

Anti–interleukin-4 therapy

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Interleukin-4 (IL-4) mediates important pro-inflammatory functions in asthma, including Th2 lymphocyte differentiation, induction of IgE production, up-regulation of IgE receptors, expression of vascular cell-adhesion molecule 1 (VCAM-1), promotion of eosinophil transmigration into the lungs, inhibition of T-lymphocyte apoptosis, and mucus hypersecretion. The role of IL-4 in the pathogenesis of asthma is supported by identification of polymorphisms linked to asthma in the IL-4 gene promoter and proteins involved in IL-4 signaling. Several approaches to IL-4 antagonism are or have been in clinical development, including soluble recombinant human IL-4 receptor (IL-4R), IL-4 mutein, humanized anti-IL-4 monoclonal antibody, and human anti-IL-4R monoclonal antibody. This article examines IL-4 and the antagonists that have been developed. Early trial results and the future of anti-IL-4 therapy are discussed.

Interleukin-4 in the pathogenesis of asthma

IL-4 is a critical cytokine in the development of allergic inflammation. It has a central role in the initial differentiation of naïve T helper cell type 0 (Th0) lymphocytes into the Th2 lymphocytes that drive the allergic phenotype [1,2]. IL-4 acts by way of the IL-4R on the surface of T lymphocytes and up-regulates the expression of the IL-4R in a positive autocrine loop [3]. IL-13, which shares many biologic activities with IL-4, does not promote Th2 differentiation, because IL-13 receptors are not expressed on T lymphocytes [4]. Incubation of human T cells with IL-4 generates Th2-type lymphocyte clones and can be inhibited by anti-IL-4. These Th2-type cells secrete additional IL-4 and other

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Th2 cytokines, including IL-5, IL-9, and IL-13. They also lose the ability to produce the Th1 cytokine interferon γ [5].

Other important activities of IL-4 are induction of the IgE isotype switch, mitogenic stimulation of B-cell growth, and up-regulation of class II major histocompatibility complex (MHC) on B cells [6,7]. IL-4 enhances IgE-mediated immune responses because of its ability to up-regulate IgE receptors on the cell surface: the low-affinity IgE receptor (Fc ϵ RII; CD23) on B lymphocytes and mononuclear phagocytic cells and the high-affinity IgE receptor (Fc ϵ RI) on mast cells and basophils [7]. IgE-dependent activation of mast cells that is induced by IL-4 has a pivotal role in the development of immediate allergic reactions. Levels of leukotriene mediators and their receptors also are increased by IL-4 exposure [8–10].

IL-4 further promotes cellular inflammation in the asthmatic lung by the induction of VCAM-1 on vascular endothelium [11]. Through the interaction with VCAM-1, IL-4 directs the migration of T lymphocytes, monocytes, basophils, and eosinophils to inflammatory loci. IL-4 promotes eosinophilic inflammation by inducing eosinophil chemotaxis and activation and by inhibiting eosinophil apoptosis through increased expression of eotaxin [12].

In addition to driving the initial differentiation of Th0 lymphocytes toward a Th2 phenotype, IL-4 is important in maintaining allergic immune responses by preventing apoptosis of T lymphocytes. Activation of these cells results in rapid proliferation and secretion of cytokines. In the absence of an appropriate signal, activated T helper lymphocytes rapidly become apoptotic and are eliminated. Several cytokines, including IL-2, IL-4, IL-7, and IL-15, are effective in preventing the death of activated T cells. Of these cytokines, IL-4 and IL-15 are the most effective [13]. Inhibition of apoptosis by IL-4 may be mediated partially by the ability of this cytokine to maintain levels of the survival-promoting, anti-apoptotic protein Bcl-2 in T cells [13]. Apoptosis of T lymphocytes can be induced through signals mediated by FasL through the Fas (CD95) receptor that is expressed on these cells. T cells from asthmatic lungs show defective expression of Fas, which may give them increased resistance to apoptotic cell death. Coculture of T cells with IL-4 down-regulates cell-surface expression of Fas. The IL-4 in the asthmatic lung and secondary lack of Fas expression may explain a persistence of inflammatory cellular infiltrates in allergic asthma that is caused by inhibition of apoptosis. Apoptosis of Th2 lymphocytes (and eosinophils) could represent a pathway whereby IL-4 blockade will produce rapid clinical benefits in asthma.

Corticosteroids normally cause apoptosis of mature T helper cell lines. Induction of cell death is prevented by IL-2 in Th1 cells and by IL-4 in Th2 cells [14]. IL-2 and IL-4 synergize to render lymphocytes refractory to the anti-inflammatory influences of corticosteroids. This action is a result of alternative splicing of glucocorticoid receptor (GCR) mRNA transcripts in the presence of IL-2 and IL-4, thereby generating GCR- β . GCR- β cannot bind to glucocorticoids but can bind to and antagonize the transactivation induced by GCR- α in the setting of glucocorticoid therapy. Through these mechanisms, the autocrine

production of IL-4 by Th2 cells in the asthmatic lung may render these cells refractory to the anti-inflammatory influences of corticosteroids [15]. Synergistic benefits of IL-4 inhibitory therapy and inhaled corticosteroids may be expected in patients with asthma. In one study, glucocorticoid therapy induced a Th2 phenotype in such patients [16]. This finding shows an additional mechanism by which IL-4 inhibition may synergize with inhaled corticosteroids in the treatment of asthma.

The use of new approaches, such as microarray gene profiling, offers the opportunity to discover novel pathways of IL-4 regulation that may be important in asthma and allergic reactions. One study found that IL-4 induced expression of squamous cell carcinoma antigens 1 and 2 [17]. Elevated levels of both proteins were found in the serum of patients with asthma, as compared the serum levels of controls, but the exact role of these proteins in the mediation of disease remains to be determined.

Clinical observations of interleukin-4 in allergic disease

IL-4 levels are increased in the serum and bronchoalveolar lavage of allergic individuals [18], and studies of bronchoalveolar lavage have demonstrated increased numbers of T cells that express mRNA for IL-4 [19]. Peripheral blood mononuclear cells from atopic asthmatics generate augmented IL-4 production in response to dust mite antigen [20]. Nebulized administration of exogenous IL-4 to patients with mild asthma resulted in a significant increase in airway hyper-responsiveness that was associated with an influx of sputum eosinophils [21]. A variety of findings suggest that atopic individuals have altered regulation of IL-4 production. In one study, CD4⁺ T-cell clones from atopic individuals produced IL-4 and IL-5 in response to bacterial antigens, whereas CD4⁺ T-cell clones from nonatopic individuals produced Th1 cytokines [22]. Atopic subjects have a higher number of IL-4-producing T cells than do normal subjects [23], and T-cell clones generated from cord blood lymphocytes of newborns with atopic parents produced higher IL-4 concentrations than neonatal lymphocytes of newborns with nonatopic parents [24].

Another mechanism by which IL-4 contributes to airway obstruction in asthma is through induction of mucin gene expression and mucus hypersecretion [25]. One study found that in asthmatic subjects, but not in normal controls, IL-4-stimulated bronchial epithelial cell explants released significantly increased levels of transforming growth factor α , which stimulates epithelial and fibroblast proliferation and mucus production [26]. IL-4 increases the fibroblast expression of eotaxin, other inflammatory cytokines, and type I procollagen, which might contribute to inflammation and lung remodeling in chronic asthma [27,28]. A more direct mechanism of IL-4-induced remodeling could be caused by the increased fibroblast proliferation that has been observed in response to IL-4 and IL-13 [29] and the ability of these cytokines to induce a myofibroblastic phenotype [30].

Interleukin-4 signaling

Optimal signaling by IL-4 [31] involves its interaction with a receptor complex that consists of a heterodimer of the high-affinity IL-4R α (IL-4R α) protein and the common γ -chain (shared with IL-2, IL-7, IL-9, IL-15, and IL-21) or the IL-13R α_1 chain (Fig. 1). IL-4R α chains are expressed ubiquitously. IL-13

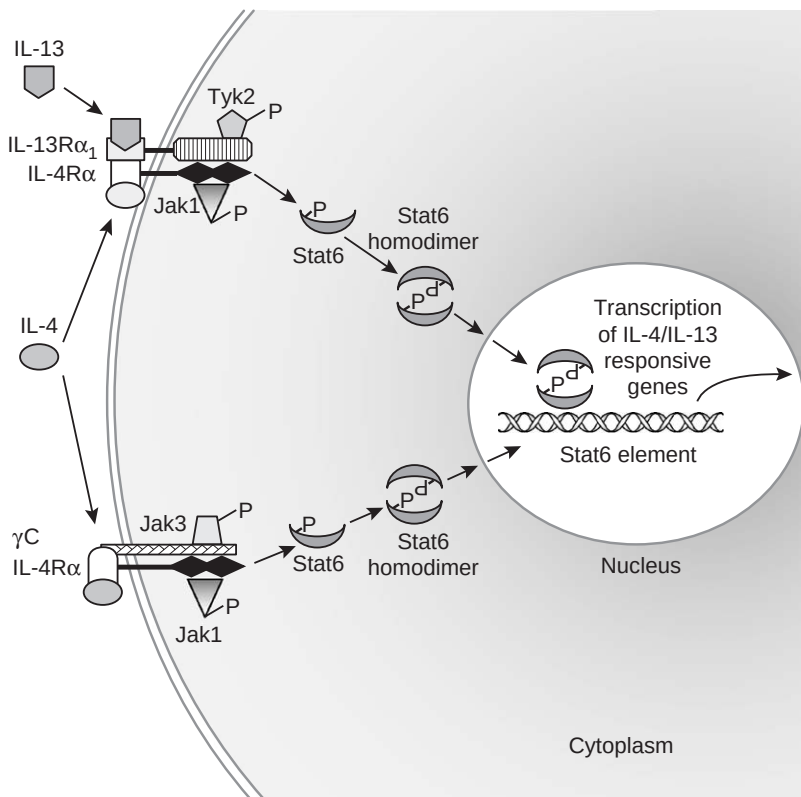


Fig. 1. IL-4 signals by way of a heterodimeric receptor composed of high-affinity IL-4R α protein and the common γ -chain (γ C; T cells and others) or the IL-13R α_1 chain (endothelial cells and others). IL-13 signals through the heterodimeric receptor consisting of IL-4R α and IL-13R α_1 . A putative inhibitory receptor for IL-13 that consists of IL-4R α and IL-13R α_2 has been described. A family of related cytoplasmic protein tyrosine kinases, termed Janus kinases (Jaks), function in IL-4 and IL-13 signaling: Jak1, Jak3, and Tyk2. On binding of the ligand, the Jaks physically associate with the receptor and mediate tyrosine kinase activity. Jak1 and Jak3 are activated in response to IL-4 when bound to the IL-4R α and common γ C receptor, respectively. Signaling by IL-13 or IL-4 bound to the IL-4R α and IL-13 α_1 heterodimer involves Jak1 and Tyk2. Activation of the cytokine receptor leads to tyrosine phosphorylation of the receptor and receptor-associated Jaks. The next step in signal transduction involves tyrosine phosphorylation of cytoplasmic factors (Stat6). After activation, these proteins become phosphorylated, form homodimers, and migrate to the nucleus, where they bind to regulatory sequences in the promoters of IL-4 and IL-13 responsive genes to activate transcription. P (also inverted P), phosphorylated residue.

mediates its biologic activities through a heterodimer consisting of the IL-4R α and the IL-13R α_1 proteins. IL-13R α_1 expression is limited to hematopoietic cells, including B cells, mononuclear phagocytes and basophils (but not T cells or mast cells), and endothelial cells. This more limited distribution of IL-13R α_1 expression explains the pattern of shared biologic activities of IL-4 and IL-13 and the unique ability of IL-4 to induce Th2 lymphocyte differentiation and leukotriene production by mast cells. The two IL-13R α chains that have been described include the active form of IL-13R α_1 and the putative inhibitory receptor IL-13R α_2 , which lacks the motif required for the binding of Janus kinases [32].

The tyrosine kinases Jak1 and Jak3 constitutively associate with IL-4R α and the γ -chain, respectively. Engagement of the IL-4R leads to the activation of Jak1 and phosphorylation of the cytoplasmic region of the IL-4R, which in turn phosphorylates the signal transducer and activator of transcription 6 (Stat6). After phosphorylation, Stat6 molecules dimerize and translocate to the nucleus, where they bind in the promoter region of responsive genes and activate gene transcription. Stat6 is necessary for IL-4-dependent expression of IL-4R α , ϵ heavy chain, MHC class II, CD23, and mucin [31]. The phosphorylation of the IL-4R α cytoplasmic region also provides a docking site for the insulin receptor substrate 1 (IRS-1) and its homolog IRS-2 [33]. IRS-1 and IRS-2 regulate cellular proliferation and protect cells from apoptosis [31]. Although the IRS-1 and IRS-2 pathway is generally responsible for cell proliferation and the Stat6 pathway is responsible for regulation of gene expression, studies in Stat6 knockout mice demonstrate that the altered expression of growth factors contributes to reduced proliferation of these cells in the presence of IL-4. IL-4 activates Stat6, leading to the development of a Th2 response, whereas IL-12 activates STAT4, leading to a Th1 response [34]. Inhibition of Stat6 signaling offers a potential therapeutic opportunity; however, targeting an intracellular mediator is a challenge. An endogenous inhibitor of Stat6, referred to as suppressor of cytokine signaling 1, inhibits IL-4 signaling [35]. Bcl-6 is a transcription factor that counteracts the stimulatory effects of Stat6 by binding to Stat6-responsive promoter elements, acting as a competitive inhibitor for Stat6 binding, and suppressing gene transcription. Bcl-6 suppresses Stat6-dependent, IL-4-induced production of IgE [36]. Inhibitors of intracellular signaling offer potential approaches to IL-4 inhibition.

Asthma and genetics of interleukin-4

Asthma is a complex genetic disorder (reviewed in a study by Steinke et al) [37]. Abnormal production of IL-4 or hyperresponsiveness to this cytokine resulting from inherited defects may contribute to the pathophysiology of this disorder in a subset of asthmatics. Individuals genetically programmed to be hyperresponsive to IL-4 may demonstrate enhanced treatment response to an IL-4 antagonist. Asthma has been associated with polymorphisms in the IL-4,

IL-4R α , IL-13, IL-13R α_1 , Stat6, and Bcl-6 genes. The naturally occurring polymorphisms that are linked to atopy and asthma and that may influence IL-4 production and signaling are summarized in Table 1.

Genome searches and candidate gene approaches have linked atopy and asthma to chromosome 5q31-33, a locus for Th2 cytokines, including IL-4 [38,39]. The best characterized polymorphism described for the IL-4 gene is a C-to-T exchange at –590 base pairs (bp) from the open reading frame of the IL-4 gene that correlated with higher levels of total serum IgE and asthma [40,41]. This association was confirmed in studies that included association of the 590T allele with asthma [42] and asthma severity [43]. This variant is associated with enhanced strength of the IL-4 promoter and increased binding affinity by nuclear transcription factors to a nuclear factor of activated T cells (NFAT) site in the IL-4 promoter [44]. Additional polymorphisms that may influence promoter activity have been reported within the IL-4 gene promoter; however, these latter polymorphisms have not been confirmed in several populations, and their significance is unclear.

Candidate gene and genome-wide searches also have linked atopy to chromosome 16p12.1, which includes the IL-4R α chain. Numerous polymorphisms have been identified in the IL-4R gene, and linkages to atopy and asthma are associated with three of them (isoleucine-to-valine mutation at amino acid 50 [Ile50Val], arginine-to-glutamine mutation at amino acid 576 [Arg576Gln], serine-to-proline mutation at amino acid 503 [Ser503Pro]) [45,46]. Ile50Val is associated with a higher prevalence of asthma [46]. Ile50-bearing mononuclear cells demonstrate augmented IL-4-mediated CD23 expression, Stat6 activation, T-cell proliferation, and transcriptional activity of the ϵ heavy chain promoter [46]. Arg576Gln lies in the cytoplasmic domain of IL-4R α that is necessary for optimal activation of Stat6 [45,47,48]. Patients with this mutation demonstrated enhanced phosphorylation of Stat6 and CD23 induction in response to IL-4 [45]. This mutation correlates with the presence of severe atopic eczema [45] and is a risk factor for poorer lung function in asthmatic patients [49]. Ser503Pro is present in the IRS-activating motif of the IL-4R and is associated with elevated total IgE levels [50]. In the concomitant presence of Arg576Gln, Ser503Pro is

Table 1

Genes associated with interleukin-4 and interleukin-4 signaling linked to asthma and allergies

Gene	Polymorphism	Location	Chromosome	Reference
IL-4 promoter	A-to-G	–81	5q31-33	[64]
	C-to-T	–285		[64]
	C-to-T	–590		[40]
IL-4R α chain	Ile-to-Val	+50	16p12.1	[46]
	Ser-to-Pro	+503		[50]
	Gln-to-Arg	+576		[45]
IL-13R α chain	A-to-G	+1398	Xq13	[51]
Stat6	G-to-A	+2964 (3' UTR)	12q13-14	[65]
Bcl-6	HindIII	5' UTR	3q27	[66]

Abbreviation: UTR, untranslated region.

associated with increased phosphorylation of IRS-1 and IRS-2. This increased phosphorylation could augment propagation signals mediated by IL-4, which would influence B-cell proliferation and IgE concentration.

In addition to the shared γ -chain, IL-4R α can form a functional receptor for IL-4 with IL-13R α_1 [31]. In one study, a noncoding A-to-G exchange at +1398 of the IL-13R α_1 gene was associated with higher IgE levels [51]. This association was not confirmed in a different cohort, and no association with asthma was detected in either population [51]. The IL-13R α gene is located on Xq13, which suggests X-linked inheritance of asthma and allergies and may explain some of the maternal influence in these conditions.

Many of these studies suffer from weak associations with allergy and asthma, lack of confirmation in unrelated populations, and the presence of polymorphisms in regions of the gene that would not be predicted to have a biologic effect. Many of these associations may be shown to be specious; however, it remains plausible that some polymorphisms are in linkage disequilibrium with unidentified variants in important regulatory or coding regions or with variants in adjacent genes. Other areas that are beginning to be explored include the ability of multiple genes to interact to increase an individual's susceptibility to asthma and atopy and the role the environment in patients with susceptible genetic backgrounds.

Safety concerns of anti-interleukin-4 therapy

Theoretical questions have been raised regarding the consequences of IL-4 inhibition in the immune system. One critical role for IL-4 is believed to be in the immune defense against parasitic diseases. Studies showed that IL-4-deficient mice did not demonstrate increased susceptibility to parasitic disease, possibly reflecting the IL-4-independent pathway for differentiation of Th2 lymphocytes and non-Th2-dependent mechanisms for protection against parasites [52,53]. The impact on humans is predicted to be less important, because asthma is most prevalent in regions where parasitic disease is not endemic. There also exists a reproductive concern for anti-IL-4 therapy, as the IL-4 that is expressed in the placenta is believed to contribute to maintaining the state of immune privilege. Normal reproduction in Stat6-deficient mice suggests that this issue is not significant. IL-4 knockout mice, IL-4R knockout mice, and transgenic IL-4R overexpressor mice had normal growth, lifespan, and reproduction without evidence of disease. IL-4 originally was cloned as a cofactor involved in B-cell proliferation. Through its induction of Th2-type responses, IL-4 may be important in humoral immunity. IL-4-deficient mice do not demonstrate diminished humoral responses. In clinical trials with the soluble IL-4R, subjects immunized with tetanus booster showed no inhibition of IgG response [54]. There is concern that IL-4 antagonism might promote immune deviation from Th2- to Th1-type responses, and this change could exacerbate autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, or multiple sclerosis.

The use of an IL-4 antagonist did not augment disease manifestations or worsen outcome in a murine model of multiple sclerosis (Immunex Corporation, unpublished data, 1999).

Therapeutic approaches to neutralizing interleukin-4 in asthma

Several drugs have been developed as a biologic therapy that interferes with the activity of IL-4 in asthma and other allergic diseases (Table 2). Trials of these agents vary in their routes of administration, including one inhaled preparation (soluble human IL-4R) and several injectable agents (humanized anti-IL-4, IL-4 mutein, human anti-IL-4R). Inhaled and oral routes of administration have the advantage of increased patient acceptance and compliance compared with injected routes.

Soluble recombinant human interleukin-4 receptor

The anti-IL-4 drug that progressed furthest in clinical development is the soluble recombinant human IL-4R Nuvance (Immunex Corporation). Soluble recombinant human IL-4R is the extracellular portion of the α -chain of the human receptor for IL-4 that has been cloned and produced in a mammalian expression system. Secreted forms of IL-4R α occur naturally and are expressed in allergic inflammation [55]. Soluble IL-4R can interact with IL-4 even though it lacks the transmembrane and cytoplasmic domains. Because soluble IL-4R does not induce cellular activation but instead binds and sequesters IL-4, it serves as an anti-inflammatory mechanism that can counter the effects of IL-4 and may represent an endogenous autoregulatory or homeostatic mechanism. Its ability to act as a decoy to bind and neutralize circulating cytokine and the high specificity and high affinity of binding for the cytokine makes the soluble receptor promising as a cytokine antagonist.

Table 2
Pharmacologic agents with potential interleukin-4 inhibitory activity

	Soluble human IL-4R	IL-4 double mutein (R121D, Y124D)	Humanized anti-IL-4 antibody	Human anti-IL-4R antibody	IL-4 transcription inhibitors (RASONS, SOCS-1)
Inhibition of IL-4	Yes	Yes	Yes	Yes	Selective
Inhibition of IL-13	Unlikely	Yes	No	Yes	Possibly
Potential immunogenicity	Low	Low to moderate	Low	Low	Unknown
Half-life	5 d	4–6 h	18–21 d	18–21 d	—
Route of administration	Inhaled	Injection	Injection	Injection	—

Abbreviations: RASON, respirable antisense oligonucleotides; SOCS-1, suppressor of cytokine signaling 1.

Clinical experience

Several initial studies suggested the safety of soluble IL-4R in the treatment of patients with asthma [54,56]. In a phase 1 study, subjects with mild or moderate persistent asthma stopped use of inhaled corticosteroids and randomly were assigned to receive placebo or IL-4R at 0.5 mg or 1.5 mg by nebulizer [54]. There were no significant adverse events related to use of the study drug, and no patients developed antibodies to IL-4R. Pharmacokinetic analysis demonstrated systemic delivery and a serum half-life of approximately 5 days, making once-weekly therapy feasible. After acute discontinuation of inhaled corticosteroid use, no subjects in the 1.5-mg group withdrew for asthma exacerbations, compared with three of eight subjects in the 0.5-mg group and two of eight subjects in the placebo group. Treatment with 1.5 mg of IL-4R was associated with significantly better forced expiratory volume in 1 second (FEV₁) 2 hours after treatment and on days 2, 4, and 15 ($P < 0.05$). Statistically significant differences in asthma symptom score ($P < 0.05$) and β_2 -agonist use ($P < 0.05$) were associated with IL-4R treatment. Scores on the third section of the Asthma Quality of Life Questionnaire (patient's perception of general health and physical functioning) worsened in the placebo group and improved in the 1.5-mg group ($P < 0.05$). Methacholine testing showed decreased sensitivity in six of eight patients in comparison to placebo group in the 1.5-mg group, and exhaled nitric oxide scores improved significantly among patients receiving IL-4R ($P < 0.05$), a finding that is consistent with an anti-inflammatory effect.

In a phase 1/2, double-blind, placebo-controlled study of IL-4R, 62 patients with moderate persistent asthma were randomized to receive 12 weekly nebulizations of placebo or 0.75, 1.5, or 3.0 mg of IL-4R or placebo [56]. Before the study, patients documented dependence on inhaled corticosteroids by an exacerbation in asthma induced by stepwise 50% reductions in dose at 2-week intervals. After being restabilized on inhaled corticosteroids for 2 weeks, patients discontinued inhaled corticosteroid use and began use of the study medication. IL-4R treatment was safe and well tolerated. Antibodies to IL-4R occurred in one patient, but the antibodies did not have neutralizing activity and produced no symptoms. In the absence of inhaled corticosteroid treatment, patients receiving IL-4R had a stable FEV₁ (−0.09 L, −2% predicted) compared with the significant decline observed in placebo recipients (−0.35 L, −13% predicted; $P = 0.05$ over the 3-month treatment period). Efficacy in IL-4R recipients was demonstrated by a stable daily morning FEV₁ measured at home using a handheld device (−0.1 L, −4% predicted), compared with the significant decline observed in placebo recipients (−0.5 L, −18% predicted; $P = 0.02$ over the 3-month treatment period). The evening FEV₁ for patients receiving the highest IL-4R dose also improved significantly and was 19% better than that for placebo recipients. The efficacy of IL-4R was confirmed further by the absence of an increase in asthma symptom score (Δ 0.1) in the 3.0-mg group compared with the placebo group (Δ 1.4 over 1 month; $P = 0.075$).

In two studies of patients with asthma who were taking only β -agonists, soluble IL-4R that was administered by nebulizer at weekly or daily doses did not

demonstrate clinical efficacy. In these two studies, the patient population or drug regimen may not have been optimal for demonstrating the clinical effects of IL-4 inhibition (Immunex Corporation, unpublished data, 2001). In a third study, the use of daily inhaled corticosteroids was tapered during soluble IL-4R treatment (Immunex Corporation, unpublished data, 2001). Such treatment was ineffective, and no further studies of this agent are planned.

Interleukin-4 mutein

Interleukin-4 mutein (BAY 16-9996; InterPenkin-4) is a competitive IL-4 and IL-13 receptor antagonist that is under development for the treatment of asthma (Bayer Pharmaceutical Corporation). The protein is expressed in *Escherichia coli* K12 as a 15 kDa double mutant (mutein) in which arginine 121 and tyrosine 124 of IL-4 are replaced by aspartic acid residues. The molecule retains high-affinity binding to the IL-4R α chain, which is a component of the IL-4 and IL-13 receptors. Despite binding, BAY 16-9996 has no discernible agonist activity. The molecule is a full antagonist for all analyzed cellular responses, including IL-13–dependent B-cell responses [57–59]. Some of the biologic activities of IL-4 are shared by IL-13, and IL-4 and IL-13 antagonism may be required for optimal inhibition of the disease process in asthma.

Clinical experience

BAY 16-9996 phase 1 clinical trials have been completed. Two clinical studies enrolled a total of 116 adults with stable mild-to-moderate allergic asthma. Stable use of low-dose inhaled corticosteroids and monitored β -agonist was permitted; other asthma therapies were excluded. An initial dose-ranging (0.07–20 mg) study investigated single subcutaneous injection of BAY 16-9996. A second study investigated multiple doses of BAY 16-9996 that were administered over an extended period (subcutaneous administration of 20 mg every other day for three doses followed by weekly injections for 3 weeks). Initial data indicated that BAY 16-9996 is safe and well tolerated at single and multiple doses. Additional testing of this novel asthma therapy is not being performed because of the short half-life of the drug (approximately 6 hours) and the absence of a noninjectable preparation [59].

Humanized antibodies

SB 240,683 (pascolizumab) is a humanized anti-IL-4 monoclonal (IgG₁) antibody that was developed for the treatment of asthma (Protein Design Labs, GlaxoSmithKline). SB 240,683 targets human IL-4, blocking interaction of IL-4 with its receptor and inhibiting early-phase events of chronic asthma. As a humanized monoclonal antibody, SB 240,683 can be expected to have high-affinity binding, low immunogenicity, and a long half-life, which lead to reduced dosing requirements. The treatment was constructed by grafting the complementarity-determining regions from the parent murine antibody 3B9 into human

heavy- and light-chain frameworks. SB 240,683 and murine antibody 3B9 are highly specific for a conformational epitope on human IL-4 and do not recognize bovine or murine IL-4. In pharmacologic studies, SB 240683 bonded to human IL-4 with a high affinity (K_d , 45 pM at 25°C with an off rate of $2 \times 10^{-4} \text{s}^{-1}$) [60].

Biologic activity of the anti-IL-4 antibody has been demonstrated in laboratory studies. IL-5 synthesis by human peripheral blood mononuclear cells that were stimulated with a recall antigen (tetanus toxoid) increased significantly in the presence of exogenous recombinant human IL-4. In this model, antigen-driven, IL-4-dependent synthesis of IL-5, Th2 activation, and up-regulation of IgE expression was inhibited by monoclonal antibody 3B9 ($IC_{50} = 730 \text{ pM}$), providing evidence that antibody neutralization of IL-4 can down-regulate Th2 cytokine production [60].

Clinical experience

The safety and pharmacokinetics of single-dose intravenous administration of SB 240683 in adults with asthma was determined in a phase 1, randomized, double-blind, placebo-controlled, dose-escalation study [61]. Twenty-four patients with mild-to-moderate asthma (age range, 19–45) were administered a single intravenous dose of placebo or SB 240683 at one of four dose levels (0.5, 1.5, 4.5, or 10 mg/kg). In each dose cohort, five patients were randomized to receive active drug, and one patient was randomized to receive placebo. Patients were followed for 55 days after infusion. SB 240683 was safe and well tolerated, and no dose-limiting adverse events occurred. The half-life of drug elimination was 18 to 21 days. Neutralizing antibodies were not observed against SB 240683 at day 55 in all dose cohorts, and no evidence of antibodies in the highest-dose group was found after 5 months of infusion. A large-scale, multidose, phase 2 study of this agent failed to show evidence of efficacy, and studies with this agent have been discontinued.

Human monoclonal antibodies against interleukin-4 receptor α

Because the IL-4 and IL-13 receptors contain the IL-4R α subunit, a monoclonal antibody directed against IL-4R α should inhibit signaling of IL-4 and IL-13. A human anti-IL-4R α receptor antibody is under development (Amgen Corporation). Early studies using a murine model of asthma demonstrated inhibition of eosinophil influx, airway hyperreactivity, and Th2 cytokine production in treated mice [62]. Further preclinical studies are being conducted.

What went wrong and what is the future

Every anti-IL-4 therapy has failed during clinical evaluation with the exception of human anti-IL-4R antibody, which is under preclinical development. All of the drugs were safe, resulted in no adverse reactions, and displayed no evidence of autoantibody production. Why did the trials with these drugs fail

to show a significant improvement in asthma? One obvious concern is the choice of outcome measures and length of time employed in the studies to document patient improvement. The mechanism of anti-IL-4 therapy is believed to involve immune deviation and result in a decrease in the number of Th2 cells and a decrease in the activity of Th2 cells that remain. For soluble IL-4R, the initial studies showed a significant improvement in FEV₁ 2 hours after treatment [54]. This timeframe is inconsistent with the theory that the drug acts through immune deviation. Continued improvement of FEV₁ was observed at days 2, 4, and 15 of the first study and at 3 months in the follow-up study [54,56]. Although 15 days and 3 months begin to approach a timeframe that immune deviation might be expected to occur, the effect on FEV₁ at shorter times likely reflects the drug preparation's possible secondary effect on mast cells, histamine release, and endothelial and smooth muscle cells. A better measure of immune deviation would be investigation of changes in antigen-specific T-cell populations and tissue and circulating eosinophil counts. Because soluble IL-4R was inhaled in these studies, it may have acted locally on the airways and may not have been absorbed systemically. Systemic absorption is needed for immune deviation to occur at the sites of T-cell development. It is possible that IL-4 is necessary to generate the Th2 cells that are important in asthma, but once these cells are formed, IL-13 has a more important role in mediating the inflammatory responses associated with asthma. Because soluble IL-4R and the humanized anti-IL-4 monoclonal antibody act by inhibiting IL-4 and not IL-13, they may not be effective in inhibiting responses in patients with established asthma. The IL-4 mutein and anti-IL-4R α antibody inhibit IL-4 and IL-13, providing dual inhibition. This added benefit did not translate into a better drug, as trials with the IL-4 mutein have been stopped because of lack of efficacy (Bruce Davidson, personal communication, 2004). This result partly may have been caused by the short half-life of the drug (4–6 hours) and the requirement for the drug to be injected. T cells signal through an immune synapse that consists of the T-cell receptor and co-stimulatory molecules [63]. It is believed that IL-4 acts through this immune synapse by delivering a local burst of IL-4 rather than secreting large quantities to act on surrounding or more distant cells. To work, soluble IL-4R and the humanized anti-IL-4 monoclonal antibody would have to be present within the T-cell junction when the immune synapse forms to block IL-4 action. Because the IL-4 mutein and anti-IL-4R α antibody bind to the IL-4R, they already would be present when the immune synapse forms and would inhibit IL-4 action. Given its prolonged half-life, the ability to block IL-4 and IL-13, and the ability to act within the immune synapse, the human anti-IL-4R α antibody has the best potential future as an asthma therapy.

Further clinical trials will clarify the target population of IL-4-based drugs for optimal inhibition of IL-4. The potential capacity for infrequent dosing with IL-4 antagonists may improve patient compliance, which represents one of the greatest challenges to the effective treatment of asthma. By inhibiting inflammation at a central regulatory point, IL-4 antagonism may impact long-term asthma progression. Other atopic disorders, such as allergic rhinitis and

atopic dermatitis, are believed to be mediated by IL-4 and represent areas for future investigation.

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