

Advances in mechanisms of asthma, allergy, and immunology in 2011

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2011 was marked by rapid progress in the identification of basic mechanisms of allergic disease and the translation of these mechanisms into human cell systems. Studies published in the *Journal of Allergy and Clinical Immunology* this year provided new insights into the molecular determinants of allergenicity, as well as the environmental, cellular, and genetic factors involved in sensitization to allergens. Several articles focused on mechanisms of allergen immunotherapy and the development of novel strategies to achieve tolerance to allergens. Additional studies identified substantial contributions from T_H17-type cells and cytokines to human disease pathogenesis. Finally, new therapeutic applications of anti-IgE were identified. The highlights of these studies and their potential clinical implications are summarized in this review. (*J Allergy Clin Immunol* 2012;129:335-41.)

Key words: Galactose- α -1,3-galactose, cyclic AMP, calcitonin gene-related peptide, dendritic cell, genome-wide association study, facilitated antigen binding, facilitated antigen presentation, interferon, lipopolysaccharide, toluidine diisothiocyanate, Toll-like receptor, vascular endothelial growth factor

It is now clear that allergic disease results from the subversion of the innate immune system by allergens and their associated chemical constituents to promote sensitization. The likelihood that such subversion will occur is dictated by the timing of exposure, the age and genetic makeup of the subject, and the milieu of factors that can also influence the nature of the immune response, including the diversity of commensal microbes.

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Abbreviations used

α -Gal:	Galactose- α -1,3-galactose
DC:	Dendritic cell
FAP:	Facilitated antigen presentation
GSDMA:	Gasdermin A
ITGB3:	β_3 integrin gene
NADPH:	Reduced nicotinic adenine dinucleotide phosphate
ORMDL3:	Orosomucoid 1-like 3 gene
slanDC:	DC that expresses the 6-sulfo-LacNAc residue on P-selectin glycoprotein ligand 1
SNP:	Single nucleotide polymorphism
TDI:	Toluidine diisothiocyanate
TLR:	Toll-like receptor
Treg:	Regulatory T

A challenge for the specialty of allergy is to harness this knowledge for the treatment and prevention of disease. This review summarizes the studies in the areas that represent, in the opinion of the editors, the most substantive advances in mechanisms of allergic disease published in the *Journal of Allergy and Clinical Immunology* during 2011 (Table I). These include the structures integral to allergens that permit them to break tolerance and cause sensitization, the dose and timing of exposure that are optimal for sensitization, and host factors and innate defense systems that facilitate sensitization and subsequent disease. Additionally, substantial progress was made in understanding the mechanisms for the efficacy of immunotherapy, as well as the development of engineered recombinant molecules that might ultimately lead to improved immunotherapeutic strategies.

MOLECULAR DETERMINANTS OF ALLERGENICITY Direct activation of keratinocytes by dust mite allergens

Transcutaneous sensitization to allergens might be a critical step in the pathogenesis of atopy. Dai et al¹ demonstrated that primary human keratinocytes responded to extracts from both *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* by releasing preformed IL-1 β and IL-18, cytokines that characteristically are secreted in response to activation of the Nlrp3 inflammasome. By knocking down the individual components of the inflammasome through RNA interference, the authors determined that the release of IL-1 β and IL-18 depended on the Nlrp3 inflammasome. The active components of the dust mites that were responsible for activating the inflammasome were cysteine proteases. This study suggests that protease-dependent mechanisms for inflammasome activation might be especially relevant to dust mite sensitization and dust mite-induced inflammation in the skin.

Pollen-associated adenosine as a modulator of dendritic cell activation

Gilles et al² used a metabolomic analysis of a water-soluble fraction of birch pollen (and other tree pollen) extracts to identify adenosine as the component responsible for stimulating the accumulation of cyclic AMP in LPS-stimulated dendritic cells (DCs). The ability of the aqueous pollen extracts to induce cyclic AMP accumulation and to suppress the production of IL-12p70 by LPS-stimulated human monocyte-derived DCs was blocked by treatment of the extracts with adenosine deaminase or by pharmacologic blockade of adenosine-specific A₂ receptors. DCs derived *in vitro* from nonatopic donors that were exposed to aqueous pollen extracts suppressed IFN- γ production and enhanced IL-10 production by naive allogeneic T cells in an adenosine deaminase-sensitive manner. In contrast, pollen extract treatment of DCs from atopic donors failed to potentiate IL-10 production. Thus pollen-associated adenosine might facilitate the induction of tolerance in nonatopic subjects through effects on DCs. The molecular basis for the lack of this facilitation in cells from atopic subjects remains to be determined.

Dose and timing of exposure determine risk of sensitization to murine allergens

Peng et al³ studied the evolution of mouse-specific IgE and IgG₄ levels in a prospective cohort of 179 new employees at a mouse facility. Exposure to murine allergen (Mus m 1) was monitored by using ELISA measurements from extracts obtained from filters used on personal air samplers taken every 6 months. At the end of 24 months, 23% of the workers had mouse-specific IgE, as determined by skin prick test responses, whereas only 8% had mouse-specific IgG₄. The risk of IgE sensitization was highest in workers who had an intermediate average level of exposure to airborne Mus m 1, with much lower levels of sensitization among workers with either very low or very high levels of exposure. Additionally, subjects with the lowest levels of variability in Mus m 1 exposure had higher rates of sensitization than those with greater levels of variation. In contrast, the appearance of mouse-specific IgG₄ was most likely in subjects with the highest levels of Mus m 1 exposure and with the highest degree of intermeasurement variability of exposure. Thus both the total dose and the variability of dosing are important determinants of whether an airborne allergen promotes a pathogenic T_H2 response or confers a tolerogenic response.

Sensitization and pulmonary inflammation in response to toluene diisothiocyanate requires oxidative stress

Toluidine diisothiocyanate (TDI) is a low-molecular-weight sensitizing agent that is the most frequent cause of occupational asthma.⁴ Liu et al⁵ studied the role of oxidative stress in a murine model of TDI-induced pulmonary inflammation. Mice lacking reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme needed for leukocyte respiratory burst, were compared with wild-type C57BL/6 control animals in a model of pulmonary inflammation induced by intrapulmonary sensitization to TDI, followed by inhalation challenge. TDI induced pulmonary inflammation, oxidative stress, airway hyperresponsiveness, TDI-specific IgE, and the production of IFN- γ and IL-17A in the lung. All of these features were attenuated or

eliminated by the deletion of NADPH oxidase or by treatment of the mice with the superoxide scavenger N-acetyl cysteine. In contrast, ovalbumin-induced pulmonary pathology occurred independently of NADPH oxidase and reactive oxygen species. The pathologic changes in the lung and the airway hyperresponsiveness depended on the induction of IL-17A and T cells. This study suggests that antioxidants can be useful in the treatment or prevention of TDI-induced lung disease.

NOVEL ASPECTS OF IgE IN DIAGNOSTICS AND IMMUNOTHERAPY

Efficacy of omalizumab in patients with chronic urticaria

In a study by Maurer et al,⁶ 42 subjects with IgE autoantibodies to thyroperoxidase who were still symptomatic despite aggressive therapy with H₁ antihistamines were given standard asthma omalizumab dosing in a double-blind placebo-controlled study. At 24 weeks, 70% of subjects were wheal free with the active drug compared with 5% of subjects receiving placebo. Interestingly, more than 50% of this improvement occurred by week 2, and less than 10% of patients receiving the active drug still had severe disease compared with more than 30% receiving placebo by the end of the study.

Saini et al⁷ enrolled 90 patients with inadequately controlled chronic urticaria independent of any clinical or immunologic parameters that were inadequately responsive to H₁ antihistamines. This was a single-dose study in which placebo or omalizumab was administered at 3 set amounts (75, 300, or 600 mg) independently of baseline IgE levels, which ranged from 2 to more than 3000 IU/mL. At week 4, the 300-mg dose worked best in that 60% of patients showed 75% improvement in their clinical scores compared with 10% of patients showing this degree of improvement after placebo. More than half of the subjects experienced their maximum improvement within 1 week of dosing, which coincided with peak plasma levels. For both the Maurer et al⁶ and Saini et al⁷ studies, omalizumab was well tolerated.

New insights into approaches and mechanisms of immunotherapy

James et al⁸ studied subjects who had been treated for 2 years with grass pollen immunotherapy, at which time they were randomized to either 2 more years of continued active immunotherapy or 2 years of placebo. At the end of 4 years, they measured grass pollen-specific IgG₁ and IgG₄ levels, as well as results of facilitated allergen binding, an *in vitro* assay for IgG-dependent inhibitory activity against IgE-allergen complex binding to B cells through CD23. Grass pollen symptoms remained well controlled in both groups, even though levels of grass pollen-specific IgG₁ and IgG₄ returned to near-baseline levels in the 2-year placebo group but persisted in the 4-year active group. Most importantly, using their facilitated antigen-binding assay, they showed persistent blocking activity in both groups. Thus 2 years of immunotherapy generates a small amount of allergen-specific blocking IgG that could be mediating clinical tolerance.

Subcutaneous allergen-specific immunotherapy has an early onset of action but has a limited use in pediatric patients because of the need for repeated injections and safety concerns. Meanwhile, the improved safety profile of the sublingual route is becoming an accepted alternative despite its relatively late onset

TABLE I. Summary of selected key advances in mechanisms of asthma, allergy, and immunology reported in the *Journal of Allergy and Clinical Immunology* in 2011

Topic	Advances and observations
Molecular determinants of allergenicity	● House dust mite–derived proteases activate the Nlrp3 inflammasome in keratinocytes. ● Tree pollen–associated adenosine promotes a tolerogenic response in DCs from nonatopic but not atopic donors.
Novel aspects of IgE in diagnostics and immunotherapy	● Anti-IgE is efficacious in the treatment of chronic urticaria. ● IgE to α-Gal develops after tick bites and cross-reacts with cat IgA (Fel d 5), creating false-positive serum IgE test results to cat.
Cytokine biology	● T_H17 cytokines are found in brain lesions and peripheral blood lymphocytes from patients with Behçet disease. ● Human T_H17 cells express the IL-13Rα1 chain and respond to IL-13 with suppression of IL-17 production.
Genetic and environmental factors involved in susceptibility to allergic disease	● Variants of the <i>ITGB3</i> gene influence the risk of atopic sensitization to molds. ● Diversity of enteric flora in infancy is inversely related to risk of subsequent atopic sensitization, allergic rhinitis, and eosinophilia.

of action. Keles et al⁹ report the efficacy and safety of immunotherapy using a combination of the subcutaneous route in the build-up phase and sublingual maintenance in comparison with the sublingual or subcutaneous routes alone. In a study of 51 pediatric patients with house dust mite sensitization, the subcutaneous immunotherapy route, either alone or in combination with sublingual therapy, decreased the number of asthma attacks or inhaled corticosteroid use. Mechanistically, increases in the levels of regulatory and T_H1 cytokines were observed both in the subcutaneous and sublingual groups. Antigen-specific IgG₄ levels were only increased when subcutaneous immunotherapy was used alone or with sublingual therapy. Notably, clinical symptom scores correlated positively with IL-5 levels and negatively with antigen-specific IgG₄, IFN- γ , and TGF- β levels. This report demonstrates the potential attractiveness of immunotherapy that is composed of early subcutaneous doses followed by the addition of sublingual therapy.

Dreschler et al¹⁰ analyzed the number and characteristics of blood DC subsets in patients with Hymenoptera venom allergy before and after initiation of specific immunotherapy. Accordingly, blood myeloid and plasmacytoid DCs were analyzed in 20 patients with Hymenoptera venom allergy (bee or wasp venom) by using 4-color flow cytometry. They found that in patients with Hymenoptera venom allergy, the number of plasmacytoid DCs transiently decreased after immunotherapy. However, myeloid DC numbers were increased in patients with Hymenoptera venom allergy before, during, and after immunotherapy. There were notable changes in the expression of function-associated surface molecules on myeloid DCs (eg, Fc γ receptor 2 and Toll-like receptor [TLR] 2) during immunotherapy. These findings begin to unravel the mechanisms of venom immunotherapy.

Genetically engineered allergens for immunotherapy

Niespodziana et al¹¹ created a new potential reagent for cat allergen immunotherapy based on Fel d 1 designed to eliminate both allergic and T cell–mediated side effects. They engineered a recombinant fusion protein made up of N-terminal pieces of Fel d 1 chain 1 and C-terminal pieces of Fel d 1 chain 2 fused to the PreS domain of the hepatitis B virus, the latter having

been used safely in human subjects in other studies to provide enhanced T-cell help to immunization. This structure failed to bind IgE in the sera of many donors with cat allergy, suggesting good safety potential, whereas injection into animals resulted in strong IgG antibody responses. The IgG blocked IgE binding to Fel d 1 by about 60% and created a 1000-fold reduction in the ability of Fel d 1 to mediate basophil CD203C upregulation, representing encouraging preclinical safety and efficacy characteristics.

Wallner et al¹² created a recombinant Bet v 1 homologue (termed BM4) containing a 7-amino-acid substitution with a sequence derived from the structurally similar region of the apple allergen Mal d 1. This substitution resulted in a molecule that lacked a characteristic fold and that did not form the oligomers or high-molecular-weight aggregates typical of native Bet v 1. Moreover, BM4 exhibited markedly lower affinity binding to IgE than native Bet v 1 and impaired the ability to stimulate the activation of basophils from donors with Bet v 1 allergy. Despite impaired IgE-binding capabilities, BM4 elicited stronger proliferative responses in PBMCs obtained from sensitized patients than Bet v 1. When used to immunize BALB/c mice, BM4 elicited both T_H1 and T_H2 immune responses, whereas native Bet v 1 elicited only T_H2 responses. BM4 was a more potent stimulus for the maturation of murine bone marrow–derived DCs than Bet v 1 and was also more resistant to degradation by DC-derived endolysosomal enzymes. Thus a subtle change in the amino acid sequence of a classical allergen can markedly alter its physicochemical properties, in turn leading to dramatic alterations in the nature of the recognition by the innate immune system and its consequent effect on the adaptive response. These findings might carry implications for the design of recombinant allergens for immunotherapy.

Application of regulatory T-cell induction for immunotherapy

Regulatory T (Treg) cells are now regarded as *bona fide* suppressor cells involved in regulating homeostatic, inflammatory, and autoimmune states. Schwarz et al¹³ aimed to determine whether Treg cells can be induced by UV radiation in sensitized mice and manipulated to suppress the elicitation of immune responses. Treg cells inhibited the elicitation of cutaneous hypersensitivity responses in sensitized mice when stimulated by means of

an antigen-specific boost through the skin. This required cutaneous antigen-presenting cells that altered the migratory behavior of Treg cells and drove them out of the lymph nodes into the skin. Because this process was achieved entirely *in vivo* without invasive interventions, these findings have implications for strategies aiming to induce and use Treg cells in therapeutic settings.

IgE biology and diagnostics

Holm et al¹⁴ used a unique repertoire of IgE reagents to study facilitated antigen presentation (FAP), the process by which soluble IgE-allergen complexes can be captured and internalized by CD23 on antigen-presenting B cells, leading to presentation of allergen peptide to CD4⁺ T cells with subsequent T-cell activation and proliferation. Although allergen-IgE complex formation is particularly influenced by IgE clonality, FAP activity correlates with IgE affinity and IgE complexity, meaning that the higher the IgE affinity and the greater the diversity of IgE and epitopes recognized, the greater the T-cell proliferative FAP response. Such IgE characteristics might help to explain differences in both acute and chronic allergic disease manifestations among patients.

A cluster of cases of delayed-onset anaphylaxis after the ingestion of beef, lamb, or pork were described in association with the presence of IgE antibodies to the mammalian protein-associated oligosaccharide galactose- α -1,3-galactose (α -Gal).¹⁵ Commins et al¹⁶ reported a potential explanation for the remarkably geographically restricted pattern of α -Gal sensitization. The presence of α -Gal-specific IgE in sera was strongly associated with self-reported tick bites, and the prevalence of α -Gal-specific IgE antibodies in serum samples correlated markedly with the geographic distribution of the tick *Amblyomma americanum*. The levels of IgE antibodies to α -Gal correlated strongly with the levels of IgE antibodies to *A americanum* in the same serum samples. Three subjects who were bitten by *A americanum* were followed prospectively, and all 3 had α -Gal-specific IgE antibodies. This study suggests that the adaptive immune response to carbohydrate structures on ticks and other ectoparasites has the potential, through cross-reactivity, to cause allergic diseases.

In addition to delayed anaphylaxis, antibodies to α -Gal might affect diagnostic testing to other allergens. Grönlund et al¹⁷ recently reported that cat IgA (Fel d 5) is an allergen that contains α -Gal. This raised the question of whether diagnostic tests for IgE to cat dander detect IgE to Fel d 5, α -Gal on Fel d 5, or both. Arkesteal et al¹⁸ reported that most serum samples obtained from parasite-infected subjects in Zimbabwe have IgE to α -Gal and Fel d 5, with a strong correlation between reactivity to cat dander extract and both α -Gal and Fel d 5 rather than Fel d 1. In contrast, most serum samples from nonparasitized subjects with cat allergy contained IgE to Fel d 1, and IgE levels to cat dander extract correlated with IgE levels to Fel d 1 but not to Fel d 5 or α -Gal. One important conclusion of this work is that when testing for anti-cat IgE in parasitized patients, some of the anti-cat IgE might be directed to α -Gal on Fel d 5. Another curious aspect of this work is that the African subjects with IgE to α -Gal do not report any food-related anaphylaxis when ingesting mammalian meat.

Basic mechanisms of anaphylaxis

IgE-dependent reactions obey a diurnal variation pattern. Nakamura et al¹⁹ studied the role of *Period2*, a gene that regulates

circadian function, in a model of passive cutaneous anaphylaxis. The diurnal rhythm that is normally present for IgE-mediated immediate-type skin reactions in mammals was absent in mice deficient in *Period2*. The similar loss of diurnal rhythm in adrenalectomized and aged mice (which lose diurnal variation in glucocorticoid secretion) and the loss of sensitivity to the inhibitory effects of dexamethasone in *Period2*-deficient mice suggest that *Period2* might act by controlling the rhythm of adrenal secretion of glucocorticoids, mast cell responses to glucocorticoids, or both.

ADVANCES IN CYTOKINE BIOLOGY

T_H17 cells in human disease

T_H17 cells represent a lineage of effector T cells that play important roles in murine models of autoimmune disease. Geri et al²⁰ found increases in both T_H17 cell numbers and IL-21 secretion in inflammatory brain lesions of patients with Behçet disease. *In vitro* anti-CD3/anti-CD28 stimulation of peripheral blood lymphoid cells from patients with Behçet disease induced more T_H17 cells and fewer Treg cells than did similar stimulation of peripheral blood lymphoid cells from healthy subjects. Serum from patients with Behçet disease contained increased IL-21 levels and promoted T_H17 differentiation and suppressed Treg cell differentiation by normal lymphocytes; this effect was inhibited by an IL-21 antagonist.

Psoriasis might be promoted by a population of DCs that express the 6-sulfo-LacNAc residue on P-selectin glycoprotein ligand 1 (sLanDCs). Hansel et al²¹ demonstrated that sLanDCs secrete large amounts of IL-12, IL-23, IL-1 β , IL-6, and TNF when stimulated with nucleic acid-containing ligands for TLRs. sLanDCs were found in large numbers in psoriatic skin lesions and were likely recruited to the skin by C5a, CX3CL1, and CXCL12. Importantly, sLanDCs program the T_H17/T_H1 T cells that contribute to the pathogenesis of psoriasis by secreting IL-17, IL-22, TNF, and IFN- γ .

Two additional articles demonstrated mechanisms that can limit T_H17 responses in human subjects. In one article Maddur et al²² demonstrated that intravenous IgG acted through its F(ab')₂ moiety to inhibit the *in vitro* differentiation and expansion of human T_H17 cells and production of their characteristic cytokines. In a second article Newcomb et al²³ demonstrated that human *in vitro*-generated T_H17 cells, but not other CD4⁺ T cells, express the IL-13R α 1 chain of the type 2 IL-4 receptor, which allows them to respond to IL-13. The presence of IL-13 during the differentiation or restimulation of T_H17 cells decreased IL-17A production and inhibited the expression of T_H17-associated transcription factors. Thus the dominant effector of T_H2 immunity, IL-13, might downregulate the contributions from the T_H17 pathway.

Relationship between T_H17 and other T_H cell responses in murine models

Raymond et al²⁴ demonstrated that intratracheal or intravenous administration of ovalbumin-loaded bone marrow-derived DCs to mice promoted the development of ovalbumin-specific CD4⁺ T cells in the lungs and mediastinal lymph nodes that shared T_H2 and T_H17 characteristics. These cells induced airway inflammation that was characterized by infiltration of both neutrophils and eosinophils and was IgE independent and steroid resistant.

Albrecht et al²⁵ demonstrated that T_H17 cells in the lungs can perpetuate and expand T_H1 and T_H17 responses. IL-17A-secreting T_H17 cells that are specific for one antigen were shown to act in the lungs to promote the generation of a T_H1 and T_H17 response to a second antigen that is inoculated simultaneously with their cognate antigen. This expands a similar paradigm previously demonstrated for T_H2 responses and explains how the presence of a characteristic inflammatory response in the lungs can induce additional antigens to stimulate the same type of inflammation.

Homeostatic control of T_H2 responses and IL-4-dependent pathobiology

Two studies carried out in mice provided insight into the mechanisms that modulate the strength of IL-4-dependent effector pathways. Schneider et al²⁶ reported that serotonin, a prominent product of mast cells and platelets, acts through the organic cation transporter 3 to suppress the secretion of IL-4 by basophils *in vivo* and *in vitro*. This pathway prominently suppressed a basophil-dependent T_H2 response *in vivo*. Mathias et al²⁷ demonstrated that mice in which IL-4 receptor α signaling was increased by a mutation that inactivated an inhibitory pathway, but not wild-type mice, had food allergy to ovalbumin in response to being fed this antigen. Both bone marrow-derived and non-bone marrow-derived cell expression of the hyperactive IL-4 receptor were involved in this process. Subsequent oral challenge with ovalbumin induced systemic anaphylaxis that was IgE and Fc ϵ RI α dependent.

Cytokines in late-phase cutaneous reactions

A study of allergic skin disease also showed evidence of cytokine influences. Human edematous late-phase skin reactions were characterized by infiltration with cells that secrete 2 cytokines that induce vasodilatation and increase permeability: calcitonin gene-related peptide and vascular endothelial growth factor. Calcitonin gene-related peptide was produced predominantly by neutrophils and T cells, whereas vascular endothelial growth factor was produced predominantly by eosinophils, neutrophils, and macrophages.²⁸

GENETIC AND ENVIRONMENTAL FACTORS INVOLVED IN SUSCEPTIBILITY TO ALLERGIC DISEASE

Variations in pattern-recognition receptor function

In addition to their critical role in innate defense against microbes, pattern recognition by TLRs plays a key role in sensitization to allergens. Reece et al²⁹ examined the expression and function of TLRs on cord blood hematopoietic progenitors in relation to atopic risk. High-atopic-risk infants had significantly lower cord blood CD34⁺ cell expression of TLR2, TLR4, and TLR9. Interestingly, CD34⁺ progenitor cells from high-risk infants gave rise to significantly more eosinophils and basophils *in vitro* than did cells from control subjects. Although LPS costimulation induced eosinophil and basophil development, this response was significantly decreased in the progenitor cells from high-risk infants. As such, the authors have presented evidence that neonatal cord blood hematopoietic progenitor cell TLR expression and functional responsiveness are altered in atopic at-risk infants. It seems possible that maternal allergic

sensitization might modulate hematopoietic progenitor TLR expression and function *in utero* to modulate risk for subsequent allergy.

Mold-associated glucans can be recognized by monocytes through a complex formed from TLR2 and the β_3 integrin subunit.³⁰ Smit et al³¹ examined the influence of single nucleotide polymorphisms (SNPs) in the β_3 integrin gene (*ITGB3*) on mold sensitization among patients with and without SNPs in the *TLR2* gene in a large French cohort consisting of 524 patients with asthma and 719 nonasthmatic control subjects. In addition to verifying a previously recognized association with the *TLR2*/+596 C allele and asthma risk, this study also identified significant associations between 11 different *ITGB3* SNPs and mold sensitization (*Alternaria*, *Aspergillus*, and *Cladosporium* species) among asthmatic patients bearing 2 copies of the *TLR2*/+TT allele, with significance for 7 persisting after correcting for multiple comparisons. The *ITGB3* SNPs did not influence the risk of mold sensitization in subjects bearing the *TLR2*/+596 C allele but did influence the risk of asthma. Although this study did not determine the influence of the *ITGB3* SNPs on the expression or function of β_3 integrin or its association with TLR2 on antigen-presenting cells, it suggests substantial gene-gene interactions in the TLR2/ β_3 integrin complex that might significantly alter the likelihood of sensitization to molds.

Genome-wide association studies and functional correlates

Wan et al³² used a genome-wide association study approach to examine genetic susceptibility to atopy, which was defined as increased specific IgE levels, positive skin prick test responses, or both, within a large discovery cohort and 3 additional white populations. The British 1958 Birth Cohort (1083 cases and 2770 control subjects) was probed on the Illumina 550K Array (Illumina, San Diego, Calif). A single SNP on chromosome 13q14 met genome-wide significance, and a further 6 loci showed weaker evidence for association with increased specific IgE levels. No genetic variant was associated with atopy. As such, it seems that large-scale analyses with results from multiple populations will be needed to reliably identify key genetic factors underlying atopy predisposition.

Prior genome-wide association studies have implicated genetic variants on chromosome 17q21 as strongly associated with asthma and orosomucoid 1-like 3 gene (*ORMDL3*) expression. Yet the mechanism by which this genetic linkage associates with asthma is not well understood. Lluís et al³³ examined cord blood mononuclear cells for expression of genes at 17q21 and their association with genetic risk variants within 17q21. Notably, increased *ORMDL3* and gasdermin A (*GSDMA*) expression showed a genotype-expression relationship. Children homozygous for 4 risk alleles for 17q21 tagging SNPs showed increased expression for *ORMDL3* after Der p 1 stimulation and increased expression for *GSDMA* after PHA stimulation. Of note, no correlation between 17q21 polymorphisms and Treg, T_H2, or T_H1 lineages was detectable. However, 17q21 risk allele carriers showed significantly increased IL-17A secretion. These results suggest an association of 17q21 polymorphisms with *ORMDL3* and *GSDMA* expression and IL-17 secretion early in life. These observations might imply a functional role of the 17q21 locus affecting T-cell development during immune maturation.

Early-life immune responses and asthma risk

Another way to assess early steps in the development of allergic disease is to immunologically phenotype lymphocytes in young children and study whether they subsequently predict disease development. Accordingly, Rothers et al³⁴ assessed the relationship between first-year adaptive immune cytokine production with asthma and total IgE levels through age 5 years. Notably, relationships of cytokines to total IgE levels and asthma were strikingly different. Total IgE levels through age 5 years positively correlated with 12-month IL-4, IL-5, and IL-13 levels and were inversely associated with 12-month IFN- γ levels. Active asthma through age 5 years was positively associated with 3-month IL-13 levels and inversely associated with 3-month IFN- γ levels. As such, total IgE levels and active asthma through age 5 years are associated with adaptive cytokine production in early life, although relations vary temporally and with regard to the importance of individual cytokines.

Diversity of enteric flora influences susceptibility to allergic sensitization

Bisgaard et al³⁵ applied both conventional culture methodology and a molecular approach to examine the extent of diversity in the intestinal microbiome in a birth cohort of 411 infants at high risk for the development of allergic disease. The investigators studied the relationship between intestinal microbial diversity in infancy and the development of atopic sensitization and disease at 6 years of age. Reduced intestinal microbial diversity was significantly associated with allergen-specific IgE levels and positive skin prick test responses, the presence of peripheral blood eosinophilia, and the development of allergic rhinitis but not asthma or atopic dermatitis. This study supports the concept that factors that alter the composition and diversity of endogenous intestinal flora have the potential to alter the risk of allergic disease as a component of the hygiene hypothesis.

CONCLUSION

In summary, the mechanistic studies published in the *Journal of Allergy and Clinical Immunology* in 2011 saw a strong emphasis on the identification of cellular, genetic, and environmental predictors of atopy; the molecular properties of allergens that facilitate sensitization; and the potential ability to manipulate these properties for therapeutic purposes. The development of engineered allergens that lack the ability to stimulate an IgE-facilitating response is a major step toward improving immunotherapy. The efficacy of anti-IgE for indications other than asthma both validates the importance of IgE and IgE receptors in the pathobiology of human disease and suggests that there will likely be additional opportunities for therapy. It is anticipated that each of these areas highlighted by the *Journal* has strong potential to change paradigms of treatment and pathophysiology.

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