

Advances in upper airway diseases and allergen immunotherapy

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The purpose of this review is to highlight recently published important articles on upper airway diseases and immunotherapy. We review articles on rhinitis, sinusitis, conjunctivitis, and immunotherapy. New insights into epidemiology, pathophysiology, diagnosis, and therapy are described for each of the above diseases. Regarding immunotherapy, we discuss numerous clinical trials on sublingual and subcutaneous immunotherapy, mechanisms of immunotherapy, safety, and use of modified allergens and biological agents for immunotherapy. (*J Allergy Clin Immunol* 2011;127:342-50.)

Key words: Review, rhinitis, sinusitis, conjunctivitis, allergen immunotherapy

The purpose of this review is to highlight important articles on upper airway diseases and allergen immunotherapy published between 2008 and 2010. We start with the key advances in upper airway diseases which are summarized in [Table I](#).¹⁻¹⁷

RHINITIS Epidemiology

Several risk factors are related to atopic diseases, including allergic rhinitis. Use of antibiotics in the first year of life increased the risks for asthma, allergic rhinitis, and atopic eczema at ages 6 to 7 years by 56% to 82% based on results of the International Study of Allergies and Asthma in Childhood 3 study.¹⁸ Cigarette smoking induces an inflammatory response in epithelial cells¹⁹ and can increase up to 5 times the risk of developing asthma over 10 years in adults with allergic rhinitis.¹ Birth by cesarean section can double the risk of allergic rhinitis at age 9 years in children with at least 1 atopic parent.² Particulate matter and volatile organic compounds arising from petrochemical plants, but not from high traffic density, were associated with worse respiratory health in children.³ Prolonged breast-feeding and multiple children in the household were protective, whereas IgE sensitization and endotoxin levels in house dust increased the risk of allergic rhinitis at age 3 years in African Americans living in Cincinnati.⁴

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Abbreviations used

BAFF:	B cell-activating factor
CRAC:	Ca ²⁺ release-activated Ca ²⁺
CRS:	Chronic rhinosinusitis
CRSwNP:	Chronic rhinosinusitis with nasal polyps
FP:	Fluticasone propionate
LTC ₄ :	Leukotriene C ₄
NO:	Nitric oxide
ppb:	Parts per billion
SCIT:	Subcutaneous immunotherapy
SLIT:	Sublingual immunotherapy
Treg:	Regulatory T

The burden of allergic rhinitis in US children was assessed in detail in a nationwide telephone survey of more than 35,000 households.⁵ The prevalence of health practitioner-diagnosed allergic rhinitis in children aged 4 to 17 years was 13%, with the most bothersome symptoms being nasal congestion, repeated sneezing, runny nose, and watering eyes. The prevalence of asthma was 39% in children with allergic rhinitis and 13% in non-allergic children.

In Sweden, a cross-sectional evaluation of children in 1996 at age 7-8 years, and again in 2006, revealed that the prevalence of IgE sensitization based on skin tests increased from 21% to 30% during this period, whereas the prevalence of asthma (12%) and allergic rhinitis (15%) did not change.²⁰

The natural history of patients with nonallergic rhinitis includes development of IgE sensitization and new airway diseases. In a cohort of 180 adults with nonallergic rhinitis followed for 3 to 7 years, 40% of subjects experienced no change in disease severity, whereas 52% had worsened disease. About 24% had new conditions, such as asthma (prevalence increased from 32% to 55%), conjunctivitis (28% to 43%), IgE sensitization to aeroallergens (0% to 24%), and chronic rhinosinusitis (CRS; 0% to 7%).⁶

Pathogenesis

Patients with local allergic rhinitis (or “entopy”) have a history of rhinitis and negative skin allergy test results but have evidence of local IgE sensitization in nasal cavities and a similar response to allergen nasal provocation as that seen in patients with allergic rhinitis. Rondon et al²¹ showed that 30% to 40% of patients with local allergic rhinitis to grass demonstrated increases in nasal lavage tryptase, eosinophil cationic protein, and levels of specific IgE to grass after nasal provocation. This supports a role for IgE, mast cells, and eosinophils in this disease. Together with previous evidence of IgM to IgE immunoglobulin class switching in submucosal nasal airways, the evidence indicates a high degree of local immune competency and organ specificity in the upper airway immune system. As

TABLE I. Key advances in rhinitis, sinusitis, and conjunctivitis

1. Risk factors for allergic rhinitis in childhood include exposure to cigarette smoking, birth by cesarean section, petrochemical plants' pollutants, and endotoxin levels in house dust of inner city homes. ¹⁻⁴
2. Prevalence of health care provider–diagnosed allergic rhinitis in the United States is 13%. ⁵
3. Patients with nonallergic rhinitis can develop allergic rhinitis, asthma, and chronic rhinosinusitis within 3–7 years. ⁶
4. The intracellular caveolar system might be responsible for transportation of allergens across epithelial cells in the nasal mucosa. ⁷
5. Apolipoprotein A-IV levels increase after SLIT, and this inhibits basophil histamine release. ⁸
6. Acute exacerbations of chronic rhinosinusitis tend to occur in the fall and winter. ⁹
7. Nasal polyps in European patients are associated with T _H 2 inflammation and asthma, whereas nasal polyps in Chinese patients are more associated with neutrophilic inflammation. ¹⁰
8. Nasal mucosal inflammation in patients with CRS is characterized by increased TGF- β activity. ^{11,12}
9. New features of inflammation in nasal polyps include BAFF ¹³ and S100 proteins. ^{14,15}
10. Decreased nasal nitric oxide levels might help in the diagnosis of acute sinusitis. ¹⁶
11. In patients with vernal and atopic keratoconjunctivitis, there is formation of new tertiary lymphoid tissue containing lymphocytes and local IgE immunoglobulin class switching. ¹⁷

mentioned, a proportion of patients with nonallergic rhinitis will have allergic airway diseases over a few years.⁶ This suggests that some patients might first experience IgE sensitization localized to the airways (entopy), followed within a few years by systemic IgE sensitization when skin test results become positive (allergic rhinitis).

Several publications made new advances regarding our understanding of airway physiology. Patients with allergic rhinitis have impaired lung physiology because they do not bronchodilate with deep inspiration, as occurs in the airways of healthy subjects.²² Serum IL-17 levels were 10-fold higher in subjects with allergic rhinitis monosensitized to birch pollen compared with those seen in healthy control subjects, and levels were directly related to disease severity.²³ During the season and after nasal allergen challenge, IFN- γ levels increase in nasal fluid cells.²⁴ Levels of free immunoglobulin light chains κ and λ are increased in nasal secretions of subjects with allergic rhinitis and are found within the epithelium and on submucosal mast cells.²⁵ Further investigations are needed to determine whether free immunoglobulin light chains are an epiphenomenon or whether they have a pathological role in airway inflammation. Anaphylatoxin receptors C3aR and C5aR are overexpressed in the nasal mucosa and submucosa of subjects with allergic rhinitis. C5aR was detected on inflammatory cells and C3aR on gland epithelium and endothelial cells.²⁶ Nasal epithelial cells express costimulatory molecules B7-H1 and B7-DC upon rhinovirus infection through Toll-like receptor 3 signaling,²⁷ a detector of double-stranded RNA. Long double-stranded RNA is not usually formed in cells except during replication of RNA viruses, which defines most respiratory tract viruses. Nasal epithelial cells are 20 times more resistant to rhinovirus infection than bronchial epithelial cells.²⁸ Transport of allergen across airway-pseudostratified epithelium can occur through the caveolar system, an intracellular system that uses the cytoskeleton for transport across the cell.⁷ Based on proteomics in sera from subjects with allergic rhinitis undergoing immunotherapy for Japanese cedar pollen allergy, Makino et al⁸ found that apolipoprotein A-IV levels increased after SLIT and that this inhibited basophil histamine release. All of these mechanisms identify new targets that might lead to novel treatments for patients with allergic airway diseases.

Diagnosis and therapy

Identification of allergen triggers for subjects with allergic rhinitis requires allergy testing. Relying only on patient report or a

structured allergy history results in false identification of allergen triggers (false-positive rate) in 32% (animal allergy) to 75% (dust allergy) of patients when compared with allergy skin testing results as the gold standard. Thus objective assessment of IgE sensitivity improves accuracy in identifying allergenic triggers.²⁹

The efficacy of omalizumab therapy on suppressing skin test reactivity requires almost complete elimination of free serum IgE. Reduction of omalizumab dose increases serum free IgE levels, resulting in return of skin reactivity to allergen within 4 months.³⁰

Application of carbon dioxide gas intranasally for 60 seconds twice improved seasonal allergic rhinitis symptoms for 24 hours.³¹ However, the treatment causes tearing and nasal stinging, probably because of transient formation of carbonic acid,³² which could be attenuated by carbonic anhydrase inhibitors. This is a potential future treatment for rhinitis.

Laropiprant (up to 300 mg administered orally daily for 3 weeks), an inhibitor of DP1, one of 2 receptors for prostaglandin D₂, the main prostaglandin produced by mast cells, was not efficacious in improving FEV₁ in patients with asthma, or in decreasing nasal symptoms in patients with allergic rhinitis based on 2 randomized, placebo-controlled, double-blind clinical trials. Perhaps inhibiting DP2 might result in therapeutic benefit because this receptor mediates chemoattraction of T_H2 lymphocytes.³³

Parasitic helminth infections are associated with reduction in the risk of atopy in epidemiologic studies, and helminth infections attenuate airway inflammation in experimental asthma. Bager et al³⁴ administered orally 2,500 ova of *Trichuris suis* every 21 days for 8 doses to subjects with grass-induced allergic rhinitis in a randomized, placebo-controlled, double-blinded clinical trial. About 32% of subjects who received ova experienced diarrhea and eosinophilia from the parasitic infection, but experienced no attenuation in their nasal seasonal allergy symptoms.

A combination of probiotic (live bacteria: lactobacilli, bifidobacteria, and propionibacteria) and prebiotic (nutrient that promotes growth of probiotic bacteria: galacto-oligosaccharides) treatments administered to mothers during the last month of pregnancy and infants until 6 months of age³⁵ did not alter their offspring's prevalence of IgE sensitization (52%), atopic eczema (24%), allergic rhinitis (20%), or asthma (13%) during their first 5 years of age. However, treatment did decrease IgE-associated allergic diseases from 40.5% to 24.3% ($P < .035$) in cesarean-delivered children (number needed to treat, 6). This result raises the hypothesis that brief exposure to maternal vaginal flora through vaginal birth can reduce the risk of atopic disease by 40%.

Although most patients with upper airway diseases respond to treatment, others do not and continue to experience severe chronic upper airway disease. They might have underlying nonallergic rhinitis, allergic rhinitis, or CRS. Leading investigators in the field have identified priorities for research in these patients³⁶ and have documented the significant impact in quality of life associated with their severe chronic upper airway diseases.³⁷

Reviews of interest in rhinitis include updates on the practice parameters on the diagnosis and management of rhinitis³⁸ and on the Allergic Rhinitis and its Impact on Asthma guidelines,³⁹ as well as reviews of the use of complementary and alternative medicine in patients with allergic rhinitis and other allergic conditions.^{40,41}

SINUSITIS AND NASAL POLYPS

Epidemiology

Acute exacerbations of CRS have a seasonal pattern in Olmsted County, Minnesota. They peak in fall and winter and are at their nadir from May through August based on medical record data indicating International Classification of Diseases, Ninth Revision, codes for sinusitis plus escalation of therapy (antibiotics, oral corticosteroids, and consideration of surgical treatment).⁹

African Americans tend to have lower serum vitamin D levels than whites. Only in African Americans were vitamin D levels significantly lower in patients with CRS than in healthy race-matched control subjects.⁴² However, the authors did not assess whether serum vitamin D levels correlated with disease severity.

Pathogenesis

Nasal allergen challenge causes early- and late-phase inflammatory responses inside the maxillary sinuses, including increases in histamine levels, eosinophil counts, albumin levels, and eosinophil cationic protein levels in sinus wash samples.⁴³

Several new studies have shed more light onto the pathogenesis of CRS. In a comparison of patients with chronic rhinosinusitis with nasal polyps (CRSwNP) from Belgium (white subjects)⁴⁴ and China (Asians), Zhang et al⁴⁵ found that they were similar in terms of symptoms, computed tomographic scans, and rhinoscopic findings, as well as a similar reduction in forkhead box protein 3 (a marker of regulatory T [Treg] cells) expression and TGF- β 1 protein content in polyps.⁴⁴ However, Belgians with CRSwNP had a higher prevalence of asthma and of eosinophil counts and T_H2 markers (total IgE, IgE to staphylococcal enterotoxins, IL-5, and eotaxin) in polyp tissues.⁴⁶ In contrast, Chinese subjects had more neutrophils and markers of T_H1 (IFN- γ) and T_H17 (IL-6 and IL-17A) inflammation in polyp tissues. IL-17A is produced by dispersed nasal polyp cells stimulated by staphylococcal enterotoxin B, a phenomenon that is inhibited by IFN- γ and diclofenac, and is stimulated by IL-23 and prostaglandin E₂.¹⁰ Another group of investigators also reported that nasal polyps in Chinese patients have decreased Treg cells (forkhead box protein 3 mRNA) and more diverse inflammation in nasal polyps (T_H1, T_H2, and T_H17 markers), although this was based on only mRNA markers because cytokines were not detectable.⁴⁷ In the United States, nasal polyp tissues from patients with CRSwNP have mostly unchanged IL-6 levels, but increased levels of soluble glycoprotein 130, an inhibitory IL-6 receptor, and evidence of decreased IL-6 receptor signaling because phosphorylation of signal transducer and activator of transcription 3 seemed decreased.⁴⁸ In addition, IL-17A levels were not increased. These results suggest

that nasal polyps have impaired Treg cell function, that nasal polyps have mostly eosinophilic T_H2 inflammation in European and US patients, and that in China, many patients have T_H17 inflammation in nasal polyps. The diverse predominance of inflammatory types in polyps might result from several factors that will need to be explored in future studies, such as environmental exposures (eg, allergens, infection, pollution, and endotoxin), medications taken before polypectomy, and smoking status.

Patients with CRSwNP have increased TGF- β activity in the ethmoidal sinus mucosa.^{11,12} Their ethmoidal mucosa has other signs of increased TGF- β activity, such as increased amounts of TGF- β 1 and TGF- β 2, TGF- β receptors I and III, and collagen and increased phosphorylated SMAD2, which is activated downstream of the TGF- β receptors.

Involvement of B cells in inflammation of CRSwNP might be driven by epithelial cells. Epithelial cells can secrete B cell-activating factor (BAFF), which induces B-cell proliferation and immunoglobulin class switching to IgA and IgE. Levels of BAFF are increased in nasal polyps, and these levels correlate with B-cell numbers and IgA levels in polyp tissues.¹³

S100 proteins, such as S100A7 (psoriasin) and S100A8/A9 (calprotectin), are involved in epithelial differentiation, epithelial barrier and also have antimicrobial activity. Their levels are decreased in nasal lavage fluid of patients with CRSwNP and CRS without nasal polyps. However, calprotectin levels are increased in nasal polyp tissue, where it strongly correlates with neutrophils, also a source of calprotectin.^{14,15} In patients with CRS, sinus epithelium shows enhanced apoptosis and shedding, a process that can be induced by IFN- γ from T_H1 cells.⁴⁹

Stimulation of cell membrane Ca²⁺ release-activated Ca²⁺ (CRAC) channels in mast cells leads to production of leukotriene C₄ (LTC₄) through a pathway involving Ca²⁺-dependent protein kinase C. In autocrine and paracrine actions, LTC₄ activates CRAC channels through cysteinyl leukotriene receptor 1. A combination of the CRAC channel inhibitor lanthanum ion (La³⁺) and the cysteinyl leukotriene receptor 1 inhibitor montelukast markedly inhibited Ca²⁺ signaling and production of LTC₄ in mast cells *in vitro*,⁵⁰ suggesting CRAC as a potential new therapeutic target in CRSwNP because nasal polyps have increased mast cells and LTC₄.

The interpretation of the clinical relevance of these findings is limited to association with different CRS phenotypes. More effort should be placed into correlating new biological markers with different domains of disease severity (eg, different symptoms [congestion and mucus production], quality of life, computed tomographic scores, and histologic features) and with the natural history of CRS. Another observation is that some significant results seem to be driven by a subgroup of patients with high levels of a given mediator, suggesting the existence of several CRS phenotypes.

Diagnosis and therapy of sinusitis

Nitric oxide (NO) in the nasal cavity reaches a few thousands parts per billion (ppb), whereas it decreases drastically toward the lower airways, where it is less than 45 ppb. Lanz et al¹⁶ noticed that in patients with acute sinusitis confirmed by means of computed tomographic scans, nasal NO levels were low, about 275 ppb, and they improved to normal levels of 1,000 to 1,500 ppb after antibiotic therapy. This suggests that in patients with acute sinusitis, sinus ostia may be blocked and sinus NO cannot diffuse

TABLE II. Key advances in immunotherapy

1. The updated primer on allergen immunotherapy was written. ⁶⁷
2. Allergen immunotherapy induces accumulation of IL-10–producing Treg cells in nasal mucosa. ⁶⁸⁻⁷⁰
3. Various trials demonstrated the efficacy of grass pollen SLIT for rhinoconjunctivitis and asthma in adults and children. ^{71,74-76} Benefits can last for 7-8 years after discontinuing immunotherapy. ^{72,73}
4. Multiallergen extract SLIT appears to be safe but might be inferior in efficacy compared with SLIT monotherapy. ^{77,78}
5. In the absence of dose adjustment, previous local reactions do not predict future local reactions. ⁷⁹
6. Modified proteins with decreased allergenicity but preserved immunogenicity are being developed for birch pollen and dust mite allergen immunotherapy. ^{80,81}
7. <i>In vitro</i> experiments and murine models demonstrated that DNA and RNA allergen vaccines can be feasible future approaches for immunotherapy. ^{82,83}

into nasal cavities. This might become a useful bedside test to diagnose acute sinusitis and to monitor the response to therapy.

Several trials have revealed new insights in CRS therapy. Patients with nasal polyps applying mometasone intranasally experienced improvement in nasal symptoms within 2 to 5 days (eg, rhinorrhea, congestion, and postnasal drip) and improvement in smell within 13 days.⁵¹ In a small double-blind, placebo-controlled study, 47 patients with CRSwNP were randomized to methylprednisolone, doxycycline, or placebo for 20 days and followed for 12 weeks. Both methylprednisolone and doxycycline reduced polyp sizes, symptoms, and markers of inflammation. Methylprednisolone had a greater effect that lasted 8 weeks, whereas doxycycline's benefit lasted 12 weeks.⁵² A pilot uncontrolled study suggested that 0.5 mg of budesonide in more than 100 mL of nasal saline for rinsing of nasal cavities twice a day can improve symptoms and computed tomographic sinus scores.⁵³

A case report described a patient with aspirin triad whose asthma, chronic sinusitis, nasal polyps, and chronic mastoiditis all improved after omalizumab therapy.⁵⁴ Clinical trials are currently under way to investigate the efficacy of omalizumab in patients with CRSwNP.

Useful reviews in sinusitis include one on nasal endoscopic procedures in the allergist's office,⁵⁵ a primer on rhinitis and sinusitis,⁵⁶ and another on when to refer patients to an otorhinolaryngologist.⁵⁷

CONJUNCTIVITIS

Nasal allergen challenge worsens symptoms of allergic conjunctivitis,⁵⁸ a phenomenon that is attenuated by nasal corticosteroids. Indeed, several intranasal corticosteroids can reduce symptoms of allergic conjunctivitis, which is therefore a class effect.^{59,60}

Animal models of allergic ocular conditions have suggested new mechanisms for these diseases. A murine model of allergic conjunctivitis to ragweed pollen was used to show that prostaglandin E₂ attenuated the late-phase reaction through prostaglandin E receptor 3 (EP3),⁶¹ that blocking Toll-like receptor 3 attenuated the conjunctival response to allergen,⁶² and that mast cells and IgE play important roles in mediating both early and late conjunctival allergen responses.⁶³ In a rat model of allergic conjunctivitis to ragweed pollen, Fukuda and Nishida⁶⁴ showed that corneal damage increases conjunctival inflammation to allergen challenge and, reciprocally, that this inflammation delays corneal healing compared with that seen in nonallergic rats. This pathological interaction between cornea and conjunctiva can occur in patients with vernal and atopic keratoconjunctivitis.

In giant papillae of patients with vernal and atopic keratoconjunctivitis, there is formation of new tertiary lymphoid tissue

containing T and B cells, plasma cells, follicular dendritic cells, lymphatic vessels, and evidence of local IgE immunoglobulin class switching.¹⁷ This mirrors previous evidence of local IgE immunoglobulin class switching in upper and lower airway submucosae. Patients with vernal conjunctivitis also have eosinophilic inflammation in their nasal cavities, although they might not experience much nasal symptoms.⁶⁵

New computerized methods to analyze images of allergic shiners provided an objective method⁶⁶ to assess this important cosmetic adverse effect of rhinitis. This tool could be used to assess efficacy of investigational therapies for allergic shiners.

IMMUNOTHERAPY

Key advances in immunotherapy are listed in Table II.⁶⁷⁻⁸³

Several clinical pearls articles focused on the practice of immunotherapy. Weber⁸⁴ reviewed plant systematics (taxonomy) and pollen cross-reactivity. The increasing precision of plant taxonomy, the analysis of biochemical and DNA data, and the ability to clone recombinant allergens has augmented the ability to determine cross-reactivity, which can guide selection of pollen extracts for immunotherapy. Cox⁸⁵ reviewed the advantages and disadvantages of various accelerated allergen immunotherapy schedules, risk of reactions, need for premedication, and efficacy of each schedule based on immunologic responses. Esch⁸⁶ reviewed data on allergen extract mixing and stability. Other important reviews for clinicians are the American Academy of Allergy, Asthma & Immunology proposal to standardize a grading scale for SCIT systemic reactions,⁸⁷ an updated primer on allergen immunotherapy,⁶⁷ a World Allergy Organization position paper on immunotherapy,⁸⁸ and the Global Allergy and Asthma European Network and European Academy of Allergy and Clinical Immunology guide on immunotherapy.⁸⁹

Mechanism of action

Specific allergen immunotherapy induces Treg cells that produce IL-10 (T_R1 cells) and inhibit allergen-specific proliferation of PBMCs. Several new insights were revealed in this mechanism over the past few years. Radulovic et al⁶⁸ observed that successful timothy grass pollen SCIT for 2 years increased numbers of forkhead box protein 3–positive CD4⁺CD25⁺ (T_R1 cells) in the nasal mucosa in subjects with hay fever. In addition, SCIT caused a further increase in nasal T_R1 cells during the pollen season together with a decrease in nasal mucosal eosinophil and IL-5–expressing cell numbers. Francis et al⁶⁹ administered timothy grass cluster SCIT for 2 months and monthly maintenance (20 µg of Phl p 5) for 10 months and noticed a significant increase in IL-10 production of PBMCs to grass allergen stimulation very

early at 4 weeks. This was followed by a significant inhibition of basophil histamine release to grass allergen at 6 weeks and increases in grass-specific IgG4 and IgA levels together with suppression of grass-induced skin responses at week 12. The early increase in allergen-specific IL-10 production might be responsible for inducing allergen-specific IgG4 and IgA. Yamanaka et al⁷⁰ noticed that SLIT with Japanese cedar pollen (Cry j 1 and Cry j 2) induced cedar-specific T_R1 cells, but there was no clonal expansion of T_R1 cells based on T-cell receptor diversity analysis. In addition, T_R1 cells also inhibited T-cell proliferation to nonspecific stimuli (anti-CD3/CD28 antibodies), suggesting a mechanism by which allergen-induced T_R1 cells can inhibit T_H2 responses to other allergens not included in the immunotherapy injections. Finally, Allam et al⁹⁰ studied oral mucosal biopsy fragments *ex vivo* and observed that after uptaking timothy grass Phl p 5 allergen, oral Langerhans cells decreased their maturation capacity, but increased their migratory capacity and their production of TGF- β 1 and IL-10, cytokines known to promote differentiation of Treg cells. Taken together, these articles shed light on several steps of the mechanism of generation and function of Treg cells as a result of specific allergen immunotherapy.

SLIT

SLIT trials. Although SLIT has been the most extensively studied form of immunotherapy in the last few years, it has only been significantly incorporated into clinical practice in Europe.⁹¹ The US Food and Drug Administration has not approved any SLIT extract in the United States as of January 2011. SLIT can start to alleviate symptoms of allergic rhinoconjunctivitis after 4 weeks of therapy.⁹²

An industry-sponsored, double blind, placebo-controlled phase III trial evaluated daily timothy grass tablet SLIT (15 μ g of Phl p 5/d, GRAZAX; ALK-Abelló A/S, Hørsholm, Denmark) or placebo for 3 years in subjects with allergic rhinoconjunctivitis (n = 351). After 2 years of SLIT,⁷¹ patients experienced reductions in rhinoconjunctivitis symptom scores by 36% and in medication scores by 46%. Clinical improvements were paralleled by significant changes in grass pollen-specific IgG₄ levels. No serious adverse events occurred. One year after the end of the 3 years of therapy,⁷² rhinoconjunctivitis and medication scores were persistently reduced compared with those after placebo at 26% and 29%, respectively, demonstrating the durability of immunotherapy's benefits 1 year after its discontinuation.

SLIT's effects can last for many years. Marogna et al⁷³ studied adolescents and adults with allergic rhinitis, asthma, or both monosensitized to dust mites and noted that SLIT for 3 years provided more than 50% symptom reduction for 7 years, whereas SLIT for 4 to 5 years reduced symptoms for 8 years. SLIT for 4 to 5 years was also more effective in reducing the risk of new allergies.

Two European groups investigated the efficacy and safety of SLIT monotherapy for grass in children. Bufe et al⁷⁴ completed a multicenter, randomized, parallel-group, double-blind, placebo-controlled trial of daily sublingual *Phleum pratense*, 75,000 SQ-T/28,00 BAU (approximately 15 μ g of Phl p 5), initiated 8 weeks before and continued through the timothy grass season in 253 patients aged 5 to 16 years with grass pollen-induced rhinoconjunctivitis and asthma. Patients were excluded for severe asthma (Global Initiative for Asthma step 4) and FEV₁ of less than 80%. The treatment group demonstrated improved daily

rhinoconjunctivitis symptom scores (24%) and asthma symptom scores (64%) compared with those in the placebo group.

Wahn et al⁷⁵ conducted a multicenter, randomized, double-blind, placebo-controlled study of daily sublingual 5-grass-pollen (orchard, meadow, perennial rye, sweet vernal, and timothy grass) therapy dosed at 300 IR (approximately 20 μ g) of group 5 major allergens initiated 4 months before and continuing through the grass season in 278 patients aged 5 to 17 years with grass pollen-induced allergic rhinitis. The treatment group experienced a 39% reduction in rhinoconjunctivitis total symptom scores. In both studies no serious adverse events secondary to the treatment were reported. Combined with previous experience,⁹³ these studies^{74,75} extend the safety and efficacy data of SLIT in children.

SLIT was compared with inhaled corticosteroids in an unblinded, parallel-group, randomized controlled trial of 51 adults monosensitized to grass pollen with mild asthma and rhinitis restricted to the pollen season. Subjects were titrated to 10,000 RU/mL grass allergen 3 times a week (approximately 70 μ g of Phl p 1 cumulative dose per year) for 5 years or received 400 μ g of inhaled budesonide twice daily in the grass pollen season.⁷⁶ Upper and lower airway scores, use of rescue short-acting bronchodilators, use of intranasal steroids, nasal eosinophilia, and airways hyperreactivity were all improved in the SLIT arm at years 3 and 5 compared with those in the budesonide arm.

One of the first SLIT trials in the United States showed modest efficacy in patients with ragweed-induced rhinoconjunctivitis. Glycerinated short ragweed pollen extract SLIT (4.8 μ g or 48 μ g of Amb a 1/once daily) was evaluated in a randomized, double-blind, placebo-controlled trial involving 115 adults with ragweed allergy.⁹⁴ SLIT caused a nonsignificant small reduction of 15% in rhinoconjunctivitis symptom scores in both the medium- and high-dose groups. Only when scores were adjusted for baseline values were there significant reductions in symptom and medication scores, although only in the high-dose group compared with the placebo group. SLIT was safe, but caused more oral mucosal adverse events compared with placebo, as expected. SLIT induced significant increases in ragweed-specific IgG₄ and IgA levels. This trial was disappointing when compared with the more impressive efficacy results from SLIT trials for grass allergy conducted in Europe.

The average SCIT prescription in the United States contains 10 different extracts, but the vast majority of SLIT trials have examined 1 or 2 allergens at a time.⁹⁵ Amar et al⁷⁷ conducted the first randomized, double-blind, placebo-controlled trial in adults using a multiallergen extract SLIT preparation. Patients with allergic rhinitis caused by timothy grass were titrated to 10 months of daily therapy with SLIT 0.25-mL monotherapy extract (100,000 BAU/mL in a 10-mL vial, approximately 19 μ g of Phl p 5 per 0.25 mL) or multiallergen SLIT extract with the same 19 μ g of Phl p 5 of timothy grass per dose plus 9 other nonstandardized extracts (1:20 wt/vol) in 50% glycerin (maple, ash, juniper, American elm, cottonwood, Kochia, ragweed, sagebrush, and Russian thistle), or placebo. Subjects were not required to be sensitized to the various components of the multiallergen extract other than timothy grass. Because of a mild grass pollen season during the study, there was no difference among groups for the primary outcome of symptom score. Timothy grass monotherapy attenuated significantly the response to timothy grass nasal challenge and to skin test titration, and it increased serum timothy grass-specific IgG₄ levels compared with multiallergen therapy, which only showed a modest improvement in skin test titration.

The authors concluded that multiple allergen extracts might decrease efficacy for an individual allergen, perhaps because of limited sublingual absorption or competition for antigen-presenting cells. It is noteworthy that this is another SLIT trial in the United States showing unimpressive clinical efficacy.

SLIT safety. Regarding the safety of SLIT, Agostinis et al⁷⁸ examined data from 433 children who received more than 40,000 doses of SLIT in a clinical practice setting. Mild-to-moderate adverse events occurred in about 40% of patients regardless of whether SLIT contained 1 or multiple allergens. The most common events were oral pruritus or burning, throat irritation, and oral/tongue edema. Although none needed emergency treatment for adverse events, there are several reports of systemic reactions to SLIT, including anaphylaxis.⁹⁶⁻⁹⁸ The allergist should be cautious, particularly with very sensitive patients.

SLIT meta-analyses. Given the mixed results of various SLIT trials, several meta-analyses⁹⁹⁻¹⁰⁴ have been completed, and the evidence supports the efficacy of SLIT in patients with rhinoconjunctivitis and allergic asthma. Nieto et al¹⁰⁵ examined 5 meta-analyses and challenged their findings, citing data discrepancies and possible publication bias that might lead to overstatement of treatment effects. Later, Casale et al⁹¹ formulated a position paper on trial design for SLIT therapy. They cited that most studies have been of short duration (<12 months) and small number (<100 subjects). Overall, however, 80% of studies have shown positive results. They formulated specific recommendations regarding allergen vaccine, patient selection, and study design to standardize future SLIT trials.

SCIT

Safety, prognosis, and indication for SCIT were studied in the past 2 years. In the LOCAL study Calabria et al⁷⁹ retrospectively reviewed immunotherapy records and found that in the absence of dose adjustment, a previous local reaction was not predictive of a local reaction on the next injection. Only 26% of local reactions were followed immediately by another local reaction, and only 6% of large local reactions were followed immediately by another large local reaction.

Prognosis of specific immunotherapy was examined retrospectively in monosensitized patients who completed 4 years of SCIT or SLIT in Italy.¹⁰⁶ Good response to immunotherapy was predicted with 97% sensitivity and 88% specificity by a baseline specific IgE/total IgE ratio greater than 16.2%. These findings suggest that for successful immunotherapy, clinicians might need to include enough allergens so that the sum of their individual serum specific IgE concentrations is 16.2% or higher of the total serum IgE concentration. These results will need to be confirmed in prospective studies and in polysensitized patients.

Venom immunotherapy was found to reduce large local reactions to insect stings.¹⁰⁷ Subjects with a positive venom skin test and a local reaction larger than 16 cm during sting challenge showed a 42% and 53% reduction in the size and duration of their local reactions after 11 weeks of venom immunotherapy compared with an 18% reduction in the unblinded placebo group, who received no injection. Difficulty in subject recruitment suggests that only a finite group of patients would undergo venom immunotherapy for this indication.

Montelukast was found to diminish the efficacy of SCIT in children aged 6 to 12 years.¹⁰⁸ In a small study, 36 children with asthma monosensitized to dust mite were randomized to 5 mg of

montelukast or placebo daily, starting before the initiation of SCIT. Inhaled corticosteroid dose was decreased 16.7% more in the placebo group compared with that seen in the montelukast group. The authors presented evidence that the diminished efficacy of SCIT in the setting of montelukast therapy might be due to impaired induction of Treg lymphocytes. In the per-protocol analysis, placebo-treated patients had a higher dropout rate because of poorly controlled asthma and thus, were not included in the analysis. In the intent-to-treat analysis, no difference between groups was observed. Larger confirmatory studies are required before a conclusion can be drawn regarding montelukast and specific immunotherapy.

SCIT can spare inhaled corticosteroid dose. Zielen et al¹⁰⁹ studied 65 asthmatic children with asthma of stage II or III severity based on Global Initiative for Asthma Guidelines and whose symptoms were well controlled with inhaled corticosteroids. Subjects were randomized to either inhaled fluticasone propionate (FP) plus SCIT, or FP only. After 2 years of unblinded therapy and attempts to taper FP, children treated with SCIT were able to taper 54% of the initial FP dose, whereas children who did not get SCIT tapered only 29% of their FP. Despite being unblinded, this study suggests a steroid-sparing benefit from SCIT therapy in children with asthma.

Modified extracts and novel delivery systems

Two fusion proteins that work through negative signaling were described in 2008. GE2, a human fusion protein in which of the Fc portions of IgE and IgG₁ are linked, was developed as a long-term, subcutaneous, allergen-nonspecific therapy for any IgE-mediated disease.¹¹⁰ The authors demonstrated that it blocks the effector phase of the IgE response *in vitro* in mice and human subjects, and *in vivo* in the skin and airway and systemically in mice and monkeys. They also investigated a human cat chimeric fusion protein (GFD), a genetically engineered chimeric human Fcγ1 joined to Fel d 1. The allergen portion, Fel d 1, binds specifically to IgE on FcεRs, whereas the Fcγ portion coaggregates inhibitory FcγRIIb, thus blocking allergic reactivity. GFD was found to block Fel d 1–induced allergic reactivity of human mast cells *in vitro*. *In vivo* in murine models, it functions as an immunogen, but not as an allergen.

Modified proteins that have reduced allergenicity with preserved or improved immunogenicity remain promising. A multicenter, randomized, double-blind, placebo-controlled trial comparing recombinant birch pollen allergen vaccine (rBet v 1a), licensed birch pollen extract, natural purified birch pollen allergen, and placebo was performed in 134 adults with allergic rhinoconjunctivitis to birch pollens.⁸⁰ Subjects received 12 weekly injections followed by monthly injections of maintenance dose (15 μg of Bet v 1) for 2 years. Significant reductions in symptoms were found in the 3 active treatment groups compared with the placebo group. No severe systemic adverse events occurred in the rBet v 1–treated group.

A recombinant precursor of the major dust mite allergen Der p 1 (proDer p 1) was expressed and purified in *Escherichia Coli*.⁸¹ This recombinant allergen, ProDer p 1 coli, was enzymatically inactivated and lacked the 3-dimensional structure of Der p 1 and thus bound specific IgE very weakly compared with native allergen. *In vitro* stimulation of T cells from Der p 1–sensitized subjects with ProDer p 1 coli produced similar response to that of Der p 1. A murine model demonstrated that vaccination with

ProDer p 1 coli before sensitization significantly limited IgE production, enhanced IgG2a production, biased the immune response toward a T_H1 response, and reduced airway eosinophilia and hyperreactivity to Der p 1 challenges.

DNA-gene vaccination to treat IgE-mediated disease continues to garner attention. Behnecke et al⁸² created a fusion protein consisting of the human CHE2-4 linked to a polylysine tail. This positively charged tail binds to negatively charged DNA, such as plasmid DNAs. As a result, a construct fusion protein bound to plasmid encoding allergens or cytokines can be targeted to cells expressing FcεRI, such as dendritic cells and mast cells. However, encoding a dendritic cell-specific fascin promoter in the plasmid allows plasmid DNA transcription only in dendritic cells. The construct did not provoke mediator release from mast cells and basophils because it did not cross-link IgE. This is a promising therapeutic approach for allergic diseases.

Concerns regarding DNA vaccination include incorporation of plasmid DNA into the host genome, adverse immunopathological effects, and uncontrolled antigen exposure.

Roesler et al⁸³ created a viral replicase-based and non-replicase-based mRNA vaccine encoding timothy grass pollen (Phl p 5). In a murine model, vaccination with both constructs before sensitization diminished IgE production, skewed the immune response toward a T_H1 response, and alleviated allergic lung inflammation and airways hyperreactivity in a dose-dependent fashion. These data were replicated with 28 other allergens and shown to be as effective as dose-equivalent DNA vaccines on most parameters. Although mRNA vaccines lack risk regarding incorporation in the host genome and do not require a viral replicase, DNA vaccines do induce higher levels of antigen for longer durations.

CONCLUSION

Publications in upper airway disease further elucidated risk factors for allergic rhinitis, including antibiotic use in the first year of life, tobacco exposure, and cesarean delivery; described the natural history of nonallergic rhinitis and new IgE sensitization; and identified the contribution of local IgE production in the nasal cavity for allergic rhinitis in the absence of positive skin test results. Investigations into CRS identified a reduction in markers of Treg cells, a different inflammatory pattern depending on country of origin (Belgium vs China), and increased production of BAFF and decreased S100 proteins, implicating the epithelium in disease pathogenesis. Decrease in nasal NO levels was found to be a potential marker of acute sinusitis, and short courses of methylprednisolone and doxycycline were found to be efficacious in the treatment of CRSwNP. Investigations into the mechanism of immunotherapy continue to identify a central role of Treg cells and production of IL-10. Trials of SLIT monotherapy with grass pollen show efficacy in adults and children and a durable benefit. However, trials in the United States showed unimpressive clinical efficacy with ragweed monotherapy and multiextract SLIT. Lastly, new delivery systems and modifications of allergens are under investigation and show promise for future innovative allergen immunotherapy.

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