

A molecular perspective on T_H2-promoting cytokine receptors in patients with allergic disease

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The cytokines IL-4, IL-13, and thymic stromal lymphopoietin play a key role in allergic disease by virtue of their ability to initiate, maintain, and augment T_H2 responses. These molecules mediate their effects through type 1 cytokine receptors, which bind cytokines with a characteristic structure. Receptors are expressed on a broad array of immune cell types and are integral to complex cytokine networks operating in health and disease. T_H2-promoting cytokines bind different configurations of receptors. Receptor subunits can exist in surface-bound or soluble forms, as well as in isolation or in partnership with other subunits. Sharing of receptor subunits among different cytokine receptor complexes adds to the intricate landscape. This article describes the characteristics of receptors for IL-4, IL-13, and thymic stromal lymphopoietin and their respective ligands from a structure-function perspective. We detail the mechanisms of receptor complex assembly, the interrelated nature of these receptors, and the effect on allergic inflammation. The ability for novel and atypical types of receptors to modulate inflammatory processes is also discussed. We highlight current and emerging treatments that target T_H2-promoting receptor complexes. Understanding the molecular features of these receptors provides insight into different disease phenotypes and the variable clinical outcomes arising from targeted therapies. These considerations can be used to inform future directions for research and creative strategies for treating individual patients. (*J Allergy Clin Immunol* 2013;■■■:■■■-■■■.)

Key words: Thymic stromal lymphopoietin, IL-4, IL-13, receptors, cytokines, T_H2, allergy, asthma treatment

Cytokines are small secreted proteins that act in an autocrine or paracrine fashion. Binding of cytokines to surface receptors allows cells to respond to external cues that are vital to immune cell function. These processes manifest as cell survival, growth, and differentiation, all of which, under normal circumstances, are precisely regulated biological events. The T_H2-promoting cytokines IL-4, IL-13, and thymic stromal lymphopoietin (TSLP) are overexpressed at sites of allergic inflammation, including the skin, respiratory tract, and gut. IL-4 and IL-13 mediate a broad array of functions, including IgE antibody class-switching by B cells and bronchial hyperresponsiveness. In addition, they target a range of effector cell types, including mast cells and basophils, which themselves release IL-4 and IL-13 on activation. Recently, the epithelial cell-derived cytokine TSLP has been spotlighted for its ability to initiate T_H2 differentiation through priming of dendritic cells.^{1,2} T_H2-promoting cytokines are pleiotropic and have overlapping effects. These properties arise from their ability to bind to different receptors and through sharing of receptor subunits among different receptor complexes (Fig 1). Here we explore these molecular relationships and their influence on mechanisms of cytokine action, disease phenotype, and response to treatment.

MOLECULAR FEATURES OF T_H2-PROMOTING CYTOKINES

TSLP, IL-4, and IL-13 each belong to the IL-2 subfamily of the “4-helix-bundle” cytokine family, which also includes IL-2, IL-7, IL-9, IL-15, and IL-21. These cytokines bind their receptors through polar or charged amino acids on their surface, resulting in specific high-affinity interactions and formation of stable signaling complexes.³⁻⁵ T_H2-promoting cytokines exhibit limited amino acid sequence similarity (10% to 13% identity); however, their structures are highly conserved, comprising 4 short amphipathic α -helices that organize to form a hydrophobic core (Fig 2, A, and see Fig E1 in this article’s Online Repository at www.jacionline.org).⁶ Three helices (designated A, C, and D) are involved in receptor binding, and regions of high sequence similarity map to these regions (see Fig E1). These conserved structural elements allow different cytokines to interact with the same receptor subunit.⁵

RECEPTORS

Extracellular domain

Receptors for the T_H2-promoting cytokines belong to the type 1 cytokine receptor family. These molecules share similarities in

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Abbreviations used

FNIII: Fibronectin III
IL-4R: IL-4 receptor
IL-7R: IL-7 receptor
IL-13R: IL-13 receptor
JAK: Janus kinase
MMP: Matrix metalloproteinase
STAT: Signal transducer and activator of transcription
TSLP: Thymic stromal lymphopoietin
TSLPR: Thymic stromal lymphopoietin receptor chain

amino acid sequence (approximately 20% identity) and structure. Conventional receptors for IL-4, IL-13, and TSLP exist as heterodimers, which consist of an extracellular cytokine-binding domain, a transmembrane portion, and an intracellular signaling platform (Fig 1). The extracellular domain of each monomer contains tandem fibronectin III (FNIII)-like domains, each comprising 7 β -strands.⁷ The junction of these domains forms an “elbow” configuration that is involved in cytokine binding (Fig 2, B).

As is typical of type 1 cytokine receptors, each receptor subunit contains a modified WSXWS motif in the membrane-proximal FNIII domain and 4 conserved cysteine residues in the membrane-distal FNIII domain. The WSXWS motif was recently reported to act as a molecular “switch” to drive dimerization subsequent to ligand binding.⁸ On the other hand, cysteine residues fulfill a structural role by forming disulfide bonds between different β -strands.⁹

Signaling machinery

In general, T_H2 -promoting cytokine receptors transmit signals through pathways involving Janus kinase (JAK) proteins and signal transducer and activator of transcription (STAT) proteins (Fig 1). The cytoplasmic tail of each receptor subunit contains conserved binding motifs for JAK proteins (designated box 1 and box 2) and tyrosine residues, which are critical to signaling.¹⁰ Phosphorylation of tyrosine residues recruits STAT proteins, which in turn are phosphorylated by JAK proteins (Fig 1). This results in STAT dimerization, translocation to the nucleus, and binding to DNA elements that regulate gene transcription.

A few basic principles are worth noting in relation to T_H2 -promoting pathways. First, the same cytokine can activate different JAK/STAT pathways, resulting in different outcomes within the same cell (Fig 1),¹¹ and second, the same cytokine might activate a different repertoire of JAK/STAT molecules depending on the cell type.² In relation to these aspects, partnering of the same subunit with different subunits that each bind their own array of signaling molecules allows differential signaling by receptor complexes containing shared subunits.¹² Moreover, the same subunit within different receptor complexes can itself trigger discrete JAK/STAT pathways within the same cell.¹³ Thus each T_H2 -promoting cytokine typically triggers multiple intracellular pathways that depend on the nature of the receptor complex and cell type.

Receptor assembly

Understanding how cytokine receptor complexes form is fundamental to the design of therapeutics for blocking T_H2 -promoting pathways in allergic disease (Fig 3). Receptor

complexes assemble in a stepwise fashion. The conventional view is that a cytokine first binds the subunit for which it has high affinity, and additional subunits are then recruited to form the functional ternary complex. This has been termed the driver-trigger concept. In this model cytokine engages the first chain of the receptor complex, termed the driver (usually the α subunit), whereas interaction with the second chain provides the trigger.⁴ The driver imparts ligand specificity and initiates formation of the complex, whereas recruitment of the trigger into the complex establishes signaling potential.

The type I receptor complex for IL-4 provides an example of this process. This receptor is a heterodimer of IL-4 receptor (IL-4R) α and the common cytokine receptor gamma chain (γ_c) (Fig 1). Recent work suggests that IL-4 initially binds IL-4R α (the “driver”), and the γ_c chain (the “trigger”) is then recruited for ternary complex signaling (Fig 2, B, upper panel).⁴ In this complex IL-4R α binds the surface of IL-4 formed by the A and C α -helices through a region of the receptor, termed site I. The γ_c chain engages surfaces formed by the A and D α -helices of IL-4 through site IIa of the receptor, and the membrane-proximal FNIII domain of IL-4R α (site IIb).

In some cases, receptor assembly might involve recruitment of receptor chains from other types of complexes that can form in the absence of ligand. IL-4R α , IL-7 receptor (IL-7R) α , IL-2 receptor (IL-2R) β , IL-9 receptor (IL-9R) α , and γ_c , can each form homodimers independent of ligand.¹⁴⁻¹⁷ Furthermore, nonsignaling heterodimers containing γ_c have been identified.¹⁷ Such “ligand-free” dimers might position receptor components in microdomains of the cell membrane for efficient recruitment to the receptor complex. Alternatively, these complexes might stabilize receptor subunits that are inactive.^{14,18}

SHARED RECEPTORS

γ_c is shared by multiple receptors

Receptor complexes for IL-4, IL-13, and TSLP each contain shared subunits. The most notable example is γ_c , which not only participates in the type I receptor complex for IL-4 but also binds IL-2, IL-7, IL-9, IL-15, and IL-21. The overarching role of γ_c in the immune system is reflected in X-linked severe combined immune deficiency, which arises from a lack of T cells and natural killer cells because of a loss of function in γ_c . The general view is that γ_c provides the trigger to stabilize different receptor complexes. In the receptor assembly process, cytokines that are already complexed with their high-affinity driver subunit engage with γ_c through a depression formed by the highly conserved D α -helix in a configuration that has been likened to a “knob in a hole.”^{4,7}

Sharing of IL-4R α chain by receptors that bind IL-4 and IL-13

The IL-4R α chain participates in 2 distinct receptor complexes that mediate the effects of IL-4 and IL-13 and thus are fundamental to allergic inflammatory processes. IL-4R α partners with γ_c to form the type I receptor and also couples with IL-13 receptor (IL-13R) $\alpha 1$ to form the type II receptor (Fig 1).¹⁹ Whereas both receptors bind IL-4, the type II receptor also binds IL-13. Within the type II complex, both IL-4R α and IL-13R $\alpha 1$ can each act as both driver and trigger, depending on which cytokine is bound. Thus while IL-4R α is the driver of the complex when IL-4 is bound, it acts as the trigger when IL-13 is part of the complex.

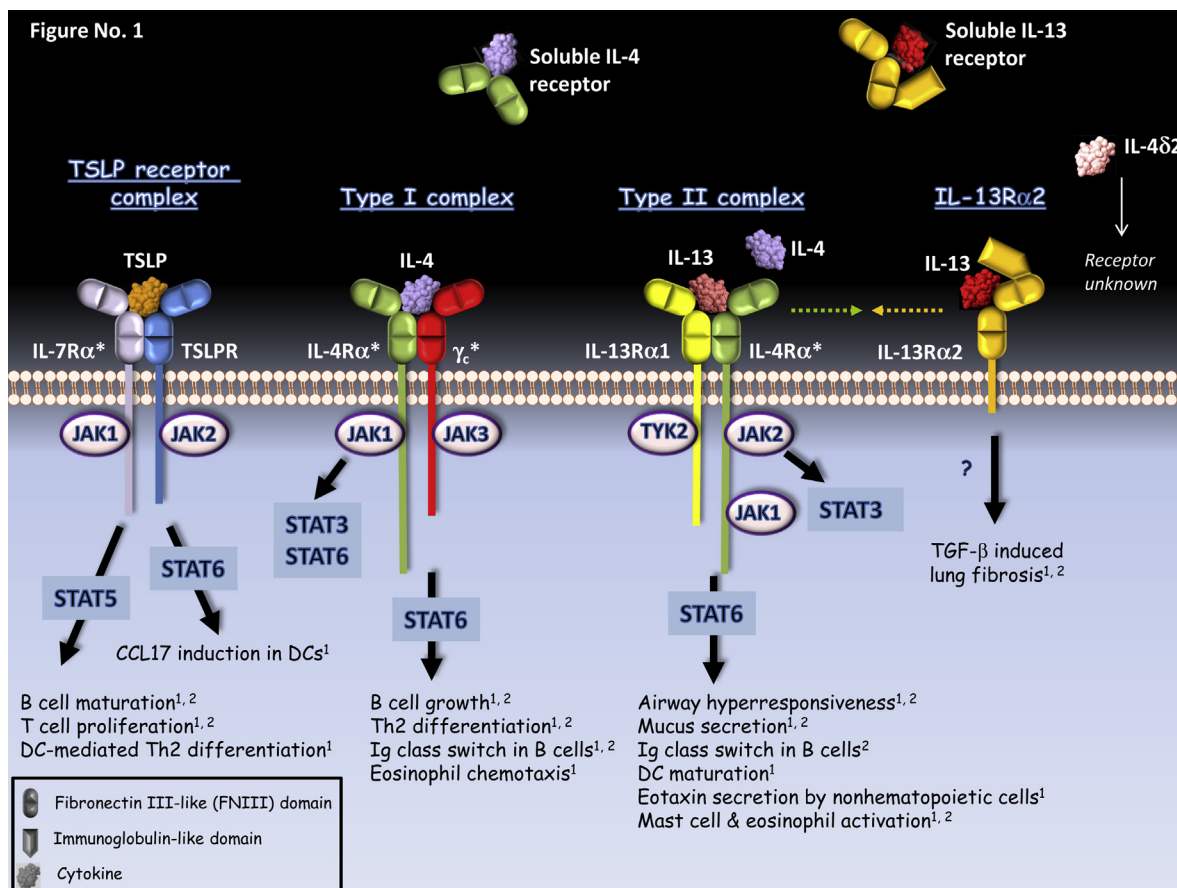


FIG 1. Receptor configurations for TSLP, IL-4, and IL-13. Asterisks indicate subunits shared among different receptor complexes. Green and gold arrows denote putative interactions between IL-4R α and IL-13R α 2 to form a signaling-inert complex. Functional outcomes are listed for human subjects (1) and mice (2). DC, Dendritic cell.

Conversely, on binding of IL-13, IL-13R α 1 acts as the driver of the type II complex but provides the trigger when IL-4 is bound. Notably, each receptor chain always binds cytokine in the same orientation. That is, IL-4R α binds IL-4 through a site I interaction and IL-13R α 1 binds IL-13 through site IIa, irrespective of function as a driver or trigger.⁴

Assembly of the type II receptor complex containing IL-13 appears to be favored over IL-4. This has been attributed to the presence of an additional N-terminal immunoglobulin-like domain (site III) within IL-13R α 1 (Fig 2, B, lower panel). Specifically, this region enhances the efficiency of binding of IL-13/IL-13R α 1 (driver) to IL-4R α (trigger) compared with binding of IL-4/IL-4R α (driver) to IL-13R α 1 (trigger).^{4,20} These structural aspects, along with the concentration of available ligand and the relative abundance of IL-4R α and IL-13R α 1 within the cell membrane, are key determinants in the type of receptor assembled and thus provide a mechanism for shaping the response to IL-4 and IL-13 *in vivo*.^{4,21}

Expression of IL-4R α and IL-13R α 1 differs among different cell types. Only the type I receptor is expressed on hematopoietic cells, such as T and B cells; however, both receptors are found on myeloid cells, including macrophages, monocytes, and fibroblasts. By contrast, nonhematopoietic cells, including epithelial cells and smooth muscle cells, predominantly express the type II receptor. Such tissue specificity explains, at least in part, the

discrete functions of IL-4 and IL-13. In animal models IL-4 signaling through the type I complex drives T_H2 differentiation of CD4⁺ T cells,²² IgE class-switching in B cells,²³ and eosinophil chemotaxis.²⁴ By contrast, IL-13 binding to the type II complex mediates the development of airway hyperresponsiveness and mucus overproduction by epithelial cells (Fig 1).^{25,26} However, the situation might be more complex in human subjects. Recent work using a variant of IL-4 that was engineered to bind IL-13R α 1 with high affinity revealed a role for IL-4 signaling through the type II complex in the differentiation of human monocytes into dendritic cells.²⁷ Such observations suggest that the biologic effects of IL-4 acting through the type II complex might be underappreciated in allergic disease.

IL-7R α chain is integral to TSLP receptor complex

IL-7R α is a component of both the IL-7 receptor complex (IL-7R α / γ_c) and TSLP receptor complex (TSLP receptor chain [TSLPR]/IL-7R α).^{28,29} Receptors for IL-7 are expressed on immature B cells and on T cells of various developmental stages.^{30,31} By contrast, the TSLP receptor complex is expressed on human dendritic cells and eosinophils and is upregulated on activated T cells, including T_H2 cells (Fig 1).^{1,2,32-34} The TSLPR is the high-affinity ligand-binding subunit of the TSLP receptor complex.³⁵ Thus whereas IL-7R α is the driver of the IL-7 receptor

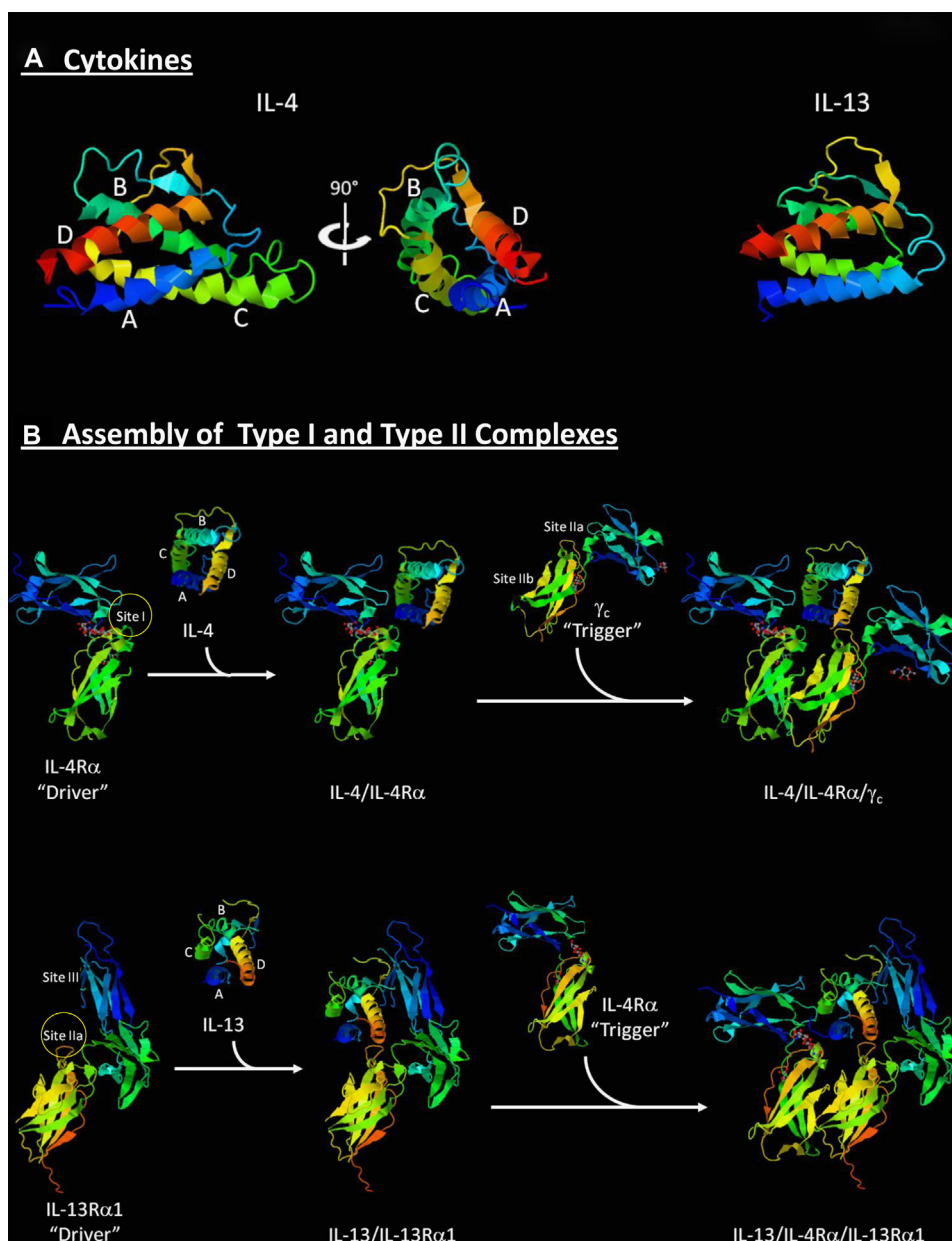


FIG 2. Assembly of type I and type II receptor complexes. **A**, Ribbon diagrams for IL-4 (PDB: 1ITM) and IL-13 (PDB: 1IJZ). **B**, Assembly of the IL-4 type I receptor and the IL-13 type II receptor (upper and lower panels, respectively). Upper panel, Blue and green regions denote membrane-distal and membrane-proximal FNIII domains, respectively, of IL-4Rα. The circle denotes site I. Lower panel, Immunoglobulin-like domain (blue) and membrane-distal and membrane-proximal FNIII domains (green and mixed, respectively) are shown for IL-13Rα1. The circle denotes site IIa. Models were constructed by using x-ray crystal structures of extracellular domains (IL-4/IL-4Rα/γ_c: PDB, 3BPL; type II receptor complex: PDB, 3BPO).

complex, it acts as the trigger in the TSLP receptor complex. Functional overlap of IL-7 receptor and TSLP in B-cell differentiation and proliferation has been attributed to participation of IL-7Rα in receptors for each of these cytokines.^{36,37}

Although the structure of the TSLP receptor complex remains unsolved, several observations support a model similar to the type II receptor bound to IL-13. On the basis of the premise that receptor chains always bind cytokines in the same orientation, IL-7Rα is predicted to engage TSLP through a site I interaction in the TSLP receptor complex (trigger). By extension, TSLPR

chain might be expected to bind TSLP through site IIa (driver), which is similar to the IL-13/IL-13Rα1 interaction. Interestingly, although the TSLPR chain is a homolog of γ_c, it also exhibits similarity with IL-13Rα1.³⁸ Sequence similarities between IL-13 and TSLP lend credence to the proposed model of TSLP receptor complex formation. Specifically, IL-13 and TSLP each contain basic amino acids in a "hot spot" for receptor contact located within helix D (see Fig E1).^{39,40} Together, these aspects suggest that receptor complexes containing TSLP and IL-13 might bear resemblance.

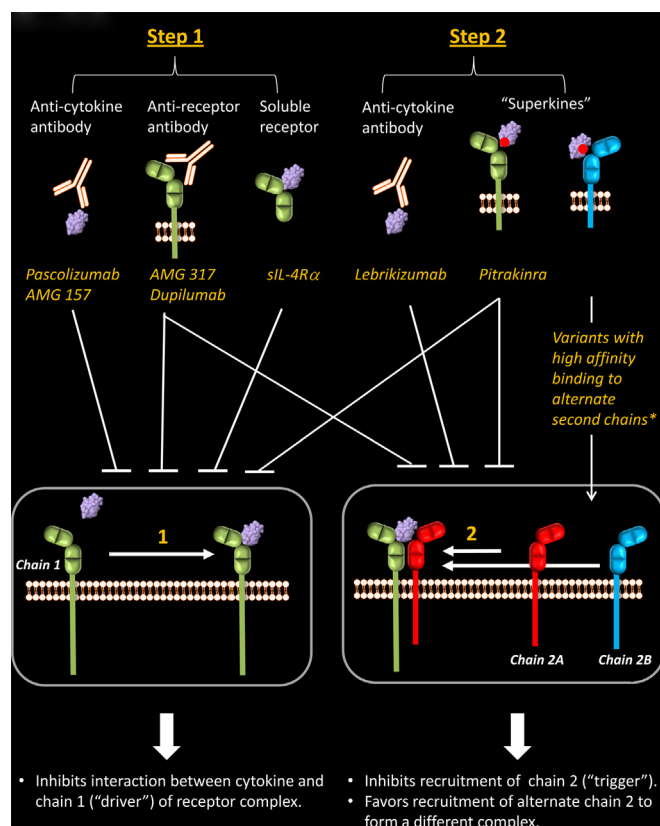


FIG 3. Treatments targeting T_H2 -promoting pathways. Drugs are categorized according to how they act in steps of the receptor assembly process. *Molecules in the research phase.

ALTERNATIVE RECEPTORS AND CYTOKINES

Soluble receptors

Soluble cytokine receptors have the potential to modulate T_H2 pathways and thus provide targets for the treatment of allergic disease. These receptors arise either from alternative splicing of pre-mRNA encoding the cytokine receptor or from proteolytic cleavage of membrane-bound receptor extracellular domains in a process known as shedding. Modulation by alternative splicing is poorly defined, although recent observations indicate involvement of the RNA cleavage and polyadenylation factor CPSF1 in human subjects.⁴¹ Generation of soluble receptors by shedding is regulated by matrix metalloproteinases (MMPs). Interestingly, members of this protein family have also been implicated in the pathogenesis of asthma and atopic dermatitis.^{42,43}

In human subjects alternative splicing of IL-4Rα pre-mRNA generates a truncated soluble secreted protein product that lacks the transmembrane and cytoplasmic tail but retains the ability to bind IL-4 (Fig 1).⁴⁴ Soluble IL-4Rα is also produced through MMP-dependent release of the IL-4Rα extracellular domain from the surface of activated human T cells.⁴⁵ Studies of a recombinant soluble human IL-4Rα showed its ability to enhance or else neutralize IL-4 activity *in vitro*, depending on its concentration.⁴⁶ However, the actions of this receptor *in vivo* remain enigmatic. Recent work on novel soluble IL-4 receptor variants in zebrafish might shed new light on soluble forms of IL-4Rα. Soluble variants displayed tissue-specific expression profiles. Furthermore, administration of soluble IL-4Rα blocked IL-4-induced B-cell proliferation and antibody production *in vivo*. This work implied an effect of soluble

receptor on the CD154-CD40 costimulatory axis, which promotes T_H2 induction by B cells.⁴⁷

IL-13Rα2, which has been proposed to act as a "cytokine trap" for IL-13 (ie, a decoy receptor), can exist in membrane and soluble forms (Fig 1).⁴⁸ Whereas in mice distinct forms of this receptor are generated by means of alternative splicing, in human subjects the soluble form arises from cleavage of membrane receptor by MMP-8.^{49,50} Protease allergens from dust mites can solubilize IL-13Rα2, and soluble receptor has been detected in bronchoalveolar lavage fluid of asthmatic patients, although at very low levels.⁵¹ In contrast to mice, soluble IL-13Rα2 appears to be absent in human blood.⁵²

The affinity of IL-13Rα2 for IL-13 is several orders of magnitude greater than that of IL-13Rα1 because of more extensive contacts with the cytokine.⁴⁰ This feature, coupled with the capacity for IL-13Rα2 to efficiently internalize IL-13 without inducing signaling events,⁵³ points to an ability for IL-13Rα2 to sequester IL-13 from its signaling-competent type II receptor. Consistent with this view, an IL-13 variant (Arg110Gln) linked to bronchial asthma that binds IL-13Rα2 with reduced affinity appears to be cleared less efficiently by IL-13Rα2 *in vitro*.⁵⁴ However, evidence that IL-13Rα2 exerts a modulatory effect in allergic disease in human subjects remains scant.

Adding to the complex landscape, recent evidence in mice suggests that membrane IL-13Rα2 might actually contribute to allergic inflammation.⁵⁵ Thus IL-13Rα2 could play key roles beyond its decoy activity. IL-13Rα2 can be induced on fibroblasts, smooth muscle cells, and keratinocytes.⁵⁶⁻⁵⁹ Its expression is highly regulated by IL-4 and IL-13, as well as by the type II interferon IFN-β. Notably, induction of IL-13Rα2 in human bronchial fibroblasts by double-stranded RNA through a mechanism involving IFN-β implicates this receptor in modulating responses to respiratory tract viruses.⁵⁹ There is also evidence that binding of IL-13 and signaling through IL-13Rα2 contributes to TGF-β-induced lung fibrosis.⁶⁰ Other work suggests that IL-13Rα2 can partner with IL-4Rα to form a signaling-inert complex capable of regulating the response to both IL-4 and IL-13.⁶¹ Thus the functions of membrane-bound IL-13Rα2 might extend well beyond its ability to provide a "sink" for IL-13 in the lungs.

Cytokine variants

Splice variants of both TSLP and IL-4 have been identified. Long and short transcripts encoding the same form of TSLP protein are expressed in human bronchial epithelial cells.⁶² The long transcript is preferentially upregulated in human keratinocytes by Toll-like receptor ligands and by T_H2 -associated cytokines.⁶³ There is also evidence that TSLP can be modified at the posttranslational level. Proteases expressed in nasal polyps generate a form of TSLP that enhances IL-5 production by mast cells, supporting a role for this molecule in the pathogenesis of chronic rhinosinusitis.⁶⁴

Alternative splicing of IL-4-encoding pre-mRNA yields at least 2 transcripts that give rise to full-length IL-4 and a truncated form (IL-4δ2).⁶⁵ The kinetics of expression of these variants differs in activated T cells from asthmatic patients, with IL-4 secretion peaking at 24 hours and IL-4δ2 secretion peaking later. There is evidence that these variants fulfill different functional roles and that IL-4δ2 can engage a range of receptors; however,

further work is necessary to elucidate how these variants might act in allergic disease.⁶⁶

AVENUES FOR RECEPTOR DISCOVERY

The role of alternate forms of cytokines and receptors, as well as their participatory complexes, has largely been overlooked in allergic disease. This is striking, given that these molecules might provide valuable therapeutic targets based on their potential to counterbalance T_H2 -driven inflammation. IL-482 provides an important example in this regard given its ability to inhibit both IL-4-induced production of polarized T_H2 cells and IgE synthesis.^{66,67} The availability of bioinformatics tools, including genome browsers and molecular modeling algorithms, makes it feasible to mine existing data for putative variants.⁶⁸ However, distinguishing among protein variants expressed in experimental systems using available mAbs might be problematic because of shared epitopes. Furthermore, it is important to consider whether novel receptor configurations, including homodimers, exist that can form functional complexes.^{69,70}

Establishing the relevance of “alternate” forms of T_H2 -promoting receptors and cytokines to the pathogenesis of allergic disease remains a significant challenge. Testing for expression of mRNA corresponding to molecular variants at inflamed sites is straightforward; however, detecting protein is problematic, especially for secreted molecules that might be rapidly degraded or sequestered at inflamed sites. In addition, certain features of cytokine receptor expression could be unique to specific cell types at sites of allergic inflammation. Single-cell transcriptomics, which involves analysis of the complete set of mRNA transcripts within a given cell, provides a powerful tool for tissue-specific receptor discovery.⁷¹ Because only a single type of cell is analyzed, this allows a determination of which gene products might partner with each other to form complexes. This is important because the tissue specificity of variants for some T_H2 -promoting cytokine receptor subunits might be underappreciated. Nonetheless, expression of mRNA components within the same cell does not guarantee formation of a functional receptor. Thus receptor expression must be confirmed at the protein level to establish relevance to disease.

CYTOKINE NETWORKS AND ALLERGIC DISEASE

The T_H2 -promoting cytokines likely evolved to serve protective roles in the immune system. For example, IL-4 provides the principal signal for IgE class-switching necessary for antihelminthic responses. On the other hand, TSLP, which is secreted by epithelial cells in response to bacteria and viruses, is pivotal to the innate response. TSLP can regulate helminth infection and colitis through a variety of mechanisms that involve constraint of damaging effector T cells, enhancement of regulatory T cells, and inhibitory factors.⁷²⁻⁷⁴

The overlapping functions and multiple actions of T_H2 -promoting cytokines result in complex cytokine networks. A variety of regulatory mechanisms operate within these networks to control pathogenic processes. Differential expression of functional receptors on specific cell types provides one such mechanism. The integration of shared subunits into different receptor configurations capable of binding different cytokines with discrete affinities provides an additional level of molecular dexterity and control that is quite remarkable. Generation of

receptor variants, including soluble forms, provides further regulation by modulating ligand engagement or else receptor triggering. The abundance of receptor chains on the cell surface and the concentration of ligand in the milieu are also key determinants of the type of receptor assembled and hence the type of signal generated.^{12,21} Perturbation of these myriad processes results in exaggerated T_H2 -driven responses and allergic inflammation.

Dysregulation of T_H2 pathways in patients with allergic disease can be initiated at the epithelial barrier. This is supported by the propensity for bronchial epithelial cells from atopic asthmatic patients to express higher levels of TSLP and its receptor in response to virus compared with cells from nonatopic donors.^{75,76} After T_H2 initiation, this process is perpetuated through upregulation of cytokine receptors on effector cells, including mast cells, basophils, dendritic cells, and T cells, in response to a variety of stimuli, including allergen.^{77,78} In turn, enhancement of T_H2 cytokine pathways has the capacity to augment allergen-triggered events through upregulation of IgE receptor, thereby creating a vicious cycle of inflammation.⁷⁹ Although it is easy to envisage how disruption of regulatory networks can occur, their complexity presents challenges for future research and treatment strategies.

TREATMENTS TARGETING T_H2 -PROMOTING PATHWAYS

The clinical benefits of targeting receptors for IL-4, IL-13, and TSLP are already being realized. This is accomplished by using molecules that intervene in the receptor assembly process to inhibit cytokine binding or else block the interaction of receptor subunits (Fig 3). However, not all approaches have been successful. Treatment modalities that inhibit IL-4 itself have been disappointing.^{80,81} By contrast, IL-13 antagonists have proved beneficial in patients with asthma. Interestingly, the IL-13-specific mAb lebrikizumab is more effective for treatment of asthma associated with high levels of periostin,⁸² a protein implicated in asthmatic airway eosinophilia.⁸³ Recent molecular observations indicate that the high-affinity interaction between lebrikizumab and IL-13 sterically hinders IL-13 from binding IL-4R α in the type II complex, thereby precluding receptor assembly and signaling.⁸⁴

It has been proposed that the lack of clinical benefit observed by targeting IL-4 relates, at least in part, to the redundancy between IL-4 and IL-13. Thus drugs that inhibit both IL-4 and IL-13 pathways simultaneously (so-called dual antagonists) might be advantageous. The IL-4 variant pitrakinra is one such drug. Pitrakinra is engineered to contain 2 single-point mutations that allow it to competitively bind to IL-4R α , thereby preventing formation of both type I and type II complexes (Fig 3).⁸⁵⁻⁸⁷ Inhalation of this molecule was shown to diminish allergen-induced late-phase asthmatic responses in patients with atopic asthma.⁸⁸ In subsequent studies pitrakinra inhibited exacerbations in patients with moderate-to-severe asthma.^{89,90} Interestingly, this latter effect was restricted to those patients who had specific single nucleotide polymorphisms in the 3' untranslated region of the gene encoding IL-4R α . Thus genetic polymorphisms, which are common in loci encoding T_H2 cytokine receptors, might have predictive value for the efficacy of therapeutics that target these receptors.⁹¹ Such

considerations have to be weighed when evaluating outcomes for mAbs that target IL-4/IL-13 pathways through IL-4R α .

Two antibodies have been developed that bind with high affinity to IL-4R α (known as AMG 317^{92,93} and dupilumab [REGN668]).⁹⁴ Thus similar to pitakinra, these antibodies block the biologic activities of both IL-4 and IL-13 through effects on type I and type II receptor complexes (Fig 3). Although AMG 317 was shown to be safe and well tolerated among patients with moderate-to-severe atopic asthma, no significant effect was observed in symptom scores in treated patients.⁹³ However, results in the same study showed a decrease in exacerbations in a group of patients receiving a higher dose of AMG 317 and increased response to treatment in patients with more severe asthma. More recently, dupilumab was reported to decrease asthma exacerbations among patients with persistent asthma associated with eosinophilia after withdrawal of inhaled glucocorticoids and long-acting β -agonist therapy.⁹⁴ In that study lung function was improved, and markers of T_H2-mediated inflammation in the blood (thymus and activation-regulated chemokine, eotaxin-3, and IgE) were significantly reduced.

As already mentioned, not all attempts to inhibit T_H2-promoting pathways have proved fruitful. The use of soluble receptors is a notable example in this regard. In early studies recombinant soluble IL-4R α provided promising results^{95,96}; however, subsequent work did not confirm these beneficial effects. This might be explained by the fact that soluble IL-4R α does not work effectively as a “cytokine trap.”⁹⁷ For a soluble receptor to optimally block cytokine binding, it should bind cytokine with higher affinity than its membrane-bound counterpart. These molecular considerations, coupled with the overlapping effects of IL-4 and IL-13, likely conspire to undermine the efficacy of soluble IL-4R α as a treatment modality.

Newer therapies are on the horizon that might be exploited to modify T_H2-promoting pathways. Engineered cytokines that bind with high affinity to specific receptor chains are currently under development that might redirect receptor signaling by preferentially favoring the assembly of specific receptor complexes. Recent work has shown that an IL-4 variant with high affinity for IL-13R α 1 favors activation through the type II receptor complex (IL-4R α /IL-13R α 1), as opposed to the type I receptor complex (IL-4R α / γ _c).²⁷ Thus using these so-called super-kines to exploit the variability in numbers of second chains (ie, IL-13R α 1 and γ _c) in certain cell types could provide a modality to redirect signaling both within and among specific cell types (Fig 3).

On the basis of the complexity of molecules engaged in cytokine networks, it is perhaps surprising that any clinical benefit is attainable by inhibiting a single molecule. With this in mind, blocking cytokines, such as TSLP, which operate upstream in the allergic inflammatory cascade, might be predicted to optimize clinical outcomes. Moreover, in contrast to IL-4 and IL-13, which appear to exert differential roles in certain asthma phenotypes, TSLP might be involved in multiple asthma phenotypes through its critical role in the innate phase of allergic responses. An anti-TSLP mAb that blocks the interaction of TSLP with TSLPR (AMG 157) is currently being tested as a treatment for asthma in phase 1b trials (Fig 3).

CONCLUSIONS AND FUTURE DIRECTIONS

We have highlighted the shared and discrepant molecular features of T_H2-promoting cytokines and their receptors with a

view to understanding how these aspects inform their function in allergic disease and the response to treatment. Moving forward, it will be important to understand the molecular basis of different asthma phenotypes to interpret variability in the response to new treatments that target T_H2-promoting cytokine pathways. Genetic polymorphisms have the ability to alter the interactions between receptors and their ligands and to modify downstream signaling events through effects on protein structure. Alternatively, more subtle effects can occur at the posttranscriptional level, which could affect the generation of splice variants, disease phenotypes, and therapeutic outcomes.

Environmental triggers, including allergens, feed into T_H2-promoting cytokine networks by modulating receptor expression. Thus combined therapies that mitigate the adverse effects of allergen exposure (eg, specific immunotherapy) and dampen T_H2-promoting pathways (eg, receptor antagonists) might optimize clinical benefit. This dual approach could be tailored according to the individual patient's sensitivity to allergens and disease phenotype.

Finally, it should be noted that the T_H2-promoting cytokines that contribute to the pathogenesis of allergic disease also fulfill critical immune functions related to B-cell growth and proliferation, as well as induction of regulatory T cells. Thus long-term blockade of these cytokines might not be without risk.

In summary, the relationships among T_H2-promoting cytokines and their receptors are complex. The molecular underpinnings that dictate receptor configurations, their relative abundance, and the resulting immune outcomes are shaped by genetics of the individual and environmental exposures. Studies that continue to query the nature of cytokine-receptor interactions in human subjects will not only serve to advance our knowledge of these structures but also hasten the development of new treatments that provide maximal clinical benefit to the individual patient.

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Helix A	
IL-2	-TKKTQLQLEHLLDQMIINGI
IL-7	----KQESVIMVSDQLLDS-
IL-4	-----ITLQETIKTNSLTE--
IL-13	----PSTALREIEIVNITQ--
TSLP	FEKIKAAVLSISKDITYM---
Helix C	
IL-2	-----PRDLISNINVIVLEIK---
IL-7	-----FDLHLKLVSEGTTLIN---
IL-4	AQQFHRHKQLPFLKRLDRNLWGLA
IL-13	-----AIEXTQRMISGFC
TSLP	-----AKEMGAMKTKAATAIW---
Helix D	
IL-2	---IVEFTNRWITFCQSIIST--
IL-7	----CFIKRRLQELKTCWNKIL
IL-4	---LENFTERIKTIMFEKYSKC-
IL-13	KIEVAQFVKDILLHLKLFRE--
TSLP	-NKCLOVSOQLWRRFNRPIL

FIG E1. Sequence similarity among the structurally conserved regions of TSLP, IL-4, and IL-13. Sequence alignments for TSLP, IL-4, and IL-13 and related IL-2 subfamily members (IL-2 and IL-7) within the α -helices A, C, and D. α -Helices were determined from x-ray structural data for IL-4 (PDB: 1ITM) and IL-13 (PDB: 1IJZ) and from prediction of the secondary structure for TSLP (JPred prediction algorithm). *Coloring* denotes sequence identity or similarity. *Black highlighting* denotes conserved hydrophobic core residues. *Underlining* denotes a region containing 3 basic residues (KKR) corresponding to a “hot spot” for IL-13 binding to IL-13R α 1 and IL-13R α 2. TSLP contains 3 basic residues at this site (RRR).