

Advances in mechanisms of asthma, allergy, and immunology in 2010

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2010 was marked by rapid progress in our understanding of the cellular and molecular mechanisms involved in the pathogenesis of allergic inflammation and asthma. Studies published in the *Journal of Allergy and Clinical Immunology* described advances in our knowledge of cells associated with allergic inflammation (mast cells, eosinophils, dendritic cells, and T cells), as well as IgE, cytokines, receptors, signaling molecules, and pathways. Studies used animal models, as well as human cells and tissues, to advance our understanding of mechanisms of asthma, eosinophilic esophagitis, food allergy, anaphylaxis and immediate hypersensitivity, mast cells and their disorders, atopic dermatitis, nasal polyposis, and hypereosinophilic syndromes. Additional studies provided novel information about the induction and regulation of allergic inflammation and the genetic contribution to allergic inflammation. Critical features of these studies and their potential effects on human atopic disorders are summarized here. (*J Allergy Clin Immunol* 2011;127:689-95.)

Key words: Allergic conjunctivitis, allergic inflammation, anaphylaxis, asthma, asthma genetics, atopic dermatitis, cytokines, eosinophils, food allergy, immediate hypersensitivity, mast cells, nasal polyposis, polymorphisms

2010 was marked by rapid progress in understanding the cellular and molecular mechanisms in the pathogenesis of asthma and other allergic disorders, as reflected by a large number of excellent articles published in the *Journal of Allergy and Clinical Immunology*. Many of the observations described in these articles

Abbreviations used

CSS: Churg-Strauss syndrome
cysLT: Cysteinyl leukotriene
DC: Dendritic cell
FOXP3: Forkhead box protein 3
HES: Hypereosinophilic syndrome
IPEX: Immune dysregulation, polyendocrinopathy, X-linked
ITIM: Immunoreceptor tyrosine-based inhibitory motif
LT: Leukotriene
NO: Nitric oxide
PAF: Platelet-activating factor
PG: Prostaglandin
SNP: Single nucleotide polymorphism
TSLP: Thymic stromal lymphopoietin

have potentially important clinical implications. Brief descriptions of some of the most important of these articles are now provided.

EOSINOPHILS AND INFLAMMATION

Cellular activation

Cell-free eosinophil granules are present extracellularly as intact membrane-bound organelles in sites associated with eosinophil infiltration, including asthma, but have unknown functional capabilities. Neves et al¹ reported that receptors for cysteinyl leukotrienes (cysLTs), cysLT1R, cysLT2 receptor, and the purinergic P2Y12 receptor, are expressed on eosinophil granule membranes. Notably, leukotriene (LT) C₄ and extracellularly generated LTD₄ and LTE₄ stimulated isolated eosinophil granules to secrete eosinophil cationic protein. Inhibitors of the P2Y12 receptor and cysLT1R inhibited these effects. These studies identify previously unrecognized sites of localization, the membranes of intracellular eosinophil granule organelles, and the function for cysLT-responsive receptors that mediate cysLT-stimulated secretion from within eosinophil granules, including those present extracellularly.

Migration

Although much progress has been made with understanding eosinophil recruitment into tissues, relatively little is known about mechanisms that inhibit eosinophil migration. Konya et al² reported the role of endothelial cell-derived prostaglandin (PG) I₂. PGI₂ markedly attenuated the migration of eosinophils *in vitro*, and this effect was inhibited by a PGI₂ receptor antagonist, as well as the adenylyl cyclase inhibitor SQ22536. Furthermore, when endothelial cells were treated with a COX inhibitor, eosinophil

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adhesion and migration were blocked. Inhibition of PGI₂ biosynthesis decreased the electrical resistance of endothelial monolayers and compromised the texture of adherent junctions. As such, these findings demonstrate that endothelium-derived PGI₂ might be fundamental for the maintenance of endothelial barrier function against infiltrating cells. These results suggest that selective PGI₂ receptor agonists might have beneficial effects in allergic inflammation.

Extending the role of endothelial cells in regulating eosinophil responses, Asosingh et al³ examined the ability of endothelial progenitor cells to induce eosinophil recruitment. They showed that circulating endothelial cell progenitors of asthmatic patients and mice with experimental asthma secreted high levels of eotaxin-1 and promoted eosinophil recruitment *in vivo*.

Diseases

Hypereosinophilic syndromes (HESs) are a heterogeneous group of disorders that have received recent attention because of active advances in understanding disease pathology and new approaches to therapy. Crane et al⁴ reported the crude incidence of HES as 0.035 cases per 100,000 and an age-adjusted rate of 0.036 per 100,000 person-years. These findings are likely underestimates and mainly limited to the more malignant variants of HES, such as the myeloproliferative types. In another article Helbig et al⁵ reported the characteristics and molecular causes of 88 patients with HES from Poland. They found marked patient heterogeneity, with 18% having molecular rearrangement involving the imatinib-inhibited activated tyrosine kinase fusion protein FIPIL1-PDGFR α .

Eosinophilic asthma has been proposed to be associated with asthma severity and represents a phenotype that is likely amenable to therapy with anti-IL-5 therapeutics. Al-Samri et al⁶ reported variability of sputum inflammatory cells in asthmatic patients receiving corticosteroid therapy using a prospective study. The investigators found substantial fractions of patients with moderate and severe asthma with sputum eosinophilia, neutrophilia, or both and noted that the inflammatory cells were not stable in nearly two thirds of the subjects over a 1-year period.

Nair et al⁷ were able to dissociate sputum eosinophilia from exhaled nitric oxide (NO). They reported the lack of correlation between sputum eosinophil percentages and exhaled NO levels in patients with prednisone-dependent asthma and sputum eosinophilia who participated in a clinical trial of anti-IL-5 mAb (mepolizumab).

Kahn et al⁸ reported a novel approach to treating Churg-Strauss syndrome (CSS) by studying a single patient with antineutrophil cytoplasmic antigen-negative disease treated with mepolizumab. They found a remarkable improvement in asthma symptoms, eosinophilia, and chest computed axial tomographic scan findings, highlighting the potential pivotal role of IL-5 and eosinophils in patients with CSS. Extending these findings, Kim et al⁹ reported that mepolizumab was effective in reducing steroid doses and eosinophil levels in 7 patients with CSS.

Furthering the focus on mepolizumab, Conus et al¹⁰ examined its effect on duodenal eosinophils in patients with eosinophilic esophagitis. The treatment had no effect on the duodenal infiltration of eosinophils, T cells, and mast cells in subjects participating in a small placebo-controlled clinical study.

Although typically associated with eosinophils, Abonia et al¹¹ demonstrated the presence of mastocytosis and mast cell

degranulation in patients with eosinophilic esophagitis. In addition, the investigators identified an esophageal mast cell-associated transcriptome that was significantly divergent from the eosinophil-associated transcriptome, with carboxypeptidase A3 mRNA levels serving as the best mast cell surrogate marker, and provided evidence for the involvement of KIT ligand in the pathogenesis of eosinophilic esophagitis. Extending the involvement of mast cells in eosinophilic esophagitis, Aceves et al¹² reported selective accumulation of mast cells in the esophageal smooth muscle. Furthermore, these investigators demonstrated that these mast cells produced TGF- β , which had the potential to induce smooth muscle contractility *in vitro*.

Swallowed glucocorticoids are known to be effective for the treatment of eosinophilic esophagitis, but the mechanism of their action has not been tested. Now Caldwell et al¹³ have presented evidence that swallowed steroids induce local effects in the esophagus based on the identification of corticosteroid-induced genes in the esophagus after treatment. Focusing on one steroid-induced transcript, the authors distinguish fluticasone responders from untreated patients with active eosinophilic esophagitis and patients without eosinophilic esophagitis. In addition, they determine that one steroid-induced transcript, FKBP51, reduces glucocorticoid-mediated inhibition of IL-13 signaling in epithelial cells *in vitro*, suggesting that FKBP51 might influence fluticasone propionate responsiveness. Additionally, they propose that esophageal FKBP51 levels have diagnostic and prognostic significance in patients with eosinophilic esophagitis.

DENDRITIC CELLS AND T LYMPHOCYTES

Dendritic cell promotion of inflammation

Comparison of inflammatory (CD11c⁺CD1c⁻) and resident (CD11c⁺CD1c⁺) skin dendritic cells (DCs) from patients with psoriasis by Zaba et al¹⁴ demonstrated increased expression of inflammation-related proteins, including TRAIL, Toll-like receptors 1 and 2, S100A12/ENRAGE, and CD32 by the CD1c⁻ DC population. These molecules, which are ligands for keratinocyte receptors, could allow inflammatory DCs to promote inflammatory disease through direct effects on keratinocytes. Esser et al¹⁵ demonstrated that nanovesicles (exosomes) released from human macrophages and DCs can contribute to inflammation by producing eicosanoids in response to appropriate stimuli and can synthesize LTC₄ from LTA₄.

Association of DC subpopulations with inflammation

Dua et al¹⁶ demonstrated that allergen challenge of asthmatic subjects increases the sputum numbers of both inflammation-associated myeloid DCs and tolerance-associated plasmacytoid DCs. Because DC expression of the $\alpha\gamma_2$ form of Fc ϵ RI facilitates DC allergen uptake and presentation, Chand et al¹⁷ evaluated the effects of 12 weeks of omalizumab (anti-IgE antibody) treatment on myeloid and plasmacytoid DCs in bronchial tissue of asthmatic subjects. They demonstrated that this treatment induced a selective decrease in airway myeloid DCs, with no decrease in plasmacytoid DC numbers.

Schroeder et al¹⁸ demonstrated that treatment of subjects with cat allergy with omalizumab (anti-IgE mAb) for 3.5 months decreased DC IgE and Fc ϵ RI expression and the ability of

allergen-exposed DCs to stimulate T-cell proliferation and increased the ability of allergen-exposed plasmacytoid DCs to suppress T_H2 and anti-inflammatory cytokines. This suggests that IgE “facilitates allergen presentation by dendritic cells *in vivo* and ... regulates DC-dependent T-cell cytokines during effector phases of allergic disease.”

Use of modified DCs to suppress inflammation

Suzuki et al¹⁹ showed that bone marrow–derived DCs in which DC CD40 expression was silenced by a small interfering RNA approach could be used to both prevent and suppress established disease in an antigen-specific manner in a murine model of allergic rhinitis.

Antigen presentation by DCs to T cells

Hulse et al²⁰ demonstrated that Fel d 1 modified to target it to DC FcγRI increased DC expression of the receptor for the T_H2 -promoting cytokine thymic stromal lymphopoietin (TSLP). Furthermore, a combination of TSLP and modified Fel d 1 synergistically stimulated a T_H2 cytokine response by T cells from subjects with Fel d 1 allergy, whereas modified Fel d 1 by itself stimulated these T cells to secrete IL-5 and IL-10. These observations demonstrate that FcγRI ligation can promote TSLP induction of allergic responses and raise cautions about the use of immunotherapeutics that are targeted to cellular receptors.

Studies by Mutschlechner et al²¹ of DC processing of the major birch pollen allergen Bet v 1 demonstrated that this allergen is relatively resistant to digestion by endosomal enzymes. Both early digestion products of Bet v 1 and Bet v 1–derived peptides eluted from DC MHC class II “matched known T-cell epitopes and showed T cell-activating capacity.” This suggests that the limited susceptibility of Bet v 1 to digestion is an important determinant of its antigenic epitopes.

Singh et al²² found that human rhinovirus infection–induced exacerbations of chronic obstructive pulmonary disease were associated with increased T_H1 and T_H2 cytokine production and found that human rhinovirus–encoded proteinase 2A–activated DCs induce both T_H1 and T_H2 responses by human T cells *in vitro*. Intranasal inoculation of mice with the same protein similarly induced airway hyperreactivity, lung inflammation, and T_H1 and T_H2 cytokine production. This suggests that adjuvant activity of proteinase 2A might contribute to chronic obstructive pulmonary disease exacerbations.

T-cell subpopulations

Both T_H2 and T_H17 cytokines are thought to contribute to the pathogenesis of bronchial asthma. Cosmi et al²³ detected a $CD4^+$ memory T-cell population that could secrete both T_H2 and T_H17 cytokines, demonstrated that this population is increased in the blood of subjects with chronic asthma, and showed that IL-4 can induce T_H2 cytokine production by T_H17 cells. This mixed T_H2/T_H17 T-cell population might be important in asthma pathogenesis.

Although the T_H2 -promoting cytokine TSLP is thought to act predominantly on DCs, Reefer et al²⁴ demonstrated that the receptor for this cytokine is present on a small subset of T cells. TSLP receptor is preferentially expressed on T cells that are undergoing mitosis and express IL-4. This suggests that a “direct action of TSLP on T cells could amplify T_H2 responses at sites of allergic inflammation.”

Statistical analysis of single nucleotide polymorphisms (SNPs) by Bottema et al²⁵ provided evidence that IL-2 receptor α (*IL2RA*), Toll-like receptor 2 (*TLR2*), TGF- β receptor 2 (*TGFBR2*), and forkhead box protein 3 (*FOXP3*), all genes that are involved in regulatory T-cell development and function, “associate with atopy and asthma by gene-gene interaction.”

Migration of T cells to inflammatory lesions

Ohmatsu et al²⁶ used $\beta7$ integrin–deficient mice, anti- $\alpha4\beta7$ integrin antibody, and a model of contact hypersensitivity to show that “ $\alpha4\beta7$ integrin contributes to contact hypersensitivity responses by regulating T-cell migration to inflammatory skin.”

Hasegawa et al²⁷ developed an *in vivo* imaging system and used it to analyze accumulation of antigen-specific $CD4^+$ T cells in the lungs of mice with allergic airway disease. Studies with this system demonstrated that intercellular adhesion molecule 1/vascular cell adhesion molecule 1–dependent foci of these antigen-specific cells develop in the lungs 6 to 20 hours after antigen inhalation and that these cells later colocalize with areas of eosinophil infiltration.

Regulation of T-cell responses

Sonkoly et al²⁸ observed increased expression of the microRNA miR-155 in infiltrating cells in atopic dermatitis lesions and showed that miR-155 downregulates T-cell expression of cytotoxic T lymphocyte–associated antigen 4. Because cytotoxic T lymphocyte–associated antigen 4 downregulates T-cell responses, the increased miR-155 expression in patients with atopic dermatitis might promote the severity and chronicity of this disease.

Ring et al²⁹ used a murine model of contact hypersensitivity to demonstrate that regulatory T cells that migrate to draining lymph nodes block the induction of $CD8^+$ T cell–dependent inflammation “by interfering with T-cell stimulatory activity of DCs.” Interference resulted from a gap junctional intercellular communication that downregulated DC surface expression of costimulatory molecules.

McMurchy et al³⁰ transduced human $CD4^+$ T cells with wild-type *FOXP3* or 3 point mutant forms of *FOXP3* that are associated with the immune dysregulation, polyendocrinopathy, X-linked (IPEX) syndrome to evaluate whether IPEX development could be explained solely by the inability of the mutant forms to suppress cytokine transcription and promote regulatory T-cell development. The results disproved this hypothesis, suggesting that additional factors are involved in IPEX pathogenesis.

MAST CELLS, BASOPHILS, AND IgE

Consequences of basophil and mast cell activation

Kajiwara et al³¹ presented convincing data that human mast cells express a receptor for platelet-activating factor (PAF), a molecule recently implicated as a major mediator in human anaphylaxis. They showed that PAF is capable of inducing mediator release from mast cells derived from cultures, as well as from human lung tissue, but not skin mast cells. Thus PAF might enhance anaphylaxis by directly activating mast cells.

Basheer et al³² showed that infusion of small peptides similar to pieces of fibrinogen (so-called haptides) caused hypotensive shock in rats and that this effect could be blocked by a combination of H1 and H2 antihistamines. These haptides caused degranulation of mast cells through serotonin type 2 receptors. A potentially clinically important aspect of this finding is that these haptides,

TABLE I. Proposed criteria for the diagnosis of mast cell activation syndrome*

1. Episodic symptoms consistent with mast cell mediator release affecting two or more organ systems evidenced as follows:
 - a. Skin: urticaria, angioedema, flushing
 - b. Gastrointestinal: nausea, vomiting, diarrhea, abdominal cramping
 - c. Cardiovascular: hypotensive syncope or near syncope, tachycardia
 - d. Respiratory: wheezing
 - e. Naso-ocular: conjunctival injection, pruritus, nasal stuffiness
2. A decrease in the frequency or severity; or resolution of symptoms with anti-mediator therapy: H1 and H2 histamine receptor antagonists, anti-leukotriene medications (cysLT receptor blockers or 5-LO inhibitor), or mast cell stabilizers (cromolyn sodium)
3. Evidence of an elevation in a validated urinary or serum marker of mast cell activation: Documentation of elevation of the marker above the patient's baseline during a symptomatic period on at least two occasions; or if baseline tryptase levels are persistently >15ng, documentation of elevation of the tryptase above baseline on one occasion. Total serum tryptase is recommended as the marker of choice; less specific (also from basophils) 24 hour urine histamine metabolites, or PGD₂ or its metabolite 11- β -prostaglandin-F₂.
4. Rule out primary and secondary causes of mast cell activation and well defined clinical idiopathic entities.

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*Mast cell activation syndrome for now remains an idiopathic disorder; however, in some cases it could be an early reflection of a monoclonal population of mast cells, in which case with time it could meet the criteria for MMAS as 1 or 2 minor criteria for mastocytosis are fulfilled.

which can be generated during streptokinase fibrinolysis of clots, might cause hypotension through mast cell activation.

Akin et al³³ proposed diagnostic criteria for "mast cell activation syndrome," for which there has been no consensus definition. Mast cell activation syndrome, in short, is characterized by symptoms consistent with mast cell mediator release, clinical improvement with antimediator therapy, lack of primary and secondary causes of mast cell activation, and evidence of an increase in at least 1 serum or urine marker of mast cell activation (Table I). It will be interesting to see whether these proposed criteria become widely accepted and permit further study and delineation of mast cell pathophysiology.

Eckman et al³⁴ reported data that implicated the basophil, rather than the mast cell, in acute nasal challenge responses in human subjects. These challenges were performed with cat allergen in subjects with cat allergy receiving omalizumab but were done at a time point relatively early on in omalizumab therapy when basophil, but not mast cell, responses were diminished. A 50% to 60% decrease in allergen-induced nasal symptoms and sneezes was seen with omalizumab at a time when only basophil function was reduced.

Finally, Qi et al³⁵ report that human basophils are a major source of amphiregulin, a cytokine in the epidermal growth factor family. This finding was rather unexpected because in the mouse T_H2 cells are the primary source of amphiregulin. Amphiregulin production by basophils was strongly enhanced by IL-3, which was a stronger stimulus than IgE receptor activation. The potential significance of this finding is that basophil-derived amphiregulin could be involved in tissue remodeling associated with chronic allergic diseases.

IgE and allergy

Bønnelykke et al³⁶ challenged existing dogma that IgE in cord blood is produced by the fetus and is as a marker of risk for atopy by reporting that for nearly half of cord blood samples with an increased IgE level, this presence was probably because of maternal-fetal transfer, and therefore prior data interpretation might be flawed. Hong et al³⁷ examined SNPs in genes involved in T_H2 biology and cord blood IgE levels. They identified several SNPs in the *IL13* gene, IL-13 receptor (*IL13R*) gene, and signal transducer and activator of transcription 6 (*STAT6*) gene, as well as SNPs in the *GATA3* and *IL5* gene, that were influenced by ethnicity. Their conclusion is that there might be *in utero*

genetic influences on postpartum IgE production and therefore the propensity for atopic diseases.

Milovanovic et al³⁸ reported that the active form of vitamin D (calcitriol) negatively regulates IgE production at the level of the ϵ -germ line gene promoter. They showed *in vitro* that this is due to the ability of calcitriol to alter chromatin compacting around a region containing the ϵ -germ line gene. Whether vitamin D supplementation reduces IgE production *in vivo* remains to be seen.

Schouten et al³⁹ followed up on previous work suggesting that at least in murine models certain forms of allergic sensitization elicit a strong IgE response, whereas others instead elicit the production of so-called immunoglobulin free light chains. They now show that these molecules, but not IgE, are induced in casein-sensitized mice, whereas mice sensitized to whey had traditional IgE antibodies. Some children with cow's milk allergy had increased levels of these immunoglobulin free light chains, but their exact role diagnostically and therapeutically remain to be elucidated.

Finally, Wang et al⁴⁰ defined the role of mast cells, cell-surface receptors, and cytokines in a murine model of peanut gastrointestinal allergy manifesting with diarrhea and weight loss. Mice deficient in Fc ϵ RI had decreased goblet cells and intestinal inflammation along with decreased levels of T_H2 cytokines. The full inflammatory response was restored by passively transferring normal mast cells, but transfer of mast cells from Fc ϵ RI-deficient, IL-13-deficient, or dual IL-4/IL-13-deficient mice failed to restore the response.

Novel mechanisms learned from studies targeting mast cells, basophils, IgE, and their mediators

Massanari et al⁴¹ studied whether omalizumab would make specific injection immunotherapy safer. Subjects receiving omalizumab experienced about half as many allergic reactions to immunotherapy as those receiving placebo. The number of reactions affecting the airway was reduced by about 75%, even though 2 subjects in each group had severe reactions. Use of omalizumab made it slightly more likely that subjects would successfully reach maintenance doses. Whether omalizumab use is practical, cost-effective, and leads to better long-term immunotherapy outcomes remains unknown.

Staevska et al⁴² explored the usefulness of up to 4 times the recommended doses of levocetirizine and desloratadine in subjects with severe urticaria. Encouragingly, about 35% of subjects became

TABLE II. Summary of selected key advances in mechanisms of asthma, allergy, and immunology reported in the *Journal of Allergy and Clinical Immunology* in 2010

Topic	Advances and observations
Antihistamines and chronic urticaria	Using 4 times the recommended dose of either desloratadine or levocetirizine improved quality of life without worsening drowsiness in 75% of subjects with refractory chronic urticaria.
Anti-IgE and DCs	Treatment of human asthmatic subjects with omalizumab (anti-IgE mAb) for 12 weeks decreased the percentage of myeloid DCs, which are associated with lymphocyte activation, but not plasmacytoid DCs, which are associated with lymphocyte suppression, in bronchial tissues. This might be an additional mechanism for suppression of allergic disease by omalizumab.
Basophils and sneezing	Basophils, rather than mast cells, appear to be the cellular source of mediators triggering sneezes in acute nasal allergen challenge responses in human subjects.
Eosinophils	Eosinophil cytoplasmic granules generate cysLTs.
IL-9	Studies in murine models suggest that anti-IL-9 limits the extent of anaphylaxis associated with food allergy but not allergy induced by systemic antigens.
Immunotherapy	A naive Bayes prediction model using 18 genes demonstrated a profile that was present in patients with successful venom immunotherapy.
Mast cells	- Mast cell involvement in patients with eosinophilic esophagitis has been identified. - A link has been defined between mast cells, TGF-β, and smooth muscle in patients with eosinophilic esophagitis.
TSLP and T _H 2	The receptor for TSLP is preferentially expressed on a small subset of CD4⁺ T cells that are dividing and expressing IL-4. This suggests that TSLP promotes a T_H2 response by stimulating these cells in addition to its well-established effects on DCs.

symptom free on 2 to 4 times the recommended doses. Among the remaining patients, they reported that about 25% nonresponsive to high-dose desloratadine were responsive to levocetirizine, even though none of the levocetirizine nonresponders improved when switched to desloratadine. Overall, 75% responded to either desloratadine or levocetirizine at 4 times the recommended dose and with improved quality of life without worsening drowsiness.

As part of the Eckman study mentioned previously, Zaidi et al⁴³ used this same cohort of subjects to explore the effect of omalizumab on Fc ϵ RI subunits and the consequences of this regulation on downstream intracellular signal transduction molecules, such as Syk and others. The authors found that basophils from subjects receiving omalizumab displayed an enhanced degranulation response to anti-IgE, even though cat allergen-induced responses were blunted. This 2-fold increase in histamine induced by anti-IgE antibody while receiving omalizumab was associated with about a 2-fold increase in levels of Syk per basophil but not in other signaling elements. There was also a nearly two-thirds reduction in the ratio of the β chain to the α chain in the high-affinity receptors. This suggests that omalizumab results in an increase in overall basophil stimulation sensitivity, despite the reduction in circulating and cell-surface IgE.

CYTOKINES AND ALLERGIC INFLAMMATION IL-4

Recent studies have demonstrated inconsistent benefit in human asthmatic subjects when targeting either IL-4 or the IL-4 receptor α (which blocks both IL-4 and IL-13 pathways). Studies by Kirstein et al⁴⁴ using mutant mice selectively deficient in IL-4 receptor α on smooth muscle cells demonstrated that these mice did not have any reduction in airway responsiveness after allergen challenge, suggesting that IL-4 and IL-13 signaling through smooth muscle IL-4 receptor α was not essential in inducing airway responsiveness.

In studies of the proliferative capacity of human smooth muscle, Hassan et al⁴⁵ demonstrated that proliferating nuclear antigen was a highly sensitive marker of proliferation in biopsy specimens and that heparin-binding epidermal growth factor was upregulated in airway smooth muscle in biopsy specimens from subjects with severe asthma.

In contrast to results of studies with mutant mice deficient in IL-4 receptor α on smooth muscle cells, Tachdjian et al⁴⁶ demonstrated an important role for immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic tail of IL-4 receptor α in contributing to airway inflammation and airway responsiveness. They used targeted mutagenesis of the murine IL-4 receptor α to inactivate an ITIM domain. These IL-4 receptor α cytoplasmic tail Y709 ITIM mutant mice exhibited exaggerated allergen-induced eosinophilic airway inflammation and hyperreactivity, suggesting a physiologic negative regulatory role for the Y709 ITIM in signaling through the IL-4 receptor α . Thus both murine and human studies provide variable evidence regarding the benefit of targeting the IL-4 receptor α in asthma.

IL-5

Although initial human studies with anti-IL-5 suggested minimal benefit in asthmatic subjects, more recent studies have demonstrated a benefit of anti-IL-5 as a corticosteroid-sparing agent in subjects with HES, as well as a benefit in a subset of patients with asthma associated with significant eosinophilia. In a phase I study Busse et al⁴⁷ demonstrated the safety of administration of a single dose of an anti-IL-5 receptor α antibody in reducing eosinophil levels for 2 to 3 months. In studies in nonhuman primates Kolbeck et al⁴⁸ demonstrated that the anti-IL-5 receptor α antibody depleted eosinophil precursors in the bone marrow. *In vitro* studies demonstrated that the anti-IL-5 receptor antibody inhibited IL-5-mediated cell proliferation and induced antibody-dependent cell-mediated cytotoxicity of eosinophils,⁴⁸ an effect not observed with antibodies targeting IL-5 rather than the IL-5 receptor. Further studies are needed to determine whether targeting the IL-5 receptor α will be similar or more effective than targeting IL-5 in subjects with asthma and other eosinophil-associated disorders.

IL-6

Studies by Peters et al⁴⁹ demonstrated that IL-6 protein levels were significantly increased in subjects with chronic rhinosinusitis with nasal polyps compared with those seen in control groups

(subjects with chronic rhinosinusitis without nasal polyps and healthy control subjects). In addition, levels of the soluble IL-6 receptor were also increased in nasal polyps. Despite increased levels of IL-6 and its receptor, there were no increased levels of phosphorylated signal transducer and activator of transcription 3 (through which IL-6 signals) in nasal polyps. Detection of increased nasal polyp levels of soluble gp130,⁴⁹ a known inhibitor of IL-6 signaling, might thus reduce IL-6 signaling in nasal polyps and limit the ability of IL-6 to be an inflammatory stimulus in polyps.

IL-9

Studies by Osterfeld et al⁵⁰ demonstrated differential involvement of the IL-9/IL-9 receptor pathway in subjects with systemic anaphylaxis compared with oral antigen-induced anaphylaxis using murine models. Oral ovalbumin-induced anaphylaxis was IgE dependent and attenuated in IL-9- or IL-9 receptor-deficient mice. In contrast, systemic ovalbumin-induced anaphylaxis was independent of the IL-9 or IL-9 receptor pathway. These studies suggest that anti-IL-9 might be a useful adjunct in limiting the extent of anaphylaxis associated with food allergy but not with allergy induced by systemic antigens, such as insect venoms.

IL-13

Studies by Semlali et al⁵¹ suggest that IL-13 expression by epithelial cells might play an important role in airway epithelial cell proliferation and epithelial injury repair, with TSLP being an important stimulus for induction of IL-13 expression by epithelial cells. Wadsworth et al⁵² demonstrated that IL-13 also triggers matrix metalloproteinase 7-mediated Fas ligand cleavage from bronchial epithelial cells, providing another mechanism for IL-13 to contribute to airway epithelial damage and inflammation. In addition to airway epithelial cells being an important source of IL-13, as well as TSLP, Prefontaine et al⁵³ demonstrated that airway epithelium is an important source of IL-13, an inducer of T_H2 responses.

GENES AND ALLERGIC INFLAMMATION

Several studies investigated the importance of gene SNPs, gene methylation, and gene expression profiles in allergic inflammation. For example, Wise et al⁵⁴ demonstrated that an SNP in the chemokine receptor *CCR3* gene ablates receptor export to the plasma membrane and that targeting this pathway might provide a novel way to inhibit *CCR3* function.

Studies by Munthe-Kaas et al⁵⁵ investigated associations between CD14 methylation, *CD14* SNPs, and soluble CD14 levels in a birth cohort followed to age 10 years. CD14 methylation increased significantly from age 2 to age 10 years and was inversely correlated with soluble CD14 levels at age 10 years. Further studies are needed to determine the role of epigenetic effects on gene-environment interactions in allergy.

Studies by Niedoszytoko et al⁵⁶ used gene expression profiles of whole blood to predict the long-term effect of venom immunotherapy. A naive Bayes prediction model using 18 genes demonstrated a gene expression profile that was present in all patients with successful venom immunotherapy (n = 17) but absent in all subjects with failure of venom immunotherapy (n = 12). Further prospective studies are needed to validate these results and determine their value in immunotherapy with allergens other than venom.

Conclusion

The articles we have discussed highlight the important advances in our understanding of mechanisms of allergic inflammation published in the *Journal of Allergy Clinical Immunology* in 2010 that contribute to insights into potential novel therapies and biomarkers of allergic diseases (Table II).

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