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Commentary

IL-13 as a therapeutic target for respiratory disease

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ABSTRACT

Interleukin-13 (IL-13) is a critical mediator of asthma pathology. On B cells, monocytes, epithelial cells, and smooth muscle cells, IL-13 acts through the IL-13Rα1/IL-4Rα complex to directly induce activation responses that contribute to atopic disease. In human populations, genetic polymorphisms in IL-13, its receptor components, or the essential signaling element STAT6, have all been associated with increased risk of atopy and asthma. Animal studies using IL-13 deficient mice, IL-13 transgenic animals, and IL-13 neutralization strategies have confirmed an essential role for this cytokine in driving major correlates of asthma pathology, including airway hyperresponsiveness (AHR), lung eosinophilia, mucus generation, and fibrosis. Ongoing studies continue to define both overlapping and distinct roles for IL-13 and the related cytokine, IL-4, in promoting asthmatic changes. Furthermore, new evidence concerning the role of the ''decoy'' receptor, IL-13Rα2, has prompted re-evaluation of the receptor forms that underlie the numerous activities of IL-13. In this review, we summarize the essential role of IL-13 in asthma, compare the relative contributions of IL-13 and IL-4 to key aspects of the asthmatic phenotype, and outline novel therapeutic strategies to target this critical cytokine.

1. Introduction

IL-13 is a Th2 cytokine that has emerged as a critical regulator of inflammatory immune responses, with key roles in asthma and parasite immunity [1,2]. *In vitro*, IL-13 drives many cellular responses relevant to asthma, including epithelial cell maturation and mucus production, generation of extracellular matrix proteins, and enhanced contractility of airway smooth muscle cells [2]. Generated by activated Th2 cells, NKT cells, mast cells, and basophils, IL-13 shares approximately 20% amino acid sequence identity, and a range of cellular activities, with IL-4 [1,3]. Both IL-4 and IL-13 were originally described based on their ability to promote IgE switch recombination, and remain the only cytokines known to possess this activity. The genes encoding both cytokines are localized within the cytokine gene cluster on human chromosome 5q31 [4], which also includes the genes encoding IL-5 and IL-3. The common activities of IL-13 and IL-4 are mediated through the IL-4Rα/IL-13Rα1 receptor complex shared by both cytokines. Because IL-4 may also utilize the IL-4Rα/γ common receptor complex, it has additional activities which are not shared by IL-13, including effects on T cell maturation and skewing to Th2 [3].

A wealth of data supports a role for IL-13 in mediating asthma pathology. IL-13 can be detected in the bronchial tissue [5], nasal lavage fluid [6], and induced sputum [5] of asthmatics. Following segmental allergen challenge, bronchoalveolar lavage (BAL) fluid contains IL-13 mRNA [7] and IL-13 protein [8], confirming that the cytokine is generated in the

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Abbreviations: AHR, airway hyperresponiveness; BAL, bronchoalveolar lavage; NHP, nonhuman primate.

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lung in response to respiratory provocation. Similar observations have been noted in animal models. In mice immunized and given lung challenge with ovalbumin or house dust mite, significant increases in IL-13 mRNA and protein can be found in lung tissue and BAL fluid [9].

Numerous additional findings demonstrate a role for IL-13 in mediating pathology in the lung. Pulmonary delivery of IL-13 to mice [10–12] or targeted overexpression of IL-13 to the lung [13,14] induces multiple correlates of asthma pathology, including airway eosinophilia, mucus cell metaplasia, airway fibrosis, eotaxin production, and AHR. Despite their shared activities, studies in animal models have pointed to a preferential role for IL-13 over IL-4 in driving asthma pathology [11,15]. In the following sections, we will explore the evidence, summarized in Table 1, linking IL-13 to key disease parameters. In addition, we will review IL-13 receptor interactions, and summarize ongoing strategies to target IL-13, IL-4, or both for the treatment of asthma.

2. Airway hyperresponsiveness and inflammation

Both IL-4 and IL-13 contribute to AHR and inflammation in animal models. Initial observations demonstrated that pulmonary delivery of IL-4 induces AHR and mucus production in mice, but not airway inflammation [11,16,17]. Paradoxically, animals with IL-4 transgenically targeted to the lung developed airway inflammation, mucus, and high serum IgE, but not AHR [18,19]. IL-4-deficient mice were protected from airway eosinophilia, and exhibited reduced bronchial hyperresponsiveness [20]. Although lung eosinophila and AHR were impaired in mice lacking IL-4, they were not abolished, indicating that IL-4 is not essential for these responses. The residual inflammation could be overcome by neutralization of IL-5 [21], but some degree of AHR persisted, even in the combined absence of IL-4 and IL-5 activity [22]. Following the demonstration that IL-13 could contribute to AHR and eosinophilia in mouse asthma models independently of IL-4 [10,11], the factor responsible for this residual activity was identified as IL-13. Antibody-mediated neutralization of IL-4 in IL-13-deficient mice effectively blocks all residual airway inflammation, in addition to AHR, and goblet cell hyperplasia [23]. Interestingly, initial observations that IL-13 deletion failed to completely abrogate allergic airway responses in mice [23] were traced to inheritance of a polymorphic variant form of IL-4Rα, such that airway responses persisted only in IL-13−/− mice expressing a high IL-4-binding variant of IL-4Rα [24]. In accordance with this, mice lacking IL-13 can be driven to develop AHR under conditions promoting IL-4-dependent responses. Following inhalation of a long-acting form of IL-4 complexed to antibody (IL-4C), IL-13-deficient mice, which would normally be protected [15], developed airway inflammation, and goblet cell hyperplasia [25]. Mice with targeted deletions in both IL-4 and IL-13 genes [23,26–28], or those lacking the shared receptor component, IL-4Rα [26,29], are severely impaired in Th2 responses, including development of specific IgE, AHR and pulmonary inflammation. Thus, IL-4 and IL-13 appear to have redundant roles in promoting airway eosinophilia and AHR, such that complete ablation of these response requires neutralization of both cytokines.

3. Mucus production

Mucus generation responses are impaired in mice lacking IL-13, but not in those lacking IL-4 [23,30]. This activity could be critical for promoting asthma pathology, as IL-13 responsiveness restricted to epithelial cells appears sufficient to drive AHR and mucus production [12]. The molecular basis for the greater dependence of mucus production on IL-13 over IL-4 remains to be determined. In human bronchial epithelial cells, both cytokines increase goblet cell density, mucin gene expression, mucin glycosylation, and mucus secretion [31]. IL-13 generation may be preferentially induced under conditions driving mucus production, such that Th2 cells or Th2 cytokines amplify IL-13 secretion, which in turn mediates epithelial cell mucus production [30]. Alternatively, IL-4 may induce expression of genes that down-modulate IL-13 function [32], suggesting a rationale for the relatively lower

Table 1 – Responses in animal models correlating with human asthma disease parameters

Model	AHR	Mucus	Inflammation	IgE	Fibrosis	References
Allergen	++	++	++	++	++	[10,11,91]
Pulmonary IL-13	++	+++	++	++		[10–12,92]
Pulmonary IL-4	++	++	+			[11,16,17]
Anti-IL-13	–	–	–	–	–	[91,93–95]
Anti-IL-4	+/−	++	++	–		[84,23]
sIL-13Rα2-Fc	–	–	–	–	–	[10,11]
IL-13 KO	–	–	+/−	+/−	–	[15,26,30,35,36]
IL-4 KO	+/−	++	+/−	+/−	++	[20,21,26,34]
IL-4/13 KO	–	–	–	–	–	[23,26–28]
IL-4Rα KO	–	–	–	–	+/−	[26,29,41]
IL-13Rα1 KO	−	–	++	–	–	[33]
IL-13Rα2 KO		+++	+++	+++	+++	[48,65]
STAT6 KO	++	+/−	+/−	–	+/−	[27,40]
IL-13 TG	+++	+++	+++	+++	+++	[12–14]
IL-4 TG	+	+++	+++	+++	+++	[18,19,34]

Response level: +++, strong; ++, moderate; +, mild; +/−, weak; −, undetectable.

capacity for IL-4 to induce goblet cell hyperplasia and AHR in asthma. Recently, examination of mice deficient in IL-13Rα1 demonstrated that AHR and mucus hypersecretion were abolished in an asthma model, emphasizing the importance of responses mediated through the IL-13Rα1/IL-4Rα receptor complex (type II) shared by both IL-4 and IL-13, as opposed to the IL-4Rα/γcommon (type I) receptor utilized exclusively by IL-4 [33].

4. Fibrosis

IL-13 also appears more critical than IL-4 in driving fibrotic responses *in vivo* [1]. In models of murine lung fibrosis induced by bleomycin or FITC, IL-4 has proved to be non-essential [34], whereas IL-13-deficient animals were protected from fibrotic changes [35,36]. In an inducible model of transgenic lung IL-13 expression, fibrosis was initiated upon IL-13 induction and persisted even following withdrawal of the cytokine, demonstrating that IL-13 has the capacity to drive irreversible remodeling processes in the asthmatic lung [37]. Both IL-4 and IL-13 can mediate direct activation of lung fibroblasts *in vitro*, inducing eotaxin release, TGF-β production, and collagen gene expression [38]. Both cytokines, and the receptor forms IL-13Rα1 and IL-4Rα, are induced in mouse lungs following bleomycin administration [39]. Therefore, the basis for the greater dependence of fibrosis on IL-13 over IL-4 is not apparent. The demonstration that STAT6-deficient [40] or IL-4Rα-deficient [41] mice can still develop IL-13-dependent fibrosis upon chronic antigen exposure, raises the possibility that IL-13-induced fibrotic effects are mediated through a novel receptor form or a novel signaling cascade [40]. IL-13Rα2 expression is induced in fibroblasts upon activation *in vitro* [42], and in mouse lungs upon induction of fibrosis *in vivo* [39]. This form of receptor binds IL-13 but not IL-4, and it has been proposed that putative IL-13Rα2-mediated signaling pathways could help to account for the pro-fibrotic activity of IL-13 [43]. This issue remains controversial, however, as IL-13Rα2 expression is generally associated with reduced IL-13 activation responses, consistent with a decoy function [42,44–46]. IL-13Rα2 induction on fibroblasts, acting in concert with IL-10 release, has been found to limit AHR, mucus production, and fibrosis in mice [47]. Conversely, IL-13Rα2-deficient mice exhibit enhanced pro-fibrotic responses, consistent with characterization of IL-13Rα2 as an antagonist, rather than a mediator, of IL-13-induced fibrosis [48]. Additional work will be required to resolve the receptor interactions supporting the critical role of IL-13 in pro-fibrotic responses.

5. IL-13Rα1/IL-4Rα binding and signaling interactions

IL-13 bioactivity is mediated through a receptor complex consisting of IL-13Rα1 and IL-4Rα chains. Of the four alpha helices that comprise IL-13, the C-terminal alpha helix D contains key residues for interaction with both IL-13Rα1 and IL-13Rα2, whereas helices A and C appear primarily responsible for IL-13 interaction with IL-4Rα [49,50]. Recently, crystal structures of ternary complexes consisting of IL-4 or IL-13 interacting with IL-13Rα1 and IL-4Rα chains have been solved [51]. Analysis of these structures offers insights into distinct cytokine interactions with a shared receptor, helping to account for the different affinities and potencies of cellular responses to IL-4 and IL-13 [51].

IL-13 first binds to the IL-13Rα1 chain on the surface of B cells, monocytes, epithelial cells, and other cell types, with an affinity of approximately 10^{-8} to 10^{-9} M in both mouse and human systems [52]. IL-4Rα is recruited upon IL-13 interaction with IL-13Rα1, to form the high affinity ($\sim 10^{-10}$ to 10^{-11} M) receptor complex [52]. Heterodimerization of cell surface IL-13Rα1 and IL-4Rα receptor chains initiates IL-13 signaling via recruitment of Jak1, Jak2 and Tyk2, resulting in the phosphorylation of STAT6, a critical step in IL-13 and IL-4 dependent signaling [53]. Mice deficient in STAT6 fail to develop AHR or airway inflammation in response to immunization and lung challenge with ovalbumin, and lack detectable titers of IgE or IgG1 specific for ovalbumin [54]. The IL-13Rα1/IL-4Rα complex can also trigger phosphorylation of IRS1/2, recruitment of the adaptor protein Grb2, and activation of the PI3kinase pathway [55]. IL-13 and IL-4 binding and signaling interactions are summarized in Fig. 1.

In some cases, IL-13 responsiveness has been reported in the absence of IL-4Rα, suggesting the existence of alternate receptor forms. Adoptively transferred OVA-specific IL-13 producing T cells induced AHR, airway mucus production, and lung eosinophilia in mice lacking IL-4Rα, but not in those lacking STAT6 [56]. In a chronic OVA challenge model, airway inflammation, fibrosis, and mucus cell hyperplasia, were eliminated in mice lacking IL-13 [41], but persisted in animals deficient in IL-4 [57] or IL-4Rα [41], suggesting that IL-13 may act through an IL-4Rα-independent pathway in this chronic asthma model [41]. Other experiments in the mouse question the role of STAT6. In a model of chronic fungal asthma, AHR, fibrosis, and goblet cell hyperplasia, but not lung eosinophilia, were maintained in STAT6−/− animals, but eliminated by administration of an IL-13-conjugated immunotoxin, suggesting that IL-13 acted in a STAT6-independent manner to support asthmatic changes [40]. Similarly, in a chronic model of OVA sensitization and challenge, AHR, eosinophilia, and lung fibrosis were maintained in STAT6-deficient mice, but greatly reduced in mice lacking both IL-4 and IL-13 [27], again suggesting the involvement of a STAT6-independent activation pathway under conditions of chronic antigen stimulation. In B cells, monocytes, and fibroblasts, IL-4 and IL-13 may induce phosphorylation and activation of STAT1 and STAT3, in addition to STAT6, through the shared receptor [58]. Roles for p38, JNK, and ERK have recently been implicated in IL-13-induced eotaxin production, independent of STAT6 [59]. Thus, additional signaling pathways downstream of IL-13 receptor interactions continue to be elucidated.

6. IL-13 interactions with IL-13Rα2

IL-13Rα2 is inducibly expressed on fibroblasts, keratinocytes, epithelial cells, macrophages, and certain tumor cells, and binds IL-13 with high affinity ($\sim 10^{-11}$ M) [45,60]. Although it has been proposed to mediate AP-1-dependent signaling responses under certain activation conditions [43], IL-13Rα2

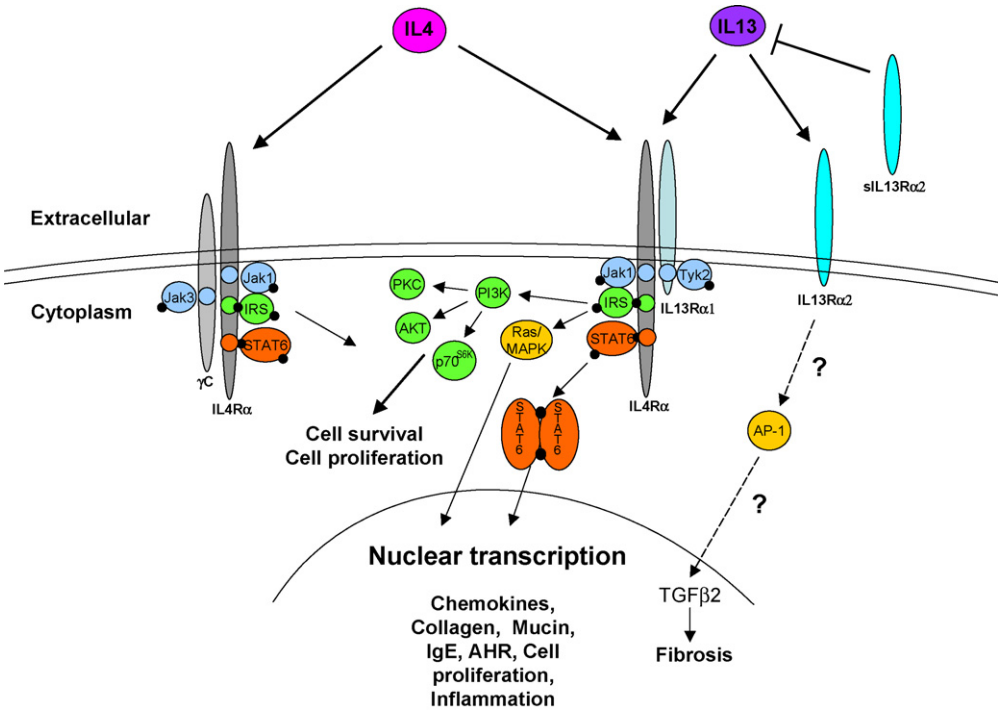

Fig. 1 – Signaling pathways downstream of IL-13 and IL-4. IL-13 binds to two IL-13 receptors. Binding of the ligands IL-13 or IL-4 to the IL-4Rα/IL-13Rα1 receptor (Type II) activates the phosphorylation of Janus kinase (Jak) 1 associated with the IL-4Rα and Tyk2 on the IL-13Rα1 and their subsequent phosphorylation of other Tyr residues on the IL-4Rα (phosphorylation denoted by black circles). These phosphorylated sites recruit both insulin receptor substrates (IRS) 1/2 and signal transducer and activator of transcription (STAT) 6 leading to their subsequent phosphorylation. IRS activates phosphotidylinositol 3 kinase (PI3K) which subsequently activates protein kinase C (PKC), AKT (protein kinase B), and p70-S6 kinase (p70S6K), leading to cell survival and proliferation. IRS also activates the Ras/MAP kinase pathway stimulating nuclear transcription. Phosphorylated STAT6 dimerizes and translocates to the nucleus where it activates transcription of a number of target genes [100], leading to IgE production, fibrosis, eosinophilia, and AHR. IL-4 also stimulates similar responses via a second heterodimeric IL-4 receptor in hematopoetic cells (Type I) containing the IL-4Rα/γ common chain, via Jak1 and Jak3, resulting in STAT6 activation. IL-13 interaction with soluble or cell surface forms of IL-13Rα2 has typically been associated with inhibition of IL-13 functional responses, but recent data suggests IL-13Rα2-mediates induction of pro-fibrotic activity through an indirect mechanism involving activation of AP-1, and subsequently stimulates the synthesis of transforming growth factor β2 (TGFβ2) [43].

is thought to act primarily as a ''decoy'' receptor, sequestering IL-13 from the IL-13Rα1/IL-4Rα complex, and thus inhibiting its function [44]. Cell surface IL-13Rα2 is normally absent on resting cells, but can be induced in response to high concentrations of IL-4 or IL-13, and regulated by TNFα and IFNγ [42]. Evidence suggests the existence of an intracellular pool of receptor, capable of rapidly populating the cell surface in response to inducing agents [61]. The cell surface form of IL-13Rα2 is competent to mediate internalization of bound IL-13 [62], but has a short cytoplasmic region, which lacks known signaling motifs [60]. By binding IL-13 with higher affinity than IL-13Rα1, IL-13Rα2 may act as a sink for the cytokine. In both mouse and human systems, expression of cell surface IL-13Rα2 results in ablation of IL-13 responses [42,48]. These observations strongly support the model of cell surface IL-13Rα2 acting as an antagonist of IL-13 bioactivity.

Recent findings suggest the possibility that IL-13Rα2 may abrogate responsiveness to IL-4 in addition to IL-13 [45,63,64]. In human astrocytes, induction of IL-13Rα2 moderated STAT6 phosphorylation responses not only to IL-13, but surprisingly, also to IL-4 [64]. It was speculated that the short intracellular domain of IL-13Rα2 could directly interact with IL-4Rα, by as yet uncharacterized mechanisms [64]. Similarly, observations with primary human fibroblasts demonstrated that IL-4Rα could be co-immunoprecipitated with IL-13Rα2, supporting the model of direct interaction between these receptor components, resulting in diminished responsiveness to both IL-4 and IL-13 [45]. In human bronchial smooth muscle cells, calcium mobilization responses to acetylcholine, triggered by either IL-4 or IL-13, were reduced upon induction of IL-13Rα2 expression [63]. Further work will be required to uncover the mechanism and regulation of IL-4Rα interaction with IL-13Rα2.

In murine systems, a soluble form of IL-13Rα2 (sIL-13Rα2) appears to act as a natural antagonist of the cytokine, and mice lacking IL-13Rα2 have exaggerated responses to IL-13 [48,65]. Interestingly, these mice also have increased levels of IL-13 in tissues and reduced titers in the circulation, indicating that IL-13Rα2 may also function as a carrier for the cytokine [65]. Several models have been proposed to account for

generation of the soluble form of IL-13Rα2. IL-13Rα2 may be enzymatically cleaved from the cell surface to generate the soluble form, possibly catalyzed by house dust mite allergens [66] or MMPs [67]. Additional data indicates that sIL-13Rα2 may be encoded by an alternatively spliced transcript. Short alternative transcripts of IL-13Rα2 have been described in mice [60] and in humans [68]. Recently, a short transcript in the mouse was characterized as lacking the transmembrane region, thus encoding sIL-13Rα2, and its expression was preferentially induced over the transmembrane form in a mouse asthma model [69].

Soluble IL-13Rα2 can be reliably quantitated in the sera of mice [65,70], but this form of receptor has not been well-validated in human systems [71]. Although the mouse and human IL-13Rα2 proteins share 59% amino acid homology [60], and alternatively spliced transcripts of human IL-13Rα2 can be found [68], it is not yet clear whether any of these transcripts encodes a form lacking the transmembrane region. The splice variants identified in the human IL-13Rα2 gene involve alternative usage of the first 4 (noncoding) exons, located in the 5′ UTR [68]. Whether this could influence translation efficiency or further post-transcriptional processing to generate a soluble form remains to be determined.

7. Genetic associations with human asthma

Several IL-13 genetic polymorphisms have been linked with susceptibility to develop atopic disease. In populations throughout the world, the R110Q variant has been associated with high serum IgE titers, allergy, and atopic dermatits [72,73]. Residue #110 of IL-13 lies in the region of the molecule thought to interact with IL-13Rα1 and IL-13Rα2 [50]. Several hypotheses have been proposed to rationalize the association of this polymorphic variant IL-13 with tendency to develop atopic disease, including decreased affinity for binding to the negative regulatory element, IL-13Rα2 [46], increased functional activity mediated through IL-13Rα1 [74], and enhanced stability in plasma [75]. In addition to the R110Q variant, associations have been found between atopic susceptibility and the IL-13 promoter polymorphisms −1055 C/T and −1112 C/T [4,72]. Genetic polymorphisms in genes encoding IL-4Rα, IL-13Rα1, or STAT6 [4,73] may also predispose toward development of atopy. Taken together, human genetic data strongly support a role for the IL-13 pathway in contributing to risk of developing atopic disease.

8. Therapeutic strategies targeting both IL-13 and IL-4

Despite the wealth of preclinical data validating the importance of IL-4 and IL-13 in asthma models, the pathway has been difficult to effectively target therapeutically. One promising strategy has taken advantage of the finding that a single amino acid mutation, Y124D, converts human IL-4 to a potent IL-4 antagonist [76], and a double mutant, R121D/Y124D, blocks all signaling responses through IL-4Rα [77]. By binding to the IL-4Rα without apparent signaling capacity, these mutants inhibit cellular responses to both IL-4 and IL-13 [76,77]. A murine analog of the double mutant, IL-4.Q116D/Y119D (QY), inhibited Th2 responses and specific IgE production in mice [78]. A plasmid encoding this mutant reduced AHR, lung inflammation, and IgE production upon OVA challenge [79]. Similarly, adenoviral delivery of plasmid DNA encoding a truncated form of murine IL-4, lacking reside Y119D, inhibited development of AHR, BAL eosinophilia, and mucus production [80]. Pitrakinra (Aerovant™) is a mutant form of IL-4 being developed as a human therapeutic. In a phase IIa study in asthmatics, pitrakinra preserved lung function, reduced asthma-related adverse events, and reduced the use of rescue medication following allergen challenge [81]. These promising findings serve to validate the potential therapeutic utility of targeting the IL-4/IL-13 pathway in asthma.

IL-13 receptors are also being targeted by antibody-based approaches. Recently, a panel of scFv molecules specific for the extracellular domain of the human IL-13Rα1 was described [82]. *In vitro*, these reagents inhibited IL-13 responses in cell lines and in primary human monocytes, and represent a novel potential strategy for therapeutic development [82]. A cytokine trap, composed of the extracellular domains of IL-13Rα1 and IL-4Rα, was also described as an antagonist of both IL-4 and IL-13 responses [83], but clinical findings have not been reported.

9. Therapeutic strategies targeting IL-4

Antibody to IL-4 initially appeared to be a promising strategy for treatment of asthma. In preclinical testing, monoclonal anti-IL-4 blocked development of specific IgE and AHR to ovalbumin in mice, but did not abrogate eosinophilia [84]. Humanized antibody to IL-4 (pascolizumab) effectively blocked IL-4 responses *in vitro*, and showed a favorable *in vivo* pharmacokinetic profile in cynomolgus monkeys [85]. Nevertheless, phase II testing in steroid-naïve asthmatics showed no apparent clinical benefit compared to placebo, and further development was discontinued [86].

An alternative approach involved the delivery of sIL-4Rα to antagonize responses to IL-4. In mice, targeting IL-4Rα through inhaled delivery of antisensene RNA [87], intranasal or i.p. administration of sIL-4Rα [88], or administration of monoclonal antibody to IL-4Rα [89], all proved to be effective strategies to reduce allergic sensitization, AHR, airway eosinophilia, goblet cell metaplasia, and cytokine production following immunization and airway challenge with ovalbumin. As a result of IL-4 antagonism, sIL-4Rα administration may indirectly down-regulate IL-13 production, through reduced Th2 development and activation [87]. In early clinical testing, soluble recombinant human IL-4 receptor (IL-4R; Nuvance™; altrakincept) showed promising results, with better maintenance of lung function and improved symptom scores in treated subjects as compared to controls given placebo [90]. Subsequent testing with larger patient populations did not support the early findings, however, and further development was discontinued. Subsequent research has questioned the effectiveness of sIL-4Rα as an antagonist of IL-13 responses, suggesting that IL-13 responses may even be enhanced by sIL-4Rα under suboptimal activation conditions [77].

10. Therapeutic strategies targeting IL-13

In murine systems, IL-13 blockade by sIL-13Rα2-Fc [10,11] or by antibody [91] effectively limits asthmatic responses, including AHR, eosinophilia, mucus production, IgE generation, and fibrosis. A number of monoclonal antibodies targeting IL-13 are being developed for the treatment of asthma, with several more in preclinical development. Results of clinical studies are not yet available, but preclinical data indicates a promising therapeutic profile. In mice, AHR, airway eosinophilia, and mucus production can be initiated by administration of human IL-13, and these activities were effectively blocked by anti-human IL-13 antibody, CAT-354 [92]. In sheep naturally sensitized to the roundworm parasite, *Ascaris suum*, airway challenge with *Ascaris* antigen triggers early and late phase bronchoconstriction responses, and airway hyperresponsiveness to carbachol. Anti-human IL-13 antibody IMA-638, delivered by i.v. infusion 24 h prior to antigen challenge, protected the animals from late phase bronchoconstriction following airway challenge with *Ascaris* antigen, and inhibited *Ascaris*-induced airway hyperresponsiveness [93]. At high dose, the antibody also significantly reduced the early phase response [93]. In *Ascaris*-sensitized cynomolgus monkeys, segmental lung challenge with the antigen induces an eosinophilic airway inflammation. IMA-638, delivered i.v. 24 h prior to challenge, reduced the inflammatory infiltrate [94]. IL-13 antibody treatment also affected serum titers of IgE anti-*Ascaris*, and modulated the IgE-dependent basophil histamine release response following *ex vivo* challenge of peripheral blood with *Ascaris* antigen [95], suggesting the potential for long-term modulation of atopic sensitivity following IL-13 neutralization.

Recently, peptide-based vaccines for IL-4 and IL-13 have been developed and utilized in mouse asthma models. Structural analysis of the IL-4/IL-4Rα complex suggested a peptide epitope of IL-4 that lies at the contact site with IL-4Rα. This peptide was conjugated to truncated hepatitis B core antigen as carrier, and used to immunize mice, which developed antibodies to IL-4 [96]. Upon OVA immunization, these mice displayed reduced titers of IgE antibodies to OVA, along with reduced BAL inflammation, mucus production, and diminished AHR to methacholine [96]. Similarly, a peptide corresponding to a phylogenetically conserved region of IL-13 helix A, which is thought to contain a receptor interaction site, was conjugated to truncated heptatitis B core antigen, and used to immunize mice [97]. Upon OVA immunization and challenge, the IL-13-immune mice displayed reduced IgE titers, airway inflammation, mucus metaplasia, and AHR to methacholine [97]. These experiments demonstrate that in mice, asthmatic responses can be well controlled by the presence of antibody to either IL-4 or IL-13.

11. Conclusion

IL-13 neutralization by sIL-13Rα2-Fc [10,11,48], siRNA [98], or antibody [91,93–95] effectively blocks signs of asthma pathology, including airway hyperresponsiveness, lung inflammation, mucus production, fibrosis and increased serum IgE, in murine, NHP, and sheep models of respiratory disease. IL-13 has been described as "necessary and sufficient" for development of allergic asthma in animal models [10,12,15]. Several strategies are currently being investigated to directly target IL-13 for the treatment of asthma. Additional strategies would antagonize IL-13 generation by targeting IL-4 or other Th2-modulating cytokines, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 [99].

After years of preclinical development, several IL-13 antagonists, including monoclonal antibodies to IL-13 and its receptor components, are currently undergoing clinical evaluation in asthmatics. The efficacy of these agents in modulating pulmonary function, disease exacerbations, emergency room admissions, or oral steroid use will test predictions made on the basis of animal models, and will ultimately provide insights into the role of IL-13 in pathogenesis of the human disease. Only then can the efficacy of IL-13 antagonists be benchmarked against existing therapies, including anti-IgE antibody (Xolair®), the only protein therapeutic currently approved for treatment of asthma.

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