

Advances in upper airway diseases and allergen immunotherapy

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Evidence of an increased prevalence of rhinitis in patients with asthma, and asthma in patients with rhinitis, supports the 1 airway concept. However, there are basic differences between the upper and lower airways, such as the virtual absence of remodeling in the nose compared with the bronchi, despite the presence of similar inflammation. Etiologic factors in chronic rhinosinusitis (CRS) attract increasing interest. Peripheral blood monocytes from patients with CRS release IL-4, IL-13, and IFN- γ on stimulation with fungal antigens, especially those from *Alternaria*. This is not seen with cells from normal controls. However, a double-blind trial of intranasal amphotericin in CRS was negative. Evidence continues to accumulate of the pivotal role of regulatory T-lymphocytes secretion of IL-10 in the response to allergen immunotherapy. In patients with asthma and house dust mite allergy who are receiving appropriate pharmacotherapy and have instituted environmental controls, house dust mite immunotherapy provides marginal additional benefits in asthma control. Immunotherapy with cat dander extract at a maintenance dose containing 15 μ g Fel d 1 produces a more consistent immunologic response than with maintenance doses containing 3.0 μ g, whereas doses containing only 0.6 μ g are no more effective than placebo. Sublingual immunotherapy for seasonal grass allergy can be safely administered by general practitioners, but symptom relief begins only in the second season of therapy. Sublingual immunotherapy for seasonal grass allergy in children reduced symptoms and onset of new asthma symptoms but, again, beginning only in the second year of treatment. A course of 6 weekly injections of ragweed Amb a 1 bound to cytosine phosphorothionate guanosine containing DNA produced a shift from T_H2 to T_H1 cytokine release both in peripheral blood cells and in the nose after allergen challenge. No symptom improvement was seen the first year, but symptoms were reduced the second year without further treatment. (J Allergy Clin Immunol 2005;115:676-84.)

Key words: Sinusitis, allergic rhinitis, immunotherapy, skin testing, one airway

Abbreviations used

AAAAI:	American Academy of Allergy, Asthma and Immunology
AFS:	Allergic fungal sinusitis
AIC:	Amb a 1-immunostimulatory DNA sequence conjugate
CpG:	Cytosine phosphorothionate guanosine
CRS:	Chronic rhinosinusitis
HDM:	House dust mite
HRQOL:	Health-related quality of life
HRS:	Hypertrophic rhinosinusitis
OME:	Otitis media with effusion
PDC:	Plasmacytoid dendritic cell
PGLA:	Biodegradable poly(lactide-co-glycolide)
SLIT:	Sublingual immunotherapy

UPPER AIRWAY

One airway

The concept of 1 airway, with immunological as well as anatomical connections between the upper and lower airway, continues to be a subject of investigation and to gain support (Table I). Data on the coexistence of asthma and rhinitis were collected from subjects age 20 to 44 years as part of the European Community Respiratory Health Survey.¹ On the basis of a postal questionnaire completed by 3000 respondents, asthma was present at the various centers in between 1.0% and 6.0% of respondents without rhinitis and between 7.6% and 22.6% of respondents with rhinitis. Rhinitis prevalence at the various centers ranged between 10.5% and 36.2% for respondents without asthma and between 50.0% and 77.1% for respondents with asthma. Among 600 of the respondents who underwent further testing, asthma was present in 2.0% of those without rhinitis and 13.4% of those with rhinitis (odds ratio, 6.63). In respondents with rhinitis, there was a 3-fold increase in bronchial hyperresponsiveness compared with respondents without rhinitis. The risks for both asthma and rhinitis were significantly associated with a parental history of asthma, levels of total IgE, and atopic sensitization. However, after adjusting for these and other confounding factors, rhinitis remained significantly associated with a higher risk of asthma and for bronchial hyperresponsiveness in subjects without asthma. Thus, the

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association between rhinitis and asthma does not appear to be explained fully by a common atopic predisposition.

The similarities and differences between rhinitis and asthma were explored from the standpoint of remodeling.² Even though inflammation is similar in rhinitis and asthma, there is far less remodeling seen in the nose in rhinitis than in the bronchi in patients with asthma. Epithelial damage is minimal, and the reticular basement membrane does not have the pseudothickening characteristic of asthma. Two possible explanations were offered for the differences between remodeling in the nose and lung: changes in the bronchi may be a result of cytokine production by smooth muscle cells, or differences may relate to the differing embryonic origin, because the nose is of ectodermal origin, and the bronchi are of endodermal origin.

Otitis media with effusion (OME) is a common, chronic inflammatory disease of the middle ear space. Epidemiologically, patients with OME have an increased prevalence of atopic conditions such as allergic rhinitis, eczema, and asthma. To explore the possibility that OME may have an inflammation similar to that found in rhinitis, specimens were obtained of middle ear effusion, and of adenoidal and eustachian tube tissue during adenoidectomy from 45 children with OME (age 2-18 years).³ Eleven (24%) of the children were atopic as defined by at least 1 positive skin prick test result. In these atopic children, the middle ear effusions had significantly higher levels of eosinophils, T lymphocytes, and IL-4 mRNA⁺ cells and significantly lower levels of neutrophils and IFN- γ mRNA⁺ cells compared with nonatopic patients. Similar differences were found in the eustachian and adenoidal tissue. The authors concluded that their data support the concept that the middle ear may be part of the united airway in atopic individuals.

Rhinitis therapy has been reported to improve subjective and objective measures of asthma. To examine the effect of rhinitis treatment on asthma exacerbations, patient data were analyzed from a large managed care organization.⁴ To be eligible, patients were identified as having both asthma and rhinitis. Records were searched for emergency department visits or hospitalizations. A nested case control analysis was then conducted of 361 subjects and 1444 control patients. Patients who used nasal corticosteroids had a significantly lower risk of both asthma-related emergency department treatment and hospitalization (adjusted odds ratios, 0.75 and 0.56, respectively). Nonsedating antihistamine use was associated with a nonsignificant trend toward reduced visits. However, patients using both nasal corticosteroids and antihistamines had a further reduction over patients using nasal corticosteroids alone in the risk for both emergency department visits and hospitalizations.

Rhinosinusitis

A combined faculty of allergist/immunologists, infectious disease specialists, radiologists, and otolaryngologists met in a workshop to discuss rhinosinusitis research and patient care.⁵ The resulting document, *Rhinosinusitis*:

TABLE I. Key advances in upper airway disease

1. Data from a large European study confirmed the increased prevalence of rhinitis in patients with asthma and asthma in patients with rhinitis. The association could not be completely explained by coexistent atopy.
2. Supporting a possible role of an immunologic reaction to fungi in CRS, peripheral blood monocytes from patients with CRS, on exposure to fungal extracts, particularly *Alternaria*, released IL-4, IL-13, and IFN- γ , whereas the cells of normal individuals did not.
3. Not supporting a possible role of an immunologic reaction to fungi in CRS was a double-blind study of intranasal amphotericin that showed no benefit in patients with CRS.
4. Studies in allergic rhinitis suggest that quality of life is affected by patients' confidence that they can cope with the disease as well as by the symptoms.
5. Inability to concentrate and daytime drowsiness in allergic rhinitis may be part of the pathophysiology of the disease rather than a result of interference with sleep.
6. In a large, 8-year follow-up study from Denmark, 17% who had had symptoms of allergic rhinitis had remitted.

Establishing Definitions for Clinical Research and Patient Care, appeared as a supplement to the December 2004 issue of the Journal and in the December 2004 issue of *Otolaryngology—Head and Neck Surgery*. This report first addressed the causative factors in rhinosinusitis: bacterial, fungal, allergic/immunologic, and nonimmunologic. There was a discussion of the histological features of chronic rhinosinusitis (CRS) and of nasal polyps. The article then addressed the approach to the diagnosis and assessment of rhinosinusitis that should be used in future studies, including symptoms, quality of life assessments, rhinoscopy, imaging, and challenges. Guidelines were provided for future studies of intervention in rhinosinusitis, including precise definitions of the disease state being studied. An editorial accompanying the workshop report highlights the importance of CRS: 18 million cases, 30 million courses of antibiotics, and 40,000 patients per year undergoing sinus surgery.⁶ Thus, the importance that future research address the role of bacterial infection and the indications for surgery in this eosinophilic inflammatory disease.

The etiology of CRS was addressed in an article from the Mayo Clinic.⁷ The authors noted that antibiotics and surgery are usually ineffective in long-term treatment; that the histological hallmark is a chronic inflammatory infiltrate of lymphocytes, plasma cells, and eosinophils similar to that in the bronchial mucosa of patients with asthma; and that the patients have increased numbers of CD4⁺ T lymphocytes positive for cytokines such as IL-5, IL-13, and IFN- γ in their sinus mucosa, suggesting an immunologic mechanism. However, less than 40% of patients with CRS are atopic. Pursuing previous suggestions of a role of common fungi in the etiology of this disease, the authors cultured PBMCs with extracts of 4 common fungi: *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium*. The PBMCs of patients with CRS showed increased cytokine production compared with normal controls on exposure to *Alternaria*, *Cladosporium*,

and *Aspergillus*. Most striking was the response to *Alternaria*, to which PBMCs from 16 of 18 CRS patients produced IL-5, compared with 0 of 15 normals. PBMCs from all of the CRS patients produced IL-13 on stimulation with *Alternaria* compared with none of the controls, and they produced 5.5 times as much IFN- γ . On the other hand, only 28% of the patients with CRS had specific IgE directed toward *Alternaria*. Because fungal antigens can be isolated from the nasal secretions of most patients with CRS and the cytokines released by stimulation of patients' PBMCs with these fungi are the same as those expressed in the airways of patients with CRS, the authors suggest that the response to these ubiquitous airborne fungi may explain both the chronic airways inflammation and the concomitant asthma symptoms in patients with CRS.

Two uncontrolled studies had reported that treatment of patients with CRS with intranasal washes containing amphotericin had resulted in the disappearance of nasal polyps and a decrease in symptoms.^{8,9} A randomized, placebo-controlled study was undertaken in Ulm, Germany, to assess the response of patients with CRS to this treatment.¹⁰ Seventy-eight patients with nasal polyps, symptoms, and positive sinus computed tomography were randomized to receive 200- μ L nasal sprays with either amphotericin solution or saline 4 times daily for 8 weeks. Sixty completed the study (6 in the amphotericin group discontinued because of intolerance of the medication). There was no difference between active and placebo groups in the follow-up sinus CT scores, whereas the median posttreatment symptom score was significantly worse in the amphotericin group than in subjects who had received placebo. The authors concluded that treatment with amphotericin was ineffective and was not indicated in patients with severe CRS. It should be noted, however, that in this study, amphotericin was administered as a 200- μ L spray rather than as a 20-mL wash, which had been used in the studies reporting a favorable response to amphotericin.

Polymorphic MHC class II molecules on the surface of antigen-presenting cells have been implicated in the immunopathogenesis of several chronic inflammatory diseases, including rheumatoid arthritis, type I diabetes, multiple sclerosis, and celiac disease. Recently, allergic bronchopulmonary aspergillosis has been reported to have HLA-MHC class II associations. This led to a study of MHC class II associations with allergic fungal sinusitis (AFS; $n = 44$) and chronic hypertrophic rhinosinusitis (HRS; $n = 30$).¹¹ Patients with AFS and HRS were similar in having increased prevalence of HLA-DQB1*03 alleles over normal. Although 66% of patients with AFS and 50% of patients with HRS had positive results for at least 1 allele, the 2 groups differed in their frequency of DQB1*03 allelic variants. These findings suggest potential roles for MHC class II molecules in the immunopathogenesis of both chronic HRS and AFS.

Nasal polyps are almost always a manifestation of CRS, and like CRS, they are characterized histologically by an inflammatory cell infiltration of eosinophils, lymphocytes, and plasma cells. DNA microarray technology consists of

a matrix with attached sequences that allow simultaneous analysis of expression of panels of human genes. Comparison of profiles of genes expressed by diseased and healthy tissues often highlights the involvement of both expected and unsuspected pathologic pathways. In a study from Johns Hopkins, expression microarrays containing approximately 10,500 genes were used to compare gene profiles of nasal polyp tissue from 10 patients and normal sphenoid mucosal samples from 4 patients who had undergone pituitary surgery.¹² Compared with normal sinus tissue, 192 genes were upregulated at least 2-fold and 156 genes were downregulated by at least 50% in the nasal polyp samples. It is hoped that this information will ultimately prove useful in providing new insight into the pathogenesis and treatment of nasal polyps and CRS.

It had been previously shown that an ongoing allergic response in the nose augmented bacterial sinusitis in a mouse model. To eliminate the possible contribution of the injected alum, studies were repeated with passive sensitization with T_H2 lymphocytes followed by nasal allergen challenge.¹³ Mice who were passively sensitized and intranasally challenged with allergen followed by infection showed an increase in the number of recovered bacteria and an increase in sinus inflammation compared with those given infection alone or those passively sensitized with T_H1 lymphocytes, followed by intranasal allergen challenge and infection. The results suggest that the release of T_H2 cytokines or the subsequent response of cells such as eosinophils interferes with the ability of the mouse to clear an infection during an ongoing allergic reaction.

Allergic rhinitis

The occurrence rate of remissions in patients with allergic rhinitis was studied in Copenhagen, Denmark.¹⁴ Fifteen hundred eighty-one subjects, half selected randomly and half selected because they reported rhinitis or asthma symptoms on allergen exposure, were queried regarding nasal symptoms on exposure to plants, animals, or house dust mites (HDMs). If they reported symptoms within the previous 12 months and had at least a class II RAST to the relevant allergen, they were diagnosed as having allergic rhinitis. Eight years later, 734 (69%) of those eligible were re-evaluated by using the same questionnaire. In 1990, allergic rhinitis was reported by 198 subjects, with a total of 257 cases. On follow-up in 1998, 36 subjects had remission in 43 cases. Remission rates were 12% for pollen, 19% for animals, and 38% for HDMs, for a mean rate of 17%. In 78% of subjects experiencing symptom remission, the specific IgE remained stable. Overall, 22% of the remitting subjects and 7% of the nonremitting subjects had a decline in specific IgE. Immunotherapy had no effect on the rate of remission. Thus, for the most part, allergic rhinitis is a persisting disease.

Symptoms may not fully reflect the effect of rhinitis on an individual's health-related quality of life (HRQOL). The elements contributing to the Rhinosinusitis Disability Index, a measure of HRQOL in rhinosinusitis, were

evaluated in a telephone interview with 106 randomly selected individuals with a physician diagnosis of allergic rhinitis, sinusitis, or chronic postnasal drip.¹⁵ In a multivariate model, 3 components were found to be independent predictors of the HRQOL. They were a Rhinitis Symptom Score consisting of 11 questions pertaining to eye, nose, and sinus symptoms, each graded 0 to 4; a Perceived Control of Rhinitis Questionnaire consisting of 8 questions regarding an individual's perception of their ability to deal with rhinitis, graded 1 to 4; and the Center for Epidemiologic Studies Depression Scale, with 20 items intended to assess depression and psychological distress. These 3 questionnaires accounted for 62% of the variance in the HRQOL (symptom score, 36%; the other 2 together, 26%). The Medical Outcomes Study Short Form (SF) 12 (an abbreviated SF-36) and medication use were not independent contributors to the rhinosinusitis HRQOL. It was concluded that disease severity accounts for only a portion of the variation observed in rhinitis-specific HRQOL. Psychological factors appear to play a nearly equal role, particularly the individual's perceived ability to deal with the condition. This emphasizes the importance of educating patients to cope with their rhinitis effectively. Strategies to achieve this include teaching patients environmental controls and proper use of their medication (especially timing).

In addition to the classical ocular/rhinitis symptoms, patients with allergic rhinitis often complain of impairment of their daytime performance, daytime sleepiness, and diminished quality of life. These latter symptoms are often attributed to impairment of sleep because of rhinitis, but this has not often been studied. Twenty-five subjects with seasonal allergic rhinitis and 25 normal subjects were followed with assessment of symptoms, daytime sleepiness, and quality of life (SF-36) during the pollen season and polysomnography before and during the season.¹⁶ During the pollen season, the subjects with allergic rhinitis had significant increases in their sleepiness scores and deterioration in the physical function and role physical parameters of the quality of life assessments. These changes were largely limited to subjects with moderate or severe rhinitis symptoms. Statistically significant differences between groups were also found for selected parameters of the sleep studies, but the changes were minimal, and all values were within normal ranges. Thus, daytime sleepiness seems to be related to rhinitis itself rather than to an impairment of nocturnal sleep. A somewhat different conclusion was reached in the supplement to the November 2004 issue of the Journal, *Allergic Rhinitis After Hours: The Relevance and Consequence of Nighttime Symptoms*.¹⁷ Evidence was reviewed supporting the conclusion that the pathophysiology of allergic rhinitis often disrupts sleep, leading to fatigue, irritability, memory deficits, excessive daytime somnolence, and depression, thereby further reducing the quality of life.

Allergic rhinitis is characterized by the epithelial layer accumulation of mast cells and eosinophils linked to the local generation and release of chemotactic factors. Those

of relevance to the tissue eosinophil accumulation have been extensively studied, but scant attention has been given to those factors relevant to epithelial mast cell accumulation. To address this need, immunohistochemical analysis of the expression of mast cell chemoattractants and their receptors was made in nasal biopsies from symptomatic atopic patients with perennial and seasonal allergic rhinitis.¹⁸ Mast cells were significantly increased in the epithelium of both rhinitis groups. Immunoreactivity for TGF- β 1, TGF- β 2, and TGF- β 3 was observed, predominantly localized to the superficial columnar epithelial cells. It was significantly increased in both rhinitis groups. Similarly, immunoreactivity for the receptors TGF- β R1, TGF- β R2, and TGF- β R3 was significantly increased predominantly in the epithelial layer of both rhinitis groups. The number of epithelial mast cells significantly correlated with immunoreactivity levels for TGF- β 1, TGF- β 2, TGF- β R1, and TGF- β R2. Immunoreactivity for eotaxin was increased in the perennial rhinitis group, and C-kit positive cells were increased in the seasonal group, whereas CCR3 and stem cell factor were not increased in the patients with rhinitis. These results are consistent with the involvement of TGF- β in either the recruitment or retention of mast cells within the epithelium in patients with allergic rhinitis.

Rhinitis treatment

Levocetirizine is the R or active enantiomer of cetirizine, which is itself an active metabolite of hydroxyzine, a first-generation antihistamine. Levocetirizine is reported to have less cerebral histamine receptor binding than the racemic compound, which would result in reduced central nervous system side effects. A 6-month placebo-controlled study of levocetirizine was conducted in Europe in 551 subjects with allergic rhinitis.¹⁹ The symptomatic response to active treatment was rapid and sustained. At 4 weeks, reduction in total nasal symptom score with levocetirizine (including congestion) was 18% more than that in the placebo group, despite the use of less rescue medication. The Juniper rhinitis-specific quality of life score also improved by 0.48 compared with placebo (0.50 is considered a clinically meaningful change). Improvement in individual symptoms generally paralleled total symptom score except for nasal congestion, which improved significantly in the levocetirizine group only from 3 months on. The only difference in side effects was with somnolence, which was reported by 1.8% of the placebo and 6.8% of the levocetirizine subjects. However, further analysis revealed that the total duration of combined fatigue, asthenia, and somnolence in the 2 groups was similar, 3.26 days per 100 days in the placebo group and 3.72 days per 100 days in the levocetirizine group.

Levocetirizine was compared with desloratadine and placebo in a crossover nasal challenge study in 24 subjects.²⁰ Single doses of each drug were administered, followed by a nasal challenge with increasing doses of grass pollen extract. Both antihistamines were more effective than placebo ($P < .001$). Desloratadine increased the threshold by 1.93 allergen doses, whereas levocetirizine

TABLE II. Key advances in immunotherapy

1. Evidence continues to accumulate of the pivotal role of regulatory T cells secreting IL-10 in the response to allergen immunotherapy.
2. In patients with asthma and HDM allergy who are receiving appropriate pharmacotherapy and have instituted environmental controls, HDM immunotherapy provides marginal additional benefits in asthma control.
3. Immunotherapy with cat dander extract at a maintenance dose of 15 µg Fel d 1 produces a more consistent immunologic response than maintenance doses containing 3.0 µg, whereas doses containing only 0.6 µg are no more effective than placebo.
4. Sublingual immunotherapy for seasonal grass allergy can be safely administered by general practitioners, but symptom relief begins only in the second season of therapy.
5. Sublingual immunotherapy for seasonal grass allergy in children reduced symptoms and onset of new asthma symptoms beginning only in the second year of treatment.
6. A course of 6 weekly injections of ragweed Amb a 1 bound to CpG containing DNA produced a shift from T_H2 to T_H1 cytokine release both in peripheral blood cells and in the nose after allergen challenge. No symptom improvement was seen the first year, but symptoms were reduced the second year without further treatment.

increased it by 2.63 doses ($P = .02$, levocetirizine vs desloratadine). Neither drug had any effect on nasal congestion or IL-5, IL-8, eotaxin, or eosinophil cationic protein levels in nasal lavage fluid at 24 hours after challenge.

The monoclonal anti-IgE, omalizumab, has been shown to be effective in the treatment of allergic asthma and allergic rhinitis. A study using nasal allergen challenges was undertaken to determine the time to onset of action.²¹ Subjects underwent baseline nasal challenge with measurement of nasal volume decrease by acoustic rhinometry. They received omalizumab at 0 and 28 days, whereas nasal allergen challenge was repeated at days 7 and 14, 21 and 28, and 35 and 42. As expected, 3 days after omalizumab, the mean free IgE level had decreased by 96.1%. By 14 days, circulating basophil RceRI expression had decreased 73%, and it remained near that level throughout the study. The 30% decrease in nasal volume observed with nasal challenge at baseline was reduced to 20.4% when first measured after 7 to 14 days, remained unchanged at 21 to 28 days, and fell further to 12.2% at days 35 to 42 after the second dose of omalizumab on day 28. The results imply that patients with seasonal rhinitis would have measurable protection with a single dose of omalizumab given 2 weeks before an anticipated pollen season. They also suggest that 2 doses given 1 month apart would provide greater protection.

Inhaled heparin inhibits the bronchoconstrictor response to a variety of stimuli, including allergen, exercise, and adenosine. It is likely that this protective effect is mediated through an inhibitory action on mast cells. Adenosine is known to cause release of mast cell mediators through its reaction with a mast cell surface receptor. The model of nasal adenosine challenge was

therefore used to study the mechanism of the protective effect of heparin.²² Nasal adenosine challenge produced sneezing and a transient increase in histamine and tryptase release. Compared with placebo, inhaled heparin significantly attenuated the release of histamine and tryptase. The results confirm that inhaled heparin plays its protective role by inhibition of mast cell activation. Further studies are required to determine whether there is any potential for therapeutic use in allergic rhinitis.

Ocular therapy

Vernal keratoconjunctivitis is a serious childhood ophthalmologic condition characterized by eosinophilic infiltration within giant conjunctival follicles and in the limbal Trantas dots. CD4⁺ T cells with a T_H2 phenotype are abundant in tissue and scrapings. In view of this histology, a group in Thailand conducted an open trial of tacrolimus ointment in 10 children with recalcitrant vernal keratoconjunctivitis.²³ The response to tacrolimus was superior to that with conventional therapy and was achieved without significant toxicity.

IMMUNOTHERAPY

The June 2004 issue of the Journal highlighted advances in allergy immunotherapy (Table II). An editorial overview of the contents of the issue was provided by Tom Casale.²⁴ Dr Philip Norman²⁵ provided an overview of advances in the field since his last review in 1998. During those 5 years there was further, although still incomplete, understanding of the underlying immunologic mechanism. Clinically important was the convincing demonstration by Stephen Durham of long-standing improvement after discontinuation of treatment.²⁵ European investigators also provided convincing evidence that adequate doses applied sublingually can alter rhinitis and asthma. During this period, several improvements in materials used for immunotherapy were investigated. These included the synthetic peptides representing T-cell epitopes, cytosine phosphorothionate guanosine (CpG) DNA bound to major allergens, and concomitant use of anti-IgE and immunotherapy. Mechanisms underlying allergen immunotherapy were further explored in a article with senior author Stephen Durham.²⁶ The authors noted that immunotherapy is accompanied by increases in allergen-specific IgG₄, which blocks not only IgE-dependent histamine release from basophils but also IgE-mediated antigen presentation to T cells. Immunotherapy also acts on T cells to modify peripheral and mucosal T_H2 response to allergen in favor of T_H1 responses. Recent studies have identified regulatory T cells secreting IL-10, which produces suppression of mast cell, eosinophil, and T-cell responses and acts on B cells to favor heavy-chain class switching to IgG₄.

Double-blind studies in human beings

In the same issue of the Journal, Sheldon Cohen²⁷ and Murray Dworetzky,²⁸ coeditors of the Allergy Archives series, recalled the monumental work of Francis Lowell

and William Franklin in establishing the validity of allergen immunotherapy with their carefully performed double-blind studies of ragweed immunotherapy.

Continuing with the model of double-blind, placebo-controlled studies of allergen immunotherapy, 2 studies looked at clinical effectiveness. In the first article, active or placebo immunotherapy was added to pharmacologic treatment and allergen avoidance in patients with asthma who were allergic to HDMs.²⁹ Treatment was continued for 3 years. Active treatment was superior to placebo in a small but significant increase in morning and evening peak expiratory flows accompanied by reduction in skin prick tests to HDMs. There were no significant differences in medication use, symptoms, lung volumes, or bronchial response to methacholine. Several possible reasons were offered for the unimpressive results despite 3 years of treatment with adequate doses of HDM extract. It was suggested that perhaps immunotherapy for HDM sensitivity in asthma adds little to adequate pharmacotherapy or that HDM sensitization is not an important trigger for asthma in patients receiving optimal medical care, including allergen avoidance measures and antiasthma drugs. Supporting the second of these suggestions are the data on HDM allergen levels in the bedrooms, which were quite low.

The second article examined the effect of immunotherapy with a range of doses of cat dander extract. Previous studies had demonstrated that immunotherapy with a maintenance dose containing 15 μ g of the major cat allergen Fel d 1 was clinically effective. A study of immunotherapy with varying doses of cat extract had demonstrated that, after first reaching maintenance after 5 weeks by using a cluster schedule, subjects receiving a 15- μ g dose had more marked and consistent immunologic responses than subjects receiving 3.0 μ g, whereas those receiving 0.6 μ g fared no better than those receiving placebo.³⁰ The study by Nanda et al³¹ extended these observations by treating a second group of subjects with the same doses, again reaching maintenance after 4 weeks, but continuing maintenance treatment for 1 year. They again observed the largest and most consistent immune response in those receiving 15 μ g, some response in those receiving 3.0 μ g, and no difference from placebo in those receiving 0.6 μ g. Furthermore, they demonstrated that the same relative effectiveness persisted after a year of maintenance immunotherapy. This study confirmed the importance of delivering an adequate dose of cat extract and showed that a short-term study could be used to predict relative effectiveness of various doses of extract.

Sublingual immunotherapy

The use of sublingual immunotherapy (SLIT) continues to be explored in Europe. A study was conducted to assess the feasibility of delivering SLIT in a United Kingdom general practice setting.³² Patients with severe grass pollen allergic rhinitis were observed for 1 year, then randomized to receive active SLIT for 2 seasons, active treatment the first year followed by placebo the second, or placebo both years. The dose administered to the active

groups was approximately 300 times that used for injection immunotherapy. One hundred thirty-six subjects completed the 3 years of the study. There were no significant differences in the 3 main symptoms of sneezing, rhinorrhea, and blockage after the first year of treatment. After the second year, there were significant reductions in rhinorrhea and sneezing in subjects who had received active treatment both years. There was significant reduction of blockage in both active treatment groups during the peak of the second pollen season. It was concluded that high-dose SLIT can be successfully and safely administered in the general practice setting. At least 2 years of treatment is required to show a benefit.

A 3-year open study was conducted in Italy in children age 5 to 14 years with grass pollen allergy who had rhinitis but not asthma.³³ Each year, for 3 pollen seasons, they received coseasonal active or no SLIT. The study used a relatively low SLIT dose of 0.5 μ g group 5 grass allergen 5 days a week (cumulative monthly dose, about 1/2 the conventional monthly maintenance injected dose). One hundred thirteen children were randomized, and 97 completed the 3 years of the study. There were no differences in symptoms or medication use the first season; the second season, both were significantly lower in the active treatment group, whereas the third year, only medication scores were significantly lower in the active treatment group. Global evaluation significantly favored active treatment the second and third years. In the active treatment group, asthma occurred in 6 the first year, 7 the second year, and 8 the third year. In the control group, the corresponding numbers were 6, 16 ($P = .06$), and 18 ($P = .04$). Thus, 3 years of coseasonal SLIT reduced rhinitis symptoms, medication use, and the occurrence of asthma, all beginning in the second year of treatment.

Sublingual immunotherapy has the advantage over subcutaneous injections of greater safety and the convenience of self-administration at home. The latter, however, makes assessment of compliance difficult. A study was undertaken to assess compliance with SLIT in a group of subjects receiving SLIT with tablets either perennially for HDM sensitivity or preseasonally for pollen allergy.³⁴ Patients were called at random and asked to count the number of tablets remaining in the blister. Compliance was assessed after 1 year of treatment for mite extract and at the beginning of the pollen season for pollen SLIT. Calculated adherence over the course of treatment to date was 96.8% for mite and 97.6% for pollen. Contributing to the high rate of adherence was the low rate of side effects of the medication.

Safety

The immunotherapy committee of the American Academy of Allergy, Asthma and Immunology (AAAAI) reported the results of a survey of deaths attributable to allergen immunotherapy and skin testing that occurred between 1990 and 2001.³⁵ Of 2404 members of the AAAAI, 25% responded, reporting a total of 20 deaths, 19 from immunotherapy and 1 from skin prick testing. As has been true in previous similar surveys, most fatalities

(15 of 17 with details) occurred in patients with asthma. Nine of the 17 patients were considered to have suboptimally controlled asthma. Three subjects were receiving injections in medically unsupervised situations. Three of the fatal reactions began more than 30 minutes after the injection. The 1 fatality with skin prick testing was in a young woman with poorly controlled asthma who reacted after the performance of 90 food skin tests, many to highly allergenic extracts, using a DermaPik device (Greer Laboratories, Lenoir, NC). Committee recommendations to avoid future fatalities included avoiding skin testing and immunotherapy in patients with poorly controlled asthma, assessing asthma and peak flows before administering injections to patients with asthma, extending the waiting period and providing self-injectable epinephrine to high-risk patients, administering immunotherapy only in fully equipped clinics with trained personnel, and being prepared to establish airway when necessary.

Another survey was conducted of 1717 members of the AAAAI and the Joint Council of Allergy, Asthma and Immunology asking whether they knew of an incorrect immunotherapy injection administered within the last 5 years in their offices.³⁶ Four hundred seventy-six allergists replied to a secure Web site. Fifty-seven percent of respondents reported at least 1 wrong injection given to their patients, and 74 percent reported at least 1 incorrect dose injection. These errors resulted in 443 systemic reactions not requiring hospital care, 59 emergency department visits, 24 hospitalizations, and 1 death. The authors made several recommendations to avoid errors in allergen administration. Among these were annually educating physicians and nurses in safe administration of allergen immunotherapy, using patient-specific vials, using 3 identity checks before each injection, and allowing only 1 patient at a time in the injection room.

Modified allergens

Two studies were reported in human beings with the major allergen of ragweed, Amb a 1, linked to a 22-base-long immunostimulatory phosphorothionate oligodeoxynucleotide (Amb a 1-immunostimulatory DNA sequence conjugate; AIC). In both studies, AIC was administered in 6 weekly injections, beginning with a dose containing 0.06 µg Amb a 1, with a final dose containing 12.0 µg. The response of PBMCs was assessed before and 2 and 16 weeks after the course of immunotherapy.³⁷ After AIC, *in vivo* ragweed-specific T_H2 responses were selectively redirected toward T_H1 responses, with significant increases in IFN-γ, CXCL9, and CXCL10 and significant decreases in IL-5, CCL17, and CCL22 at both 2 and 16 weeks after the sixth injection. Unlike the ragweed stimulated responses, cytokine and chemokine responses to the unrelated bacterial antigen streptokinase and the polyclonal activator PHA did not change. The same schedule of injections of AIC was administered to 28 ragweed-sensitive patients, whereas 29 received placebos.³⁸ A subset underwent nasal challenges with ragweed followed by nasal biopsies 24 hours later before receiving AIC and before and after the first ragweed pollen season.

All subjects were followed clinically for rhinitis and asthma symptoms through 2 ragweed pollen seasons. There were no changes in the nasal biopsies before the first ragweed pollen season. However, after the season (which was 4 to 5 months after completion of the AIC injections), 24 hours after nasal ragweed challenge, the treated patients had a significantly reduced increase in eosinophils and IL-4 mRNA-positive cells and an increased number of IFN-γ mRNA-positive cells compared with the placebo-treated group. No difference between treatment groups was observed in symptoms or medication use during the first ragweed season. During the second ragweed season, however, there was a significant decrease in chest symptoms and a trend toward reduced nasal symptoms in the AIC-treated group. It was concluded that a short course of immunotherapy with AIC can modify the response of nasal mucosa to allergen challenge. This was followed by evidence of clinical efficacy in the second ragweed season without additional AIC immunization.

The mechanism of action of the immunostimulatory oligodeoxynucleotide sequences was examined in an *in vitro* system.³⁹ Plasmacytoid dendritic cells (PDCs) and autologous CD4⁺ T cells isolated from PBMCs of patients with allergic rhinitis were cultured with or without grass pollen extract and CpG. In the presence of grass pollen extract, PDCs stimulated allergen dependent T-cell proliferations and T_H2 cytokine production. PDCs that were activated by CpG inhibited the allergen-dependent proliferation of T_H2 memory cells and markedly increased IFN-γ production in PDC/T-cell cocultures. The findings suggest that mucosal PDCs may be targets for CpG-based immunotherapy.

Fungal extracts

The status of fungal allergen extracts was assessed by Robert Esch⁴⁰ from Greer Laboratories. Currently there are commercially available extracts representing 45 genera and 75 species of fungi manufactured by 7 allergen product manufacturers. Products representing the same species produced by difference manufacturers are not interchangeable, however, because of differences in the source of the culture and in manufacturing processes. An example was given of the marked variation in commercially available *Alternaria alternaria* products for protein, carbohydrate, and Alt a 1 content, as well as relative potency by ELISA inhibition (greater than 100-fold). Similar variability has been shown from commercially available fungal extracts derived from *Penicillium notatum*, *Aspergillus fumigatus*, *Helminthosporium sativum*, and *Epicoccum nigrum*. Because of this variability, standardization of fungal extracts is unlikely to occur without outside intervention.

Animal studies of immunotherapy

Human studies have demonstrated that allergen immunotherapy induces memory suppressive responses and IL-10 production by allergen-specific regulatory T-cells. The possible role of IL-10 was investigated in a murine model of immunotherapy that shows a sustained effect.⁴¹

There was significant suppression of allergen specific IgE levels and T_H2 cytokine production and a shift in the IL-5:IL-10 ratio in bronchoalveolar lavage fluid toward IL-10. When the mice were treated with a mAb against IL-10 receptors, the beneficial effects of immunotherapy were largely abrogated. The data support an essential role for IL-10 in the response to immunotherapy in this murine asthma model.

Immunization with antigen-encoding DNA has been used experimentally to suppress allergic reactions to the antigen.⁴² The usual route of administration is by intramuscular or intradermal injection. One disadvantage of this form of treatment is the large amount of plasma DNA required. An alternative method for *in vivo* transfection of somatic cells with DNA is the bombardment of skin with DNA-coated gold particles by using the helium-powered gene gun. This latter approach was used to administer a plasmid vector encoding the antigen β -galactosidase followed by sensitization of mice to the antigen by intraperitoneal injection with alum. Preadministration by gene gun of the plasmid encoding β -galactosidase resulted in an immune deviation from a T_H2 -biased to a mixed T_H1/T_H2 immune response.

Biodegradable poly(lactide-co-glycolide) (PLGA) microspheres are a promising carrier for vaccine delivery. PLGA microspheres are targeted to dendritic cells or macrophages, which then present the encapsulated antigen to T lymphocytes. PLGA microspheres have been used to encapsulate DNA or, alternatively, DNA has been adsorbed to their surface. A study was conducted to examine the immunomodulatory capacity of presensitization subcutaneous administration of DNA-loaded and plain PLGA microspheres in a murine model of bee venom allergy.⁴³ It was found that the microspheres alone protected most mice against anaphylaxis through the induction of T_H1 -type antibody, antigen-specific T-cell suppression, and production of IL-10. Thus, they demonstrated an antigen-independent and prolonged suppression of an allergic reaction modulated by intradermal treatment with microspheres alone. The same microspheres were also tested in an allergen-specific oral approach to immunotherapy⁴⁴ taking advantage of the fact that the M cells of the intestine, but not the enterocytes, express α -L-fucose residues on their apical surface. A vehicle for oral immunotherapy was constricted by using PLGA microspheres filled with birch pollen extract and containing on the surface lectin derived from *Aleuria aurantia* that had α -L-fucose specificity. In mice previously sensitized to birch pollen, administration of these microspheres resulted in induction of IL-10 and IFN- γ , not seen with microspheres not targeting the M cells.

AEROBIOLOGY

In Allergy Archives, Dr Gregg Mitman of the Department of Medical History and Bioethics of the University of Wisconsin reviewed the rise of awareness of the role of pollen in the etiology of allergic rhinitis and

the subsequent development of aeroallergen sampling.⁴⁵ Among the colorful characters in his story were Drs Charles Blackley and Morrill Wyman. Critical to the development of a national pollen sampling network were the contributions of Oren Durham, whose activities were also reviewed by Dr Mitman, including a map of the United States clearly portraying the weed pollen burden.⁴⁶ To bring pollen and spore sampling into the current era, Jay Portnoy and Charles Barnes detailed the development of the National Allergy Bureau that today provides AAAA&I members, as well as the media and the public, with accurate regional pollen and spore prevalence data.⁴⁷

SKIN TESTING

Observing that positive skin test results to mesquite pollen extract were fairly common in San Diego despite the absence of that pollen in the air, John Kelso⁴⁸ analyzed the occurrence of isolated positive skin test results to mesquite extract. He found that these were more common than isolated skin tests to the locally significant olive tree pollen. Furthermore, those with isolated skin tests to mesquite were much less likely to have evidence of allergen-specific IgE. These findings suggest the possibility of some irritant or histamine-releasing activity in the mesquite pollen extract. The conclusion is that isolated positive skin test results to mesquite pollen should be viewed with suspicion.

New devices for prick/puncture skin testing are being continually introduced. It is important to assess objectively their performance because they may produce false-negative reactions caused by inadequate penetration of the skin. More often, excess trauma to the skin may produce unpredictably large reactions even with saline that could be falsely interpreted as positive reactions. Three new multiheaded prick/puncture devices were tested: the Greer Track (Greer Laboratories), DermaPik II (Biomedex, Inc, Spokane, Wash), and Quick Test (Panatrex, Inc, Placentia, Calif).⁴⁹ These were compared with 2 standard devices—the MultiTest II (Lincoln Diagnostics, Decatur, Ill) and a smallpox needle—by using saline and a histamine solution. In this study, all devices produced negative reactions with saline with a high rate of consistency. However, 2 of the new devices had sufficiently high rates of failure to produce positive reactions with histamine to suggest the need for performing tests with these devices in duplicate or, alternatively, performing intradermal testing to confirm negative reactions to important allergen.

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