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D Y PATIL INTERNATIONAL UNIVERSITY
AKURDI PUNE

ASTHMA THAT LEADS TO OBESITY IN CHILDREN

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ABSTRACT:

Obesity is an important public health problem. An increasing body of data supports the hypothesis that obesity is a risk factor for asthma. These data include numerous large cross-sectional and prospective studies performed in adults, adolescents, and children throughout the world. With few exceptions, these studies indicate an increased relative risk of asthma in the obese and overweight and demonstrate that obesity antedates asthma. Obesity appears to be a particularly important issue for severe asthma. Studies showing improvements in asthma in subjects who lose weight, as well as studies showing that obese mice have innate airway hyperresponsiveness (AHR) as well as increased responses to certain asthma triggers also suggest a causal relationship between obesity and asthma. The mechanistic basis for this relationship has not been established. It may be that obesity and asthma share some common etiology, such as a common genetic predisposition, common effects of in utero conditions, or that obesity and asthma are both the result of some other predisposing factor such as physical activity or diet. However, there are also plausible biological mechanisms whereby obesity could be expected to either cause or worsen asthma. These include co-morbidities such as gastroesophageal reflux, complications from sleep-disordered breathing (SDB), breathing at low lung volume, chronic systemic inflammation, and endocrine factors, including adipokines and reproductive hormones. Understanding the mechanistic basis for the relationship between obesity and asthma may lead to new therapeutic strategies for treatment of this susceptible population.

Keywords:
Obesity, Airway Hyperresponsiveness (AHR), Sleep-Disordered Breathing (SDB), Therapeutic Strategies

Introduction:

Asthma is a chronic respiratory disease characterised by episodes of wheeze, cough, and shortness of breath. Around 14% of children worldwide have a diagnosis of asthma, making it the most common chronic respiratory disease of childhood. Poor asthma control is associated with a number of negative effects on children and families. For example, they are more likely to be absent from school, have additional educational needs and have lower educational attainment. Caregivers also experience missed work days and financial challenges as a result. Some children will experience severe symptoms and life-threatening attacks.

Taking the UK as an example, paediatric asthma outcomes are poor overall with considerable associated morbidity and high rates of emergency hospital admissions, and most pertinently, there are several preventable deaths each year. Alarmingly, the National Review of Asthma Deaths (NRAD) found that in almost all paediatric cases, there were a number of significant avoidable contributing factors and that these deaths may have been preventable. There are several factors that make the diagnosis and management of asthma in children challenging. The aim of this review was to explore these issues and highlight good clinical practice in the diagnosis and management of paediatric asthma.

Nowadays, asthma and obesity represent two of the major chronic diseases in children and adolescents. The International Study of Asthma and Allergies in Childhood, an international multicenter epidemiological study, showed that asthma prevalence worldwide is slightly increasing, even if there are striking differences among countries [1]. Current wheezing (defined as at least one episode of wheezing in the last 12 months) has become a major health issue in Latin American countries, Western Europe, Tunisia, Morocco, and Algeria, whereas English-speaking countries have experienced a slight reduction in prevalence trend throughout the last decade. Similarly, obesity in children of all ages has increased over the last four decades, with an eightfold increase in 5– 19 year old individuals between 1975 and 2016 and a twofold increase in younger children between 1980 and 2015 [2]. There is relevant heterogeneity in prevalence trends among different geographic areas, with a flattening in the last decade among countries with high socio-economic status and a steeper rise in Africa, Asia, and Latin America. Traditionally, asthma in children has been associated with atopy and bronchial eosinophilic inflammation. Recent studies have shown that despite new treatment options in asthma management (including biologic drugs and new inhalant combination-strategies) there are still 5–10% of patients who present with a difficult-to-treat phenotype, characterized by more exacerbations and bronchial hyper-reactivity that is non-respondent to standard therapies. The parallel increase in the prevalence of pediatric obesity through the last decade has raised concerns about a possible correlation between obesity and asthma in children and adolescents, heading to an intensive study of the topic by many research groups all over the world.

The deeper we explore the link between asthma and obesity, the more it appears to be complex and tangled, involving many different factors such as insulin resistance, dyslipidemia, fat distribution, and dietary habits. For example, insulin resistance has been found to affect respiratory function independently from body mass index (BMI) and other obesity-related parameters. The aim of this review is to critically summarize the current evidence on the obese asthmatic phenotype in children, allowing us to describe three main molecular pathophysiological mechanisms that might hypothetically underpin different clinical subtypes.

Methodology:

IL-13 is an anti-inflammatory cytokine that plays a role in IgE production and IgE-mediated allergic responses. In the lung, IL-13 regulates eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness. IL-13 receptors are expressed on human B cells, basophils, eosinophils, mast cells, endothelial cells, fibroblasts, monocytes, macrophages, respiratory epithelial cells, and smooth muscle cells.

Endogenous glucocorticoid (GC) activation is regulated by the intracellular GC-activating and -inactivating enzymes 11β-hydroxysteroid dehydrogenase (11β-HSD)1 and 11β-HSD2, respectively, that catalyze interconversion of inert cortisone and its bioactive metabolite cortisol. Because endogenous GCs are critically implicated in suppressing the asthmatic state, this study examined the roles of the 11β-HSD enzymes in regulating GC activation and bronchoprotection during proasthmatic stimulation. Airway hyperresponsiveness to methacholine and inflammation were assessed in rabbits following inhalation of the proasthmatic/proinflammatory cytokine IL-13 with and without pretreatment with the 11β-HSD inhibitor carbenoxolone (CBX). Additionally, IL-13-induced changes in 11β-HSD isozyme expression and GC metabolism were examined in epithelium-intact and -denuded tracheal segments and peripheral lung tissues. Finally, the effects of pretreatment with CBX or 11β-HSD2-targeted siRNAs were investigated with respect to cortisol prevention of IL-13-induced airway constrictor hyperresponsiveness and eotaxin-3 production by airway epithelial cells. IL-13-exposed rabbits exhibited airway hyperresponsiveness, inflammation, and elevated bronchoalveolar lung fluid levels of eotaxin-3. These responses were inhibited by pretreatment with CBX, suggesting a permissive proasthmatic role for 11β-HSD2. Supporting this concept, extended studies demonstrated that 1) IL-13-treated tracheal epithelium and peripheral lung tissues exhibit upregulated 11β-HSD2 activity, 2) the latter impairs cortisone-induced cortisol accumulation and the ability of administered cortisol to prevent both IL-13-induced heightened airway contractility and eotaxin-3 release from epithelial cells, and 3) these proasthmatic responses are prevented by cortisol administration in the presence of 11β-HSD2 inhibition. Collectively, these data demonstrate that the proasthmatic effects of IL-13 are enabled by impaired endogenous GC activation in the lung that is attributed to upregulation of 11β-HSD2 in the pulmonary epithelium.

Human ASM cells exposed to the primary proasthmatic T helper type 2 (Th2) cytokine, IL-13, exhibited upregulated expression of 11beta-HSD1, an effect that was attributed to activation of the transcription factor, AP-1, coupled to MAPK signaling via the ERK1/2 and JNK pathways. The induction of 11beta-HSD1 expression and its oxoreductase activity by IL-13 (also IL-4) served to amplify the conversion of cortisone to cortisol by the cytokine-exposed ASM and, hence, heighten GR-mediated transcriptional activation. Extended studies demonstrated that this amplified 11beta-HSD1-dependent GC activation enabled physiologically relevant concentrations of cortisone to exert enhanced protection of ASM tissues from the proasthmatic effects of IL-13 on ASM constrictor and relaxation responsiveness. Collectively, these novel findings identify a Th2 cytokine-driven homeostatic feedback mechanism in ASM that enhances its responsiveness to endogenous GCs by upregulating 11beta-HSD1 activity, thereby curtailing the adverse effects of the proasthmatic cytokine on airway function.

Structure:

IL-4 and IL-13 are type 2 secreted glycoprotein cytokines that engage heterodimeric cytokine receptor complexes. Structurally, each cytokine comprises a bundle of 4 alpha helices in an up-up-down topology, typical of the short-chain cytokine family. As such, both cytokines contain 4 juxtaposed alpha helices (denoted A, B, C, and D) connected by 3 interhelical loops, including 2 long end-to-end loops (AB and CD), a short hairpin loop (BC) stabilized by a disulfide bond,36 and an antiparallel beta-sheet in the AB and CD loops that sits against helices B and D Human IL-4 is slightly larger than human IL-13. IL-4 has 3 disulfide bonds, IL-13 contains only 2 disulfide bonds. Despite their structural homology, the two cytokines share only ≈25% similarity in their amino acid sequences. IL-4 engages two types of receptor complexes: the type I receptor complex, which is generally expressed

on lymphocytes and myeloid cells; and the type II receptor complex, which is typically found on myeloid and all non-hematopoietic cells, whereas IL-13 signals through only the type II receptor complex. Both IL-4 receptor complex types include the IL-4 receptor α chain (IL-4Rα, 140 kDa), but whereas the type I receptor complex includes the shared common gamma chain γc (60 kDa) as its co-receptor, the type II receptor complex utilizes the IL-13Rα1 chain (65–70 kDa).

The WSXWS motif is crucial for receptor function because it maintains the proper receptor conformation to allow for cytokine binding. Together, the type III fibronectin domains and the WSXWS motif form the elbow-shaped "cytokine binding homology region" (CHR), which binds the helical faces of the cytokine. LaPorte et al determined the molecular structure of IL-4 and IL- 13 cytokine/receptor complexes, revealing the detailed interfaces between the cytokines and their respective receptor complexes. There are 3 receptor-binding epitopes on IL-4 that engage the type I and/or type II receptor complexes, referred to as site I, site IIa, and site III. Site I refer to the high affinity interaction between the A and C helices of IL-4 and the EF loop of the IL-4Rα receptor subunit in both the type I and type II receptor complexes. Site IIa denotes the lower affinity interaction between the A and D helices of IL-4 and either the FG2, BC2, and EF1 loops of γc or the FG2 and BC2 loops of IL-13Rα1 in the type I and type II receptor complexes, respectively. Site III designates the interaction between the CD loop of IL-4 and the *c*' strand of the IL-13Rα1 receptor, and is thus unique to the type II receptor interaction. There are also 3 receptor-binding epitopes on IL-13, also called site I, site IIa, and site. Interleukin-4 cytokine/receptor complex structures. (A) The IL-4 cytokine is an up-up-down-down 4-helix bundle cytokine with 3 disulfide bonds and 2 long and 1 short interhelical loops (*left*, *top*). IL-13 has a similar overall structure to IL-4 but only has 2 disulfide bonds (*left*, *bottom*). Cysteines (C) that form disulfide bonds are shown, color coded to illustrate pairing. Structural data (PDB ID: 3BPL) demonstrate that the helices pack together in a bundle (*middle*), presenting two faces (*right*), AC and AD, which interact with the receptor complexes. (B) The molecular structures of the IL-4 type I (PDB ID: 3BPL), IL-4 type II (PDB ID: 3BPN), and IL-13 type II (PDB ID: 3BPO) cytokine/receptor complexes. IL-4 interacts with sites I and IIa in type I complexes. An additional interaction between IL-4 and IL-13 occurs in type II complexes at site III on the D1 domain of the IL-13Rα1 subunit. The co-receptors interact at site IIb in both the type I and type II III, although this cytokine only engages the type II receptor complex. Analogous to IL-4, site I on IL-13 refers to the interaction between the IL-13 A and C helices and the FG2 loop of the IL-4Rα receptor subunit. Site IIa indicates the interaction between the A and D helices of IL-13 and the FG2 and BC2 loops of IL-13Rα1. Site III specifies the interaction between the CD loop of IL-13 and the *c*′ strand of IL-13Rα1 for IL-13 cytokine/receptor complex engagement, the sequence

of assembly of the receptor chains is reversed compared to IL-4 cytokine/receptor complex assembly IL-13 first binds to
IL-13Rα1 with moderate affinity ($KD \approx 30$ nM.The IL-13/IL-13Rα1 complex then engages with the co-receptor, IL-
4Rα ($KD \approx 20$ nM IL-13Rα1 has a narrower ligand specificity than does the γc subunit and contains an extra N-terminal
Ig-like domain, D1 which is required for binding to IL-13 (although not for binding to IL-4). As YKL-40 orchestrates multiple inflammatory processes and its expression is dysregulated in many disease settings, further research is needed to understand the many roles of this enigmatic protein, which lacks enzymatic activity, in Th2 responses downstream of IL-13Rα2. Although IL-4 and IL-13 signal through membrane-embedded receptors, the presence of soluble IL-4 receptor chains produced by either alternative splicing or surface shedding through limited proteolysis may explain observed neutralization of cytokine therapies in clinical trials Soluble IL-4Rα functions as an antagonist of IL-4 in humans. Soluble IL-13Rα2 is believed to have distinct biologic functions compared to the membrane-bound receptor chain in mice. Soluble IL-13Rα2 inhibits sequesters IL-13, preventing it from binding the lower affinity IL-13Rα1, but has no effect on IL-4 signaling. Meanwhile, membrane-bound IL-13Rα2 inhibits IL-13 by sequestering the cytokine and promoting its internalization. In addition, IL-13Rα2 is also thought to inhibit

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IL-4 signaling by physically interacting with IL-4Rα. Exploiting the capacity of the soluble receptors to sequester IL-4 and IL-13 may serve as a potential approach to control the action of these cytokines to mitigate their effects allergic and fibrotic diseases.

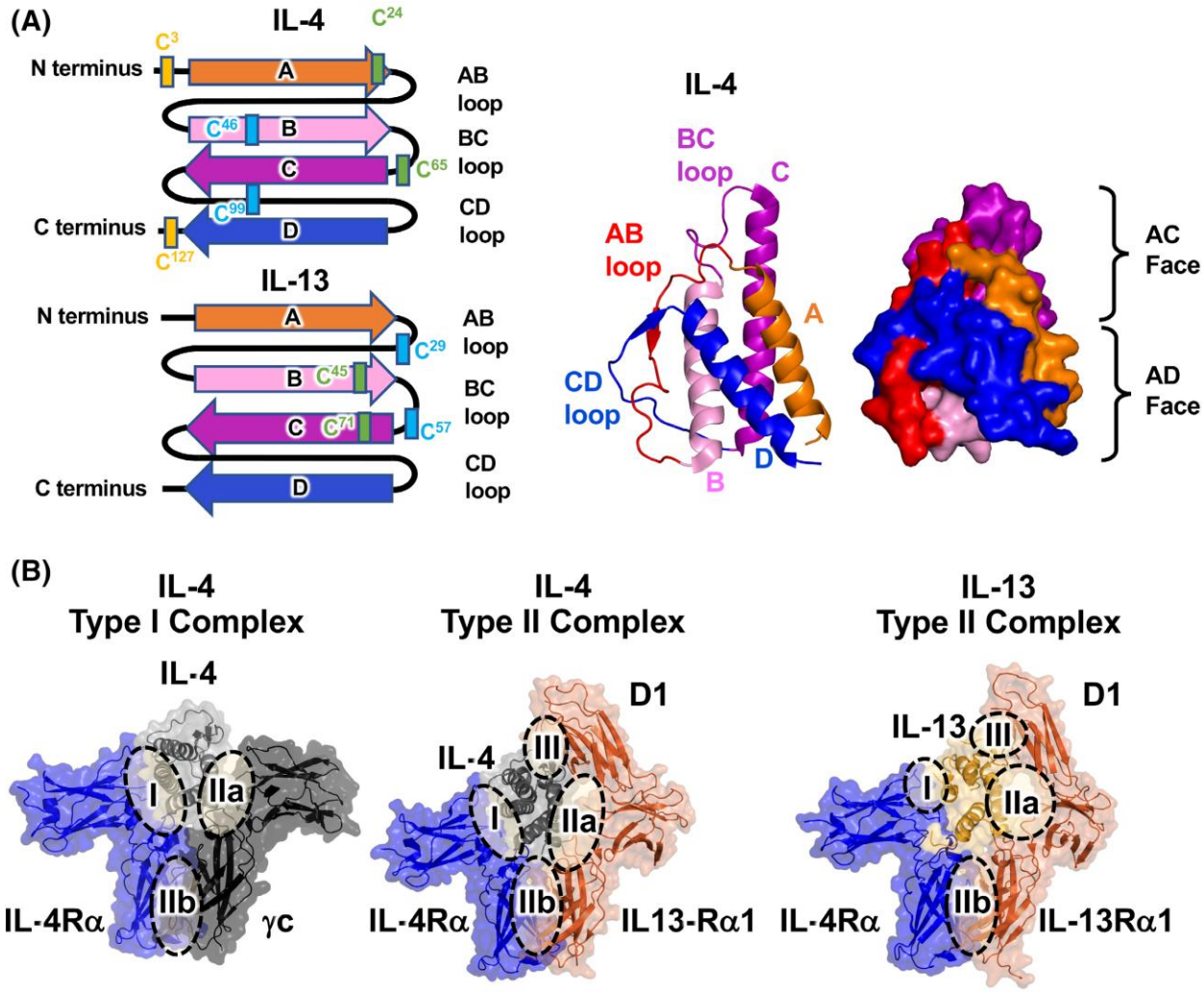

FIGURE 1 Interleukin-4 cytokine/receptor complex structures. (A) The IL-4 cytokine is an up-up-down-down 4-helix bundle cytokine with 3 disulfide bonds and 2 long and 1 short interhelical loops (*left*, *top*). IL-13 has a similar overall structure to IL-4 but only has 2 disulfide bonds (*left*, *bottom*). Cysteines (C) that form disulfide bonds are shown, color coded to illustrate pairing. Structural data (PDB ID: 3BPL) demonstrate that the helices pack together in a bundle (*middle*), presenting two faces (*right*), AC and AD, which interact with the receptor complexes. (B) The molecular structures of the IL-4 type I (PDB ID: 3BPL), IL-4 type II (PDB ID: 3BPN), and IL-13 type II (PDB ID: 3BPO) cytokine/receptor complexes. IL-4 interacts with sites I and IIa in type I complexes. An additional interaction between IL-4 and IL-13 occurs in type II complexes at site III on the D1 domain of the IL-13Rα1 subunit. The co-receptors interact at site IIb in both the type I and type II receptor complexes.

SIGNALING OUTCOMES:

IL-4 and IL-13 engage with their cognate receptor subunits to initiate intracellular signaling, but the receptor chains themselves lack endogenous kinase activity and thus utilize receptor-associated kinases, such as the Janus family tyrosine kinases (JAKs) and others,

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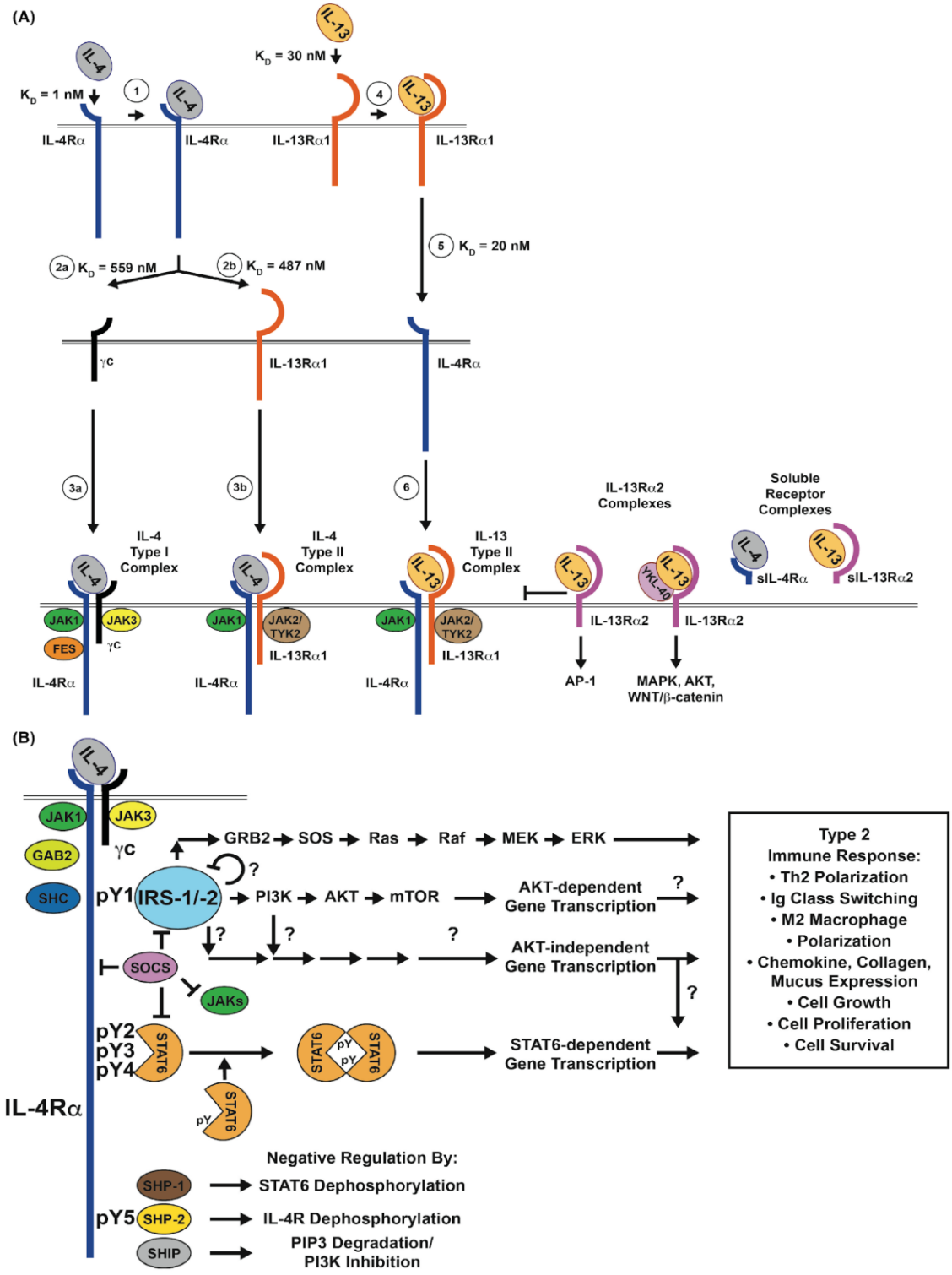
to enable signal transduction. Cytokine-mediated heterodimerization of IL-4Rα with either the γc chain (type I) or the IL-13Rα1 chain (type II) leads to the initiation of signal transduction. Because IL-4 and IL-13 share the type II receptor complex, the signal transduction pathways activated in response to each cytokine have been intensively studied to understand the shared and unique roles of these cytokines in functional outcomes on different cell types and in Type 2 immune responses. These phosphotyrosines serve as docking sites for signaling proteins that are recruited through their Src-homology 2 (SH2) or phosphotyrosine-binding (PTB)39 domains, and activate downstream signaling.



IRS pathway:

IL-4 and IL-13 also induce tyrosine phosphorylation in two members of the IRS protein family, IRS-1 and IRS-2. IRS proteins are large cytoplasmic adaptor proteins that are activated in response to insulin, insulin-like growth factor (IGF)-1, IL-4, and IL-13, as well as additional γc chain family cytokines, such as IL-2, IL-7, IL-9, and IL-15. IRS phosphorylation mainly occurs in response to IL-4 engagement of the type I receptor complex.We found that type II receptor engagement by IL-4 or IL-13 did not activate IRS-2 as significantly as type I receptor engagement, even at saturating concentrations of cytokines that induced equivalent STAT6 phosphorylation by type I and type II receptor complexes. IRS proteins do not translocate to the nucleus like STAT6, but instead serve as adaptor proteins to activate many other signal transduction pathways.

SHP-1/SHP-2/SHIP pathway:

Like SHP-1, SHIP is hematopoietically restricted, and it downregulates IL-4 signaling by removing the 5′-phosphate from PIP3. Removal of this second messenger from the membrane inhibits AKT activation and PI3K activity, limiting
IL-4 signaling.

Additional pathways:

Although GRB2 binds SOS constitutively and can activate Ras and ERK signaling in insulin responses, IL-4 and IL-13 stimulation
do not generally result in ERK phosphorylation. One notable exception is in airway smooth muscle cells isolated from human lungs,
wherein treatment with IL-4 and IL-13 activates ERK. In addition, the scaffolding/ docking protein, GRB2-associated binder (GAB2), associates with the IL-4Rα in IL-4-stimulated T cells, suggesting that other pathways such as Crk signaling can be initiated in response to IL-4 in some cell types.

FUNCTIONAL OUTCOME:

1) Gene **expression:**

As noted above, STAT6 is the major transcription factor pathway activated in response to IL-4 and IL-13 stimulation; thus, many IL-4-responsive genes contain STAT6 binding sites in their promoters. A key transcription factor in M2 differentiation, c-myc, is inhibited by the MEK5/ERK5 pathway in IL-4-activated macrophages. The AP-1 transcription factor is activated in response to IL-13 engagement of the IL-13Rα2 decoy receptor in the presence of tumor necrosis factor α (TNF-α) in human monocytes and monocyte– macrophage cell lines.

Functional differences between IL-4/IL-13:

IL-4	IL-13
No IgE production	IgE production
Allergic inflammation of the lungs (humoral response): • Th2 cell proliferation and survival • IgE and IgG1 production	Allergic inflammation of the lungs (pathophysiological features): • Mucus secretion • Goblet cell metaplasia • Smooth muscle cell contraction • Airway hyperresponsiveness (AHR) • Subepithelial fibrosis

Control of IL-4-induced gene transcription is not only dependent upon activation of specific transcription factors but also upon epigenetic control through histone modifications. IL-4-responsive genes and regulates key biological processes such as cell growth and development through the acetylation of four core histones and other transcription factors.

2) **Functional responses:**

IL-4 plays a significant role in regulating immune activity and is crucial for the development of Th2-mediated responses such as parasitic infection clearance and allergy.In lymphocytes, IL-4 polarizes CD4+ T cells towards the Th2 phenotype following antigen stimultion, prompting the cells to produce type 2 cytokines (including IL-4 and IL-13), while also strongly suppressing differentiation into interferon γ (IFNγ)-producing Th1 cells. Other IL-4 functions in hematopoietic tissues include increased expression of CD23 (the "low affinity" receptor for IgE) and IL-4Rα on B cells. IL-4 also plays a critical role in tissue adhesion and inflammation. In conjunction with TNF-α signaling, IL-4 induces expression of vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelial cells. IL-4 regulates chemokine and mucus secretion, which can contribute to airway inflammation and cell migration. In cellular studies, IL-4 was also shown to induce hypercontractility in human airway smooth muscle cells and to promote release of chemokines that recruit immune cells linked to allergic response.IL-4 and IL-13 also affect innate immune cells. For example, IL-4 has been shown to increase phagocytosis and killing of the protozoan parasite, *Trypanosoma cruzi.*

Taken together, IL-4 coordinates the functions of both adaptive and innate immune cells to produce effective Type 2 immune responses. In addition, both IL-4 and IL-13 regulate Th1 and Th17 driven T-cell inflammation. IL-13 is considered the major driver of pathogenic tissue fibrosis in both schistosomiasis and asthma. Whereas IL-4 is a regulator of lymphocyte/immune cell function, IL-13 is generally viewed as an effector cytokine that regulates the function of non-hematopoietic cells. For example, in helminth worm infections of mice, IL-13 is essential for mucus production, goblet cell hyperplasia, and worm expulsion, whereas IL-4 is critical for IgE production and mast cell activation. Similarly, in allergic lung inflammation, IL-13 is critical for airway hyperreactivity (AHR), smooth cell muscle contraction, goblet cell metaplasia, and mucus production, whereas IL-4 is essential for Th2 cell. differentiation and IgE and IgG1 production. While STAT6 phosphorylation and signaling are equally induced at saturating concentrations of IL-4 and IL-13, tyrosine phosphorylation of IRS-2 in response to type I receptor engagement by IL-4 is much greater than that induced by type II receptor engagement by either IL-4 or IL-13. The amount of IRS-2 tyrosine phosphorylation was found to be correlated with the degree of M2 gene and protein expression in macrophages. Interestingly, when IRS-2 is absent, M2 gene expression in macrophages is enhanced, suggesting either IRS-2 is part of a negative regulatory feedback pathway or IRS-1 signaling dominates in the absence of IRS-2. Novel engineered versions of IL-4 that only engage type I IL-4 receptors will help clearly define signaling and functional differences between type I and II IL-4 receptors

.**DISEASES WITH AN I L- 4 -/I L-13 - DOMINANT CYTOKINE PROFILE:**

Allergic inflammation is a Th2 inflammatory response directed to an otherwise innocuous antigen, the "allergen." survival of eosinophils. The accumulation of effector Th2 cells and release of IL-4, IL-5, and IL-13 upon second exposure to the same allergen result in organ-specific manifestations of allergic inflammation, such as asthma, atopic dermatitis (eczema), food allergies, and, systemically, anaphylaxis. The consequences of IL-4 and IL-13 release have different impacts on disease pathology depending on the organ. The hallmark features of asthma, such as difficulty breathing due to airway constriction and plugging by mucus, are a result of IL-4 and IL-13 activity on various structural cells of the lung, which leads to goblet cell differentiation, mucus production and plugging of the airways, smooth muscle cell hyperplasia and hypertrophy, enhanced contractility, and epithelial chemokine secretion. IL-4 is also critical for initiating class switching to the IgE isotype, as well as B-cell proliferation and

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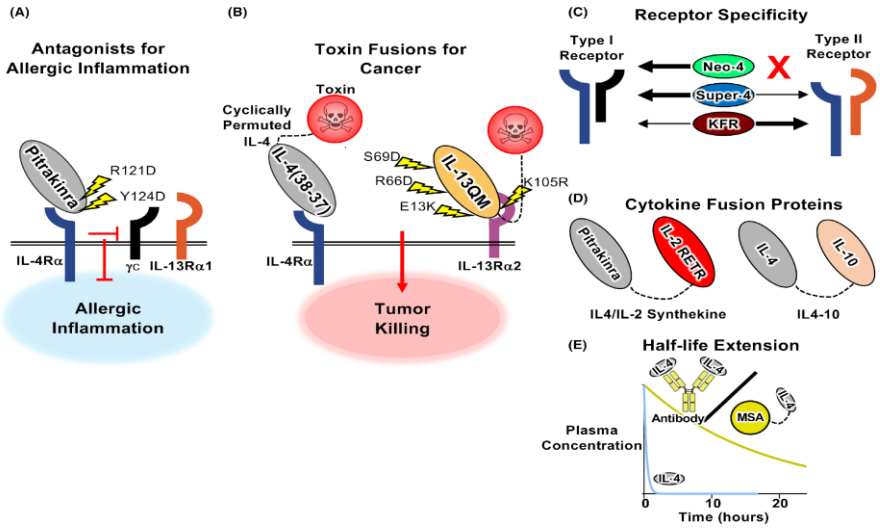
IgE secretion. Moreover, polarization of macrophages to the M2 phenotype by IL-4 and IL-13 supports Type immunity through expression of genes that regulate proinflammatory responses, promote the recruitment and function of Th2 cells, regulatory T cells (Tregs), and eosinophils, remodel ECM, and induce the proliferation of fibroblasts and endothelial cells.

Design of IL-4/IL-13 cytokine antagonists:

Engineering of the first IL-4/IL-13 antagonists began with the biophysical characterization of recombinant IL-4 and its receptor interactions. These studies found that a single amino acid substitution, Y124D, yielded a mutant version of IL-4 (termed a mutein) denoted hIL-4.Y124D that retained many biophysical properties of the natural cytokine, but did not induce detectable cell proliferation. Importantly, this molecule was competitive with hIL-4 for cell binding, suggesting that it still engaged the IL-4 receptor. Subsequent studies showed that hIL-4.Y124D, in fact, acted as an antagonist of IL-4-dependent cell proliferation in vitro.Interestingly, hIL-4.Y124D also antagonized IL-13 binding to its cognate receptors and inhibited IL-13-dependent cell proliferation in vitro, reflecting that this mutation disrupted cytokine binding to both IL-13Rα1 and γc. The antagonistic activity of hIL-4.Y124D was demonstrated in vivo through inhibition of IL-4-dependent IgE synthesis in humanized severe compromised immunodeficient (SCID-hu) mice .Y124D/R121D, commercially known as pitrakinra (Aerovant™) . Early studies of pitrakinra as an allergy therapy showed efficacy in reducing airway hyperresponsiveness and inflammation in allergen-sensitized primates. Pharmacogenetic analysis of the phase 2a trial demonstrated that only certain subpopulations of patients with polymorphisms in IL-4Rα showed clinical responses to pitrakinra.



Affinity maturation and modulation of IL-4 and IL-13 agonists:

This technology was first applied to IL-4 engineering with the goal of redirecting affinity to favor either type I and type II receptor complex engagement. Selections against γc led to a variant, denoted “Super-4,” that exhibited a 3700-fold higher affinity towards γc (when complexed with IL-4Rα) compared to native IL-4 IL-13Rα1-biased variant was generated by grafting various residues from IL-13 that are implicated in its private receptor-binding epitope onto IL-4. A triple variant, dubbed “KFR,” had a 440-fold higher affinity for IL-13Rα1 (when complexed to IL-4Rα) compared to native IL-4. Overall, these evolved mutants exhibited far greater agonistic activity than had been observed in previous mutational design efforts and provided new tools for investigating IL-4 receptor signaling and biology. In another effort investigate how IL-4/IL-13 receptor affinities modulate type II receptor dimerization and downstream signaling, type II receptor agonists with a range of affinities were engineered via yeast display-mediated directed evolution, using natural IL-13 as a template. Single-molecule microscopy studies using these IL-13 variants demonstrated that spatial organization of the plasma membrane into microdomains is a key driver of receptor dimerization, even when binding affinities are highly varied.

Synthetic and de novo cytokines:

In addition to development of muteins based upon the natural IL-4 and IL-13 cytokines, additional fusion and design approaches have

been employed to develop new molecules with distinct functional profiles. To engineer a synthetic cytokine, or "synthekine," that combined IL-2 (which signals through the IL-2Rβ and and γc chains) and IL-4 signaling, the pitrakinra IL-4 antagonist (which binds IL-4Rα but not γc or IL-13Rα1) was fused to an IL-2 receptor antagonist, denoted H9 RETR (which shows enhanced binding to IL-2Rβ compared to natural IL-2, but does not interact with γc). The first example of de novo cytokine engineering in the IL-4/IL-13 system was based on a previously developed de novo protein, denoted designed helix-protein (DHP). This molecule was used as a scaffold for structure-guided engineering to introduce IL-4Rα binding by grafting several residues implicated in the IL-4/IL-4Rα interface onto DHP.

I L- 4/I L-13 PATHWAY ANTAGONISTS:

To date, there is 1 clinical antibody targeting IL-4 (pascolizumab), 3 clinical antibodies targeting IL-13 (lebrikizumab, anrukinzumab, and tralokinumab [Adbry™]), and 1 clinical antibody targeting IL-4Rα (dupilumab [Dupixent®])

Anti-IL-4 antibody:

Pascolizumab is the only antagonistic antibody against IL-4 that has been clinically developed thus far. Pascolizumab is a humanized IgG1 antibody that binds the soluble IL-4 cytokine and prevents it from binding to cell surface receptor complexes. Pascolizumab showed promising results in cellular assays in terms of blocking IL-4-activated processes such as IL-5 synthesis, IgE production, and Th2 cell activation.

Anti-IL-13 antibodies:

Lebrikizumab is a humanized IgG2 antibody against IL-13 that blocks the cytokine from binding the type II receptor complex, thereby

disrupting downstream signaling. The binding interface of the Fab domain of this antibody in complex with IL-13 was characterized in a 1.9 Å resolution crystal structure, which revealed that the lebrikizumab Fv domain formed contacts within the B and C helices as well as the BC and CD loops of IL-13. As the IL-13 interface with IL-4Rα encompasses the A and C helices, whereas the IL-13/IL-13Rα1 interface engages with A and D helices, lebrikizumab directly competes with IL-13 binding to IL-4Rα but not IL-13Rα1. Although lebrikizumab was shown to be more effective in patients with higher levels of periostin (a matricellular protein that serves as a marker for IL-13 activity), therapeutic improvement was not significantly better than placebo. Another anti-IL-13 antibody, anrukinzumab, which is a humanized IgG2 antibody, was also shown to disrupt IL-13 engagement of its receptor and consequent downstream signaling. This antibody, also denoted as IMA-638, was shown to block the IL-13/IL-13Rα1 interaction and showed promising results in lowering allergic lung inflammation in cynomolgus monkeys. Slightly more success was found for tralokinumab, a fully human IgG4 antibody against IL-13. Tralokinumab, originally denoted as BAK 1.1, was developed through a combination of phage display and site-saturation mutagenesis. In another Phase 2b clinical trial in patients with mild to severe asthma, tralokinumab increased FEV1 in administered patients compared to placebo but did not decrease the asthma exacerbation rate in patients who received this therapeutic compared to placebo.

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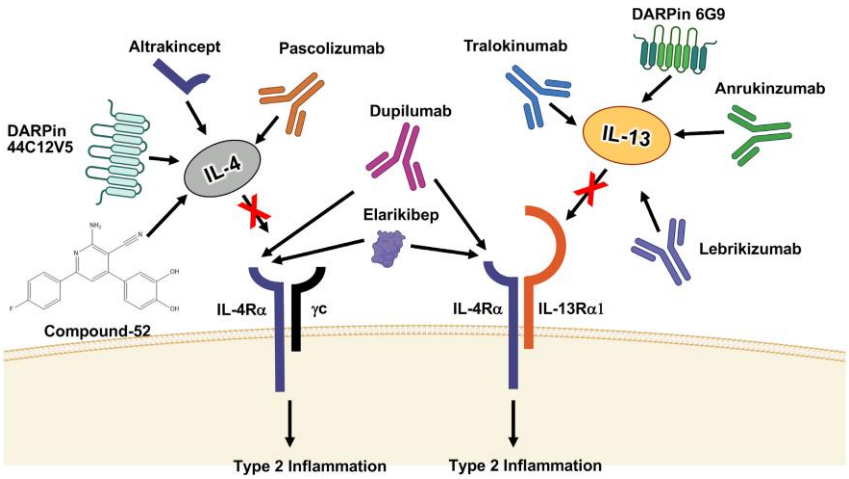

FIGURE 4 Antagonists targeting IL-4, IL-13, and their respective receptors. Antagonists against IL-4 (*left to right*: compound-52, DARPin 44C12V5, altrakincept, and pascolizumab) block signaling through the type I receptor complex, attenuating Type 2 inflammation. Antagonists against the IL-4Rα receptor subunit (*left to right*: Dupilumab and elarikibep) block signaling through the IL-4 type I receptor complex as well as the IL-4 and IL-13 type II receptor complexes, also attenuating Type 2 inflammation. Antagonists against IL-13 (*left to right*: tralokinumab, DARPin 6G9, anrukinzumab and lebrikizumab) block signaling through the IL-13 type II receptor complex, inhibiting Type 2 inflammation.

Anti-IL-4Rα antibody:

One notable example of this approach has been dupilumab (Dupixent®), a humanized IgG4 antibody that binds the IL4Rα subunit and disrupts IL-4 and IL-13 signaling through the type I and type II receptor complexes, both of which contribute to the onset of allergic inflammation. Furthermore, recent work showed that dupilumab also leads to the internalization of IL-4Rα, reducing its surface expression on various immune cells. Accordingly, dupilumab has been shown to block several events associated with allergy such as Th2 cell differentiation, IgE production by plasma cells, and macrophage activation. In addition, other secondary markers for Type 2 inflammation, such as IgE levels, eosinophil abundance, fractional exhaled nitric oxide (FeNo), and periostin levels were lower in patients treated with dupilumab.

Other IL-4/IL-13 pathway antagonists:

One approach that has been explored is the use of soluble IL-4Rα (altrakincept) as a decoy receptor to block IL-4 signaling.

CAR-T APPLICATIONS IN THE I L- 4/IL-13 AXIS:

chimeric IL-4/IL-2 receptor was created that fused the extracellular domain of IL-4Rα to the intracellular domain of the IL-2Rβ receptor chain, which recognizes another γc cytokine, IL-2. This important proof of concept illustrates the potential for development of CAR-T cells utilizing elements of the IL-4/IL-13 axis for applications in allergic diseases.

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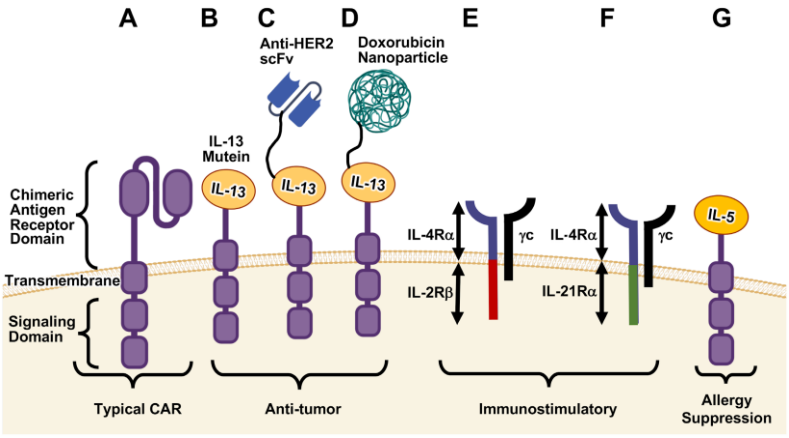
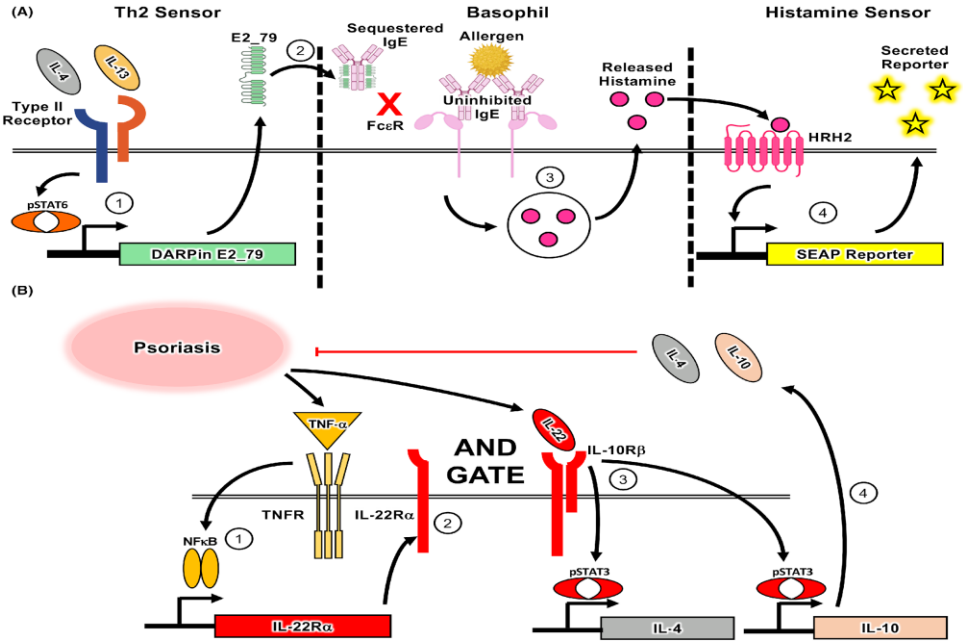


CE LL-BA SED SENSORS IN THE IL-4/IL-13 AXIS:

The type II receptor complex was transduced into human embryonic kidney (HEK) 293 cells, and a STAT6-sensitive genetic circuit was used to quantify levels of IL-4 and IL-13. In addition, to develop a sensor/actuator system that would produce a therapeutic in response to detected IL-4 and IL-13, the reporter circuit was replaced with a genetic circuit encoding a secreted DARPin, denoted E2_79. This DARPin has been shown to bind IgE and prevent subsequent formation of IgEFcεRI complexes, which drive histamine release. The resulting IL-4/ IL-13 cell sensor containing the DARPin genetic circuit was then coupled to their original histamine sensor to monitor the effectiveness of allergy attenuation. This system was highly sensitive to IL-4 and IL-13 and reduced ex vivo histamine responses in allergic patient derived blood, validating the first therapeutic cell sensor in the IL-4/ IL-13 axis. Taken together, these innovative combinations of protein and genetic engineering demonstrate the possibilities for creating biological sensors that can detect and respond to immune stimuli components of the IL-4/IL-13 pathways, in order to monitor and treat inflammatory diseases.



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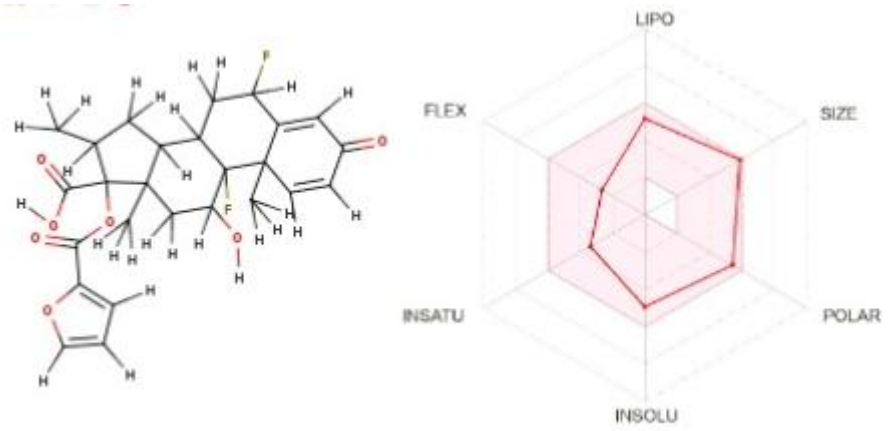
Application:

APPLICATIONS OF BIOLOGICAL THERAPY FOR SEVERE ASTHMA: Biological therapy has revolutionized the treatment of severe asthma by targeting specific inflammatory pathways. It primarily focuses on the type 2 inflammatory pathway, which involves immune cells like eosinophils and mast cells. Medications such as monoclonal antibodies against interleukins have shown significant efficacy in reducing asthma exacerbations and improving lung function. Biological therapies also target other inflammatory pathways, like IgE-mediated asthma. Personalized medicine approaches, using biomarkers to identify patients likely to respond to specific biologics, have improved treatment outcomes. Combining different biological therapies or combining them with standard asthma medications may benefit patients with complex asthma phenotypes. Overall, biological therapy offers targeted and personalized treatment options for severe asthma, improving outcomes and quality of life for patients.

RESULT:



Fluticasone furoate

Binding Affinity with Il-4 Ralpha is -8.9

PHYSICOCHEMICAL PROPERTIES	
FORMULA	C6H28F2O7
MOLECULAR WEIGHT	490.49 g/mol
NUM. HEAVY ATOMS	35
NUM. AROM. HEAVY ATOMS	5
FRACTION CSP3	0.58
NUM. ROTATABLE BONDS	4
NUM. H-BOND ACCEPTORS	9
NUM. H-BOND DONORS	2
MOLAR REFRACTIVITY	119.52
TPSA	114.04

DRUGLIKENESS	
LIPINSKI	YES; 0 VIOLATION
GHOSE	NO; 1 VIOLATION:MW>480
VEBER	YES

EGAN	YES
MUEGGE	YES
BIOAVAILABILITY SCORE	0.56

MEDICINAL CHEMESTRY	
PAINS	0 ALERT
BRENK	0 ALERT
LEADLIKENESS	NO; 2 VIOLTION: MW>350, XLOGP3>3.5
SYNTHETIC ACCESSIBILITY	5.84

CONCLUSIONS/PERSPECTIVES:

Similarly, although IL-13Rα2 was initially dubbed as a "decoy" receptor, the ligands and downstream signaling pathways associated with IL-13Rα2 remain to be fully elucidated, as do its interactions with canonical type I and type II receptor complex signaling. Furthermore, there is growing interest in how sex hormones differentially modulate immune cell phenotypes. In the IL-4/IL-13 axis, these differences most notoriously manifest as women suffering from a greater incidence of allergy. Although estrogen receptor has been shown to modulate the IL-4/IL-13 signaling pathways, the exact nature of these interactions, through directly or indirectly modulating transcription, protein interactions, and/or immunometabolism, are still unclear. Moreover, the interplay of androgen receptor with the IL-4/IL-13 axis also remains unknown. Deconvolution of the type I and type II receptor complex signaling pathways may lead to new therapeutic targets for the various diseases in which IL-4 and IL-13 are implicated.

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