

Molecular mechanisms in allergy and clinical immunology

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IL-12/IL-13 axis in allergic asthma

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Allergic airway diseases are associated with skewed T_H2 cytokine production, although the underlying cause of this aberrant immune response is not well understood. Recently, 2 double-digit cytokines, IL-12 and IL-13, have been proposed to play pivotal roles in the T_H2 -polarized immune response to inhaled allergens. IL-12 is a critical determinant of T_H1 -mediated immune responses, and it has been shown that deficiency in this cytokine can lead to T_H2 -polarized immune responses. IL-13, on the other hand, has recently been shown to be a critical mediator of the effector arm of the allergic response. Overproduction of this cytokine has been shown to induce many common features of the allergic diathesis, such as airway hyperresponsiveness, eosinophilic inflammation, IgE production, mucus hypersecretion, and subepithelial fibrosis. Substantial evidence suggests that an imbalance in the production of these 2 critical immunoregulatory cytokines occurs in the lungs of atopic and asthmatic individuals, such that IL-13 is overproduced, and IL-12 production is impaired. As a result of this imbalance, IL-13 production may go unchecked and induce the classical allergic phenotype. Although it is not entirely clear what tips the balance between these 2 cytokines, genetic studies suggest that this could indeed be a primary event. Interestingly, the genes encoding both of these cytokines reside within the chromosomal regions on 5q, which have been associated with asthma in many populations. Although no evidence exists to date to support an association between asthma and polymorphisms in the *IL12* gene, evidence is accumulating that supports an association between genetic alterations in the *IL13* gene and atopy and asthma. From these observations, we can conclude that an imbalance in the IL-12/IL-13 axis may contribute to the development of allergic diseases, such as asthma. Studies to more fully examine the yin-yang relationship between these 2 critical immunoregulatory cytokines are clearly warranted. (*J Allergy Clin Immunol* 2001;107:9-18.)

Key words: Allergy, asthma, cytokine, T-cell differentiation

Asthma is an inflammatory disease of the lung that is thought to originate as a result of polarized immune responses to ubiquitous inhaled antigens. Ample evidence suggests that the asthmatic lung is populated by $CD4^+$ T cells producing a predominantly T_H2 cytokine

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
STAT: Signal transducer and activator of transcription
TGF: Transforming growth factor

pattern (ie, IL-4, IL-13, IL-5, and IL-9). The overexpression of this cytokine pattern results in recruitment and activation of a number of effector cells (mast cells and eosinophils), which leads to the functional characteristics of the disease, namely airway obstruction and airway hyperresponsiveness (AHR). Structural changes also include epithelial desquamation, mucus cell hyperplasia, muscle hypertrophy, and subepithelial fibrosis. The underlying cause of the abnormal immune response to common environmental antigens is not well understood. Recently, 2 double-digit cytokines, IL-12 and IL-13, have been proposed to play pivotal roles in the aberrant immune response to inhaled allergens. Elevations in lung levels of IL-13 and reciprocal impairments in IL-12 levels have been associated with disease. Alterations in either production of or responsiveness to either of these important immunoregulatory cytokines could result in the polarization of T-cell responses to inhaled allergens observed in the asthmatic lung (Fig 1). Herein, I review the individual role of these 2 cytokines in allergic disease and the evidence for their respective dysregulation and then speculate on how an imbalance in production of these 2 cytokines may occur in human disease.

IL-12

IL-12 is a heterodimer (p70) composed of 2 covalently linked subunits referred to as p40 and p35.¹ These subunits are products of 2 separate genes: a heavy-chain subunit (p40), the expression of which is tightly regulated in cells that secrete the intact heterodimer, and a light-chain subunit (p35), which is constitutively expressed at low levels by most cell types that have been studied. Interestingly, the gene encoding the p40 subunit is located in the region of chromosome 5, in which linkage to human asthma has been demonstrated.^{2,3} Bioactive IL-12 is produced by activated monocytes-macrophages, dendritic cells, and other antigen-presenting cells.¹ Release of this cytokine from antigen-presenting cells directs the differentiation of T cells into T_H1 cytokine-producing cells and is a costimulus of activation of effector T_H1

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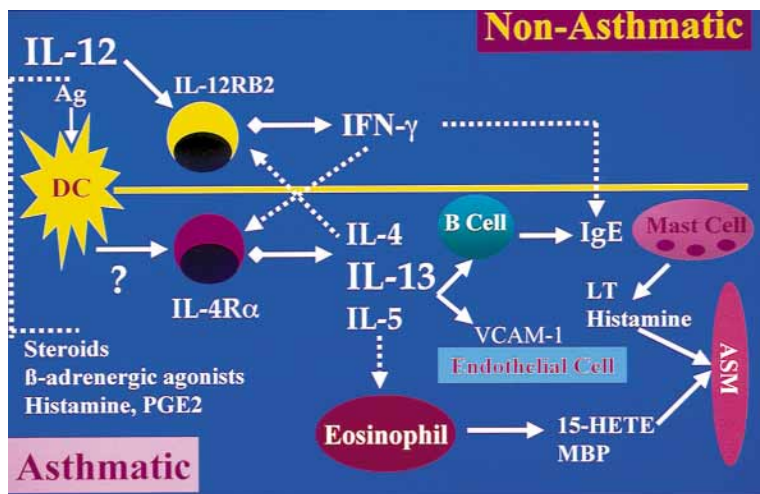


FIG 1. Schematic representation of the reciprocal role of IL-12 and IL-13 in allergic diathesis. Presentation of inhaled antigens to naive T cells results in differentiation of T cells into either T_H1 or T_H2 cytokine-producing cells. The cytokine milieu present during differentiation greatly influences the directionality of the immune response. If IL-12 is present, T cells differentiate along a T_H1 pattern. T_H1 cells produce IFN- γ , which inhibits T_H2 cell differentiation, as well as directly inhibiting a number of effector processes known to be important in allergic disease, such as IgE production and mast cell activation. Although the exact mechanisms involved in driving T_H2 cell differentiation are not known, IL-4 is thought to be important in T_H2 cell proliferation and inhibition of T_H1 cytokine production. IL-13, on the other hand, is thought to be a critical mediator of the effector arm of the immune response. Overproduction of this cytokine has been shown to induce the hallmark manifestations of the disease, such as IgE synthesis, mast cell activation, eosinophilic inflammation, and smooth muscle contractility. Substantial evidence suggests that an imbalance in the production of these 2 critical immunoregulatory cytokines occurs in the lungs of atopic and asthmatic individuals, such that IL-13 is overproduced, and IL-12 production is impaired. Although it is not clear what tips the balance between these 2 cytokines, recent evidence suggests that primary defects in either the *IL4RA* or *IL13* genes may be involved. Dashed lines, inhibition; solid lines, stimulation. DC, Dendritic cell; ASM, airway smooth muscle.

cells while suppressing T_H2 cell generation. IL-12 enhances the proliferation and cytolytic activity of natural killer and T cells and stimulates IFN- γ production by these cells. The cellular actions of IL-12 are mediated by means of a high-affinity receptor composed of 2 subunits, designated as IL-12R $\beta1$ and IL-12R $\beta2$. $\beta2$ Expression is restricted in distribution, and regulation of its expression is likely a central mechanism by which IL-12 responsiveness is controlled. It is currently thought that expression of the $\beta2$ subunit of the receptor is required for T_H1 differentiation and that expression of the $\beta2$ subunit is lost in T cells expressing a T_H2 cytokine pattern.⁴ Activation of the receptor complex results in a series of phosphorylation events leading to translocation of signal transducer and activator of transcription 4 (STAT4) to the nucleus, where it binds specific DNA sequences in the promoter of IL-12-inducible genes.⁵

The production of IL-12 is greatly influenced by the cytokine-mediator milieu present during the immune response. Its production is both positively (IFN- γ and TNF- β)⁶ and negatively (IL-4, IL-13, tumor growth factor [TGF]- β , and IL-10)^{7,8} regulated by immune mediators. The most potent stimulator of IL-12 production is IFN- γ . Although IL-12 stimulates IFN- γ production, a priming amount of IFN- γ is necessary, at least in vitro, to get mea-

surable IL-12 levels. Another antigen-presenting cell-derived cytokine, IL-10, is a powerful downregulator of IL-12 production.⁷ Although T_H2 cytokines have been thought of as inhibitors of IL-12, the relationship between these cytokines and IL-12 is more complex. For instance, although IL-4 and IL-13 suppress IL-12 production if added at the time of monocyte stimulation, long-term preincubation with either of these 2 cytokines induces elevated levels of IL-12.⁸ This dual effect likely represents the tight control on production of these 2 opposing arms of the immune response. Other mediators known to be important in the allergic diathesis, such as PGE₂,^{9,10} and histamine,¹¹ have also been shown to inhibit IL-12 production.

The ability of IL-12 to redirect T_H2 responses toward T_H1 immune responses has been demonstrated in both in vitro and in vivo studies. In particular, in vitro studies have shown that IL-12 inhibits IL-4 production in bulk cultures of peripheral blood leukocytes from allergic patients¹² and markedly suppresses IL-4-mediated IgE production by human peripheral blood monocytes.¹³ Furthermore, IL-12 suppresses antigen-specific IL-4 and IL-10 synthesis, while increasing IFN- γ production in purified antigen-primed CD4⁺ T cells from allergic subjects with rhinitis.¹⁴ Interestingly, the inhibitory effect of IL-12 on T_H2 cytokine production was not dependent on IFN- γ .

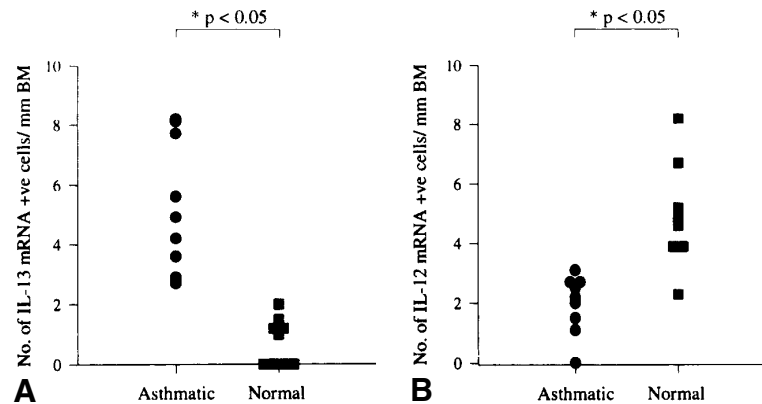


FIG 2. In situ hybridization of bronchial biopsy specimens from atopic asthmatic and normal control subjects with riboprobes for IL-13 mRNA (A) and IL-12 mRNA (B). Statistical differences were determined by using the Wilcoxon rank-sum test ($P < .05$). Reprinted with permission from Naseer T, et al. *Am J Respir Crit Care Med* 1997;155:845-51.

Numerous studies have demonstrated the importance of IL-12 in preventing T_H2 immune responses in vivo in murine models of allergic disease. For instance, Keane-Myers et al¹⁵ demonstrated that endogenous blockade of IL-12 in a normally nonresponsive murine strain (C3H/HeJ) resulted in development of T_H2 immune responses to allergen exposure (ie, airway AHR, IgE, and eosinophilic inflammation). The T_H2 -promoting effect of IL-12 blockade in this strain was accompanied by a shift in the cytokine pattern from a T_H1 cytokine pattern to a T_H2 profile. Furthermore, IL-12 knockout mice have enhanced bone marrow and bronchoalveolar lavage (BAL) eosinophilia after antigen challenge when compared with their wild-type controls.¹⁶ These studies suggest that endogenous production of IL-12 is protective against the development of the allergic airway phenotype.

Studies have also demonstrated that exogenous IL-12 treatment can markedly suppress allergic airway responses in susceptible murine strains, depending on the timing of administration. When IL-12 is administered at the time of antigen priming, it markedly suppresses IgE synthesis, BAL eosinophilia, and tracheal ring reactivity to acetylcholine.¹⁷ Delaying the treatment until the time of local allergen challenge was shown by several investigators to be effective in inhibiting AHR and eosinophilia, although it was not effective in inhibiting IgE production.^{18,19} The protection provided by IL-12 was accompanied by a shift in the cytokine pattern from a T_H2 to a T_H1 profile, with substantial increases in IFN- γ levels in the BAL fluids.¹⁸ IL-12 has been shown to reverse established T_H2 immune responses in vivo in some models¹⁸ but not others.¹⁹ Interestingly, one group has highlighted the potential utility of using IL-12 as an adjuvant in immunotherapeutic approaches to the treatment of allergic airway responses.²⁰ They showed that vaccinating mice with a fusion protein containing ovalbumin and murine IL-12 after ovalbumin sensitization significantly reduced IgE production, increased IgG2a, and greatly enhanced T_H1 cytokine synthesis, all in an antigen-specific fashion.

The inhibitory actions of IL-12 on allergic airway responses are thought to be primarily, but not entirely, mediated by its ability to stimulate IFN- γ . Studies by Gavett et al¹⁸ indicated that the inhibitory effects of IL-12 on AHR were mediated through induction of IFN- γ , whereas BAL eosinophilia was only partially dependent on IFN- γ . Brusselle and colleagues²¹ more directly examined the role of IFN- γ in the inhibitory effects of IL-12 on eosinophilia in an IFN- γ receptor knockout mouse. Not surprisingly, they demonstrated that the IFN- γ dependence of the inhibitory effect of IL-12 on allergen-induced BAL eosinophilia was dependent on the timing of the IL-12 treatment. Specifically, the ability of IL-12 to inhibit eosinophilia when given at the time of initial priming was dependent on IFN- γ . In contrast, IFN- γ -independent mechanisms were important when the cytokine was administered at the time of allergen challenge. The exact mechanisms by which IL-12 inhibits eosinophilia independently of IFN- γ remain to be determined. Collectively, these studies suggest that IL-12 is an important regulator of T_H2 immune responses in vivo and that dysregulation of this critical immunoregulatory cytokine may lead to the production of pathogenic T_H2 cytokine patterns and susceptibility to allergic airway disease.

Dysregulation of IL-12 production in human asthma

The notion that dysregulated IL-12 levels may be important in allergic airway responses is supported by several studies of human asthmatic patients. Naseer et al²² demonstrated that the number of IL-12 (p40)-expressing cells in bronchial biopsy specimens from allergic asthmatic patients is significantly less than that found in the lungs of normal control subjects (Fig 2). Furthermore, treatments that result in improved lung function, such as steroid treatment, result in significantly increased numbers of IL-12⁺ cells in bronchial biopsy specimens. These increases were associated with a concurrent reduction in the number of IL-13-expressing cells. Moreover, those

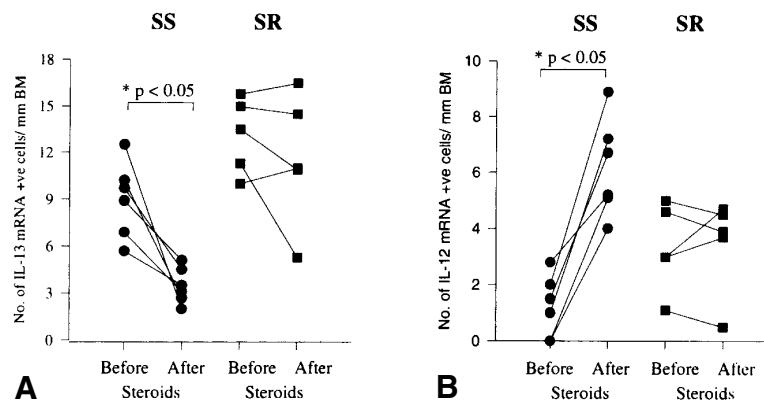


FIG 3. In situ hybridization of bronchial biopsy specimens from steroid-sensitive (SS) and steroid-resistant (SR) atopic asthmatic subjects before and 2 weeks after prednisone treatment with riboprobes for IL-13 mRNA (A) and IL-12 mRNA (B). Statistical analysis was performed by using a nonparametric Wilcoxon signed-rank test ($P < .05$). Reprinted with permission from Naseer T, et al. *Am J Respir Crit Care Med* 1997;155:845-51.

patients who were resistant to steroid treatment did not show an increase in the numbers of lung cells expressing IL-12 (Fig 3). A similar increase in skin IL-12 message levels has been observed in patients with hay fever who underwent a successful pollen immunotherapy regimen.²³ Similar impairments in IL-12 production have also been observed in whole blood cultures,²⁴ PBMCs,²⁵ and alveolar macrophages²⁶ from atopic asthmatic patients compared with atopic nonasthmatic and normal control subjects. Taken together, these studies strongly suggest that deficiencies in IL-12 production are associated with susceptibility to allergic airway disease.

Although the mechanisms that give rise to impaired IL-12 production in asthmatic subjects are unclear at the present time, several potential mechanisms exist, such as altered expression of the genes encoding either one or both of the individual subunits of the functional cytokine or overproduction of cytokines, that inhibit its production. Whether altered IL-12 production in asthmatic subjects is a primary or secondary event is not currently known; however, the fact that the gene encoding the p40 subunit is located in a region of chromosome 5q that has been associated with asthma-related traits supports the notion of a primary event.^{1,2} Indeed, rare polymorphisms in the promoter regions of both of these genes have recently been identified,²⁷ but to my knowledge, their association with human asthma has yet to be evaluated.

Alternatively, the impairment of macrophage IL-12 production in asthma may be caused by increases in mediators and cytokines known to negatively influence *IL12* gene expression, such as IL-10. Van der Pouw Kraan et al²⁴ examined the potential role of IL-10 in the suppressed levels of IL-12 in PBMCs from asthmatic subjects. They demonstrated that SAC-stimulated levels of IL-10 were equivalent between asthmatic and normal subjects, whereas IL-12 levels were reduced in asthmatic patients. Furthermore, neutralization of IL-10 in cultures of PBMCs did not influence IL-12 production in either group. They also showed that the impairment in IL-12 does not just

reflect a generalized failure of monocytes-macrophages from asthmatic subjects to produce cytokines because IL-6 levels were equivalent between normal control subjects and asthmatic patients. Despite the lack of evidence for a role for IL-10, a role for other mediators known to inhibit IL-12 production, such as histamine, PGE₂, TGF- β , IL-4, or IL-13, cannot be ruled out.

Another possible explanation for impaired T_H1 responses is a loss of responsiveness to IL-12. Recent studies in mice have indicated that loss of IL-12 responsiveness and subsequent inability of mice to mount T_H1 responses is due to altered expression of the IL-12R β 2 subunit. Furthermore, IL-12-dependent signaling in human T_H1 cells was shown to correlate with the selective expression of the transcripts encoding the signaling component of IL-12R β 2 and with the presence of high-affinity IL-12-binding sites selectively located on T_H1 cells. Thus it is proposed that not only is IL-12R β 2 a marker of T_H1 cells but that lack of expression of this receptor subunit results in an inability to mount T_H1 responses. Interestingly, various allergic conditions have been shown to be associated with altered *IL12RB2* gene expression. For example, Matsui et al²⁸ demonstrated that IFN- γ production in response to IL-12 stimulation of PBMCs was reduced in 24 of 75 atopic individuals, whereas responses to PHA were not different from those of control subjects. Ten of these 24 asthmatic subjects were heterozygous for truncated (2496 del 91) or missense (1577 A to G and 2799 A to G) mutations of the *IL12RB2* chain gene. In these same individuals IL-12-induced phosphorylation of STAT4 was reduced relative to that of control individuals. In another study, both IL-12 production and IL-12R β 2 expression in tissue biopsy specimens from patients with allergic rhinitis were reduced compared with those found in control patients.²⁹ Interestingly, although steroid treatment of these patients reversed suppression of IL-12 production, it did not restore IL-12R β 2 expression.

TABLE I. Functions of IL-12/IL-13 relevant to allergic airway diseases

Function	IL-12	IL-13
T _H cell differentiation	Induces T _H 1 and inhibits T _H 2	Inhibits T _H 1
Proinflammatory cytokine production (IL-1, IL-6, IL-8)	Induces	Inhibits
IFN- γ production	Induces	Inhibits
AHR	Inhibits (IFN- γ dependent)	Induces
IgE	Inhibits	Induces
Eosinophilia	Inhibits (IFN- γ dependent and independent)	Induces
VCAM-1	Not determined in allergic disease	Induces
Eotaxin	Inhibits	Induces
Mastocytosis	Inhibits	Induces

VCAM, Vascular cell adhesion molecule.

It has also been suggested that mainstay therapies for asthma, such as glucocorticoids and β -adrenergic agonists, may contribute to the imbalance in IL-12/IL-13 cytokine production. For example, β -agonists have recently been shown to decrease T_H1 cytokines and increase T_H2 cytokine production in human PBMCs. This shift in cytokine production is thought to be due to alterations in IL-12 levels because addition of rIL-12 prevented the β -adrenoceptor-induced changes in cytokine patterns.³⁰ Similarly, glucocorticoid treatment of human dendritic cells reduced their ability to promote the production of IFN- γ in CD4⁺ T cells.³¹ This decreased ability was likely a result of steroid effects on IL-12 because IFN- γ production could be restored by exogenous rIL-12. In addition, steroids have been shown to directly inhibit IL-12 production in monocytes³² and dendritic cells.³³ Dexamethasone has also been shown to decrease IL-12-induced IFN- γ secretion and IFN receptor factor 1 in T cells and natural killer cells. The reduced responsiveness to IL-12 appears to be due to inhibition of STAT4-dependent IFN- γ regulatory factor 1 promoter activity.³⁴ Further evidence of reduced responsiveness to IL-12 is provided by the observation that steroid treatment inhibits surface expression of both IL-12 receptor subunits (IL-12R β 1 and IL-12R β 2) on human PBMCs.¹⁰ Interestingly, the reduction in receptor expression was not mediated by IL-4, IL-10, or TGF- β .

Although the in vitro studies demonstrate that steroids reduce IL-12 levels and shift cytokine production toward a T_H2 pattern, in vivo administration of steroids to asthmatic patients is associated with increases in T_H1 cytokines and IL-12 levels in supernatants of blood cultures and in sera of patients.³⁵ This elevation in T_H1-promoting cytokine levels was associated with a significant improvement in FEV₁. Taken together, these studies suggest that although steroids may be beneficial for asthma or allergies as a result of the direct inhibitory effects of steroids on cytokine synthesis in T cells, chronic steroid therapy may indirectly exacerbate the long-term course of allergic disease.

Collectively, these studies suggest that alterations in either IL-12 production, responsiveness, or a combination thereof may contribute to aberrant immune responses to ubiquitous antigens observed in human asthma. Studies to determine the exact mechanisms of such alter-

ations in this pivotal regulatory cytokine are clearly warranted. Thus a better understanding of the mechanisms underlying dysregulation of IL-12 production and responsiveness awaits studies designed to examine IL-12 receptor expression and modulation of IL-12 production in asthma.

IL-13

IL-13 is a 12-kd cytokine cloned from activated T cells.³⁶ The human *IL13* gene is located on chromosome 5q31 in the cluster of genes encoding IL-4, IL-3, IL-5, IL-9, and GM-CSF. The gene encoding IL-13 is only 25 kb upstream of the *IL4* gene and in the same orientation, leading to the speculation that these genes arose as a duplication event during evolution. Although the IL-13 protein has approximately 25% homology with IL-4, it shares many structural characteristics with IL-4. Furthermore, it shares many, but not all, functional properties with IL-4. This overlap in function is due to the sharing of a receptor chain in their individual multimeric receptor complexes.³⁷

The functional IL-13 receptor complex is a heterodimer composed of the IL-4R α chain³⁸ and a 65- to 70-kd protein termed IL-13R α 1. There is an additional IL-13-binding protein referred to as IL-13R α 2. This receptor chain binds IL-13, but not IL-4, with very high affinity but does not seem to be important in signaling. It is currently thought to serve as a decoy receptor because it has been found in soluble form in vivo. The genes encoding the 2 IL-13 receptor chains are both mapped to the X chromosome. The unique IL-4 receptor is composed of the IL-4R α chain and the γ c chain, which it shares with IL-2, IL-7, IL-9, and IL-15. Interestingly, the receptor complex formed by IL-4R α and IL-13R α 1 also serves as a receptor for IL-4 in cells lacking the γ c chain.

IL-13R is expressed on monocyte-macrophage populations, B cells, basophils, eosinophils, mast cells, endothelial cells, fibroblasts, smooth muscle, and airway epithelium. In contrast to IL-4, no functional IL-13 receptor has been detected on human or mouse T cells or mouse B cells.³⁹

Signaling through the IL-13R complex is thought to occur through the IL-4R α chain because antibodies directed against the IL-4R α chain inhibit the binding and the

biologic activities of IL-13 and IL-4. Ligation of either cytokine receptor complex results in activation of JAK-1 and Tyk-2 kinases and induction of tyrosine phosphorylation of the IL-4R α chain and the insulin receptor substrate 2, which is the docking site for the Src homology domain containing the PI kinase in lymphoid cells.³⁷ In contrast to IL-4, IL-13 does not induce activation of JAK-3 kinase, which associates with the γ_c chain of the IL-4R complex after IL-4 binding. Phosphorylation of the IL-4R α chain after binding of IL-13 or IL-4 results in recruitment, phosphorylation, and nuclear translocation of STAT6, where it binds to specific consensus sequences found within the promoter regions of genes regulated by IL-4 and IL-13.

IL-13 is produced at relatively high levels by both T_H1 and T_H2 CD4⁺ T cells after activation. In addition, T_H0 and CD8⁺ T cells produce considerable quantities of IL-13. IL-13, unlike IL-4, is also produced by naive CD45RA⁺ T cells. Thus IL-13 does not behave as a classical T_H2 cytokine. IL-13 is also produced by a number of non-T-cell populations, such as keratinocytes, mast cells, and, in large quantities, basophils.

Biologic effects of IL-13

IL-13 has a number of actions similar to IL-4 as a result of sharing of the IL-4R α chain. Both cytokines are known to have a number of actions relevant to the allergic diathesis, such as the regulation of isotype class switching in B cells to IgE synthesis, induction of the expression of MHC II and CD23, induction of adhesion molecule expression on endothelial cells (vascular cell adhesion molecule 1), chemokine production (eotaxin), the activation of mast cells and eosinophils, and the inhibition of proinflammatory gene expression (IL-1, TNF, and IL-6) by monocyte-macrophage populations.⁴⁰ However, there are clear differences in their functions, such as the fact that in contrast to IL-4, IL-13 does not support proliferation of activated human and mouse T cells. More importantly, IL-13 is unable to directly drive differentiation of naive CD4⁺ T cells toward a T_H2 phenotype. This is likely because of the fact that functional IL-13 receptors have not been shown on T cells, although message for the receptor has been detected in activated T cells. Even though IL-13 does not have direct effects on T cells, it may affect T-cell functions and T_H1 cell differentiation indirectly through its downregulatory effects on production of proinflammatory cytokines, particularly on monocyte production of IL-12.

Role of IL-13 in experimental murine models of allergic disease

Although CD4⁺ T cells have been implicated for some time in the pathogenesis of asthma, the exact mechanisms by which they induce the allergic phenotype still remain to be determined. Initially, efforts were focused on examining the role of the paradigmatic T_H2 cytokines IL-4 and IL-5. However, recent studies suggest that perhaps these 2 cytokines are not necessarily essential for development of AHR.⁴¹⁻⁴³ In particular, several groups have shown that although antibody-mediated blockade of IL-4 during

allergen sensitization ablates the development of allergic asthma, similar blockade of IL-4 before or during antigen challenge inhibits neither allergic inflammation nor AHR.^{41,42} This strongly suggested that although IL-4 plays its well-recognized immunoregulatory role in generating T_H2 deviation in these models, it is not necessary for the expression of allergic asthma. This was further supported by the finding that transfer of T_H2 cells derived from IL-4-deficient mice was still able to confer AHR.⁴⁴ Further evidence that neither IL-4 nor IL-5 were essential for the expression of AHR was provided by the finding that mice rendered deficient in both IL-4 and IL-5 still have AHR in response to allergen sensitization and challenge.⁴³ Interestingly, Gavett et al⁴⁵ found that blockade of the IL-4R α chain before antigen provocation in sensitized mice effectively inhibited AHR, eosinophilic inflammation, and mucus hyperplasia. Furthermore, Kuperman et al⁴⁶ showed that a deficiency in the STAT6 molecule, which mediates most of the cellular actions of both IL-4 and IL-13, abolished antigen-induced eosinophilic inflammation and AHR. These results suggested that perhaps steps distal to IL-4R α ligation were clearly important in the development of allergic responses but that IL-4 did not appear to be the ligand. Because IL-13 also signals through this pathway, it was proposed that the effectiveness of IL-4 receptor blockade was due to inhibition of IL-13-mediated processes and not those mediated by IL-4. This hypothesis was supported by recent findings that blockade of endogenous levels of IL-13 in sensitized mice by the administration of a soluble form of the IL-13R α 2 chain, which binds only IL-13, reverses AHR and pulmonary mucous cell hyperplasia.^{47,48} Further evidence of its importance as an effector molecule in asthma is provided by the fact that acute administration of IL-13 itself was sufficient to recreate many features of the allergic phenotype, such as AHR, eosinophilic inflammation, and mucus metaplasia, in nonimmunized mice^{47,48} or RAG1-/- deficient mice.⁴⁸ Moreover, chronic expression of IL-13 in the murine lung results in development of these features, as well as subepithelial fibrosis and the formation of Charcot crystals.⁴⁹ Despite the ability of IL-13 to recreate the allergic phenotype in naive mice, recent studies in IL-13-deficient mice suggest that there may be some redundancies in the effects of IL-4 and IL-13 on eosinophilia and AHR but that IL-13 appears to be most important in mucus hypersecretion.⁵⁰ Thus the jury is still out on the exact roles that these 2 cytokines play in the development of allergic responses. Nonetheless, it is probably fair to say that IL-4 is essential for the initial differentiation of CD4 T cells into cells producing T_H2 cytokines, whereas IL-13 is likely the most important mediator of the effector phase of the response, depending on the existing milieu. Further studies are clearly needed to determine the exact role of these 2 cytokines in the allergic phenotype.

The possible mechanisms for differences in contribution of IL-4 and IL-13 to the effector phase of the allergic response are not well understood. However, reasonable hypotheses include the following: (1) there are differences in the kinetics of IL-4 and IL-13 production

during the immune response to inhaled antigens; (2) there are differences in either affinities or distribution of the shared receptors for IL-4 and IL-13; and (3) these receptors induce different signaling pathways. The simplest of these explanations is that perhaps IL-4 is produced primarily during the differentiation phase of the response and that IL-13 is either produced later or its production is sustained. This contention is supported by the finding that IL-13 is produced early after T-cell activation, and steady-state levels of IL-13 mRNA are still observed 72 hours after activation, whereas IL-4 production is transient and message levels are generally not detectable 12 hours after activation of human T-cell clones *in vitro*. Alternatively, the distribution and makeup of the IL-4/IL-13 receptor complex on lung cells may determine the binding affinity of the receptor complex for IL-13 or IL-4. One example of differences in IL-4/IL-13 receptor distribution is the fact that T cells do not have functional IL-13R but do, however, express the unique IL-4R. As more information becomes available on IL-13 receptor expression, more differences may be uncovered. In terms of differences in the makeup of the IL-4/IL-13 receptor complex, the presence of the γ chain (which is primarily on hematopoietic cells) lowers binding affinity of the complex for IL-13, and it is thought that the presence of IL-13R α 2 may weaken binding of the complex for IL-4.⁵¹ Thus differences in the makeup of the IL-4/IL-13 receptor complex on resident airway cells may bias the effector response toward one or the other cytokine. As discussed above, there are some differences in signaling pathways, such as the fact that IL-13 does not activate JAK-3 kinases, whereas ligation of the unique IL-4 receptor complex does. Whether these or other undiscovered signaling differences account for the nonredundant actions of these 2 cytokines remains to be discovered. Much remains to be learned about the relative contribution of IL-4/IL-13 to the allergic response.

Potential mechanisms of IL-13-induced AHR

Although the exact mechanisms by which IL-13 mediates AHR are currently unknown, several hypotheses have been put forth. IL-13 may induce AHR through direct or indirect effects achieved through mediators released by surrounding cells or infiltrating inflammatory cells.

Although CD4⁺ T cells are clearly required to initiate allergic responses, it appears that once IL-13 is produced by the T cell, they are no longer required for development of the allergic phenotype. Evidence for this is presented in RAG-/- mice, which are deficient in functional T cells.⁴⁹ In these mice IL-13 induces hyperreactivity similar to that observed in their wild-type controls. This is not surprising because T cells do not express receptors for IL-13.

IL-13, like IL-4, has been shown to induce IgE synthesis in human, but not mouse, B cells through induction of germline E transcription. Thus it may play an important role in IgE synthesis and IgE-mediated immune responses in human disease. However, IL-13-induced AHR does not appear to be dependent on IgE because rIL-13 induces AHR in animals lacking functional B cells.⁴⁹

A mechanism by which IL-13 may mediate eosinophilic inflammation and AHR is through the recruitment, homing, and activation of inflammatory cells. IL-13 has been shown to have several actions relevant to the recruitment of T cells and eosinophils into the lung. Among these actions are its role in upregulation of vascular cell adhesion molecule 1 expression, its ability to prolong eosinophil survival, and its activity as an eosinophil activation and chemotactic agent. IL-13 has been shown to induce the expression of vascular cell adhesion molecule 1 on endothelial cells in the vasculature, resulting in preferential extravasation of eosinophils into the airway spaces. In addition, IL-13 has been shown to induce eotaxin production in airway epithelial cells *in vitro*.⁵² *In vivo* confirmation of this finding has been provided in IL-13 transgenic mice.⁴⁹ Despite the importance of IL-13 in eosinophil recruitment, preliminary reports suggest that eosinophil infiltration does not appear to be required for IL-13-induced AHR (Wills-Karp M, unpublished data).

Recent reports suggest that IL-13 may induce AHR by means of its direct effects on airway smooth muscle. In this regard, Chen and Panettieri⁵³ have reported that IL-13 can augment cholinergically induced contractions of tracheal smooth muscle *in vitro*, suggesting that IL-13 may mediate AHR by means of its direct effects on airway smooth muscle. Alternatively, IL-13 has been shown to induce proliferation of airway smooth muscle in culture, potentially contributing to the muscle hyperplasia observed in human asthma. IL-13 has also been shown to activate fibroblasts and may thereby alter the balance in matrix protein deposition and lead to increased stiffness of the airways. The role of IL-13 in fibrotic responses has been clearly demonstrated in IL-13 transgenic mice.⁴⁹

IL-13 and IL-4 are potent activators of the 15-lipoxygenase enzyme, which catalyzes the production of 15-hydroxyeicosatrienoic acid in human monocytes and airway epithelial cells.⁵⁴ 15-Hydroxyeicosatrienoic acid products are known to have bronchoconstrictor and vascular effects and mucus-producing activity. Thus IL-13 stimulation of these potent mediators may contribute to the airway changes observed in allergic asthma. Although the exact mechanisms by which IL-13 induces AHR are currently unknown, future studies will undoubtedly reveal the exact mechanisms by which it mediates the effector phase of the allergic response.

IL-13 overproduction in human asthma

Numerous studies have demonstrated exaggerated IL-13 production in the lungs of human asthmatic subjects. Specifically, both message and protein levels of IL-13 are elevated in bronchial biopsy specimens^{22,55,56} and BAL cells⁵⁷⁻⁶¹ from allergic individuals when compared with those of control subjects. Interestingly, elevations in IL-13 mRNA and protein levels appear to be more associated with asthma than with atopy because levels were increased in the lungs of atopic and nonatopic asthmatic patients but not in those of atopic nonasthmatic patients when they were compared with normal subjects.⁵⁶ IL-13 levels are strongly correlated with BAL eosinophils in allergic asthma.

matic subjects after local allergen challenge, whereas IL-4 levels are not.⁶¹ Although the cellular source of IL-13 in BAL is not known, Huang et al⁵⁷ proposed that the primary source of IL-13 in BAL fluid may be alveolar macrophages. More recently, Prieto et al⁶⁰ have provided further support for this hypothesis. They enriched alveolar macrophages from asthmatic individuals after repeated low-dose allergen provocations. They found that mRNA levels for IL-13 in these cells are significantly elevated after the allergen challenges. Thus IL-13 and IL-12 produced by macrophage populations may play an important role in directing the immune response in allergic disease. A potential interplay between these 2 cytokines is illustrated by the reciprocal message levels of these 2 cytokines detected in biopsy specimens from allergic asthmatic patients compared with normal control individuals,²² with IL-13 mRNA levels being elevated and IL-12 levels being reduced. Furthermore, after steroid treatment, the reverse is seen: IL-13 levels decline, and IL-12 levels increase. Collectively, these studies strongly suggest that a yin-yang relationship exists between IL-12 and IL-13 in asthma and that overzealous production of IL-13 and impaired IL-12 production are key components of atopy and asthma.

Polymorphisms in the IL-13/IL-4R complex are associated with asthma

Overproduction of IL-13 in asthma may arise as a result of a primary defect in the *IL13* gene or a secondary effect of expanded T_H2 cells. Although there is certainly ample evidence supporting expanded T_H2 cells in asthma, recent studies suggest that primary defects in either the gene encoding IL-13 or the IL-4R α chain may be important in susceptibility to asthma and related traits. In addition to the evidence for linkage of asthma and related traits to the region of chromosome 5q, where the *IL13* gene resides, there are several population studies of different ethnic backgrounds showing evidence for associations between asthma and related traits and various polymorphisms in the *IL13* gene. Polymorphisms have been identified both in the promoter and the coding region of the gene.⁶³⁻⁶⁵ A C-to-T change at position -1055 of the promoter region has been found in 2 separate studies,⁶²⁻⁶⁴ and in one study⁶³ it has been associated with enhanced production of IL-13. One of the polymorphisms (Arg130Gln) identified in the coding region is thought to occur in a region critical for receptor ligand interactions, leading to the speculation that this polymorphism may account for enhanced IL-13 signaling.^{63,64} However, this would not likely explain the overproduction of IL-13 in asthma. As Graves et al⁶⁴ have reported, a significant level of linkage disequilibrium between the polymorphic sites in the *IL13* gene, the contribution of surrounding genes on chromosome 5q, or other as yet unidentified polymorphisms within the gene encoding IL-13 cannot be ruled out.⁶⁵

Multiple polymorphisms in the *IL4R* gene have also been identified and associated with asthma and related traits in several studies.⁶⁵⁻⁶⁷ In fact, the gene encoding IL-4R α is located on chromosome 16p12, a region that has been linked to atopy and asthma in several populations.⁶⁷

It was originally reported that a polymorphism at position Q576R of the gene encoding IL-4R α resulted in enhanced activity of the receptor⁶⁵; however, more recent studies suggest that the altered receptor activity in cells from asthmatic subjects might be due to either a different polymorphism or a combination of polymorphisms within the gene.^{66,68} Alterations in this receptor that lead to enhanced receptor activity could mediate both the IL-4-dependent differentiation of T_H2 cells and the IL-13-dependent processes mediating the effector phase of the allergic airway response. Although much work remains to be done before the exact relationships between polymorphisms in either the IL-13 or IL-4R α chain and asthma are known, these studies underscore the possibility that polymorphisms in these genes or combinations thereof may contribute to susceptibility to asthma in some individuals.

YIN-YANG: IL-12/IL-13

The studies discussed above suggest that an imbalance in IL-12/IL-13 production may underlie the T_H2-biased immune response in asthma. Ample evidence exists to support an overproduction of IL-13 and an impairment in IL-12 production in atopic and asthmatic individuals. Furthermore, treatments that shift this balance in the opposite direction are associated with improvement in lung function. Thus IL-13 working in concert with IL-4 and other T_H2 cytokines (IL-5 and IL-9), proinflammatory cytokines, and chemokines may explain many of the symptoms and manifestations of allergic disease. Its overproduction could also influence the differentiation of T cells toward a T_H2 phenotype through its ability to modulate IL-12 production from antigen-presenting cells. Whether as a result of exaggerated T_H2 cytokine production or as a primary defect, lack of IL-12 can clearly bias immune responses toward a skewed T_H2 response. Although it is not entirely clear what tips the balance between these 2 cytokines, genetic studies suggest that this could indeed be a primary event. Interestingly, the genes encoding both of these cytokines reside within the chromosomal regions on 5q, which has been associated with asthma in many populations. Although no evidence exists to date to support an association between asthma and polymorphisms in the *IL12* gene, evidence is accumulating that supports an association between genetic alterations in the *IL13* gene and atopy and asthma. Although the jury is far from in on the underlying reasons for the imbalance in production of these 2 cytokines, these 2 double-digit cytokines should hold our interest for some time to come.

REFERENCES

1. Trinchieri G. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* 1994;84:4008-27.
2. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Friedhoff LR, Ehrlich E. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
3. Postma DS, Bleeker ER, Amelung PJ, Holroyd KJ, Xu JF, Panhuysen CIM, et al. Genetic susceptibility to asthma-bronchial hyperresponsive-

- ness coinherited with a major gene for atopy. *N Engl J Med* 1995; 333:894-900.
4. Szabo SJ, Jacobson NG, Dighe AS, Gubler U, Murphy KM. Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. *Immunity* 1995;2:665-75.
5. Jacobson NG, Szabo SJ, Weber-Nordt RM, Zhong Z, Schreiber RD, Darnell JE, et al. Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat 4. *J Exp Med* 1995;181:1755-62.
6. Ma X, Chow JM, Gri G, Carra G, Gerosa F, Wolf SF, Dzialo R, et al. The interleukin-12 gene promoter is primed by interferon- γ in monocytic cells. *J Exp Med* 1996;183:147-57.
7. Meyaard L, Hovenkamp E, Otto SA, Miedema F. IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. *J Immunol* 1996;156:2776-82.
8. D'Andrea A, Ma X, Aste-amezaga M, Paganin C, Trinichieri G. Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor- α production. *J Exp Med* 1995;181:537-46.
9. Van der Pouw Kraan TCTM, Boeijs LCM, Smeenk RJT, Wijdenes J, Aarden LA. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J Exp Med* 1995;181:775-9.
10. Wu C, Wang K, McDyer JF, Seder RA. Prostaglandin E2 and dexamethasone inhibit IL-12 receptor expression and IL-12 responsiveness. *J Immunol* 1998;161:2723-30.
11. Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. *J Immunol* 1998;161:2586-93.
12. Manetti R, Parronchi P, Giudizi M-P, Piccini E, Maggi G, Trinchieri G, et al. Natural killer cell stimulatory factor (interleukin-12 [IL-12]) induces T helper type 1 (Th)-specific immune responses and inhibits the development of IL-4 producing Th cells. *J Exp Med* 1993;177:1199-204.
13. Kiniwa M, Gately M, Gubler U, Chizzonite R, Fargeas C, Delespesse G. Recombinant interleukin-12 suppresses the synthesis of IgE by interleukin-4 stimulated human lymphocytes. *J Clin Invest* 1992;90:262-6.
14. Marshall JD, Secrist H, DeKruyff, Wolf SF, Umetsu DT. IL-12 inhibits the production of IL-4 and IL-10 in allergen-specific human CD4+ T lymphocytes. *J Immunol* 1995;155:111-7.
15. Keane-Myers A, Wysocka M, Trinchieri G, Wills-Karp M. Resistance to antigen-induced airway hyperresponsiveness requires endogenous production of IL-12. *J Immunol* 1998;161:919-26.
16. Zhao LL, Linden A, Sjostrand M, Cul ZH, Lotvall J, Jordana M. IL-12 regulates bone marrow eosinophilia and airway eotaxin levels induced by airway allergen exposure. *Allergy* 2000;55:749-56.
17. Sur S, Lam J, Bouchard P, Sigounas A, Holbert D, Metzger WJ. Immunomodulatory effects of IL-12 on allergic lung inflammation depend on timing of doses. *J Immunol* 1996;157:4173-80.
18. Gavett SH, O'Hearn DJ, Li X, Huang S, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995;182:1527-36.
19. Kips JC, Brusselle GJ, Joos GF, Peleman RA, Tavernier JH, Devos RR, et al. Interleukin-12 inhibits antigen-induced airway hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1996;153:535-9.
20. Kim TS, DeKruyff RH, Rupper R, Maecker HT, Levy S, Umetsu DT. An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type 1-dominated immune response and inhibiting antigen-specific IgE production. *J Immunol* 1997;158:4137-44.
21. Bruselle GG, Kips JC, Peleman RA, Joos GF, Devos RR, Tavernier JH, et al. Role of IFN- γ in the inhibition of the allergic airway inflammation caused by IL-12. *Am J Respir Cell Mol Biol* 1997;17:767-71.
22. Naseer T, Minshall EM, Leung DYM, Laberge S, Ernst P, Martin RJ, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Respir Crit Care Med* 1997;155:845-51.
23. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durhan SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254-60.
24. Van der Pouw Kraan TCTM, Boeijs LCM, de Groot ER, Stapel SO, Snijders A, Kapsenberg ML, et al. Reduced production of IL-12 and IL-12-dependent IFN- γ release in patients with allergic asthma. *J Immunol* 1997;158:5560-5.
25. Chou CC, Huang MS, Hsieh KH, Chiang BL. Reduced IL-12 level correlates with decreased IFN- γ secreting T cells but not natural killer cell activity in asthmatic children. *Ann Allergy Asthma Immunol* 1999; 82:479-84.
26. Tang C, Rolland JM, Ward C, Li X, Bish R, Thien F, et al. Modulatory effects of alveolar macrophages on CD4+ T-cell IL-5 responses correlate with IL-1 β , IL-6, and IL-12 production. *Eur Respir J* 1999;14:106-12.
27. Pravica V, Brogan JJ, Hutchinson IV. Rare polymorphisms in the promoter regions of the human interleukin-12 p35 and interleukin-12 p40 subunit genes. *Eur J Immunogenet* 2000;27:35-6.
28. Matsui E, Kaneko H, Fukao T, Teramoto T, Inoue R, Watanabe M, et al. Mutations of the IL-12 receptor beta2 chain gene in atopic subjects. *Biochem Biophys Res Commun* 1999;266:551-5.
29. Wright ED, Christodoulouopoulos P, Frenkiel S, Hamid Q. Expression of interleukin (IL)-12 (p40) and IL-12 (beta 2) receptors in allergic rhinitis and chronic sinusitis. *Clin Exp Allergy* 1999;29:1320-5.
30. Agarwal SK, Marshall GD. Beta-adrenergic modulation of human type-1/type-2 cytokine balance. *J Allergy Clin Immunol* 2000;105:91-8.
31. Vieira PL, Kalinski P, Wierenga EA, Kapsenberg ML, de Jong EC. Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential. *J Immunol* 1998;161:5245-51.
32. Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ T lymphocytes. *J Immunol* 1997;158:5589-95.
33. Matasic R, Dietz AB, Vuk-Pavlovic S. Dexamethasone inhibits dendritic cell maturation by redirecting differentiation of a subset of cells. *J Leukoc Biol* 1999;66:909-14.
34. Franchimont D, Galon J, Gadina M, Visconti R, Zhou Y, Aringer M, et al. Inhibition of Th1 immune response by glucocorticoids: dexamethasone selectively inhibits IL-12-induced STAT4 phosphorylation in T lymphocytes. *J Immunol* 2000;164:1768-74.
35. Purello-D'Ambrosio F, Gangemi S, Merendino RA, Arena A, Guarneri F, Ricciardi L. Effect of fluticasone propionate on interleukin-12 and interferon- γ production in patients affected by allergic bronchial asthma. *J Invest Allergol Clin Immunol* 1999;9:262-7.
36. Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not T cells. *Immunol Today* 1994;15:19-26.
37. Hilton DJ, Zhang J-G, Metcalf D, Alexander WS, Nicols NA, Willson TA. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of interleukin 4 receptor. *Proc Natl Acad Sci U S A* 1996;93:497-501.
38. Aman MJ, Tayebi N, Obiri NI, Puri RK, Modi WS, Leonard WJ. cDNA cloning and characterization of the human interleukin 13 receptor α chain. *Biol Chem* 1996;271:29265-70.
39. Graber P, Gretener D, Herren S, Aubry JP, Elson G, Poudrier J, et al. The distribution of IL-13 receptor alpha 1 expression on B cells, T cells and monocytes and its regulation by IL-13 and IL-4. *Eur J Immunol* 1998;28:4286-98.
40. De Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. *J Allergy Clin Immunol* 1998;102:165-9.
41. Coyle AJ, Le Gros G, Bertrand C, Tsuyuki S, Heusser CH, Kopf M, et al. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 1995;13:54-9.
42. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Mattay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperactivity. *J Exp Med* 1996;183:109-17.
43. Hogan SP, Mattaei KI, Young JM, Koskinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperactivity in Balb/c mice independently of IL-4 and IL-5. *J Immunol* 1998;161:1501-9.
44. Cohn L, Tepper JS, Bottomly K. IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. *J Immunol* 1998;161: 3813-6.
45. Gavett SH, O'Hearn DJ, Karp CL, Patel EA, Schofield BH, Finkelman FD, et al. Interleukin-4 receptor blockade prevents airway responses induced by antigen challenge in mice. *Am J Physiol Lung Cell Mol Physiol* 1997;272:L253-61.

46. Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *J Exp Med* 1998;187:939-48.
47. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TL, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
48. Grunig G, Warnock M, Wakil AD, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-2.
49. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *Clin Invest* 1999;103:779-88.
50. Webb DC, McKenzie ANJ, Koshinen AML, Yang M, Mattes J, Foster PS. Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyper-reactivity. *J Immunol* 2000;165:108-13.
51. Kuznetsov VA, Pud RK. Kinetic analysis of high affinity forms of interleukin (IL)-13 receptors: suppression of IL-13 binding by IL-2 receptor gamma chain. *Biophys J* 1999;77:154-72.
52. Li L, Xia Y, Nguyen A, Lai YH, Feng L, Mosmann TR, et al. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression on airway epithelial cells. *J Immunol* 1999;162:2477-87.
53. Chen H, Panettieri RA. Interleukin-13 modulates carbachol- and KCL-evoked contractile responses in murine tracheal rings [abstract]. *Am J Respir Crit Care Med* 1999;159:A399.
54. Nassar GM, Morrow JD, Roberts LJ 2d, Lakkis FG, Badr KF. Induction of 15-lipoxygenase by interleukin-13 in human blood monocytes. *J Biol Chem* 1994;269:27631-4.
55. Kotsimbos TC, Ernst P, Hamid QA. Interleukin-13 and interleukin-4 are coexpressed in atopic asthma. *Proc Assoc Am Physicians* 1996;108:368-73.
56. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J Allergy Clin Immunol* 1997;99:657-65.
57. Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995;155:2688-94.
58. Ying S, Meng Q, Barata LT, Robinson DS, Durham SR, Kay AB. Association between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression and the infiltration of eosinophils, macrophages and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* 1997;158:5050-7.
59. Body KJ, Semper AE, Redington AE, Madden J, Teran LM, Holgate ST, et al. Cytokine profiles of BAL T cells and T-cell clones obtained from human asthmatic airways after local allergen challenge. *Allergy* 1999;54:1083-93.
60. Prieto J, Lensmar C, Roquet A, van der Ploeg I, Gigliotti D, Eklund A, et al. Increased interleukin-13 mRNA expression in bronchoalveolar lavage cells of atopic patients with mild asthma after repeated low-dose allergen provocations. *Respir Med* 2000;94:806-14.
61. Kroegel C, Julius P, Matthys H, Virchow JC Jr, Luttman W. Endo-bronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. *Eur Respir J* 1996;9:899-904.
62. van der Pouw Kraan TCTM, van Veen A, Boeji LCM, van Tuyl SAP, de Groot ER, Stapel SO, et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999;1:61-5.
63. Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, Gao PS, Ohshima K, et al. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet* 2000;9:549-59.
64. Graves PE, Kabesch M, Halonen M, Holberg CJ, Baldini M, Fritzsche C, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;105:506-13.
65. Hershey GKK, Friederich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the α -subunit of the interleukin-4 receptor. *N Engl J Med* 1997;337:1720-5.
66. Ober C, Leavitt SA, Tsalenko A, Howard TD, Hoki DM, Daniel R, et al. Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet* 2000;66:517-26.
67. Kruse S, Japha T, Tedner M, Sparholt SH, Forster J, Kuehr J, et al. The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. *Immunology* 1999;96:365-71.
68. Wang HY, Shelburne CP, Zamorano J, Kelly AE, Ryan JJ, Keegan AD. Cutting edge: effects of an allergy-associated mutation in the human IL-4R α (Q576R) on human IL-4-induced signal transduction. *J Immunol* 1999;162:4385-9.