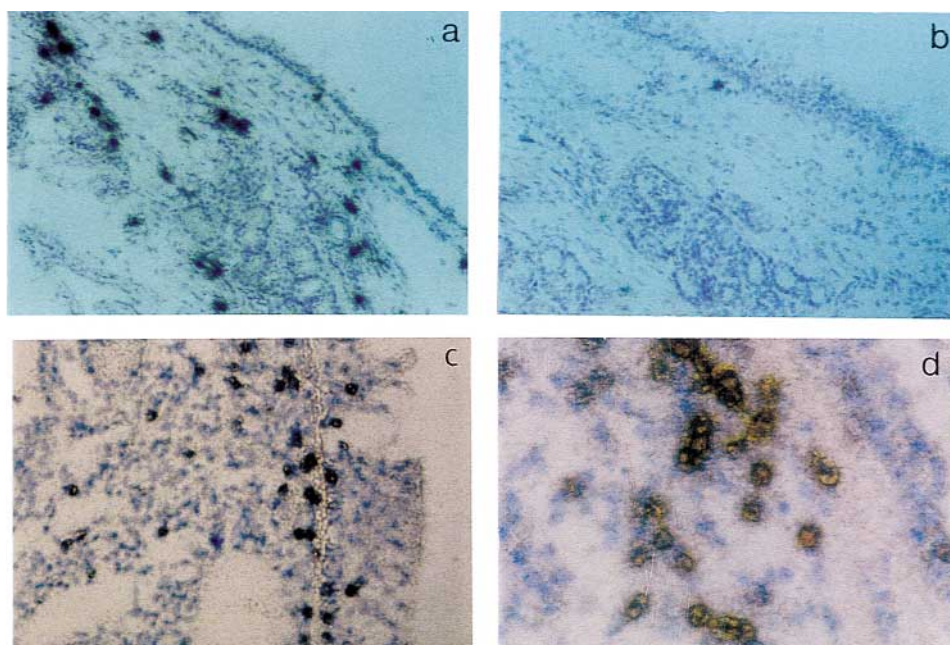


FIG 1. Representative examples of in situ hybridization of nasal biopsy specimens in allergic rhinitis. Expression of IL-4 mRNA after antigen challenge (**a**) and diluent challenge (**b**). Expression of IL-13 mRNA after allergen challenge and (**d**) colocalization of IL-4 and IL-13 mRNA after allergen challenge.

CSF mRNA⁺ cells have been observed within allergic nasal mucosa after local allergen provocation (Fig 1).^{56,57} By contrast, the T_H1-type cytokines IFN- γ and IL-12 were not increased. T cells are a major source of T_H2 cytokine expression within the nasal mucosa 24 hours after allergen challenge. Colocalization studies have demonstrated that approximately 70% to 80% of IL-4 (Fig 1), IL-5, and IL-13 mRNA⁺ cells were T cell-associated.^{56,58,59} IL-4 has been shown to induce naive T cells to commence production of T_H2 cytokines,⁶⁰ the production of NO, and the secretion of proallergic cytokines and chemokines.⁶¹⁻⁶³ Furthermore, the predisposition of inflammatory cells for IL-4 production has been suggested because PBMCs from seasonal allergic rhinitis patients produce IL-4 in response to nonspecific activation.⁶⁴ IL-3 and GM-CSF as well as IL-5 mediate eosinophil differentiation, growth, and survival within the tissue.^{65,66} IL-4 and IL-13 also play a role in the observed tissue eosinophilia because they enhance endothelial expression of vascular cell adhesion molecule-1 (VCAM-1),^{61,67} the counterligand for very late antigen-4 (VLA-4) used by eosinophils for endothelial transmigration.⁶⁸ Furthermore, IL-4 and IL-13 are important factors for allergy because the presence of at least one of these cytokines is required to initiate B-cell production of IgE.^{69,70} As such, T cells are considered to orchestrate the allergic response through cytokine production.

IL-4 and IL-5 act through heterodimeric receptors, composed of a ligand specific α subunit and a signal-transducing γ_c or β subunit, respectively. An increased number of cells expressing IL-4R and IL-5R has recently been demonstrated in allergic nasal mucosa after aller-

gen challenge.⁷¹ The IL-4 receptor signals through signal transducer and activator protein-6 (STAT-6). This transcription factor has been associated with the development of T_H2-type T cells and the production of IgE⁷²⁻⁷⁴ and has recently been reported to be increased within nasal mucosal biopsy specimens obtained from allergic rhinitis patients 24 hours after allergen challenge.⁷⁵

Inflammatory cell infiltration of the nasal mucosa is regulated by the endothelial expression of selectins and adhesion molecules as well as a chemokine gradient within the tissue. The endothelium of allergic nasal mucosa expresses E-selectin⁷⁶ and P-selectin,⁷⁷ as well as increased levels of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and VCAM-1.⁷⁸ Circulating inflammatory cells bind to these selectins and come in contact with chemokines on the surface of the endothelium,⁷⁹ leading to leukocyte activation and expression of surface molecules such as lymphocyte function antigen-1 (LFA-1) and VLA-4.⁸⁰ In vitro, IL-4, IL-13, and TNF- α up-regulate endothelial VCAM-1,^{61,67,81} which is the counterligand for VLA-4 expressed by eosinophils, basophils, and lymphocytes. Interaction between ligand-receptor pathways, such as VCAM-1/VLA-4, facilitates firm adhesion of the inflammatory cell to the vascular endothelium.⁶¹ Within the nasal mucosa of patients with allergic rhinitis there is a tendency for inflammatory cell accumulation within the epithelial layer, particularly mast cells and eosinophils.⁹ This has been attributed to the ability of the epithelial cells to generate chemokines, particularly the CC chemokines,^{82,83} with chemotactic activity for eosinophils and T lymphocytes.⁸⁴⁻⁸⁷ After antigen challenge, the nasal mucosa of seasonal allergic rhinitis

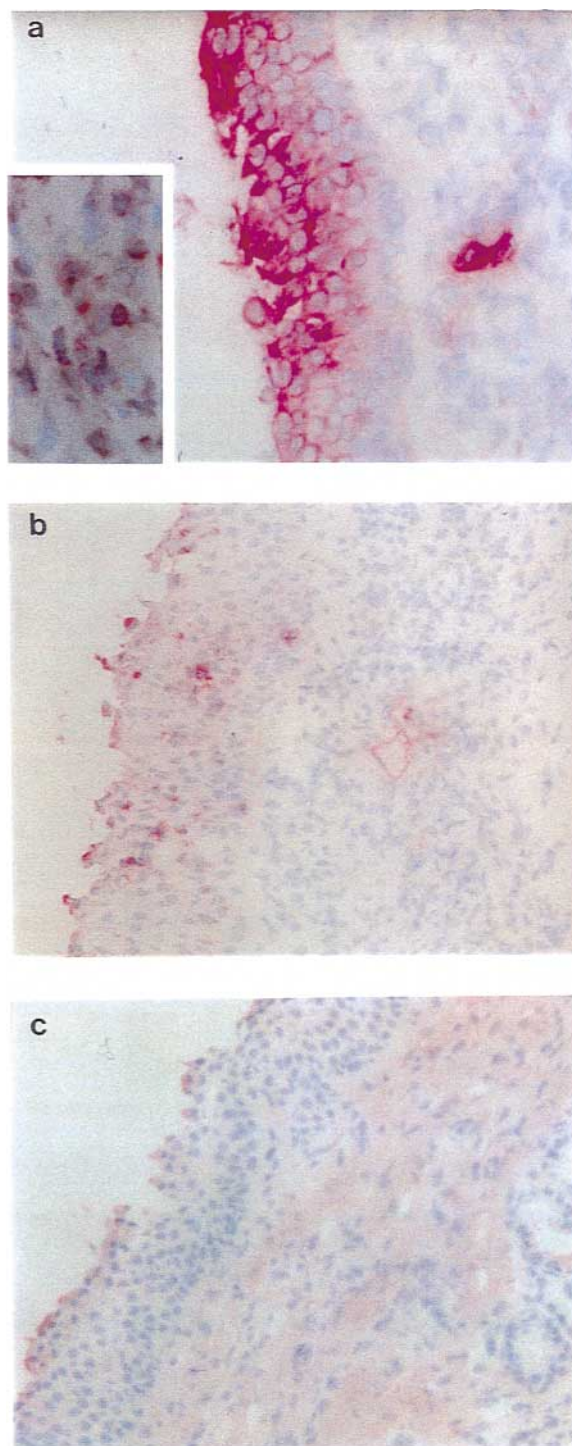


FIG 2. Representative examples of immunostaining for MCP-4 in nasal biopsy specimens from placebo-treated (**a**) and steroid-treated (**b**) patients with allergic rhinitis after allergen challenge. Inset in **a**, Colocalization of MCP-4 immunoreactivity to CD3⁺ T cells after challenge. **c**, Example of nasal biopsy specimen after antigen challenge stained with nonspecific mouse Ig (negative control).

exhibits increased numbers of RANTES, eotaxin, and the monocyte chemotactic proteins (MCPs). Colocalization studies have demonstrated that within the submucosa

mRNA coding for RANTES, MCP-3, MCP-4 (Fig 2), and eotaxin (Fig 3) is expressed mainly by macrophages, T cells, and eosinophils.^{82,83,88} Furthermore, these chemokines are induced by the cytokines IL-4, IL-13, and TNF- α .^{85,89,90} IL-16 is a cytokine with specific chemoattractant activity for CD4⁺ cells, which are primarily T cells, eosinophils, and macrophages.^{91,92} Expression of this cytokine is up-regulated in allergic nasal mucosa after antigen challenge.⁹³ IL-8 is also expressed within the nasal mucosa by leukocytes as well as the epithelium after allergen exposure. This is a CXC chemokine⁹⁴ and its expression by the epithelial layer has been suggested to mediate the recruitment of mast cells and eosinophils toward this layer, as observed in seasonal and perennial rhinitis.²⁰ Aside from being induced by cytokines,^{86,89,90} the epithelial expression of chemokines may also be a direct result of allergen-epithelial cell interaction, either from the enzymatic activity of the allergen or IgE cross-linkage. Together these molecules, selectins, adhesion molecules, and chemokines, coordinate the rolling, firm adhesion and extravasation of inflammatory cells into the inflammatory site.

INFLAMMATION IN SINUSITIS

Sinusitis is characterized by rhinorrhea, nasal congestion, and pain resulting from pressure within the sinuses and it is considered a chronic condition if symptoms persist longer than 12 weeks. Although chronic sinusitis can be derived from structural abnormalities such as a deviated septum or nasal polyps, it is most commonly associated with sensitivity to aeroallergens, as in allergic rhinitis. Chronic sinusitis is characterized by basement membrane thickening, subepithelial fibrosis and edema, goblet cell hyperplasia, and persistent inflammation (Fig 4). This can cause narrowing of the sinus ostia and obstruct mucus drainage, leading to secondary bacterial infections. Chronic sinusitis is also commonly accompanied by hypertrophy of the sinus mucosa and polypoid changes, usually resulting in nasal polyp formation. The inflammatory infiltrate in chronic sinusitis is similar to that observed in allergic rhinitis and the late-phase response to antigen challenge.^{57,95,96}

The sinus mucosa of patients with allergic chronic sinusitis is characterized by a higher number of eosinophils, T cells, and B cells^{97,98} compared with healthy control subjects.⁹⁹ The presence of activated eosinophils is associated with extracellular matrix deposition, epithelial denudation, and basement membrane disruption.⁹⁵ Kamil et al¹⁰⁰ have observed that there is heterogeneity of the inflammatory process in different sinus compartments, with the ethmoid sinus exhibiting a more severe inflammatory response than the maxillary sinus in patients with allergic chronic sinusitis, as seen by an increased CD4 helper/CD8 suppressor ratio and increased eosinophil and mast cell numbers.

Like allergic rhinitis, the pathophysiologic features of chronic sinusitis has been largely attributed to the effects of T_H2 cytokines. Increased levels of GM-CSF and IL-3

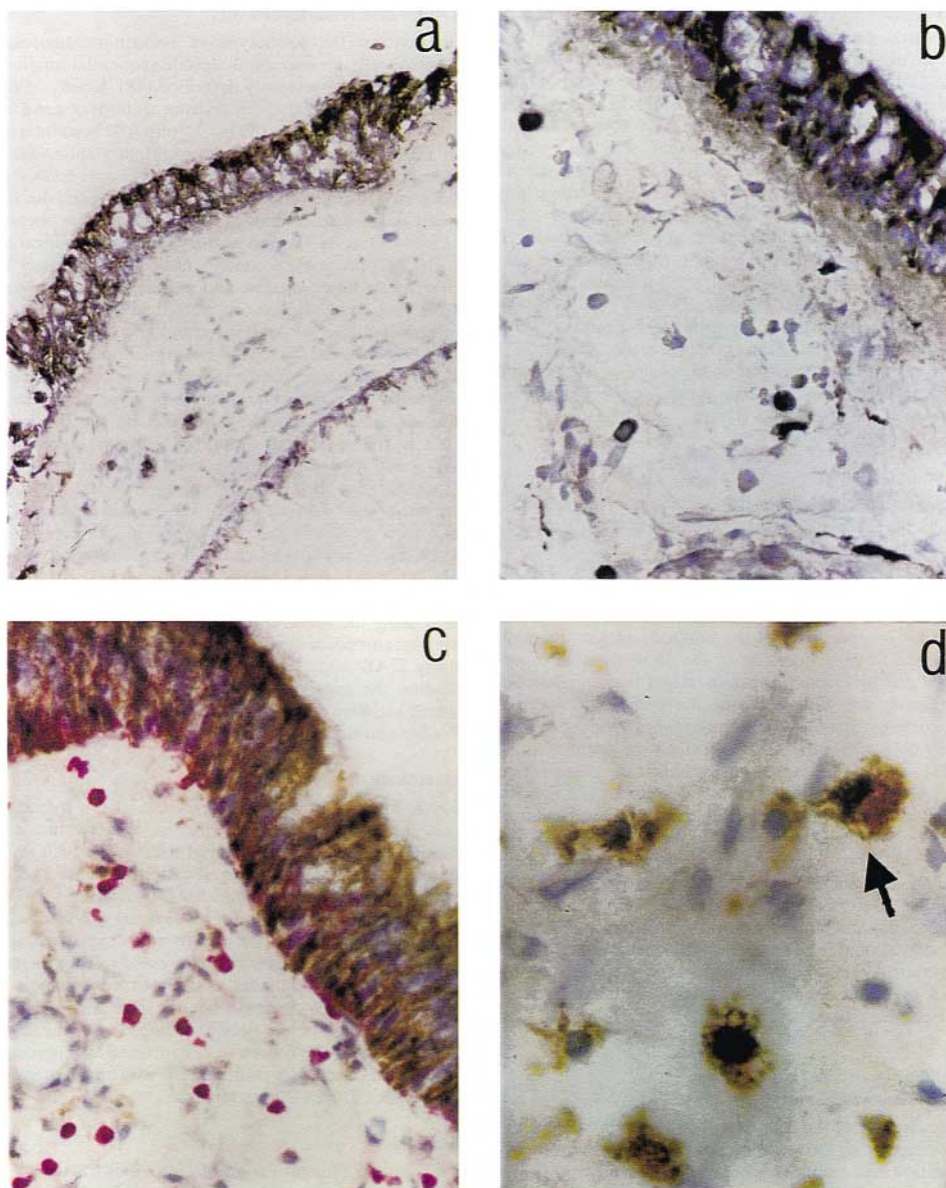


FIG 3. Representative examples of in situ hybridization of nasal biopsy specimens in (a) sinusitis and (b) allergic rhinitis after allergen challenge with use of digoxigenin-labeled antisense complementary RNA probes coding for eotaxin mRNA. Positive eotaxin mRNA cells exhibited dark purple staining. Also shown are results of double immunocytochemistry for (c) eotaxin immunoreactivity and antikeratin and (d) eotaxin immunoreactivity and CD68-positive macrophages in sections of nasal biopsy specimens in allergic rhinitis after allergen challenge. Macrophages (brown staining) were a major cellular source of eotaxin immunoreactivity (red staining). (Reproduced with permission from Minshall EM, Cameron L, Lavigne F, Leung DYM, Hamilos D, Garcia-Zepeda EA, et al. Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. *Am J Respir Cell Mol Biol* 1997;17:683-90.)

mRNA, which correlate with the density of tissue eosinophils, has been observed⁹⁸ (Figs 4 and 5). There are also higher numbers of IL-4, IL-5, and IL-13 mRNA within the sinus mucosa of patients with allergic chronic sinusitis^{98,101} compared with healthy control subjects. The expression of IL-4 mRNA, however, was shown to be higher in the ethmoid than in the maxillary sinus mucosa in chronic sinusitis.¹⁰⁰ IL-4, through induction of endothelial VCAM-1, is considered to promote eosin-

ophil infiltration to this site. IL-6 stimulates fibroblast proliferation and collagen synthesis and has been colocalized to eosinophils, macrophages, T cells, and mast cells within the sinus mucosa of patients with allergic chronic sinusitis.¹⁰² IL-13, a cytokine with functional similarities to IL-4, has been shown to be augmented in the sinus mucosa of both in allergic and nonallergic patients.¹⁰¹ Receptors for IL-4 and IL-5 are up-regulated in the mucosa of patients with allergic chronic sinusitis, whereas the GM-

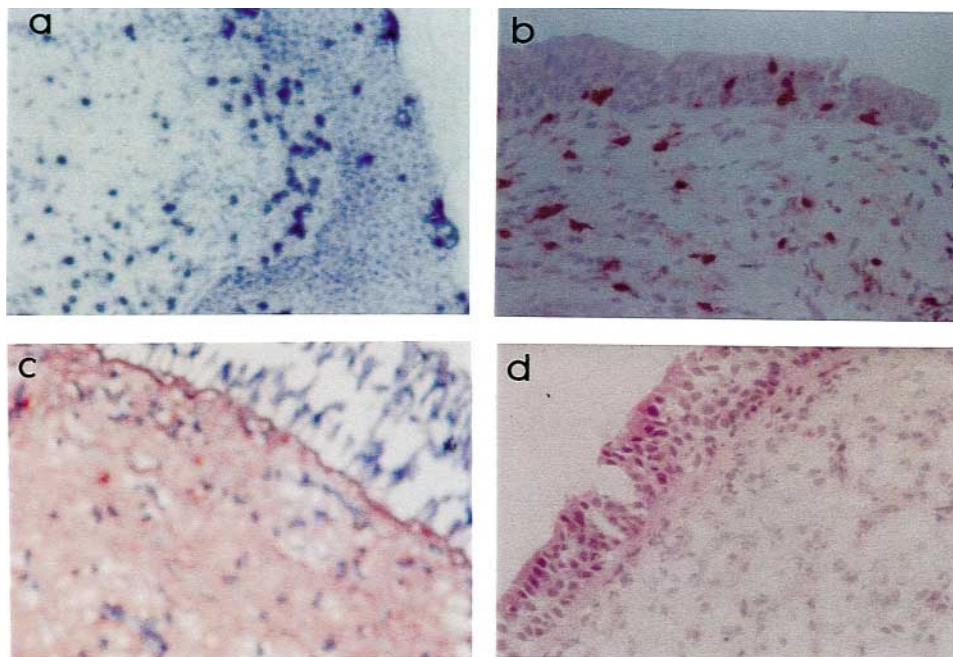


FIG 4. Representative example of immunocytochemistry and in situ hybridization staining in samples from chronic sinusitis. **a**, Expression of GM-CSF mRNA. **b**, EG2 immunoreactive eosinophils. **c**, Collagen I and III immunoreactivity. **d**, MCP-4 immunoreactivity.

CSF receptor was more predominantly expressed in nonallergic chronic sinusitis.⁷¹ Chemokines such as MCP-3, MCP-4 (Fig 4), and eotaxin are significantly increased within the sinus mucosa of both allergic and nonallergic chronic sinusitis and are associated with the accumulation of inflammatory cells, particularly eosinophils¹⁰³ (Figs 4 and 5). IL-12 is a T_H1 -associated cytokine, suggested to play a suppressive role in allergic sinonasal responses and Wright et al¹⁰⁴ have demonstrated a decreased expression of IL-12 (p40) mRNA in sinus biopsy specimens of patients with chronic sinusitis. Furthermore, IL-12R (β_2) is decreased in allergic chronic sinusitis, possibly because of the inhibitory effect of IL-4.¹⁰⁵

A large majority of subjects with chronic hyperplastic sinusitis/nasal polyposis (CHS/NP) are nonallergic on the basis of allergy skin testing and they demonstrate a different profile of cellular infiltration and cytokines despite prominent tissue eosinophilia. In fact, there is no evidence for T-lymphocyte infiltration or production of IL-4, IL-5, or IL-2 in nonallergic sinusitis. Hamilos et al¹⁰⁶ have suggested that the up-regulation of VCAM-1 through TNF- α expression and the elaboration of RANTES may be contributing factors to the marked accumulation of eosinophils in this nonallergic disorder. In both allergic and nonallergic CHS/NP, there are increased tissue densities of GM-CSF and IL-3 mRNA⁺ cells that correlate strongly with the density of tissue eosinophils in both conditions. Eosinophils have been shown to express mRNA for GM-CSF in nasal polyp tissue and in an autostimulatory fashion may promote the survival of eosinophils in sinus tissues, both allergic and nonallergic.⁹⁸

UPPER AIRWAY REMODELING

The term *remodeling* has been defined as “model again or differently, to reconstruct” (Concise Oxford Dictionary) and is a dynamic process of matrix deposition and degradation in response to insult, leading to the reconstruction of damaged tissue, and is as such critical to the wound repair process.^{107,108} However, dysregulation in the balance of deposition versus degradation, resulting in an increasingly thick subbasement membrane layer of collagen and extracellular matrix protein,¹⁰⁹ is considered a remodeling of the airways. Both allergic rhinitis and sinusitis are chronic inflammatory processes where there is a modification of the histologic and functional structure of tissue, leading to remodeling. Furthermore, subjects with chronic sinusitis with nasal polyposis reportedly regrow polyps within only a few months of sinus surgery, indicating the persistent nature of this phenomenon. Synthesis of these matrix products is attributed, at least partially, to an increased presence and activity of fibroblasts and myofibroblasts.¹¹⁰ Activated eosinophils and their products, including transforming growth factor- β (TGF- β),^{109,111,112} GM-CSF,^{113,114} and IL-11¹¹⁵ have been suggested to stimulate these cell types.¹¹⁶ Subepithelial fibrosis is seen to occur within the lungs of asthmatic individuals as well as those with sinusitis. There are relatively few data regarding the remodeling process within the nasal mucosa, although Chakir et al¹¹⁷ have demonstrated increased subepithelial deposition of types I and III collagens within the bronchial submucosa of nonasthmatic individuals with allergic rhinitis compared with healthy control subjects.

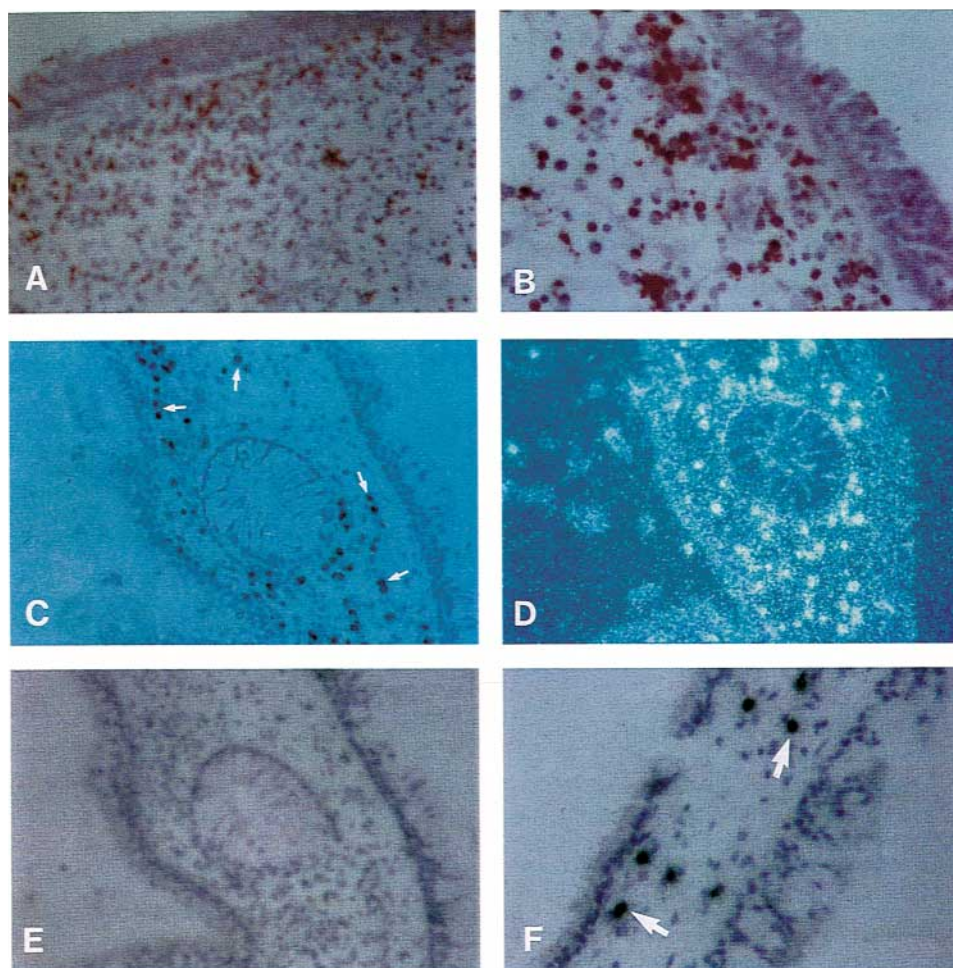


FIG 5. Examples of immunocytochemical staining and in situ hybridization in samples from CHS. **A**, Low- (original magnification ×100) and **B** high-power (original magnification ×400) light microscopic photomicrographs showing EG2 immunoreactivity in CHS with alkaline phosphatase–antialkaline phosphatase technique. Note large number of eosinophils. Autoradiographs of adjacent sections of a CHS sample hybridized with GM-CSF antisense (**C**, **D**) and sense probe (**E**). Note large number of cells with positive hybridization signal in (**C**) and (**D**) and absence of hybridization signal in (**E**). **F**, An example of IL-3 mRNA positivity in a polyp from a CHS sample. (Original magnification ×400.)

EXPLANT MODEL

Our current understanding of the pathogenesis of allergic airway disease is derived primarily from studying the events that follow *in vivo* allergen exposure, either naturally or deliberately by allergen challenge, as well as the effects of culturing inflammatory cells with allergen or other mediators of allergy. Studying the inflammatory process subsequent to *in vivo* allergen exposure provides information on the natural course of disease; however, the relative contribution of resident versus infiltrating inflammatory cells cannot be determined. Although cell culture has greatly advanced our knowledge of the consequences of cell and cytokine interactions, the problem with using this technique is that it lacks important elements of the *in vivo* situation, such as cell-cell and cell-matrix interactions as well as complex intercytokine networking. On the basis of

recent work in which rat lung explants were cultured in allergen-treated medium,¹¹⁸ *ex vivo* techniques for culturing human nasal mucosal biopsy specimens to study local events after *ex vivo* allergen challenge have recently been developed. In isolation from systemic variables, explanted nasal tissue provides a useful system for delineating local inflammatory events while maintaining normal structural and cellular interrelationships. With use of the explant system, events such as eosinophil differentiation and IgE synthesis are currently under investigation.

LOCAL EOSINOPHIL DIFFERENTIATION

Persistent infiltration of the nasal mucosa by eosinophils is thought to contribute to the underlying pathophysiologic mechanisms of allergic inflammatory diseases such as chronic sinusitis and allergic rhinitis.^{97,119}

Furthermore, the presence of these cells and their cytotoxic mediators such as MBP, ECP, and eosinophil peroxidase (EPO) has been associated with pathologic features, namely, epithelial injury and desquamation, subepithelial fibrosis, and hyperresponsiveness.^{47,120} These cells also appear to be an important source of cytokines such as IL-3, IL-5, and GM-CSF, which have been implicated in hematopoiesis and may act in an autocrine fashion to promote eosinophil differentiation and survival.¹²¹ The factors responsible for the infiltration of eosinophils in these disease states include chemokines, particularly eotaxin. Eotaxin is an eosinophil-specific chemokine that has been shown to increase after allergen challenge in allergic rhinitis and in both allergic and non-allergic sinusitis.^{82,122,123}

Although the presence of eosinophils within mucosal tissue during allergic airway disease has been primarily attributed to the *de novo* infiltration of mature cells, recent reports suggest that parallel mechanisms may also be at work. Eosinophils are derived from CD34⁺ pluripotent progenitor cells in the presence of IL-3, IL-5, and GM-CSF.^{65,124-126} Although this process is generally considered to take place within the bone marrow, CD34⁺ cells have recently been detected within peripheral blood and bronchial biopsy specimens, more in atopic asthmatics compared with controls.^{127,128} Because the α subunit of the IL-5 receptor is almost exclusively expressed by eosinophils, the colocalization of CD34 immunoreactivity with α -IL-5R is considered to marker precursor eosinophils (CD34/IL-5Ra⁺).¹²⁹ Recently these cells have been identified within the lungs of atopic asthmatics and have been seen to correlate with the number of MBP⁺ cells.¹²⁸ Although the presence of eosinophil precursor cells and the eosinophil-differentiating cytokines IL-3, IL-5, and GM-CSF within the asthmatic lung¹³⁰ indicates the potential for local eosinophil differentiation, to date no direct evidence of this phenomenon has been provided. Similar inflammatory processes are observed within allergic nasal mucosa, namely, tissue eosinophilia and increased numbers of cells producing IL-3, IL-5, and GM-CSF.⁵⁷ As such, experiments using the nasal mucosal explant technique are currently under way to determine whether this site is populated by eosinophil precursors and whether they undergo local differentiation in response to allergen exposure.

LOCAL IgE PRODUCTION

Although IgE production has long been ascribed to B cells within secondary lymphoid tissue, bone marrow, and blood, there are patients who exhibit nasal symptoms with specific IgE in nasal secretions but not in the serum¹³¹ or salivary secretions.¹³² Furthermore, IgE protein, ϵ -mRNA, and DNA switch circles, generated as a consequence of isotype switching to IgE, are present in nasal lavage fluid of allergic rhinitic patients after allergen challenge.^{133,134} IL-4, IL-13, and activation of CD40 target the ϵ promoter (I ϵ) for germline transcription, giving rise to ϵ germline transcripts.^{70,135,136} These tran-

scripts are considered to be required for the DNA recombination event that places the constant region genes coding for ϵ -mRNA (C ϵ) in a favorable position for transcription.¹³⁷ A resident population of B cells can be found within the nasal and sinus mucosa,^{138,139} and the factors required for inducing isotype switch to IgE, IL-4, IL-13, and CD40L^{70,135,140,141} are expressed within this tissue in individuals with allergic rhinitis and chronic sinusitis.^{22,56,58,101} With use of antisense riboprobes generated to detect these transcripts, increased numbers of I ϵ and C ϵ RNA⁺ cells, in the absence of a change in B-cell number, have been observed within allergic nasal mucosa after allergen challenge and natural allergen exposure^{142,143} as well as within ethmoid sinus mucosa of patients with chronic sinusitis.¹³⁹ This work indicates that resident B cells undergo ϵ germline transcription, a process necessary for DNA rearrangement and isotype switching, within the mucosal tissue during allergic upper airway disease. To confirm that these increases were not merely the result of B-cell infiltration, *ex vivo* allergen challenge with use of nasal mucosal explants is currently under way.

TREATMENT OF ALLERGIC UPPER AIRWAY DISEASE

Steroids

Corticosteroids are an effective and safe form of therapy for chronic inflammatory diseases such as allergic rhinitis and chronic sinusitis. Steroids reduce the itching, sneezing, rhinorrhea, and nasal blockage characteristic of perennial allergic rhinitis.^{144,145} They can reportedly restore the normal architecture of the nasal mucosa¹⁴⁶; long-term use of mometasone furoate has been associated with a higher percentage of pseudostratified, ciliated, columnar epithelium.¹⁴⁷ Steroid administration is also seen to coincide with a reduction in the number of inflammatory cells and T_H2-type cytokines within the nasal mucosa of patients with allergic rhinitis.

Although they do not appear to reduce T-lymphocyte number within the nasal mucosa, the allergen-induced increase in activated T cells (CD25⁺) was inhibited by steroid pretreatment.¹⁴⁸ The number of eosinophils and the level of ECP release in nasal lavage fluid resulting from allergen challenge of seasonal allergic rhinitis and in patients with perennial rhinitis is reduced after steroid treatment.^{21,42,149} Although 6 weeks of pretreatment with fluticasone propionate did not inhibit the allergen-induced increase in total eosinophil (MBP⁺ cells) within the nasal mucosa,¹⁴⁸ a 12-month course of mometasone furoate reduced the total number of eosinophils in perennial rhinitis.¹⁴⁷ Steroids also diminish basophil infiltration during the late-phase response.⁴² Although steroid treatment does not reduce baseline numbers of mucosal mast cells, the number of these cells within the epithelial layer and the level of histamine they release is reportedly lessened.^{42,150-152}

Corticosteroids have no direct effect on eosinophil chemotaxis or degranulation and as such their effective-

ness is attributed to reduced eosinophil infiltration and increased apoptosis, both resulting from the inhibition of cytokine and chemokine synthesis.^{21,150} Indeed, pretreatment with fluticasone propionate nasal spray inhibits the allergen-induced increase in mRNA coding for IL-4, IL-13, eotaxin, and MCP-4.^{56,59,82,83} In allergic sinusitis steroids have been seen to reduce the production of IL-4 and IL-13¹⁰¹ as well as the number of cells expressing receptors for IL-4, IL-5, and GM-CSF.¹⁵³ Steroids also inhibit the allergen-induced increase in I ϵ and C ϵ RNA⁺ cells within the nasal mucosa, which is considered a result of reduced IL-4 and IL-13 production,^{56,142,143,154} suggesting that steroids may also limit local IgE production and the priming of Fc ϵ R-bearing cells.²² Aside from directly reducing the synthesis of T_H2-type cytokines, steroids also increase the level of T_H1-type cytokines, particularly IFN- γ and IL-12, which can suppress the transcription of IL-4.^{155,156} In fact, Wright et al¹⁰⁴ have demonstrated that in allergen-induced rhinitis the decrease in IL-12 (p40) and IL-12R (β_2) after challenge is abrogated by pretreatment with topical corticosteroids.

Immunotherapy

Although nasal corticosteroids are effective for most patients with allergic rhinitis, there remains a group of patients who respond poorly to this treatment and for whom immunotherapy is currently recommended. Successful immunotherapy is associated with an inhibition of allergen-induced late responses and the infiltration of CD4⁺ T lymphocytes and activated eosinophils.¹⁵⁷ This inhibition appears to be mediated by the induction of T_H1 cytokine expression, such as IFN- γ and IL-12,^{157,158} leading to IgG production,¹⁵⁹ particularly the IgG₄ subclass.¹⁶⁰ Increased IFN- γ may also mediate clinical improvement by inhibiting the proliferation of T_H2 T cells,¹⁶¹ production of IL-4,¹⁵⁵ and therefore IgE synthesis.⁶⁹ It has thus been suggested that changes in serum antibodies, effector cells, and mediator secretion may occur as a consequence of an alteration in the balance between T_H1/T_H2 cytokines in favor of a prolonged T_H1 response.^{157,158} Immunotherapy has also been associated with a reduction in allergen-induced histamine release by basophils and a decreased production of histamine-releasing factors by mononuclear cells,^{162,163} which may also explain the improved clinical symptoms. Importantly, Durham et al¹⁶⁴ have recently shown under double-blind conditions that grass pollen immunotherapy for 3 to 4 years was associated with a markedly reduced response to allergen challenge (ie, a blunted T-cell infiltrate and expression of IL-4 mRNA in response to allergen challenge).

RELATIONSHIP BETWEEN UPPER AND LOWER AIRWAY PATHOLOGIC FEATURES

Asthma and rhinitis are not often considered together, although they frequently coexist. The conducting upper and lower airways have similarities and differences in their structures. The mucous membranes of both the upper and lower airways are covered by a pseudostrati-

fied, columnar, ciliated epithelium with a continuous basement membrane. For this reason they share a common mucosal susceptibility to inhaled allergens. The obvious anatomic difference is the presence of airway smooth muscle in the lower airway, whereas the prominent feature of the upper airway is the presence of large venous sinusoids and prominent glands within the submucosa. Clearly shared inflammatory pathways in the upper and lower airways may have a distinct effect depending on the nature of the effector tissue, with prominent bronchoconstriction in the lower airway and mucus secretion in the nose. Although inflammation initiated by allergen is probably similar in the upper and lower airways, the consequences of this inflammation differ on the basis of the structure and function of the airway involved (eg, sneezing vs coughing, nasal congestion vs breathlessness, rhinorrhea vs sputum production). As such, there are also important differences in rhinitis and asthma. The respiratory epithelium is disrupted in bronchial asthma, whereas it is intact in both seasonal and perennial allergic rhinitis. Similarly, the basement membrane zone is thickened with abnormal collagen in asthma but not in rhinitis. The pathogenesis of both asthma and rhinitis is complex, involving many different cell types, inflammatory mediators, cytokines, and adhesion molecules. Nevertheless, the underlying pathologic feature of both rhinitis and asthma is inflammation in which eosinophilia is characteristic.^{164,165} However, dysfunction of the upper and lower airways frequently coexist, and they appear to share key elements of pathogenesis. Although there is compelling evidence that allergic rhinitis may influence the clinical course of asthma, the mechanisms connecting upper and lower airway dysfunction have not been well established. How rhinitis may contribute to the deterioration of lung function is only beginning to be understood. For example, factors such as increased mouth breathing because of nasal blockage, which increases airway exposure to allergens, the spread of inflammatory mediators to the lungs through postnasal drip, and bronchoconstriction induced by stimulation of sensory nerves and nasobronchial reflexes may all play a role. Data from epidemiologic studies indicate that approximately 88% of patients with asthma have rhinitis and between 25% and 50% of patients with rhinitis have associated asthma.¹⁶⁶ Furthermore, patients with allergic rhinitis and no clinical evidence of asthma commonly exhibit nonspecific bronchial hyperresponsiveness. Chakir et al¹¹⁷ have reported that allergic subjects with seasonal pollen-induced rhinitis had airway alterations on bronchial biopsy samples somewhat similar to those observed in asthmatic subjects. These changes consisted of cellular infiltration, mucosal edema, increased epithelial desquamation, and focal basement membrane thickening corresponding to an irregularly distributed subepithelial fibrosis. It is difficult to determine whether rhinitis is the first manifestation of respiratory allergy for a patient who may eventually have asthma or whether nasal disease is playing a direct role in causing asthma. The observation that management of allergic rhinitis or

sinusitis also relieves symptoms of asthma has heightened interest in the link between these diseases.¹⁶⁷

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