

Targeting Interleukin-4 in Asthma: Lost in Translation?

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The first discovery that interleukin-4 (IL-4) is crucial in the development of allergic airway inflammation originates from the early 1990s. Whereas initial studies in experimental animal models provided the community with the optimistic view that targeting IL-4 would be the ultimate solution for treating asthma, the translation of these findings to the clinic has not been evident and has not yet fulfilled the expectations. Many technical challenges have been encountered in the attempts to modulate IL-4 expression or activity and in transferring knowledge of preclinical studies to clinical trials. Moreover, biological redundancies between IL-4 and IL-13 have compelled a simultaneous blockade of both cytokines. A number of phase I/II studies are now providing us with clinical evidence that targeting IL-4/IL-13 may provide some clinical benefit. However, the initial view that asthma is a purely Th2-mediated disease had to be revised. Currently, different asthma phenotypes have been described, implying that blocking specifically Th2 cytokines, such as IL-4, IL-5, and IL-13, should be targeted to only a specific subset of patients. Taking this into consideration, IL-4 (together with IL-13) deserves attention as subject of further investigations to treat asthma. In this review, we will address the role of IL-4 in asthma, describe IL-4 signaling, and give an overview of preclinical and clinical studies targeting the IL-4 Receptor pathway.

Keywords: interleukin-4; asthma; targeted therapy; murine models; asthma heterogeneity

Asthma is a chronic inflammatory disease of the airways leading to airway hyperresponsiveness (AHR) and reversible airway obstruction. Typical clinical features of asthma are recurrent episodes of wheezing, shortness of breath, chest tightness, and coughing (1). Many patients with asthma—especially those with early onset—are atopic, meaning they are genetically predisposed to generate immunoglobulin E (IgE) antibodies upon exposure to environmental allergens, such as house dust mite (HDM), pollen, and animal dander. Repeated inhalation of such allergens can subsequently induce an IgE-mediated hypersensitivity reaction, which is characterized by increased vascular permeability, vasodilatation, smooth muscle contraction, bronchoconstriction, and airway inflammation. Asthmatic airways also undergo structural changes (airway remodeling), with epithelial changes, increases in smooth muscle mass, deposition of extracellular matrix proteins, and goblet cell hyperplasia.

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Maintenance asthma therapy encompasses anti-inflammatory drugs, including inhaled corticosteroids (ICS) and leukotriene pathway inhibitors (5-lipoxygenase inhibitors and leukotriene receptor antagonists), and long-acting bronchodilators. These drugs reduce asthma manifestations and are sufficient to treat the majority of patients, but they do not cure the disease. Many breakthroughs have been made in understanding immunologic mechanisms initiating and mediating the development of asthma, specifically with regard to allergic airway responses (2). This has led to a quest to develop safe new therapeutics. Targeting interleukin-4 (IL-4) has been one of the strategies of specific (add-on) therapies, since IL-4 plays a crucial role in T-helper 2 (Th2) responses, in isotype class switching of B cells to IgE synthesis and is also involved in mast cell recruitment. We provide a state of the art on IL-4 and its potential as drug target in asthma.

ROLE OF IL-4 IN ALLERGIC ASTHMA

Nearly two decades ago, it was already hypothesized that IL-4 plays a key role in the pathogenesis of asthma (3). Individuals with asthma have elevated IL-4 protein levels in serum and bronchoalveolar lavage fluid (BALF) (4, 5), increased IL-4 mRNA and protein in bronchial biopsies (6, 7), and increased numbers of (T-)cells expressing IL-4 mRNA in BALF and bronchial biopsies (8–10). Also the protein levels of IL-4 receptor α (IL-4R α) and Stat6 (Signal Transducer and Activator of Transcription 6), two crucial components of the IL-4 signaling pathway, are elevated in bronchial biopsies and sputum samples from individuals with asthma (11–13). Allergen challenge in atopic individuals with asthma induces the release of IL-4 protein in BAL (14, 15) and from isolated peripheral blood mononuclear cells (16). Importantly, nebulization of IL-4 in individuals with mild asthma induces features of asthma, such as AHR and eosinophilia (17). In addition, genetic polymorphisms in the IL-4 gene and IL-4 receptor gene have been associated with atopy and asthma (18, 19).

Animal models have been used to elucidate the importance of IL-4 in asthma. Our research group contributed to this field by (1) establishing a murine ovalbumin (OVA) asthma model, with active immunization and allergen-aerosol challenge, and (2) testing IL-4-deficient mice in this model. We provided the experimental evidence that IL-4 plays a crucial role in the development of allergic airway inflammation, since IL-4-deficient mice showed a reduced eosinophilic inflammation and peribronchial inflammation. In addition, IL-4-deficient mice did not produce total and allergen-specific IgE and did not develop AHR (20, 21). Since then, the contribution of IL-4 (and IL-13, which shares many biological functions) in the pathogenesis of asthma has been intensively studied (reviewed in Ref. 22 and summarized below).

The development of allergic asthma can be divided into two phases: (1) an allergic sensitization phase and (2) a phase of disease development characterized by airway inflammation and remodeling. Allergic sensitization occurs in susceptible

individuals, when allergen exposure and subsequent allergen processing and antigen presentation by dendritic cells induces the differentiation of naive $CD4^+$ T cells into effector Th2 cells (Figure 1). IL-4 plays an important role in this Th2 differentiation stage. Signaling of IL-4 through IL-4R α activates transcription factor Stat6, leading to up-regulated expression of Th2 lineage-specific transcription factor GATA-binding protein 3 (GATA-3). This GATA-3 binds to target regulatory sequences of Th2 cytokine genes (*IL-4*, *IL-5*, *IL-9*, and *IL-13*), thus promoting their expression (2, 22, 23) (Figure 1). Next to this role in Th2 development, IL-4 can also induce isotype class switching of B-cells to IgE synthesis (together with IL-13) and is involved in recruitment of mast cells (together with IL-9 and IL-13). IL-4 up-regulates high-affinity IgE receptors on mast cells and low-affinity IgE receptors on B cells and mononuclear phagocytic cells, thus preparing the immune system for a subsequent antigen encounter.

In addition to the primary role of IL-4 in allergic sensitization, it also affects further development of allergic airway disease. In the early asthmatic response, allergen exposure in sensitized individuals induces crosslinking of IgE on the Fc ϵ RI receptors on mast cells and basophils, leading to the release of histamine, leukotrienes, prostaglandins (which promote vascular permeability and smooth muscle contraction), and cytokines (e.g., IL-4 and IL-13, which affect goblet cell hyperplasia and mucus production). IL-4, together with IL-13, up-regulates endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) (24, 25). This facilitates transmigration of eosinophils, T-lymphocytes, monocytes, and basophils that are attracted by mast cell-derived chemokines, thus contributing to the induction of a local inflammatory response

in the airways (late phase response). The late asthmatic response is associated with a pulmonary accumulation of inflammatory cells (typically eosinophils, but also T-lymphocytes, neutrophils, and macrophages), resulting in the release of many mediators that contribute to tissue damage, bronchial inflammation, and AHR (Figure 1). Whereas IL-4 dominates in directing Th2 cell polarization, many studies have shown that IL-13 dominates in inducing goblet cell hyperplasia, mucus hypersecretion, airway remodeling, and AHR (23, 26–31).

In the past, the pathogenesis of allergic airway responses, and more specifically asthma, was mainly explained by an imbalance between Th1 (IFN- γ -producing) and Th2 (IL-4-, IL-5-, IL-13-producing) cells. However, the picture is more complex, since also regulatory T cells (T_{Reg}, producing inhibitory IL-10 and TGF- β) and IL-17-secreting Th17 cells (implicated in neutrophilic inflammation in asthma [32]) contribute to the disease pathogenesis. This offers new opportunities for interventions, but also explains the limited success of specific Th2-directed therapies in the past.

INTERLEUKIN-4 AND IL-4 RECEPTOR SIGNALING

IL-4 is a pleiotropic cytokine that binds to high-affinity receptors expressed on many hematopoietic and nonhematopoietic cells. IL-4 is produced by Th2 cells, but may also be released by mast cells, basophils, eosinophils, and alveolar macrophages (33–36). IL-4 binds specifically to IL-4R α , which is expressed on T-lymphocytes, B-lymphocytes, eosinophils, mononuclear phagocytes, endothelial cells, lung fibroblasts, bronchial epithelial cells, and smooth muscle cells (11, 37–41).

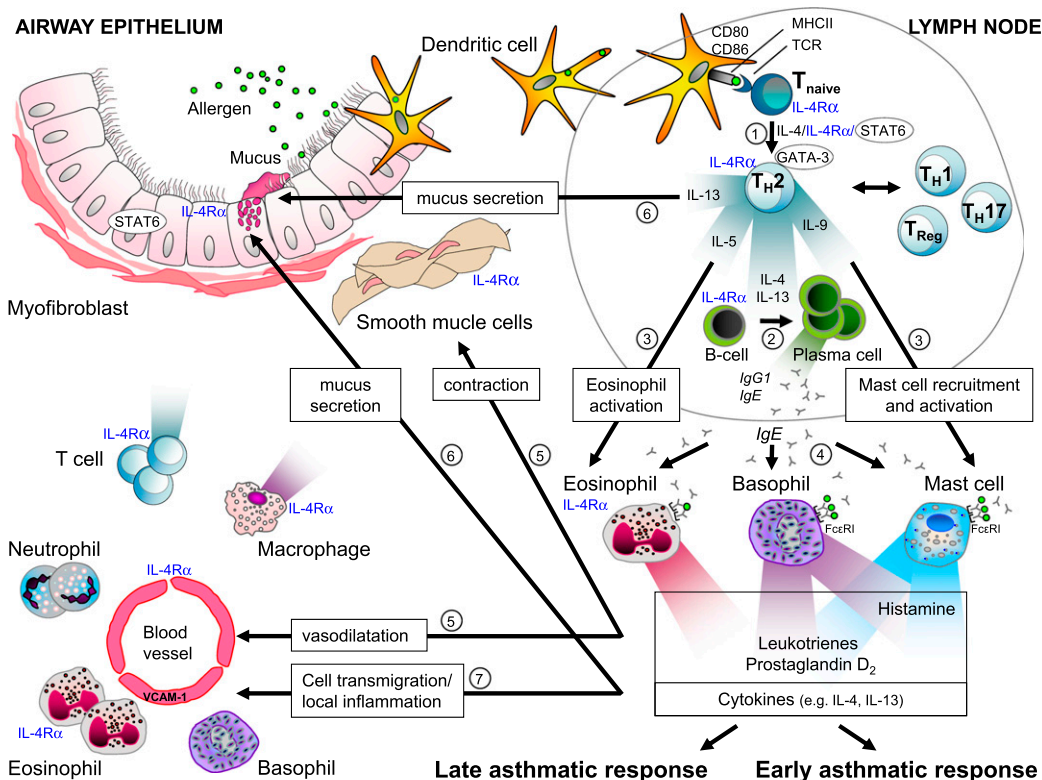


Figure 1. Role of interleukin (IL)-4 in the development of allergic asthma. The involvement of IL-4 in the different mechanisms leading to allergic airway diseases is illustrated. Major cell types producing IL-4 or affected by IL-4/IL-13 signaling (carrying receptor IL4R α , indicated in blue) are shown. Transcription factors activated by the IL-4/IL-13 signaling cascade are circled. Arrow 1 represents T cell differentiation, with IL-4 being crucial for Th2-differentiation. Arrow 2 indicates the isotype class switching of B-cells to IgE synthesis, which is mediated by IL-4 together with IL-13. Arrows 3 indicate the effect of IL-5 and IL-9 on eosinophil and mast cell function, respectively. Arrow 4 indicates that IgE, released from plasma cells binds to IgE receptors on mast cells, basophils, and eosinophils; upon allergen exposure, crosslinking of IgE results in (1) fast release of histamine (immediate), leukotrienes, and prostaglandins (after minutes); and (2) production of

cytokines (after hours). Arrows 5 indicate that histamine, leukotrienes, and prostaglandins induce smooth muscle contraction and vasodilation. Arrows 6 show that cytokines (IL-13, together with IL-4) induce mucus secretion and goblet cell hyperplasia. Arrow 7 indicates that IL-4, together with IL-13, contributes to inflammatory cell recruitment by up-regulating vascular cell adhesion molecule-1 expression, which facilitates transmigration of eosinophils, T cells, monocytes, and basophils in response to mast cell-derived chemokines (late asthmatic response). AHR, airway hyperresponsiveness; Fc ϵ RI, Fc epsilon RI, high-affinity receptor for Fc region of immunoglobulin E (IgE); IL-4R α , interleukin-4 receptor α ; Stat6, Signal Transducer and Activator of Transcription 6.

IL-4R α functions within three receptor complexes: the IL-4R α /common- γ chain (γ C)/IL-4 signaling complex (called Type I receptor), and both the IL-4R α /IL-13R α 1/IL-4 and the IL-4R α /IL-13R α 1/IL-13 signaling complexes (both called Type II receptors) (Figure 2) (42). Whereas the Type I receptor complex is solely used for IL-4 signaling, the Type II receptor complex is not only involved in IL-4 signaling, but also in IL-13 signaling by binding of IL-13 to the IL-13R α 1 subunit. This explains the overlap in biological responses toward IL-4 and IL-13. The Type I receptor is typically found on hematopoietic cells and is involved in Th2 development, whereas the Type II receptor is found on both hematopoietic and nonhematopoietic cells. Signaling of IL-4, and particularly IL-13, through this Type II receptor on nonhematopoietic airway cells, such as smooth muscle cells and epithelial cells, can directly induce AHR and mucus secretion, respectively (11, 43–45).

Ligand binding to both Type I and Type II receptors promotes the activation of the Janus family of protein kinases (Jak). More specifically, IL-4R α , γ C, and IL-13R α 1 activate Jak1, Jak3, and tyrosine kinase (Tyk2), respectively (Figure 2) (42, 44, 46). This initiates several intracellular signaling cascades by phosphorylating specific tyrosine residues in the cytoplasmatic domain of IL-4R α , resulting in the recruitment and phosphorylation of the Stat6 transcription factor. Phosphorylation of Stat6 leads to its dimerization and translocation to the nucleus, resulting in the induction of the transcription of IL-4/IL-13-responsive genes involved in Th2 responses, Ig(E)-switching, AHR, and mucus production. The IL-13R α 1 subunit in the Type II complex can amplify IL-4R α signaling, but can also initiate independent signaling pathways involving other Stat proteins (Stat3, Stat1). Such Stat6-independent but IL-13-dependent signaling affects AHR in a chronic asthma model (11, 47).

INTERLEUKIN-4 AS TARGET TO TREAT ASTHMA

The role of IL-4 in the initiation and development of allergic asthma generated the hope that blocking IL-4 signaling would result in clinical benefit, and several approaches to block either IL-4 or IL-4R α have been used. Figure 2 gives a comprehensive

overview of the different strategies, whereas Tables 1 and 2 summarize animal and clinical studies, respectively.

Blocking IL-4 Activity or IL-4 Expression

Blocking specifically IL-4 has been addressed by administering soluble IL-4R, by monoclonal antibodies or by antisense technology.

Soluble IL-4R. Soluble IL-4R (sIL-4R) is a naturally occurring secreted form of IL-4R that contains the extracellular portion of the IL-4R α chain, but lacks the transmembrane and cytoplasmic domains. sIL-4R functions as a decoy receptor by binding IL-4 and neutralizing it, without mediating cellular activation (Figure 2, Tables 1 and 2). In a murine asthma model, sIL-4R significantly inhibited AHR and IgE production when administered at the time of sensitization (48). Moreover, sIL-4R also reduced late phase pulmonary inflammation, by blocking endothelial VCAM-1 expression, airway eosinophil infiltration and mucus hypersecretion (but not AHR), when administered during allergen challenge (49), suggesting that blocking IL-4 could provide clinical benefit, even after full sensitization to an allergen.

The therapeutic potential of antagonizing IL-4 by using an inhaled recombinant human sIL-4R (Nuvance; Immunex, Seattle, WA) appeared promising in the initial phase of the clinical trials (50, 51). The prolonged half-life of sIL-4R (~ 5 d), suggested that a once-weekly inhaled therapy was feasible. A phase I/II randomized placebo-controlled trial with corticosteroid cessation demonstrated that a single nebulization of sIL-4R was safe and effective in moderate asthma, with no drug-related toxicity, a significant improvement in forced expiratory volume in 1 second (FEV₁), less requirement of β_2 -agonist rescue use, and reduced inflammation as measured by exhaled nitric oxide (NO) levels (50). A subsequent study with repeated dosing for 12 weeks to evaluate long-term safety and efficacy also demonstrated that sIL-4R was a promising therapeutic in the treatment of moderate persistent asthma (51, 52). However, in subsequent clinical trials in patients with asthma who were taking only β_2 -agonists, no clinical benefit on FEV₁ or asthma symptoms could be demonstrated (53, 54). Similar findings were

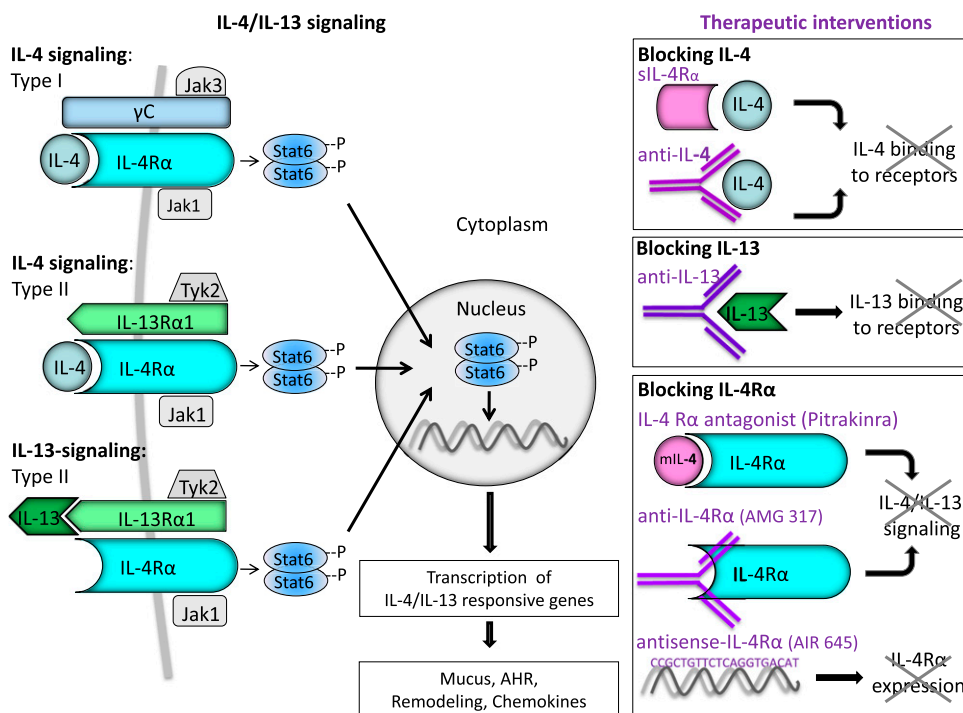


Figure 2. Overview of IL-4/IL-13 signaling and therapeutic interventions to treat allergic asthma. AHR, airway hyperresponsiveness; IL-4R α , interleukin-4 receptor α ; IL-13R α 1, interleukin-13 receptor α 1 subunit; Jak, Janus family of protein kinases; P, phosphorylation; sIL-4R α , soluble IL-4R α ; mIL-4, IL-4 mutein (Pitrakinra); Stat6, Signal Transducer and Activator of Transcription 6; Tyk, tyrosine kinase (Tyk2); γ C, common- γ chain.

TABLE 1. PRE-CLINICAL MURINE *IN VIVO* STUDIES TARGETING THE IL-4, IL-13/IL-4R α /STAT-6 PATHWAY IN ALLERGIC AIRWAY DISEASE

Target	Model	Intervention Method	Ref. No.
IL-4	OVA-asthma	Intranasal or intraperitoneal soluble IL-4R	48, 49
		Intraperitoneal anti-IL-4 mAb	31, 56
IL-13	OVA-asthma	Intravenous anti-IL-13 mAb	63
	HDM-asthma	Subcutaneous anti-IL-13 mAb	64
IL-4R α	OVA-asthma	Subcutaneous or intranasal IL-4R antagonist	84,85
		Intratracheal mouse IL-4R antagonist on plasmid*	86
		Inhaled antisense oligonucleotide [†]	100
	IL-4- or TSLP-induced airway inflammation	Intraperitoneal anti-IL-4R α mAb	29, 96
Stat6	OVA-asthma	Intranasal inhibitory peptide	104
		Intraperitoneal small molecule inhibitor	105
		Intranasal siRNA/intranasal antisense [†]	106, 107

Definition of abbreviations: HDM, house dust mite; IL, interleukin; mAb, monoclonal antibody; OVA, ovalbumin; Stat6, Signal Transducer and Activator of Transcription 6.

* Gene overexpression

[†] Gene silencing.

obtained upon reducing ICS during sIL-4R treatment (53). It was recently demonstrated that sIL-4R α can be recruited by the IL-13/IL-13R α 1 complex and stabilize IL-13 binding. This could be one reason why the clinical trials failed (55).

Anti-IL-4 monoclonal antibody. Another approach to block IL-4 was the use of a humanized anti-IL-4 mAb (Pascalizumab; Biopharma, Winchester, UK) (Figure 2, Tables 1 and 2). Pascalizumab was generated as a murine mAb (3B9) with specificity for human IL-4, which was subsequently humanized to reduce immunogenicity. Anti-IL-4 reduced IgE production and AHR in murine asthma models (31, 56). An *in vitro* study demonstrated that pascalizumab effectively neutralized IL-4 bioactivity in human cell lines and inhibited Th2 cell-related processes, such as IL-5 synthesis, and induction of IgE production (57). *In vivo* pharmacokinetic and chronic safety testing in cynomolgus monkeys demonstrated that pascalizumab was well tolerated without inducing adverse clinical responses (57). A Phase I trial with a single intravenous dose in mild to moderate asthma demonstrated that it was well tolerated and had an elimination half-life of more than 2 weeks (58). However, a subsequent large-scale, multidose phase II trial in steroid-naïve subjects with asthma was terminated because preliminary data showed that pascalizumab did not provide clinical benefit (59).

Antisense IL-4. An alternative for antibodies lays in the use of RNA-based gene-silencing strategies, which can dampen IL-4 expression (60, 61). Antisense oligonucleotides induce the degradation of target mRNA and inhibition of gene expression. This approach could be an interesting alternative for targeting a protein, since multiple copies of a specific protein are translated from each mRNA molecule (60). In a rat model of allergic asthma, systemic administration of antisense IL-4 by a recombinant adeno-associated virus reduced the levels of IL-4 in BAL and reduced the allergic inflammation and airway remodeling (62). No transfer of this approach to clinical trials has been described to date.

In summary, whereas all approaches to block biological activity of IL-4 appeared successful in preclinical models, they could not yet provide enough convincing clinical benefit in human trials.

Blocking IL-13 Activity

The predominant role of IL-13 in goblet cell hyperplasia, airway wall remodeling, and AHR has led to blocking strategies with anti-IL-13 mAbs (Figure 2). Although IL-13 is not the main topic of this review, we briefly address current status in this field.

Anti-IL-13 monoclonal antibody. Both prophylactic and therapeutic protocols have been used to test anti-IL-13 mAb in murine asthma models of ovalbumin and house dust mite

exposure (63, 64). Anti-IL-13 inhibited allergen-induced inflammation, goblet cell hyperplasia, and airway remodeling. However, whereas a prophylactic protocol reduced AHR toward metacholine, the therapeutic protocol did not inhibit AHR, although it reversed changes in baseline lung function (63, 64). Several promising studies in cynomolgus monkeys and sheep have led humanized IgG1 anti-IL-13 antibodies (IMA-638 and IMA-026) into clinical trials (65–68). The capacity of IMA-638 and IMA-026, which bind two different epitopes of IL-13, to inhibit allergen-induced airway responses was tested in atopic individuals with mild asthma (Table 2) (69–71). Whereas interaction of IMA-638 to IL-13 blocks the recruitment of IL-4R α , IMA-026 is specific for the IL-13 epitope binding IL-13R α 1 and IL-13R α 2 (72). IL-13R α 2 acts as decoy and scavenger receptor for IL-13 and is suggested to be a critical mediator of IL-13 clearance in humans (72). For both IMA-638 and IMA-026, subcutaneous dosing on Days 1 and 8 did not affect allergen-induced AHR (metacholine PC₂₀ values) or sputum eosinophilia. However, IMA-638 did transiently attenuate the allergen-induced early- and late-phase area under the curve (AUC) at Day 14 (69). However, in a more chronic study design of 12 weeks in patients with uncontrolled asthma who were receiving ICS, there was no effect of IMA-638 on symptoms, lung function, or time to exacerbation compared with placebo (69, 73).

Lebrikizumab, an IgG4 humanized mAb to IL-13, has been tested in both subjects with mild asthma and subjects with uncontrolled asthma by monthly subcutaneous administration for up to 6 months (74–77). Within the working hypothesis that anti-IL-13 therapy would benefit patients with a pretreatment profile consistent with IL-13 activity, patient subgroups were prespecified according to their baseline Th2 status (assessed with total IgE levels, blood eosinophil count, and the serum levels of periostin, a matricellular protein that is secreted by bronchial cells in response to IL-13) (75, 76). Lebrikizumab significantly improved prebronchodilator FEV₁ by 5.5% at Week 12, and a subgroup of patients with high periostin levels had even a relative increase in FEV₁ of 8.2% compared with placebo. This suggests that measuring serum periostin levels in individuals with asthma could be used as biomarker to identify potential responders toward lebrikizumab and that anti-IL-13 therapy can be targeted to susceptible patients. However, lebrikizumab treatment did not result in a significant improvement of secondary outcomes, such as asthma symptom scores or exacerbation rates (75). Replication of current observations and Phase III trials are needed to elucidate this further.

Tralikinumab (CAT-354), another IgG4 humanized anti-IL-13 mAb, has been demonstrated to be potent in preclinical

TABLE 2. CLINICAL STUDIES TARGETING IL-4/IL-13 IN ASTHMA

Product	Co.	Intervention Method	Clinical Trial	Ref. No.
IL-4 targeting				
Nuvance	Immunex corporation	Inhaled recombinant human sIL-4R	Phase I and II, in mild to moderate persistent asthma	50–52, 54
SB 240683 (pascolizumab)	NIAID PDL, GSK PDL, Biopharma	Intravenous humanized anti-IL-4 (IgG1) antibody	Phase I, in mild to moderate asthma Phase I/II, in symptomatic, steroid-naïve asthma	58 59
IL-13 targeting				
IMA-026	Wyeth (Pfizer)	Subcutaneous anti-IL-13 (IgG1)	Phase I and II, in atopic individuals with mild asthma and persistent asthma	69–71, 73
IMA-638	Genentech	Subcutaneous anti-IL-13 (IgG4)	Phase II, in mild allergic asthma and uncontrolled asthma	74–77
Lebrikizumab (MILR1444A)	Genentech	Subcutaneous anti-IL-13 (IgG4)	Phase II, in mild allergic asthma and uncontrolled asthma	74–77
Tralokinumab (CAT-354)	MedImmune	Intravenous anti-IL-13 (IgG4)	Phase I, in moderate asthma	80, 81
		Anti-IL-13 (IgG4)	Phase IIb, in uncontrolled severe asthma	82
		Subcutaneous anti-IL-13 (IgG4)	Phase IIa, in uncontrolled, moderate-to-severe, persistent asthma	83
IL-4 R α targeting				
Pitrakinra*	Aerovance	Subcutaneous IL-4R α antagonist	Phase IIa, in atopic subjects with asthma	88, 89
		Inhaled IL-4R α antagonist	Phase II/IIb, in subjects with moderate to severe asthma	88, 90, 94
AMG 317	Amgen	Subcutaneous humanized anti-IL-4R α mAb	Phase II, in moderate to severe asthma	98, 99
AIR-645	Altair Therapeutics	Inhaled antisense IL-4 α	Phase I/IIa, in mild allergic asthma	101, 103

Definition of abbreviations: GSK, GlaxoSmithKline; ICS, inhaled corticosteroids; mAb, monoclonal antibody; NIAID, National Institute of Allergy and Infectious diseases; PDL, Protein Design Labs; sIL-4R, soluble IL-4R.

*Other names for pitrakinra: AER001, IL-4 mutein, BAY 16-9996, InterPenkin-4.

models (78) and to be safe in both healthy volunteers and subjects with asthma (79–81). CAT-354 is currently tested in Phase II clinical trials, but to our knowledge, no results have been published so far (82, 83).

Blocking IL-4 Receptor α Activity or Expression

One of the reasons why IL-4-directed therapies were unsuccessful may have been the fact that IL-4 and IL-13 show redundancy in their biological activities, implicating that targeting only IL-4 may be too selective. Therefore, research has focused on targeting both IL-4 and IL-13 by blocking the IL-4R α receptor, which is shared between IL-4 and IL-13 for the induction of the Stat6-signaling pathway. Different approaches have been developed to block the IL-4R α receptor: an IL-4 mutein (pitrakinra), an IL-4R α antibody (AMG317) and, more recently, antisense IL-4R α (AIR-645) (Figure 2).

Recombinant human IL-4 (Pitrakinra). Pitrakinra is a recombinant form of human IL-4 with two functional mutations at positions 121 (arginine to aspartic acid) and 124 (tyrosine to aspartic acid). Pitrakinra has high affinity for the IL-4R α chain and acts as a competitive receptor-antagonist that blocks both IL-4- and IL-13-dependent responses by inhibiting binding of IL-4 and IL-13 to the IL-4R α receptor complexes (Figure 2). The effectiveness of such an approach was initially tested in a murine asthma model, using a murine IL-4 mutant protein (mouse IL-4 receptor antagonist, mIL-4Ra) and showed inhibition of allergen-induced Th2 cytokine production, eosinophilic inflammation, goblet cell hyperplasia, AHR, and allergen-specific IgE (84, 85). Also local transgenic overexpression of mIL-4Ra in the lungs, by intratracheal administration of the *IL-4Ra* gene on a plasmid, attenuated airway inflammation and regulated the Th1/Th2 balance in mice (86). In cynomolgus monkeys, dual IL-4/IL-13 antagonism by pitrakinra reduced AHR and tended to reduce eosinophilic inflammation (87).

The promising results from the preclinical research have led pitrakinra into the clinical test phase. Two independent phase IIa clinical trials evaluated the effect of pitrakinra (subcutaneous injection or nebulization) on the late-phase response after allergen challenge in atopic asthmatics (88–90). Irrespective of the route of administration, pitrakinra attenuated the decrease in FEV₁ in

the late asthmatic response toward allergen challenge, but inhalation dosing produced a better efficacy, suggesting that the lung is the primary site for the anti-asthmatic action (88). With the current described pharmacodynamics, pharmacokinetics, clinical efficacy, and safety/tolerability, pitrakinra could become a novel anti-inflammatory drug for asthma, but long-term studies are required (91, 92). A multicentre phase IIb study (Aero-Trial) in subjects with moderate to severe asthma evaluated whether pitrakinra can reduce exacerbation incidence upon tapering of long-acting β -agonists and inhaled corticosteroids. Pitrakinra did not have a significant effect in the entire population of subjects with moderate to severe asthma, but it did reduce asthma exacerbations by 37% in a subset of patients with eosinophilic asthma (93, 94). This is similar to the observations for anti-IL-5 (mepolizumab), which was ineffective in improving lung function in studies with a nonselected population of subjects with asthma, but which has been shown to reduce the exacerbation rate in a selected subset of patients with severe asthma with refractory eosinophilic airway inflammation despite high doses of ICS (95).

Anti-IL-4 receptor α monoclonal antibodies. AMG 317 (anti-IL4R α), a humanized mAb directed toward human IL-4R α , has become an alternative approach to tackle the biological redundancies between IL-4 and IL-13. AMG 317 (Amgen) not only prevents binding of IL-4 to IL-4R α , but also prevents signal transduction by IL-13 (Figure 2). Preclinical studies in mouse asthma models using murine anti-IL-4R α demonstrated reduced pulmonary inflammation, AHR, and goblet cell hyperplasia (29, 96).

In Phase I and II trials, the pharmacokinetics and pharmacodynamics of AMG 317 were evaluated using single and multiple dosing by the intravenous or subcutaneous route. Sustained subcutaneous dosing of AMG 317 over several months was shown to reduce total serum IgE levels (97). The phase II study in patients with moderate-to-severe asthma demonstrated that AMG 317 was safe and well tolerated. No clinical efficacy was demonstrated across the overall group of patients, but AMG 317 treatment did lead to significant clinical improvement in patients with higher baseline Asthma Control Questionnaire (ACQ) symptom scores (98, 99).

Antisense oligonucleotides against IL-4R α . Eliminating IL-4R α expression and thus IL-4 and IL-13 signaling via antisense

oligonucleotides effectively reduced several hallmarks of asthma in a mouse model (100). A Phase I study testing nebulized AIR645 (antisense IL-4R α blockade) in healthy volunteers and subjects with mild asthma demonstrated that it is well tolerated, leads to a low systemic exposure, and has the potential to be used for once-weekly treatment (101, 102). In a Phase II trial, however, AIR645 did not show enough clinical benefit (effect on FEV₁) to warrant further development (103).

Blocking STAT-6 Activity or STAT-6 Expression

Besides targeting IL-4, IL-13, or IL-4R α , another approach is targeting the Stat6 transcription factor, which is essential for the transcription of IL-4/IL-13-responsive genes. Several strategies have been applied in the preclinical models. Using a Stat6-inhibitory peptide, a small molecule inhibitor (AS1517499), an antisense RNA, and small interfering RNA (siRNA), several features of airway disease, such as allergen-induced airway inflammation, goblet cell hyperplasia, mucus production, and AHR, could be inhibited in murine models (104–107) (Table 1). Recently, siRNA was used to block Stat6 expression in human IL-4/IL-13-treated lung epithelial cells *in vitro*, indicating that mucosal cells in ongoing chronic asthma-associated lung inflammation could be responsive to this approach (108).

Although these preclinical studies suggest that Stat6 is indeed a promising target for treatment of asthma, no clinical trials have been published so far.

FUTURE DIRECTIONS AND CLINICAL RELEVANCE

Despite all existing anti-asthma therapies, there are still a considerable number of patients with a poor quality of life due to uncontrolled or partially controlled asthma (109), who could benefit from additional treatment options. Targeting IL-4 has been one of the potential treatment strategies. Although IL-4 blocking did not fulfill the expectations in clinical trials, this can be explained by several factors. First, insights into the role of

IL-4 in asthma and more specifically in Th2 differentiation have changed during the past decades. Initial *in vitro* studies generated the central hypothesis that IL-4/IL-4R α /Stat6 signaling is crucial for the development of CD4⁺ T cells and that IL-4 is the sole driver to induce differentiation to a Th2 subset. However, now it is well known that Th2 differentiation *in vivo* appears to be more complex, and that the IL-4/IL-4R α /Stat6 pathway may even be dispensable since other Th2-determining pathways (e.g., IL-25, IL-33, and thymic stromal lymphopoietin [TSLP]) exist (110, 111). Second, blocking IL-4 to prevent Th2 differentiation may appear relevant in short-term murine models, but in clinical practice, patients are already Th2-biased once they have asthma complaints and seek medical attention. Besides its role in Th2 differentiation, IL-4 and/or Stat6 are important in inducing Type 2 effector responses, such as B cell IgE class switching, eosinophilia, tissue lymphocyte accumulation, and Th2 chemokine production *in vivo* (111). The strategy to diminish these Th2 effector responses by solely blocking IL-4 has been undermined because of biological redundancy between the actions of IL-4 and IL-13.

The efforts of the scientific community are currently mainly directed toward targeting IL-4R α /Stat6 signaling. It has been successful to prevent allergic airway inflammation in preclinical models, but—up to now—the success in human trials is limited, suggesting that the clinical relevance of the tested preclinical murine models may have been overestimated (112–114). Besides the fundamental limitations of mouse asthma models (e.g., species differences in immunology, airway morphology, and epithelial responses [115]) (Table 3), the setup of most preclinical IL-4/IL-13-blocking experiments was primary or secondary prevention, instead of treatment. In addition, the predominantly used eosinophilic ovalbumin models, with up to 70% eosinophils in the BALF, apply to only a subset of patients with allergic eosinophilic asthma. Indeed, human asthma is a heterogeneous syndrome, with several different asthma phenotypes encompassing allergic versus nonallergic asthma and

TABLE 3. DISCORDANCES BETWEEN MURINE MODELS AND HUMAN ASTHMA

	Murine Asthma Model	Patient with Asthma
Timing of disease development	Adult mice with fully developed lungs	Often originates early in life, when lungs are still developing May develop in adulthood (late-onset asthma)
Species differences in morphology	Limited airway branching, no submucosal glands, not fully stratified bronchial epithelium, myofibroblasts rather than smooth muscle cells	Extensive airway branching, abundant submucosal glands, smooth muscle cells in spiral bundles
Species differences in immunology	Distinct, polarized Th1/Th2 responses	Mixed Th1 or Th2 responses
Etiology	Allergen-dependent (OVA, HDM, <i>Aspergillus</i>)	Allergic and nonallergic asthma
Genetic background	Inbred strains	Genetic heterogeneity
Localization of disease	Asthma features in BALF, airways, parenchyma, and vasculature	Asthma features mainly in airways
Timing of intervention	Mainly primary or secondary prevention: in knock-out mice or by blocking of protein/gene before or during allergen challenge and disease development	Treatment: patient has already symptoms and airflow obstruction due to chronic inflammation and damage
Endpoint: airway physiology	No airway obstruction without addition of bronchoconstrictors AHR (measured invasively after sedation)/PenH (measured noninvasively), toward bronchoconstrictors	Symptoms, FEV ₁ /FVC, variable airflow limitation AHR (noninvasive, no sedation)
Endpoint: inflammation and remodeling	Inflammation in BALF, lung parenchyma, and remodeling (post mortem)	FE _{NO} , inflammation in sputum (noninvasive), and bronchial biopsies (rarely)
Colonization/infection	Specific pathogen-free environment	Exposed to bacterial/viral infections
Environmental factors	Well-controlled; combination models of allergen and pollutant exposures exist, but only models of sole allergen exposure are used for preclinical studies	Interference of diet, exposure to tobacco smoke, and occupational and other pollutants (e.g., diesel exhaust)

Definition of abbreviations: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; FE_{NO}, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; HDM, house dust mite; OVA, ovalbumin; PenH, enhanced pause (measured by whole body plethysmography).

eosinophilic versus neutrophilic asthma. Asthma is no longer considered as a purely allergen-driven, Th2-mediated eosinophilic airway disease, but is subdivided into distinct clinical and molecular phenotypes, with varying degrees of Th2 inflammation (116–118). As a consequence, the diversity of asthma phenotypes may have contributed to the limited success of clinical trials that tested anti-Th2 cytokines as therapy in a nonselective manner.

In contrast, targeted anti-IL-5 antibody therapy significantly reduced exacerbation rate in a selected subset of patients with severe asthma with refractory eosinophilic airway inflammation (95). To give targeted treatment strategies for asthma (e.g., pitrakinra [93, 94] and lebrikizumab [75]) a fair chance, asthma phenotyping is key to accurately select potential responders within a heterogeneous patient population (e.g., assessment of Th2 status, encompassing blood eosinophil count and serum levels of total IgE and periostin).

Considering the diversity in human asthma pathogenesis and phenotypes, there is also need for diversity in murine models. Chronic models and exacerbation models, models with a mixed neutrophilic/eosinophilic inflammation, models with relevant allergens (fungi, HDM), and models combining the exposure to allergen with exposure to environmental pollutants (e.g., cigarette smoke and diesel exhaust particles [119]) are the way to go in the future.

CONCLUSIONS

The finding that blocking IL-4/IL-13 works preventively in murine asthma models has initially led researchers into the “mouse trap.” Two important issues have hampered the translation to human disease: (1) murine models have their limitations (115), including the fact that strategies of “disease prevention” are no predictors of “therapeutic benefit”; and (2) asthma heterogeneity does not support the strategy to block one single mediator in all individuals with asthma, without selection of a specific asthma phenotype. Thus, we do not believe that targeting IL-4/IL-13 will become an alternative for standard inhaled corticosteroid-based therapies. However, for individuals with severe asthma with a particular overexpression of IL-4 or IL-13, who are not well controlled despite current existing therapies, blocking IL-4/IL-13 may become an interesting targeted add-on therapy and result in better control of asthma.

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