

Mechanisms of asthma

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Airway inflammation is a key factor in the mechanisms of asthma. Articles published in the *Journal of Allergy and Clinical Immunology* this past year have highlighted the utility of investigative bronchoscopy with segmental antigen challenge and induced sputum analyses to evaluate features of airway inflammation in relationship to asthma severity. Peripheral blood cell generation of cytokines IFN- γ (T_H1) and IL-5 (T_H2) was used to evaluate the relationship of the balance of T_H1/T_H2 cytokines to asthma persistence and severity in a 42-year, longitudinal study. Chemokines, including thymus and activation-regulated chemokine, are important to the regulation of inflammation and IgE synthesis. Their potential role in asthma has also been evaluated. Finally, albuterol (R)- and (S)-enantiomers may have distinct effects on airway relaxation and regulation of inflammation, suggesting the possibility that monoisomeric therapy has therapeutic advantages. The potential contribution of genetic factors and mechanisms to airway inflammation and remodeling also continues to be an area of intense investigation. During the past year a number of articles published in the *Journal of Allergy and Clinical Immunology* have identified and clarified potential genetic mechanisms in asthma. The contribution of genetics to asthma has been examined in a wide variety of studies, ranging from epidemiologic association and twin studies all the way to molecular analysis through microarray gene expression experiments. (*J Allergy Clin Immunol* 2003;111:S799-804.)

Key words: Airway inflammation, chemokines, cytokine imbalance, genetic susceptibility, polymorphisms

Airway hyperresponsiveness, reversible airflow obstruction, and bronchial hyperresponsiveness are characteristic features of asthma. Research has focused on mechanisms of airway inflammation, regulation of these processes, and translation of these events into altered lung function. In addition, research is ongoing in an attempt to understand the contributions to and regulation of immune responses by environmental factors and allergens, the injury-repair process (now defined as airway remodeling), and the genetic regulation of these processes. Publications in the *Journal of Allergy and Clinical Immunology* from 2001 to 2002 have expanded our understanding of asthma mechanisms and genetics, and the selected publications discussed here illustrate their relevance to the state of our knowledge (Table I).

Abbreviations used

LTC₄: Leukotriene C₄

TARC: Thymus and activation-regulated chemokine

DETERMINANTS OF ASTHMA SEVERITY

Inhaled allergens are important environmental factors in the pathogenesis of asthma and most likely its persistence. Moreover, the interaction between host factors (genetics) and environmental stimuli can result in the development of airway inflammation, altered pulmonary physiology, and asthma symptoms in the susceptible host. To test the hypothesis that the intensity of airway inflammation relates to or determines asthma severity, Moore et al¹ used investigative bronchoscopy with segmental allergen challenge to compare the airway responses to allergen between subjects with mild and moderate persistent asthma. From these reactions, they attempted to determine whether the underlying disease severity parallels or determines the airway inflammatory response to allergen.

Eighteen subjects with asthma completed this study: 8 with mild persistent disease (FEV₁ 89.5% \pm 1.5% predicted) and 10 with moderate persistent asthma (FEV₁ 68.8% \pm 2.2% predicted). Baseline concentrations of cells and cytokines, including IL-4, IL-5, IL-6, IL-8, GM-CSF, TNF- α , and IL-1 β , were similar in the bronchial lavage fluid samples 24 hours after allergen challenge; both groups had similar increases in total cells (60.0 \pm 48.5 \times 10⁴ cells/mL for mild vs 50.3 \pm 15.7 \times 10⁴ cells/mL for moderate) and subpopulations of leukocytes such as eosinophils (18.1 \pm 13.1 \times 10⁴ cells/mL for mild vs 10.9 \pm 9.5 \times 10⁴ cells/mL for moderate). Concentrations of a number of cytokines, specifically IL-4, IL-5, IL-6, IL-8, GM-CSF, and IL-1 β , increased with antigen challenge, but these changes were also similar between mild and moderate asthma groups. Moore et al¹ concluded that asthma severity was neither associated with nor a determinant of an enhanced allergic response to antigen. The converse may also be true: the severity of asthma is not determined by the intensity of the allergic reaction. Moore et al¹ showed that the level of airway obstruction in asthma may not be linked to or determined solely by the intensity of the allergic reaction.

Induced sputum can also be used to analyze airway inflammation in relationship to asthma severity in terms of airflow obstruction and bronchial hyperresponsiveness. Woodruff et al² studied 205 patients with a range of clinical asthma severity as defined by the percent predicted FEV₁: FEV₁ less than 60% predicted (8%), FEV₁ of 60% to 80% predicted (37%), and FEV₁ greater than 80% pre-

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dicted (55%). Forty percent of all subjects received inhaled corticosteroids. Woodruff et al² carefully analyzed sputum samples from this large group of patients with asthma and found that "both eosinophilic inflammation and neutrophilic inflammation in sputum are independently associated with [the] degree of chronic airway obstruction." For example, sputum eosinophilia had a significant relationship with airway hyperresponsiveness, whereas sputum neutrophilia did not. In contrast, both sputum eosinophilia and neutrophilia were related to the intensity of airflow obstruction, as reflected by FEV₁ values. As Woodruff et al² pointed out, mechanisms that determine airway obstruction and responsiveness probably overlap to some degree, but there are probably distinct structural and functional changes in asthma associated with various cell types. With respect to the neutrophil, for example, the effect on lung function appears to be principally that of airway caliber. The analyses of sputum samples by Woodruff et al² have provided additional evidence that distinctive inflammatory events in the airway may be associated with specific alterations or features of lung function in asthma.

In line with this paradigm, it may be possible to gain additional insight into the significance and mechanisms of diverse inflammatory processes and how they are determinants of asthma severity. For example, Woodruff et al² also evaluated the effect of inhaled corticosteroid treatment on sputum eosinophilia and neutrophilia. Inhaled corticosteroids reduced eosinophils, but not neutrophils. There has been speculation that increases in airway neutrophils, particularly in more severe disease, are related to treatment with corticosteroids. Because this pattern of regulation was similar in both groups of asthma, these new observations suggest that this is not the case with neutrophils.

CYTOKINE IMBALANCE AND ASTHMA EXPRESSION

The relationship between cytokine imbalance and the expressions of both atopy and asthma has been of considerable interest and importance. Atopic disease is associated with a shift in immune responses away from a T_H1 (IFN- γ) pattern toward a T_H2 (IL-4, IL-5, and IL-13) profile. According to these observations, defective IFN- γ production predisposes toward the development of allergic diseases and asthma. Conceivably a correction in the imbalance between T_H1 and T_H2 would either be associated with or contribute to disease resolution. To assess this possibility, Smart et al³ determined PBMC cytokine responses in patients with persistent asthma and compared these responses with those of patients who had resolution of their asthma. The study subjects were part of the Melbourne Epidemiological Study of Childhood Asthma, which was initiated in 1964 to determine the prevalence and natural history of asthma and wheezy bronchitis in children.⁴ The cohort was initially made up of 378 wheezing children and 106 healthy subjects, who were recruited at 7 to 10 years of age and then followed up prospectively

at 7-year intervals until the age of 42 years. The interval evaluations noted clinical status and respiratory function. Eighty-nine subjects from this cohort were evaluated at 42 years of age, and PBMCs were obtained and stimulated for nonspecific (PHA) and allergen-induced (house dust mite allergen) production of T_H1 (IFN- γ) and T_H2 (IL-4, IL-5, and IL-13) cytokines. Cytokine production to these stimuli was then compared among subjects with ongoing asthma (moderate and severe), patients with resolved asthma, and control subjects.

Allergic skin test responses to a panel of antigens, including house dust mite allergen, were similar in the three asthma populations: resolved, moderate, and severe asthma. In contrast, total and house dust mite allergen-specific IgE levels were correlated with the intensity of asthma severity; that is, IgE levels were highest in those with severe disease and lowest in those with resolved asthma. Patients with severe asthma also had significantly reduced IFN- γ production in response to house dust mite allergen relative to control subjects. IFN- γ production in response to house dust mite allergen was similar between control subjects and those with resolved asthma. Patients with severe asthma had lower IFN- γ production than did those with resolved asthma. The IFN- γ response to the nonspecific stimulus, PHA, was similar in all three asthma populations and in control subjects. Patients in all three asthma categories produced increased (relative to control subjects) but similar amounts of IL-5 in response to house dust mite allergen.

A number of key findings arose from this study to shed light on the relationship, and possible importance, of the T_H1/T_H2 cytokine profile in allergic disease and asthma. First, resolution of asthma was not associated with a reduction in either polyclonal (PHA-stimulated) or allergen-induced T_H2-cytokine production (eg, IL-5). All subjects with asthma, irrespective of disease activity, had increased generation of IL-5 relative to control subjects. Smart et al³ suggested that the increased generation of IL-5 is related more to the atopic state of the patients than it is a reflection of asthma activity or severity. Second, severe persistent asthma was associated with a reduced T_H1 response to allergen. In contrast, the T_H1 cytokine generation in response to house dust mite allergen was normal in patients with resolved asthma. On the basis of these observations, Kline et al⁵ extended their findings and hypothesized that potential benefit might be gained by treatment with CpG oligonucleotides, which could switch the immune response toward T_H1 cytokine production. Third, T_H1 cytokine production by PBMCs in response to mitogen did not parallel the reduced antigen-induced T_H1 response. Previously, some of the same authors as in the original study of Smart et al³ had found reduced mitogen generation of IFN- γ in allergic asthmatic children.⁶ Because mitogen-induced IFN- γ was similar between control subjects and adult patients with asthma, their current findings suggest that the defective polyclonal IFN- γ production in childhood represents a developmentally regulated immune response that resolves with age. Therefore allergen-induced cytokine responses, particularly the T_H1

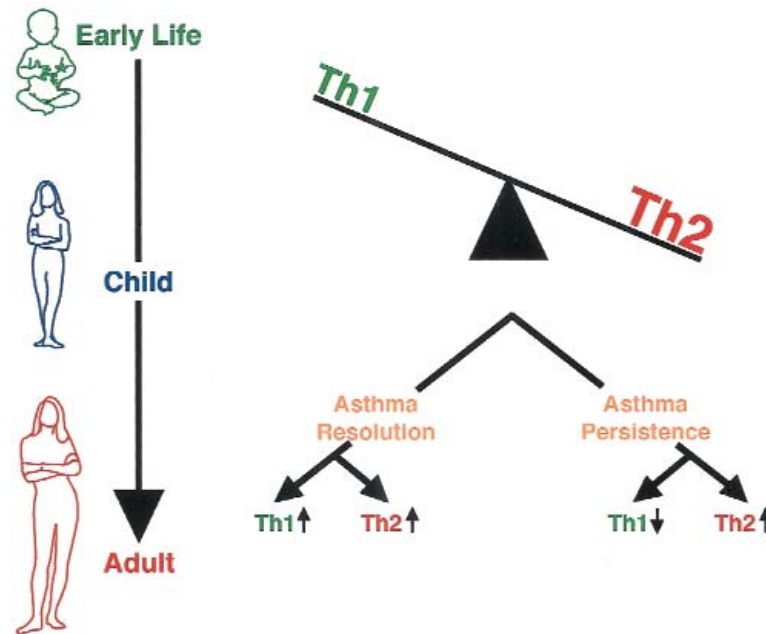


FIG 1. With Melbourne Asthma Study data (1964-1999), Smart et al³ evaluated peripheral blood cell generation of T_H1 (IFN- γ) and T_H2 (IL-5) in relationship to asthma resolution versus asthma persistence.

variety, might provide a more accurate reflection of disease activity and inflammation in asthma (Fig 1).

REGULATION OF ENDOTOXIN-INDUCED INFLAMMATION

LPS enhances eosinophilic responses in allergic disease, and allergen challenge of the airway induces eosinophilia in response to LPS, suggesting a linkage between features of allergic inflammation and LPS-associated responses. Alexis and Peden⁷ have further indicated that CD14 expression is increased in allergic asthma, suggesting that atopy may enhance CD14-mediated responses to inhaled LPS. To determine further the roles of eosinophilic inflammation and responses to LPS, Alexis and Peden⁷ treated subjects with newly recognized asthma with either fluticasone at 440 μ g or placebo twice a day for 2 weeks in a double-blind, cross-over fashion. At the end of 2 weeks of treatment, the subjects underwent a challenge with LPS. Sputum samples were obtained 48 hours before the LPS challenge and 6 hours after the challenge.

Two weeks of fluticasone treatment significantly ($P = .04$) reduced sputum eosinophils but not neutrophils. Second, fluticasone significantly reduced ($P = .04$) the increase in sputum neutrophilia to the LPS challenge. Third, expression of the LPS surface receptor CD14 on sputum monocytes and alveolar macrophages was decreased by fluticasone 48 hours before the LPS challenge and 6 hours after the challenge. In contrast, fluticasone treatment had no effect on a variety of inflammatory cytokines (IL-6, IL-8, and TNF- α). Alexis and Peden⁷ used treatment of asthma with inhaled corticosteroids to gain insight into the regulation of underlying

TABLE I. Key advances

- Asthma severity does not determine the intensity of airway allergic response.
- Sputum neutrophilia is associated with airway obstruction but not hyperresponsiveness, whereas sputum eosinophilia relates to both airway dysfunction in asthma.
- Persistent asthma is characterized by high IL-5 production and low IFN- γ generation, whereas asthma resolution is associated with normalization of IFN- γ generation in response to allergen.
- TARC is increased in asthma and associated with IgE sensitization.
- Albuterol enantiomers (R and S) may regulate cytokine generation by cell in a different fashion.
- New genetic markers and polymorphisms have been associated with features and characteristics of asthma.

inflammation, eosinophils versus neutrophils, and the potential interrelationship between the LPS receptor activity (CD14 expression) and resulting airway inflammation in response to inhaled LPS, as well as the differential susceptibility of these inflammatory markers to inhaled corticosteroids. Their data suggest that allergic inflammation affects CD14 expression and the subsequent airway response to LPS.

CHEMOKINE ACTIVITY AND ASTHMA

The contributions, concentrations, and pattern of chemokines in asthma probably influence the inflammatory response independently of or cooperatively with cytokines. Leung et al⁸ evaluated plasma concentrations of thymus and activation-regulated chemokine (TARC) in childhood asthma. TARC generation is associated with specific trafficking of T_H2 lymphocytes to the airway

through this chemokine's interaction with CCR4 receptors on lymphocytes. Earlier work found serum concentrations of TARC to correlate with the severity of atopic dermatitis.⁹ To extend these observations, plasma concentrations of TARC were measured in children with asthma and correlated with allergen sensitization as an index of asthma severity. Eighty children with asthma and 78 control subjects, aged 5 to 18 years, were studied. Twenty-six of the patients with asthma received inhaled corticosteroid treatment. As a group, the subjects with asthma had increased plasma concentrations of TARC, which were higher in the noninhaled corticosteroid-treated group. In addition, a weak but significant ($r = 0.219$, $P = .04$), correlation was found between plasma TARC values and total IgE concentrations. When subjects sensitized to cat allergen were compared with a nonsensitized population, significantly ($P = .001$) greater concentrations of TARC were found in the allergic group. Because TARC is responsible for trafficking of T_H2 lymphocytes into sites of allergic inflammation, Leung et al⁸ suggested that increases in TARC promote T_H2 trafficking and, through this activity, increase IgE sensitization and asthma severity. Additional studies will be necessary to more specifically define the role and contribution of TARC in asthma.

ALBUTEROL ENANTIOMERS AND AIRWAY DYSFUNCTION

Albuterol has two enantiomers, (R)-albuterol and (S)-albuterol, and there has been speculation that the (R)-enantiomer is the effective bronchodilating component of β -agonists. The (S)-isomer, in contrast, has been suggested to contribute to adverse β -agonist effects. In a guinea pig model, Keir et al¹⁰ compared the effects of both (R)- and (S)-enantiomers on airway function. Guinea pigs first were allergen sensitized and then had minipumps placed to receive, (R)-albuterol, or (S)-albuterol, or a mixture of the enantiomers continuously over 10 days, after which bronchoconstriction responses to intravenous histamine, bradykinin, leukotriene C_4 (LTC_4), and capsaicin were measured. The administration of these spasmogens (LTC_4 , bradykinin, and capsaicin) increased airway tone, which was greater after 10 days of exposure to mixed enantiomers and (S)-albuterol but not (R)-albuterol. Treatment with capsaicin significantly impaired the ability of mixed enantiomers and (S)-albuterol to enhance airway responsiveness to bradykinin. The prevention of enhanced airway hyperresponsiveness by capsaicin to bradykinin suggests the effects of mixed enantiomers or (S)-albuterol occurs through actions on airway sensory nerves. Because these effects with mixed enantiomers and (S)-albuterol occurred at doses that may be achieved in vivo, Keir et al¹⁰ proposed that mixed enantiomers and (S)-albuterol enantiomers could affect airway function and increase airway responsiveness. The relationship of this association with albuterol treatment in asthma has yet to be established.

To extend these observations to cellular functions, Baramki et al¹¹ evaluated the modulation of lymphocyte proliferation by albuterol enantiomers. Cell lines were

incubated either with IL-2 or tetanus toxoid, and proliferation was assessed in the presence of (R)- and (S)-enantiomers of albuterol. Only (R)-albuterol suppressed the proliferative responses, whereas the mixed enantiomers and the S-enantiomer enhanced cell-line proliferation. When cytokine generation was determined, IL-13 secretion was increased by mixed enantiomers and (S)-albuterol. Baramki et al¹¹ conclude that these in vitro studies support the possibility that different enantiomers of drugs may have differential effects on inflammatory responses and that some enantiomers could enhance inflammatory activity.

THE ROLE OF GENETICS IN MEDIATING AIRWAY CHANGES IN ASTHMA AND ATOPY

The contribution of genetics in predisposing individuals toward various pathologic processes associated with asthma and atopy has been evaluated at many levels: epidemiologic studies with twins, genome-wide searches for asthma susceptibility through genome screens, and candidate gene studies that look both statistically and mechanistically at the contribution of the many candidate genes. Potential new mechanisms that describe and identify ways in which host factors play a significant role in predisposing toward asthma can be determined. An article by Strachan et al¹² from the United Kingdom examined the concordance and interrelationship of atopic disease with markers of allergic sensitization among adult female twins. The results showed that genetic factors clearly have a major effect on aeroallergen sensitization; clinical allergic disease as assessed by clinical symptoms of hay fever, eczema, and asthma; and skin-test reactivity for specific IgE and total IgE levels as assessed by immunoassay. Despite good concordance of at least 0.59 from monozygotic twins, as opposed to 0.29 for dizygotic twins, there was still some discordance between identical twins and the development of atopy and allergic disease, suggesting that a substantial modifying role for environmental factors exists.¹² Further study on the genome-wide search for atopy and genetic susceptibility in patients with asthma and families with asthma was published by Koppelman et al.¹³ They performed a whole-genome screen to evaluate a variety of intermediate phenotypes, including total serum IgE, skin-test positivity, peripheral blood eosinophilia, and other traits. Clearly several chromosomal regions that had shown previous evidence of linkage with atopic phenotype and familial asthma (2q6p7q and 13q) also demonstrated evidence of linkage with total serum IgE. Specific regions of interest for atopic traits were detected on chromosomes 11q, 17q, and 22q. These regions potentially harbor candidate genes that have a significant role in asthma pathogenesis. A similar approach was applied to factor analysis of asthma and atopy traits in a cohort of Hispanic and non-Hispanic white children enrolled in the Tucson Children's Respiratory Study.¹⁴ Twenty-four intermediate phenotypes were evaluated, including skin-test responses, total serum IgE, asthma profile, wheezing episodes, hay fever, and cough. Factors' score coefficients were generated to determine the associ-

ation with markers in various parts of the genome. Two main factors were identified, and one of these factors was linked to human chromosome 5q31-33, where many of the genes involved in IgE regulation and other aspects of asthma and atopy reside. This study also demonstrated that a second factor outside chromosome 5q had a significant influence on the expression of atopy and IgE in these families with asthma.

In a study that examined activated gene expression scores for atopy and asthma, Brutsche et al¹⁵ obtained peripheral blood mononuclear cells of 18 atopic subjects with asthma, 8 atopic subjects without asthma, and 14 control subjects and applied microarray analysis for a wide variety of genes that may be activated in asthma and atopy. A composite atopy gene expression score was used by determining the actual association of genetic variation and expression and correlating it with this score. This expression report evaluated 10 genes that have been identified as characteristic of the asthmatic atopic phenotype, and correlations were seen to a greater degree with all 10 genes in asthma as opposed to healthy control subjects and those with atopy but no asthma. Other analyses to examine many more genes involved in this process will be necessary to fully understand this particular relationship; however, this is a first step in analysis of not only gene regulation in the pathogenesis of asthma but quantitative expression of those regulated genes on a global basis.

A variety of studies have examined candidate gene variations, or polymorphisms, in the expression of asthma. Most of these variations have been found in inflammatory or immune genes, although a number of studies have examined genetic polymorphisms with other genes involved in airway hyperresponsiveness, bronchodilation, and arachidonic acid metabolism. Kim et al¹⁶ examined the β_2 -adrenoreceptor polymorphisms that are defined at positions 16 and 27 of the receptor and play an important role in expression of nocturnal cough in atopic subjects but not in the general expression of atopy and hyperresponsiveness in patients. Konno et al¹⁷ determined that a repeat polymorphism in the nitric oxide synthase 2 promoter region was associated with the risk and enhanced risk of atopy that was independent of asthmatic status. The mechanism of this variation in the nitric oxide synthase 2 promoter region in terms of increasing susceptibility of asthma was not addressed, but there is a significant increase in the odds ratio for this gene promoter variant and its association with atopy but not asthma in a Japanese population. In a further study from Japan, Kawagishi et al¹⁸ discovered the well-described LTC₄ synthase promoter polymorphism to be associated with a higher risk of aspirin-induced asthma. Their identification of greater risk according to the genetic variations in the LTC₄ synthase promoter is consistent with some but not all reports. Nagarkatti et al¹⁹ demonstrated that a polymorphism in the IFN- γ gene is associated with asthma in the Indian population. The IFN- γ locus on human chromosome 12q21 has been identified in a number of studies as a candidate gene for asthma and with a potential role in pathophysiology and regulation of IgE. The correlation of

the IFN- γ polymorphism was examined both by total serum IgE and through the identification of approximately five genetic variants. A variety of alleles were studied, and the dinucleotide repeat polymorphism was found to be expressed at a reasonable level and to be associated with total serum IgE in patients with asthma.

The dominant expression of an alternate transcript of the IL-18 receptor α -chain was identified in a population of 39 Japanese atopic children. This variant, which included a 3-based deletion of the IL-18 receptor α -chain cDNA, as assessed from expression of peripheral blood, was associated with reduced IFN- γ production by IL-18 stimulation and higher serum IgE levels, thereby predisposing by some mechanism toward allergic diseases in these atopic children.²⁰ The role of IL-18 in regulating the induction of IFN is considered an important factor in the actual output of IFN- γ by IL-18, and its function is a critical component in the regulation of cytokine balance between T_H1- and T_H2-mediated responses. Two studies from Finland by Karjalainen et al^{21,22} evaluated IL-1 α and IL-2 β genotypes in associations with asthma and atopy in adult men and women. An IL-1 α gene polymorphism was found in association with positive skin-test results. The difference was caused by an increase in frequency of a rare IL-1 α allele, suggesting that the IL-1 gene complex is involved in the regulation of IgE-mediated atopic reactions, including positive results of skin tests. In a companion article, it was found in a relatively large population of 245 patients with asthma and 405 control subjects that the variation in the IL-1 β gene promoter polymorphism was associated with asthma, in particular with asthma in male adults. The exact mechanism of abnormal IL-1 β function in men as opposed to women was not defined, but this finding clearly points to a potential role for IL-1 in a genetic susceptibility to asthma.

In a study from Harvard Medical School, a variation in eotaxin expression in which threonine is substituted either with alanine or other amino acids at position 23 of the eotaxin structure was examined; those individuals with asthma who expressed this substitution had both decreased eosinophil counts and higher levels of lung function.²³ This variation in a large association study suggests that the threonine substitution for alanine at position 23 in the eotaxin structure may have functional significance.

Finally, the series on Molecular Mechanisms in Allergy and Immunology included three reviews related to genetics. The first centered on the functional genomics of CD14, a human chromosome 5q31-33 gene that has been linked to the potential regulation of IgE responses and asthma severity.²⁴ CD14 is an important factor in the response to endotoxin, which may represent a significant factor in the regulation of cytokine balance through the production of IFN- γ by the expression and induction of IL-12 and IL-18 through CD14-dependent mechanisms. Two articles, by De Sanctis et al²⁵ and Daser et al,²⁶ examined the genetics of airway hyperresponsiveness and allergen-induced airway inflammation with murine models and identified a number of murine correlates of the human candidate genes in associated studies.

CONCLUSION

Studies that are designed to evaluate and determine mechanisms of asthma and their relevance to disease severity are essential to gain better insight to this disease. Key areas of future research will include efforts to determine how airway inflammatory activity causes or contributes to disease severity. Furthermore, as molecules contributing to the regulation of airway inflammation are discovered, the role of cytokines and their balance in the development and evolution of disease will be important to understand asthma persistence and severity. Genetic studies have also progressed in the past year, ranging from whole-genome screens through microarray analysis, large and small association studies, and other genetic studies that aim to look at potential asthma mechanisms in airway inflammation and remodeling. These publications will help identify the significant contributions of host factors and genetics in the exquisite control of these airway processes in asthma.

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