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IL-4 and IL-13 Signaling in Allergic Airway Disease

Naina Gour and **Marsha Wills-Karp**

Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Abstract

Aberrant production of the prototypical type 2 cytokines, interleukin (IL)-4 and IL-13 has long been associated with the pathogenesis of allergic disorders. Despite tremendous scientific inquiry, the similarities in their structure, and receptor usage have made it difficult to ascertain the distinct role that these two look-alike cytokines play in the onset and perpetuation of allergic inflammation. However, recent discoveries of differences in receptor distribution, utilization/assembly and affinity between IL-4 and IL-13, along with the discovery of unique innate lymphoid 2 cells (ILC2) which preferentially produce IL-13, not IL-4, are beginning to shed light on these mysteries. The purpose of this chapter is to review our current understanding of the distinct roles that IL-4 and IL-13 play in allergic inflammatory states and the utility of their modulation as potential therapeutic strategies for the treatment of allergic disorders.

1. Introduction

Allergic asthma is a chronic inflammatory disease of the lung, which has been on the rise in recent decades. Currently asthma affects over 300 million people and one of every 250 deaths worldwide is attributed to this disease. Asthma is a reversible airway disease characterized by airway hyperresponsiveness, airway inflammation, and airway remodeling (i.e. mucus cell metaplasia, smooth muscle thickening, sub-epithelial fibrosis) and the main pathophysiological manifestation of the disease-airway hyperresponsiveness. Since the groundbreaking discovery of distinct CD4+ T cell subsets in the late 1980's [1], Th2 cytokine producing cells have been implicated in the pathogenesis of asthma. Indeed, a tremendous amount of evidence in both humans and animal models support a role for Th2 cells in the development of the prominent features of the allergic diathesis [2]. In deciphering the mechanisms by which Th2 cell derived cytokines regulate asthma pathogenesis, extensive efforts have been devoted to defining the role of the prototypical Th2 cytokines, IL-4 and IL-13. As IL-4 and IL-13 are encoded by adjacent genes (chr 5q in humans and chr11 in mice) that share a number of regulatory elements (GATA-3) and transmit signals through a shared functional receptor complex (IL-4Rα/IL-13Rα1), it was

Address correspondence to: Marsha Wills-Karp, Ph.D., Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., RM E7527, Baltimore, MD 21231, Ph: (410)-955-2452, mwkarp@jhu.edu.

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originally assumed that they would serve redundant functions in the pathogenesis of allergic asthma [3]. However, despite their many similarities, a series of *in vivo* functional experiments utilizing mice deficient in cytokines, cytokine-producing cells or lacking specific receptor subunits have shown that IL-4 and IL-13 play distinct roles in allergic asthma *in vivo*. In particular, IL-4, through its role in regulating Th2 cell proliferation and survival and IgE synthesis has been shown to be essential in the initiation of allergic airway responses and humoral responses, but it does not regulate AHR, mucus production, or subepithelial fibrosis *in vivo* in the context of allergic inflammation [4–10]. In contrast, it's look-a-like, IL-13 is thought to play a more important role in the effector phase of the response and to be sufficient to induce the main manifestations of allergic disease including AHR, mucus production, airway smooth muscle alterations, and sub-epithelial fibrosis [11–15]. The precise mechanism(s) by which these two cytokines regulate distinct features of the allergic response, and how these actions either individually or in concert induce the pathophysiological manifestations of disease, still remain a mystery. Herein, we review recent insights into the potential mechanisms underlying their distinct roles in the asthmatic phenotype, the mechanisms by which IL-13 signaling plays a central role in regulating the effector phase of the disease, and the potential of modulating these cytokines for therapeutic benefit in asthma.

2. Mechanisms of Distinct Roles for IL-4 and IL-13 in Allergic Inflammation

Although the exact mechanisms by which IL-4 and IL-13 mediate different aspects of the asthmatic phenotype are not totally understood, studies examining the contributions of the four receptor chains (IL-4Rα, IL-13Rα1, γc, IL-13Rα2) which make up the type I and type II IL-4R complexes are providing some clues. IL-4 signaling is initiated via two types of heterodimeric transmembrane receptor complexes: the type I receptor, which exclusively binds IL-4 and is comprised of IL-4Rα and γ_c subunits (the latter of which also functions as a subunit in IL-2, IL-7, IL-9, IL-15, and IL-21 receptor complexes), and the type II receptor, which binds both IL-4 and IL-13 and is comprised of IL-4Rα and IL-13Rα1 subunits. IL-13, but not IL-4, can also bind a unique receptor chain referred to as the IL-13Rα2. Engagement of both types of receptors initiates activation of the signal transducer and activator of transcription (STAT6) pathway [16] [17], but only type I IL-4 receptors activate the insulin receptor substrate (IRS)-2 pathway [18]. Consistent with the activation of STAT6 by ligation of the type II receptor, STAT6 has been shown to be essential for the majority of the features of asthma [16, 19].

The unique functions of IL-4 can be easily explained by exclusive expression of the type I receptor on specific cell types. For example, expression of the type I receptor complexes on mouse and human T cells limits responsiveness to IL-4, not IL-13. Interestingly, although the message for IL-13Rα1 is present in T cells, there is no surface expression of this chain on human or mouse T cells [20]. Consequently, IL-13 is not thought to exert any control over T cell function in either species. Likewise, IL-4's dominant role in IgE synthesis in mice is likely explained by the lack of IL-13Rα1 expression on mouse B cells. However, the IL-13Rα1 is expressed on human B cells. The type I receptor dependence of these two features of disease in mouse models likely explain the requirement for IL-4, not IL-13, in the initiation of allergic responses.

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Determination of the unique functions of IL-13 during allergic inflammation is more difficult as it shares its functional receptor (IL-4Rα/IL-13Rα1) with IL-4. As would be predicted given their use of the same receptor complex, administration of equal quantities of either recombinant IL-4 or IL-13 to naïve mice elicits the main features of disease including AHR, mucus metaplasia, and tissue fibrosis [21]. The ability of both cytokines to mediate these responses through the type II receptor has been examined in mice deficient in the type II receptor unique chain, the IL-13Rα1. As expected, allergen-, IL-4- and IL-13-induced AHR, mucus production, and profibrogenic mediator (TGF-β) production was completely dependent upon the IL-13Rα1 chain as a deficiency in this gene prevented development of these features of disease. However, consistent with the primary role of IL-4 in Th2 differentiation and IgE synthesis, IL-13Rα1 was not required for these responses. Interestingly however, signaling through the type II receptor was not sufficient to regulate eosinophil recruitment into the airways and was only partially STAT6 dependent [19] and independent of IL-13Rα1 [21] suggesting that eosinophilia may be regulated by the type I IL-4 receptor-perhaps as a result of IL-4 regulation of T cell activation and IL-5 production. This was confirmed by Heller and colleagues [22]. Alternatively activated macrophage induction appears to be dependent on both type 1 and type 2 receptors likely reflecting the expression of both type 1 and type II receptors on their surface [23, 24] [21]. The exception to this shared regulation is the chitinase gene that was shown to be solely dependent on the IL-13Rα1 chain [21]. The exception to this shared regulation is the chitinase gene that was shown to be solely dependent on the IL-13Rα1 chain [21].

Although the mystery underlying the unique functions of IL-4 and IL-13 is far from solved, multiple explanations for their apparently unique functions are emerging. First, a possible explanation for the differential functions of IL-4 and IL-13 in the asthmatic lung is that IL-13 may be produced at higher quantities under allergic inflammatory conditions. Indeed, IL-13 levels have often been shown to be elevated compared to IL-4 levels in inflamed tissues. This discrepancy has long puzzled investigators, and was thought to be due to either reuptake of IL-4 through its receptor on T cells or to differences in the half-lives of the proteins *in vivo*. However, recent elegant studies using cytokine reporter mice [25] have examined the expression patterns of these two type II cytokines in the inflamed lung and have revealed that their expression varies by cell type. Specifically, these studies have shown that during type 2 immune responses, the primary sources of IL-4 in the affected lung tissue include basophils and conventional Th2 cells [25]. In contrast, tissue Th2 cells and ILC2s are the major producers of IL-13 [26, 27]. The fact that ILC2s produce significant quantities of IL-13, but not IL-4, in response to antigen-induced IL-25 and IL-33, likely explains both the quantity and timing of expression of IL-13 during allergic airway disease. However, a predominantly quantitative explanation for a difference in IL-4 vs. IL-13 effects cannot account for the differences seen in gene expression patterns in mice stimulated with similar quantities of IL-4 or IL-13 *in vivo* [28] [29], but probably explains, to some extent, their different contributions to the allergic phenotype *in vivo*.

Secondly, several lines of evidence suggest that IL-4 may induce inhibitory pathways through the type I receptor, which limit pro-allergic effects mediated through the type II receptor [28] [29]. First, IL-4 signaling through the type I IL-4R inhibited the induction of a

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set of IL-13-specific genes (*Ccl11, Itln2, Retnlb*, Sprr2a, *Slc5a1, Aass, Scin, Agr2*), that are not upregulated by rIL-4, but are however STAT6-dependent, and γC-independent [29]. The IL-4-mediated suppression of IL-13-specific genes was associated with the ability of IL-4 to induce IL-12 and IFN-γ expression in the mouse lung [29, 30]. This inhibitory function likely reflects the ability of IL-4, but not IL-13 to signal through the type I receptor and activate an immunoregulatory immune response. This is supported by recent findings of enhanced allergic responses in γc-deficient mice [31]. Specifically, it has recently been shown that mice lacking the γc chain, reconstituted with OVA-primed CD4+ OT-II cells developed increased pulmonary inflammation and eosinophilia as compared to RAG2−/− controls. The increase in inflammation was associated with higher Th2 cytokine levels and reduced IFN-γ levels in the BAL. Taken together these results illustrate that IL-4 activation of the type 1 receptor, is clearly not required to mediate allergic airway responses, and that it may instead serve to dampen pro-allergic responses mediated through the type II IL-4R.

Thirdly, recent studies detailing the ternary structure of IL-4 and IL-13 binding to the type II IL-4R have provided evidence that engagement of the type II receptor varies in signaling potencies and kinetics when bound by IL-4 versus IL-13. This observation reflects the differences in sequential assembly of the shared subunits with consequences for the strength and duration of downstream signaling events [32]. The IL-13R (type II IL-4R) forms in a sequential manner in which IL-13 first binds to IL-13Rα1 before recruiting IL-4Ra to form a high affinity signaling complex. IL-4 on the other hand, initially interacts with the type II IL-4R by binding with relatively high affinity to IL-4Rα; the IL-4/IL-4Rα complex then recruits IL-13Rα1 to form the signaling complex. The higher affinity of the initial binding step for IL-4 than IL-13 appears to allow low concentrations of IL-4 to signal more effectively than low concentrations of IL-13, while the higher cell membrane concentration of IL-13Rα1 than IL-4Rα appears to allow high concentrations of IL-13 to signal more strongly than IL-4 through the type II IL-4R receptor [32]. The implication of these findings is that both cytokine and receptor subunit availability influence subsequent signaling.

Fourthly, recent studies suggest that IL-13-binding to its unique receptor chain, IL-13Rα2 may contribute to its singular role in allergic inflammation. It was initially believed that IL-13Rα2 was a decoy receptor as it only contains a 7-amino-acid cytoplasmic domain that lacks a conserved box 1 region that plays a critical role in signal transduction [33, 34]. This notion was further supported by the finding that a soluble form of IL-13Rα2 exists *in vivo* in mice and that administration of the soluble form of IL-13Rα2 suppressed IL-13-mediated airway inflammation, airway hyperresponsiveness, and mucus hypersecretion in allergen-challenged mice [11, 12]. Moreover, initial characterization of IL-13Rα2-deficient mice showed that they have constitutively elevated levels of serum IgE as compared to wildtype mice [35]. Despite strong evidence that IL-13Rα2 can act as a decoy, recent studies have demonstrated that under certain circumstances it may have the capacity to mediate IL-13 signaling. For example, *in vivo* silencing of IL-13Rα2 with siRNAs resulted in reduced production of TGF-β concomitant with a reduction in collagen deposition in the lungs of bleomycin-exposed mice [36]. Although the mechanisms by which IL-13Rα2 mediates IL-13-dependent signaling are unclear, one study proposed that IL-13 signals through IL-13Rα2 to stimulate an activator protein (AP-1) variant containing c-jun and fra-2, which

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initiates transcription at the transforming growth factor β1 (TGF-β1) promoter and thereby induces TGF-β1 production by macrophages [36]. Alternatively, a recent intriguing study has demonstrated that IL-13Rα2 may form a heterodimeric signaling complex with the chitinase-like protein family member, Chi3l1, and IL-13 and activate MAPK, Akt, Wnt/B-catenin signaling pathways leading to TGF-β production ([37]. The importance of this pathway in airway remodeling has recently been shown in Chi3l1 deficient mice, in which IL-13-dependent fibrosis was significantly impaired [38]. As Chi3l1 can be produced by both alternatively activated macrophages and epithelial cells, the authors went on to show that Chi3l1 overexpression in lung epithelial cells is able to fully rescue the defective IL-13 responses in Chi3l1 −/− mice.

The recent discovery that mouse IL-13Rα2 exists in both soluble and membrane forms (sIL-13Rα2 and memlL-13Rα2), encoded by distinct transcripts generated by alternative splicing [39] may provide an explanation for the apparently opposing roles for IL-13Rα2 in allergic inflammation. Of note, humans only express the membrane form (memIL-13Rα2) [40] [41]. In an *in vivo* study designed to evaluate the specific role of the membrane form in allergic inflammation, Chen and colleagues [42] have shown that allergen-driven AHR, eosinophilic inflammation, and mucus production were attenuated in memIL-13RA2 deficient mice as compared to wildtype control mice. Moreover, lung epithelial overexpression of the membrane form of IL-13Rα2 in memIL-13Rα2 deficient mice restored AHR and inflammation to the levels observed in wildtype allergen-challenged mice. These results support the contention that memIL-13Rα2 and soluble forms of IL-13Rα2 might play distinct roles in allergic inflammation. This hypothesis is supported by an intriguing study conducted in human intestinal epithelial cells isolated from ulcerative colitis (UC) or colorectal cancer (CRC) patients. The authors noted that expression of the IL-13 receptor chains (IL-13Rα1 and IL-13Rα2) were similarly upregulated in the epithelium from UC patients and CRC lesions as compared to healthy controls. However, IL-13 did not activate Stat6 in cells from UC patients. Using neutralizing antibodies and cell lines expressing a range of surface densities for IL-13Rα1 and IL-3Rα2, [43], they found that when IL-13Rα2 is expressed at a low to moderate cell surface density, it functions alone, or may heterodimerize with an unknown receptor chain, to initiate MAPK signaling, thus competing equally with IL-13Rα1 for binding to IL-13. When the surface expression of IL-13Rα2 increases dramatically over that of IL-13Rα1, as in UC, the equilibrium is shifted toward homodimerization, yielding the classic high affinity decoy receptor that competes effectively to quench both IL-13Rα1- and IL-13Rα2-mediated signal transduction. If, however, expression of IL-13Rα1 and IL-13Rα2 increase coordinately, as in CRC, then IL-13Rα1 can compete effectively for IL-13 binding and signal through the STAT6 pathway, while IL-13Rα2 remains homodimerized and is unable to signal. Taken together these results suggest that IL-13Rα2 may serve dual functions, depending on its relative expression and the local concentration of IL-13, which together modulate the balance and intensity of the signaling pathways initiated. The exact mechanisms by which IL-13Rα2 mediates activation of downstream signaling pathways remain unknown. Thus more in depth studies of its expression and regulation during allergic inflammatory responses will be required to fully elucidate the exact contribution of this receptor to IL-13-dependent processes in vivo. Collectively, these studies suggest that the greater role for IL-13 as

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compared to IL-4 in mediating allergy-related airway changes likely reflects a combination of factors including: increased local production of IL-13 relative to IL-4, increased affinity of the type II complex for IL-13 depending on the presence of other binding partners of these 2 cytokines (i.e γc, IL-13Rα2), and potential inhibitory pathways induced by IL-4 through the type I receptor complex.

3. Mechanism(s) of IL-13 Regulation of the Effector Phase of the Allergic Response

With the preponderance of evidence continuing to support a pivotal role for IL-13 in allergic disorders, attention is now turned towards understanding the mechanisms by which this cytokine may mediate the pathophysiological features of allergic disease. The emerging paradigm is that IL-13 induces features of the allergic response via a complex array of actions on resident airway cells. Below we review our current understanding of the mechanisms by which IL-13 regulates: 1) mucus cell metaplasia, 2) tissue fibrosis, and 3) lastly the main physiological manifestation of the disease, AHR.

3.1 Interleukin-13 Regulation of Mucus Hypersecretion and Epithelial Cell Function

While mucus secretion in the airways generally serves to protect the airways by infection and injury, excessive goblet cell differentiation and mucus hypersecretion are characteristic features of the allergic diathesis. Indeed, extensive mucus plugging of the airways is associated with fatal episodes of asthma [44]. Similar to human asthma, increases in the numbers of mucus containing cells have been observed in the lungs of mice sensitized to allergens [11, 12]. Support for this being a Th2 cell-dependent process is the finding that adoptive transfer of Th2 cells into the murine lung reconstituted the effect of allergen challenge on mucus cell metaplasia [45]. IL-4 and IL-13 were originally implicated in these mucus changes, as allergen driven mucus hypersecretion was absence in STAT6 deficient mice [19]. However, several lines of evidence support the contention that the overzealous production of mucus in the lungs of allergen-exposed mice is an IL-13–, not IL-4–, dependent process. First, blockade of IL-13 alone *in vivo* by either administration of the soluble IL-I3Rα2-Ig [11] or through IL-13 gene targeting in mice [14] both prevents and reverses established mucus cell changes. Conversely, administration of rIL-13 *in vivo* or overexpression of the IL-13 gene recapitulates the effects of allergen sensitization on mucus cell changes [11, 12]. A role for IL-4, has been ruled out in that adoptive transfer of Th2 cells devoid of the IL-4 gene still induce extensive goblet cell metaplasia in the murine lung [45]. Furthermore, mucus hypersecretion in transgenic mice with lung-specific overexpression of IL-4 was found to be IL-13 dependent [28]. The ability of IL-13 to regulate mucus cell changes appears to be a direct effect on airway epithelial cells as IL-13 can induce mucin gene induction and mucus secretion *in vitro* in epithelial cell lines [46]. Furthermore, STAT6−/− mice with transgenic overexpression of IL-13 did not develop mucus overproduction, but mucin gene expression and mucus metaplasia were restored after selective reconstitution of STAT6 in airway epithelial cells [16]. Collectively, these studies demonstrate that IL-13 is likely the primary regulator of mucus cell metaplasia and hypersecretion in asthma.

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Mucus hypersecretion in the context of allergic inflammation is a highly complex multi-step process that is currently not well understood. Nonetheless, it is thought to involve mucus cell metaplasia, mucin gene induction, mucus packaging in storage granules, release from granules into the lumen of the airways, and lastly changes in the viscosity of the mucus produced. IL-13 has been shown to play a pivotal role in each of these processes. The first step is believed to involve the transition of Clara (club) cells to a goblet cell phenotype through the coordinate actions of several transcription factors including Spdef, Foxa2, and TTF1. IL-13 induces the expression of the Sam pointed domain-containing ETS transcription factor (Spdef), an epithelial specific transcription factor through an unknown STAT6-dependent process [47]. Spdef, in turn, inhibits the transcription factor, Foxa2 that is required for maintenance of normal differentiation of the airway epithelium [48]. Removal of this transcriptional program, leads to spontaneous mucus cell metaplasia and the production of mucins and other factors necessary for secretory functions. Evidence for this sequence of events is provided by the observations that transgenic overexpression of Spdef in the murine airway epithelium caused spontaneous mucus metaplasia [47], while targeted deletion of Foxa2 in the airway epithelium of mice results in spontaneous mucus metaplasia [48]. These transcription factors reciprocally regulate the expression of several genes important in mucus cell function including: muc5ac, CLCA1, Arg2, Serpin3A, and 15-lipoxygenase. As neither Stat6 or Spdef directly regulate muc5ac, it is thought that Spdef-dependent genes including: CLCA1 [49], Serpin3A [50], and 15-lipoxygenase-1 (15-LO-1) [51] may regulate mucu5ac expression. Mice deficient in Serpin3A [50]and 15-LO-1 [52]have impaired allergen-induced mucus cell changes. Another IL-13-Spdef-regulated gene, the anterior gradient 2 (ARG2) homologue, which is a member of the protein disulfide isomerase family has been shown to be induced by allergen and a deficiency in the gene has been associated with a reduction in mucus production [53]. This reduction in mucus was associated with the activation of the unfolded protein response in the ER, suggesting that AGR2 may have a direct role in mucin folding or another function necessary for maintaining normal mucin production and packaging in the mucus cell ER. This cycle of events is further perpetuated by the ability of Spdef to induce the production of IL-13-promoting cytokines such as IL-33, and TSLP in the epithelium [48].

Other IL-13-inducible genes also play an important role in mucus hypersecretion including: TMEM16A, GABAA-R, MAPK13, and ezrin. TMEM16A, which is a calcium-activated chloride channel, has recently been shown to play a significant role in muc5ac expression and mucus release both in vitro and *in vivo* [54]. Surprisingly, the γ-aminobutyric acid receptor (GABAAR), which is a pentameric chloride channel, that is primarily expressed in the brain, has been shown to be expressed in the airway epithelium. It functions in the airway epithelium to regulate mucin gene induction and mucus secretion [55]. Lastly, IL-13 decreases ciliary beat frequency by interfering with the apical localization of the actin binding protein ezrin [56]. Although the exact details of the sequence of events induced by IL-13-Stat6 signaling pathways are far from complete, insights into the biology of these pathways will help guide the development of novel strategies for the treatment of hypersecretory lung diseases such as asthma.

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3.2 IL-13 Regulation of Sub-epithelial Fibrosis

One of the central components of airway remodeling in asthma is sub-epithelial fibrosis. In normal individuals, fibroblasts are generally recruited to the sub-epithelial compartment of the airway following environmental insults or injury where they initiate a wound healing response through the deposition of extracellular matrix proteins. However, in the asthmatic airway this wound healing response is dysregulated resulting in extensive fibroblast recruitment, collagen deposition and subepithelial fibrosis, which contributes to diminished lung function [57]. In the context of allergic inflammation, chronic allergen challenge results in extensive subepithelial fibrosis in the mouse lung [58]. Detailed mechanistic studies conducted with IL-4 and IL-13 inhibitors (sIL-13Ra2-Fc) and *Il-4−/−, 1113−/−, Il-4ra−/−*, and *Il13ra1−/−* mice suggest that IL-13 likely serves as the dominant inducer of Th2-dependent fibrosis in several chronic lung diseases [13, 59–67]. The exact IL-13 receptors involved in IL-13-mediated lung fibrosis are not known, however some studies show that IL-13Rα2 plays a role in tissue fibrosis In contrast, it has been shown that prevention of IL-13Rα2 expression, gene silencing or blockade of IL-13Rα2 signaling led to marked reduction in TBF-β production, and collagen deposition in bleomycin-induced lung fibrosis [68], [69]). As discussed in the section 3.1, the balance of IL-13Rα1 and IL-13Rα2 expression in the different models likely dictates whether IL-13Rα2 plays a stimulatory or inhibitory role in lung fibrosis.

Although the mechanisms by which IL-13 induces tissue fibrosis are far from understood, several recent studies have begun to shed light on the sequence of events leading to IL-13-induced lung fibrosis. IL-13 likely regulates tissue fibrosis through a series of coordinated actions on a number of cell types within tissues. First. IL-13 has been shown by several investigators to upregulate the synthesis of arginase I, likely in alternatively activated macrophages [70]. Arginase I is an enzyme which hydrolyzes L-arginine to urea and L-ornithine, which is a necessary metabolite for the production of polyamines and prolines required for collagen synthesis by fibroblasts. Evidence for an *in vivo* role for this pathway in tissue fibrosis, was first provided by Hesse and colleagues in a parasite model [71]. Subsequently in a guinea pig model of allergen-driven fibrosis, Maarsingh and colleagues [72] demonstrated that pharmacological inhibition of arginase 1 attenuated allergen-induced pulmonary hydroxyproline production and lung fibrosis. In contrast, Niese and colleagues [73] found that although arginase 1 was upregulated in the lungs of IL-13- and ovalbumin-exposed mice, it did not appear to be important in collagen deposition in the mouse lung. Whether the discrepancies in these findings are due to species differences or to off target effects of pharmacological inhibitors remain unknown. Secondly, IL-13-dependent lung fibrosis has been shown to be mediated via its ability to induce and activate the profibrotic mediator transforming growth factor-3 (TGF-β) [36]. IL-13 induces the production of TGF-β both directly in epithelial cells and indirectly in monocytes/macrophages via its ability to recruit and activate these cells. This activity is thought to occur via the interaction of MCP-1 with its receptor CCR2 on monocytes/macrophages, as both lung fibrosis and TGF-β production in IL-13 transgenic mice are diminished in mice devoid of CCR2 [74]. Further support for this argument is the fact that MCP-1 has been shown to stimulate TGF-β1 production in lung fibroblasts [75]. In addition to stimulation of TGF-β synthesis IL-13 also activates latent TGF-β through the induction of MMP9 (59). Lung fibrosis may also arise as

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a result of the ability of IL-13 to stimulate the accumulation of the nucleoside adenosine (13). Indeed blockade of adenosine levels in the lung by ADA enzyme therapy diminished IL-13-induced fibrosis [76].

IL-13 may also have a direct effect on fibrotic processes by stimulating the proliferation of myofibroblasts [77] via a STAT6-dependent process involving platelet-derived growth factor AA [77]. In support of a direct role for altered IL-13 signaling in lung fibrotic responses in human asthma, recent studies by Kraft and colleagues [78] demonstrated that IL-13 stimulated increased mobility of fibroblasts in a gel layer at significantly lower concentrations of IL-13 in asthmatic fibroblasts as compared to those derived from healthy individuals. These effects were dependent on IL-13-stimulated MMP2 and TGF-β secretion from the fibroblasts in an autocrine fashion. In the same subjects there was an inverse correlation between the methacholine PC20 and the number of IL-13-activated airway fibroblasts invading the matrix (P=0.0005). The enhanced IL-13 responsiveness of asthmatic cells was associated with an upregulation of each of the IL-13 receptor chains (IL-13Rα1, IL-4Rα, IL-13Rα2) in biopsies from asthmatics compared to healthy individuals. However, surface staining of isolated fibroblasts from these asthmatic subjects showed that IL-4Rα and IL-13Rα1 levels remained constant relative to normal cells, whereas IL-13Rα2 levels were reduced. These results suggest that the reduction in the regulatory IL-13Rα2 chain results in greater responsiveness of fibroblasts to IL-13 [78], supporting a suppressive role for IL-13Rα2 in asthmatic fibroblasts. Taken together these studies suggest that altered regulation of IL-13 signaling in asthma may lead to uncontrolled fibroblast proliferation and invasion of airway tissues. Although the exact mechanisms through which IL-13 induces subepithelial fibrosis in the asthmatic lung are not entirely understood, existing data suggest that a number of IL-13-driven processes culminate in the generation of fibrogenic processes in tissues.

3.3 IL-4 and IL-13 Regulation of Alternatively Activated Macrophage Function

IL-4 and IL-13 are major regulators of macrophage differentiation into the alternatively activated subtype, referred to as M2 macrophages. M2 macrophages are generally thought to be anti-inflammatory due to their ability to inhibit M1 production of inflammatory mediators such as TNF-γ, NO, and IL-1 [79]. They are also thought to play an important role in wound healing and fibrosis through the production of growth factors (TGFβ, PDGF) that stimulate epithelial cells and fibroblasts. Additionally they produce a variety of immunoregulatory proteins (RELMα, RETNL, FIZZ), chitinase-like proteins (YM1/2, Chi3l1), arginase 1, and chemokines (CCL2, CCL11, CCL24) REF. A role for M2 macrophages and their products is asthma is supported by the observation that asthmatic individuals have elevated numbers of M2 macrophages and chitinase proteins in their blood and lavage fluids [80, 81]. However, their functional role in asthma is controversial, with some studies suggesting that they play a role in eosinophilic inflammation [82], while others indicate no role for these cells in allergic inflammation [83]. For example, myeloid deficiency in IL-4Ra in mice lead to a reduction in M2 markers (YM-1, Arg1, Relmα) in ovalbumin-induced allergic inflammation, but this reduction resulted in only minor changes in mucus production, eosinophilic inflammation, and collagen deposition [83]. This was mirrored by studies in which a genetic deficiency in either Relmα [84] or Arg-1 [70], had only minor effects on

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parameters of allergic inflammation. However, one study showed that adoptive transfer of IL-4Rα sufficient macrophages to RAG2–/– mice resulted in an enhancement of allergen-driven lung eosinophil recruitment as compared to the transfer of IL-4Rα-deficient macrophages [82]. Although these genes may not be exclusively produced by M2 macrophages, the chitinase-like proteins (BRG-39/Ch3l1) and the human homologue YKL-40 have been strongly associated with susceptibility to human asthma (Lee CG, Da Dilva CA, Annu Rev Physiol., 2011). Moreover, mice deficient in the BRP-39 gene have attenuated allergen-driven IgE synthesis, AHR, eosinophillic inflammation, M2 macrophage numbers, and mucus production. Similar reductions in IL-13-induced lung pathology were observed in BRP-39 deficient mice suggesting that BRP-39 plays a critical role in the pathogenesis of IL-13 effector responses involved in AHR, inflammation and airway remodeling [38]. Interestingly, in the context of allergic inflammation this gene was predominantly expressed in the airway epithelium. In support of this concept, the authors show that reconstitution of YKL-40 in the lung epithelium of BRP-39 deficient mice rescued their defective Th2 response. Thus, although IL-13 and IL-4 clearly play a role in M2 macrophage function, the exact role that they play in allergic inflammation is not well understood. Further studies are clearly needed to clarify their role in the regulation of asthma.

3.4 Mechanisms of IL-13 and IL-4-induced Airway Hyperresponsiveness

Airway hyperresponsiveness or an exaggerated bronchoconstrictor response to specific or non-specific stimuli is a functional hallmark of asthma. This is typically assessed in humans by measuring changes in forced expiratory volume in 1 sec (FEV1) following inhalation of increasing concentrations of cholinergic agonists such as methacholine. In asthmatic humans, a lower dose of methacholine is required to induce changes in FEV1 as compared to healthy individuals. The exact mechanisms by which IL-13 induces AHR are currently unknown. Several lines of evidence suggest that IL-13 can induce AHR in the absence of traditional inflammatory cells. The initial IL-13Rα2Ig studies showed that IL-13 blockade inhibited AHR without affecting inflammatory cell recruitment [11, 12]. Moreover, studies in mice deficient in various cells and cytokines have definitely confirmed the contention that the traditional effector cells, T cells [12], B cells [12], and eosinophils [85] are not required for IL-13 to induce AHR. These results suggest that although IL-13 is able to direct the recruitment of inflammatory cells into the airways, they are likely not necessary for induction of AHR.

Alternatively, IL-13 may induce AHR via direct effects on resident airway cells. Airway epithelial cells, airway smooth muscle cells and alveolar macrophages have been implicated in driving AHR. However, whether AHR is mediated by direct effects on airway smooth muscle or indirectly via effects on other resident airway cells such as the epithelium is currently a matter of debate. The importance of the IL-13—epithelial cell axis was illustrated by the demonstration that AHR develops in mice that overproduce IL-13 in their lungs and express Stat6 only in the airway epithelium [16]. Interestingly, IL-13 transgenic mice expressing STAT6 only in the epithelium did not manifest eosinophilic inflammation, suggesting that eosinophils are not required for the development of AHR and that signals from the epithelium are not sufficient to regulate eosinophll recruitment. However, a

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subsequent study showed that IL-13 also induced AHR in mice that selectively lack IL-4Rα in airway epithelial cells [86]. The suggestion that other cell types may contribute to IL-13-induced AHR led Finkelman and colleagues [87] to explore the role of the IL-4Rα in mice that express IL-4Rα only on smooth muscle cells or on all cell types other than smooth muscle. Studies with these mice demonstrate that direct effects of IL-13 on smooth muscle, as on airway epithelium, are sufficient but not necessary to induce AHR. On the other hand, a study utilizing transgenic smooth muscle myosin heavy chain (cre)IL-4Ralpha(−/lox) mice deficient in IL-4Rα in smooth muscle cells, have demonstrated that expression of the IL-4Rα on airway smooth muscle is not required for the development of allergen-induced airway hyperresponsiveness [88]. As the ability of the epithelium to respond to IL-13 is intact in these mice, IL-13-induced effects are likely mediated through the epithelium in the absence of IL-13 signaling in ASM. Taken together these results suggest that IL-13-mediated processes in both the epithelium and ASM likely contribute to AHR. Additional studies are required to determine whether IL-13-induced AHR can be totally accounted for by its effects on epithelial and smooth muscle cells or by as yet unidentified pathways.

As IL-13-inducible processes in the airway epithelium likely play at least a partial role in AHR, one could envision several potential mechanisms by which it induces these effects on the epithelium may mediate AHR. First, the well-described changes in mucus production and MUC5AC induction may contribute to airway obstruction. Indeed, it has recently been shown that a deficiency in MUC5AC abolishes AHR in allergen-exposed mice concomitant with a 74% reduction in mucus occlusion suggesting that Muc5ac-mediated plugging of the airways is an essential mechanism of induction of AHR [89]. Secondly, epithelial-derived mediators such as TGF-β may contribute to increases in stiffness of the airway wall. Although the fibrotic changes induced via IL-13 stimulation of epithelium are likely important in chronic disease, Kuperman et al [16] suggest that IL- 13-induced AHR was independent of acute fibrotic changes in the airways. Alternatively, IL-13 may induce AHR through regulation of epithelial derived contractile mediators. For example, IL-13 is known to regulate the production of a number of secreted molecules that alter the contraction or relaxation of airway smooth muscle (ASM) cells. For example, IL-13 inhibits the activity of inducible nitric oxide (NO) synthase that results in a decrease in production of the bronchodilator NO, and presumably enhancement of the contractile state of the muscle. Consistent with a role for IL-13 in NO regulation, it has now been shown that both allergen- and IL-13 induced arginase I, may inhibit NO production [90]. Although several studies utilizing siRNA and chemical inhibitors have shown that blockade of arginase I suppresses allergic inflammation and AHR [72] [90, 91], genetic deletion of Arg1 in bone marrow-derived cells of chimeric mice or in mice engineered to delete IL-4Rα in lysozyme M-expressing cells had no effect on lung pathology or airway hyperrresponsiveness [70]. Other IL-13-specific genes produced by the epithelium include periostin. The extracellular matrix protein, periostin, has been identified as an IL-13-specific signature gene, which faithfully identifies asthmatic patients expressing a Th2 high phenotype [92, 93]. Comparison of periostin deficient and wildtype mice showed that periostin was required for allergen-driven GCM, inflammation, and AHR [94]. As described above IL-13-induction of the chitinase-like protein, BRP-39 by the epithelium may also contribute to AHR [38].

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Although ASM contraction may occur through the actions of epithelial-derived mediators on ASM, it is also possible that IL-13 could induce direct effects on ASM as they express the IL-13 receptor chains, including the IL-4Rα, IL-13Rα1, and IL-13Rα2 chain, but not the common γ chain. Indeed, IL-13 has consistently been shown to upregulate each of the receptor chains in isolated human ASM cells [95]. Several investigators have shown that IL-13 does not directly enhance contractile function of ASM, but that it enhances force generation in response to stimulation with a number of contractile agonists [46, 96]. Consistent with these findings, IL-13 has been shown to enhance intracellular calcium fluxes in response to stimulation with a variety of contractile agonists such as bradykinin, histamine, and acetylcholine in isolated smooth muscle cells [46]. A general mechanism by which IL-13 may alter the contractile function of ASM to various stimuli has been postulated by Panettieri and colleagues [97] [98]. Specifically, they demonstrated that IL-13 can induce calcium fluxes in ASM via increasing expression of CD38, a cell surface hydrolase and cyclase whose activation generates cyclic adenosine diphosphate-ribosyl cyclase (cADPR) activity, a putative activator of the ryanodine receptor. The importance of this pathway is supported by the observation that asthmatic-derived ASM cells have elevated CD38 expression [98]. Another mechanism by which IL-13 may enhance responsiveness to contractile agonists is through regulation of the GTP-binding protein, RhoA. Studies by *Chiba et al* reported that *in-vitro* mouse bronchial smooth muscle rings pre-treated with rIL-13 showed enhanced contraction following acetylcholine, which was dependent on STAT-6 mediated upregulation of RhoA levels [99]. RhoA is thought to induce bronchial smooth muscle contraction via increasing calcium sensitivity. Consistent with the importance of RhoA in IL-13-induced muscle hypercontractility, Sheppard & colleagues [100] demonstrated that IL-13 promotes in vitro smooth muscle contraction in tracheal rings through RhoA acting on the downstream effector protein, ROCK-2. Moreover, Shore and colleagues recently reported that mice lacking one allele of ROCK-2 (+/−) had partial abrogation of OVA-induced AHR, whereas AHR was completely abrogated in mice lacking a single allele of ROCK-1 (+/−)[101]. Evidence that IL-13 may induce a hypercontractile state in ASM, through direct activation of ROCK pathways in human ASM cells, was recently provided by Nesmith and colleagues [102]. They showed that IL-13-treated human airway muscle strips on a chip, developed hypercontractile responses to cholinergic agonist stimulation [102]. Moreover, they demonstrated that incubation of the chip muscle tissues with the ROCKII inhibitor (HA1077) prevented the hypercontractile response of the IL-13-treated tissues and promoted relaxation of tissues. Lastly, IL-13 may also impact the contractile properties of smooth muscle by altering the ability of smooth muscle to relax once contracted. For example, Laporte et al [103] demonstrated that IL-13, but not IL-4 significantly reduced β-adrenoceptor-induced relaxation of cell stiffness of human ASM cells through a mitogen-activated protein kinase-dependent pathway.

Because increases in ASM mass are one of the most commonly reported features of asthma, it is likely that hypertrophy and hyperplasia of ASM may enhance the effects of agonist-induced ASM shortening that could prominently amplify airway hyperresponsiveness in asthma. The effects of IL-13 on smooth muscle function may also be a result of significant increases in the area of ASM in the airway wall. Although IL-13 likely does not directly

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mediate smooth muscle proliferation, it may influence muscle proliferation indirectly via stimulation of Cys-LT [104]. Specifically, although IL-13 treatment of ASM cells in culture alone did not induce smooth muscle proliferation, it enhanced proliferation induced by LTD4 and TGF-β treatment of cells. The enhanced proliferation was prevented by pretreatment of the IL-13-primed cells with a selective Cys-LT receptor antagonist. The authors speculate that muscle thickening may be due to positive feedback interactions between LTs, IL-13, and TGF-β. Alternatively, IL-13 may induce smooth muscle proliferation through its ability to increase vascular endothelial growth factor from human ASM that in turn induces fibronectln expression in these cells [105]. Although the exact mechanisms through which IL-13 regulates smooth muscle function are not entirely understood, the collective data support the ability of IL-13 to alter ASM function both directly and indirectly. Taken together with the demonstrated ability of airway smooth muscle to mediate IL-13-induced AHR in the absence of IL-13 signaling in the epithelium, these results suggest that IL-13 normally mediates AHR through its combined actions on the airway epithelium and airway smooth muscle.

4. IL-4 and IL-13 in Human Asthma

The importance of IL-4 and IL-13 in allergic asthma in humans is supported by numerous reports of exaggerated IL-4 and IL-13 production in asthma. In asthma, both message and protein levels of both cytokines are elevated in bronchial biopsy specimens and BAL cells from allergic individuals when compared with those of control subjects [3, 106]. Conversely, IL-13 levels are reduced in patients with asthma undergoing allergen desensitization treatment regimes or steroid treatment. Moreover, a recent cohort study of neonates demonstrates that high IL-13 productive capacity of cord blood CD4+ T cells is a significant predictor of risk for subsequent development of atopic diseases [107]. In support of the notion that overzealous production of IL-4 and IL-13 are strongly associated with asthma, numerous investigative teams have reported strong associations between polymorphisms in the IL-4 gene (Il4-589C/T; Il4C-33T) and the IL-13 promoter (IL-13–1112T) and coding region (IL13+Arg130Gln, *IL13*+2044G>A) with asthma risk [108]. Of particular interest is the Arg130/Gln substitution in the coding region of IL-13 noted by multiple groups and detected across multiple ethnic groups. Moreover, Arima and colleagues [109] have provided evidence that the Arg110Gln variant may be functional as they demonstrate that the Arg130Gln substitution results in decreased affinity of IL-13 for IL-13Rα2, increased expression of IL-13 and phosphorylation of STAT6. Studies of the IL-13 promoter variant (–1112T) have shown that it is functional as its expression in human and murine CD4 + Th2 lymphocytes enhanced IL-13 promoter activity [110]. Interestingly, increased expression of IL-13- 1112T in Th2 cells was associated with the creation of a Yin-Yang 1 binding site that relieved STAT6-mediated transcriptional repression. Based on these findings, Vercelli and colleagues [111]went on to show that IL-13 expression in CD4+ T cells is paralleled by extensive *IL13* locus remodeling resulting in the appearance of multiple DNase I hypersensitive sites. They noted that of these sites, HS4, spans a SNP, IL-13–1512A>C [111], which is strongly associated with IgE levels in German and Korean children [112, 113]. They went on to show that the IL13–1512A>C SNP is functional, in that it significantly enhances HS4-dependent IL13 expression by creating a binding site for

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the transcription factor Oct-1 [114]. Evidence of its functionality in humans was provided by the observation that endogenous Oct-1 was preferentially recruited to the IL13–1512C risk allele in primary CD4+ T cells from IL13–1512A>C heterozygous subjects.

Polymorphisms are also present in the shared IL-4 and IL-13 receptor chain, IL4Rα [115]. In particular, a meta-analysis demonstrated that the IL4RA-Q551R variant imparts a significant risk for atopic asthma (P<0.004) [116]. The amino acid residue 55 is located in the cytoplasmic domain in the vicinity of the STAT6 binding site and is hypothesized to affect STAT6 signal transduction. Strikingly carrying combinations of cytokine and receptor variants has been shown to greatly enhance the risk of development of asthma. For example, individuals with polymorphisms in both the IL-4R gene (S478P) and the IL-13 promoter region (−1112T) have a 4.87x greater risk of developing asthma than individuals without these associated genotypes (P<0.0004) [70]. Likewise, gene-gene interactions between risk alleles of each IL13 promoter polymorphism (A-1512C and C-1112T) and IL13Ra1-A +1398G are associated with significantly higher serum levels of total IgE in children [113].

5. Clinical Trials Targeting IL-4/IL-13 Signaling in Asthma

Based on the preponderance of evidence implicating IL-4 and IL-13 in mediating asthma pathophysiology, clinical trials are currently underway to investigate the blockade of IL-4, IL-13 and/or both in the treatment of asthma. One of the first studies was designed to evaluate the efficacy of blocking both IL-4 and IL-13 signaling with a recombinant human soluble interleukin-4 receptor on asthma. Although the study was small, it suggested that IL-4Rα blockade might improve FEV1 in asthmatics [117]. In 2007, a Phase IIa clinical trial (NCT00535431) of a recombinant human IL-4 variant, pitrakinra (AER 001/BAY-16–9996, Aerovance, Bayer) that competitively binds the IL-4R complex and interferes with both IL-4 and IL-13 actions was shown to reduce the late phase response to allergen challenge in allergic asthmatics as measured by a decreased fall in forced expiratory volume in 1 second (FEV1) [118]. However, a Phase IIb double-blind randomized, dose-ranging follow up study with pitrakinra in patients with moderate to severe uncontrolled asthma failed to show clinical benefit compared with placebo (not published except in abstract form). Interestingly, a pharmacogenetic study of participants in this study showed that the IL4RA Q576R polymorphism might predict treatment response to pitrakinra in patients with asthma [119]. Despite the failure of previous approaches to blocking IL-4Rα activity in asthma, a recent randomized controlled trial, showed that treatment with a fully human monoclonal antibody to the alpha subunit of the IL-4R (dupilumab, SAR231893/REGN668, NCT01312961), was better than placebo in preventing asthma exacerbations in the context of withdrawal of long acting Β agonists and inhaled corticosteroid in patients with moderate to severe asthma [120]. As blocking IL-4Rα inhibits both IL-4 and IL-13 responses, it is unclear whether the effects of dupilumab on asthma are due to inhibiting IL-4, IL-13 or both.

The clinical efficacy of a number of antibodies directed against IL-13 alone has been evaluated in asthma, with mixed results. First, Wyeth conducted clinical trials with two separate anti-IL-13 IgG1 monoclonal antibodies, IMA-638 (NCT00339872, Wyeth/Pfizer) and IMA-026 (NCT00725582, Wyeth/Pfizer) that block binding of IL-13 to different receptor chains. IMA-638 recognizes the IL-13 epitope that binds to IL-4Rα, while

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IMA-026 binds to the epitope on IL-13, which is crucial for binding to both IL-13Rα1 and IL-13Rα2 [121]. Out of the two, IMA-638 treatment was successful in significantly reducing both the early and late phase asthmatic response following exposure to allergen. Similarly, a recent clinical trial utilizing another humanized anti-IL-13 antibody (GSK679586, IgG1, GlaxoSmithKline) targeting binding of IL-13 to both IL-13Rα1 and IL-13Rα2, did not result in any improvement in severe asthmatic patients over placebo controls [122]. Therefore, it seems that blocking binding of IL-13 to IL-4Rα maybe more promising than inhibiting binding to either IL-13Rα1 or IL-13Ra2.

In addition to IgG1 antibodies, multiple IgG4 antibodies targeting IL-13 have shown beneficial results. One such antibody Tralokinumab (a neutralizing IL-13 IgG4 antibody, CAT-354, NCT00873860, Medimmune) had no significant effect on the primary end point, Asthma Questionnaire Score (ACQ), as compared to those receiving placebo. However, it was shown that the patients receiving Tralokinumab treatment had increases in FEV1 and reductions in the usage of short-acting B2 agonists [123]. Based on the mixed results of the previous studies a recent study was designed to examine the effects of blocking IL-13 in asthma patients with evidence of an IL-13 signature *in vivo* [124]. In this randomized, double-blind, trial of asthmatic patients with poorly controlled disease, patients were stratified into two subgroups based on their baseline Th2 status [serum IgE, eosinophil counts, elevated levels of a blood marker of IL-13 activity, periostin] or a Th2-low group and treated subcutaneously with either placebo or 200 mg humanized IgG4 monoclonal antibody directed against IL-13 (Lebrikizumab, Genentech, NCT00930163) monthly for 12 weeks. The results showed that, despite the steroid treatment, Lebrikzumab treatment resulted in an improvement in FEV1 (8.2% points higher than placebo) in patients with a high Th2 phenotype, but provided no significant increases in FEV1 were observed in patients treated with Lebrikzumab with a low Th2 phenotype (1.6% points higher than placebo). These results have suggested that blocking IL-13 in patients with a documented IL-13 signature may provide benefit above that of steroid treatment. As steroid treatment in the first Lebrikzumab trial may have complicated the interpretation of the effects of IL-13 blockade, a subsequent Phase II randomized, double-blind, placebo controlled dose-ranging study of Lebrikizumab (NCT00971035) in asthmatic patients not receiving inhaled corticosteroids was conducted [125]. In this study, a total of 212 patients receiving either 125, 250 or 500 mg of lebrikizumab or placebo subcutaneously monthly for 12 weeks did not show significant improvement in pre-bronchodilator FEV1 values compared to baseline. Although, lebrikizumab was able to inhibit Th2 biomarkers [IgE levels, CCL13, CCL17, and fractional exhaled nitric oxide (FeNO)] in patients, it failed to significantly improve the disease. The results of these two studies suggest that while targeting IL-13 in combination with ICSs provided added benefit to steroid refractory patients, it did not seem to provide benefit in patients not receiving steroids (mild disease). Clearly additional studies are needed to determine whether therapies targeting IL-13 alone, IL-4 or both will provide clinical benefit beyond steroids in specific subsets of patients with asthma.

6. CONCLUSION

In conclusion, the preponderance of information generated in animal models of asthma support the conclusion that the type II cytokines IL-4 and IL-13, in tandem, play an

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important role in the pathogenesis of allergic asthma. IL-4 playing a pivotal role in Th2 cell proliferation and cytokine production and IgE synthesis, while IL-13 plays a pivotal role in the pathological features of disease (mucus production, AHR, and collagen deposition). Although we do not fully understand the mechanisms by which these cytokines elicit different responses through their shared receptor, new studies identifying unique cellular sources of IL-13 (ILC2s), and differences in assembly/affinity of the type II receptor for these two cytokines, and inhibitory actions of IL-4 mediated through the type I receptor likely explain the dominant role for IL-13 in asthma pathogenesis. Although after almost 20 years since the original studies implicating these cytokines in allergic disease were conducted, we still do not know the precise mechanisms by which IL-13 induces disease. However, great progress is being made in identifying new downstream targets of IL-13 in resident lung cells such as the airway epithelium (BRP-39, MUC5AC, periostin), airway smooth muscle (RhoA, CD38), rather than in the traditional effector cells of the allergic response (i.e. eosinophil, mast cells). Clinical trials examining the efficacy of blocking IL-4 or IL-13 using biologic approaches show promise, but paint a complex picture of IL-13-responders and non-responders, and potential interactions between IL-13 and glucocorticoids. Future studies will undoubtedly unravel the remaining mysteries of the mechanisms of IL-13-induced disease. These insights should inform the further development and testing of reagents designed to modulate IL-13 and/or IL-4 or their downstream signaling molecules for the treatment of asthma.

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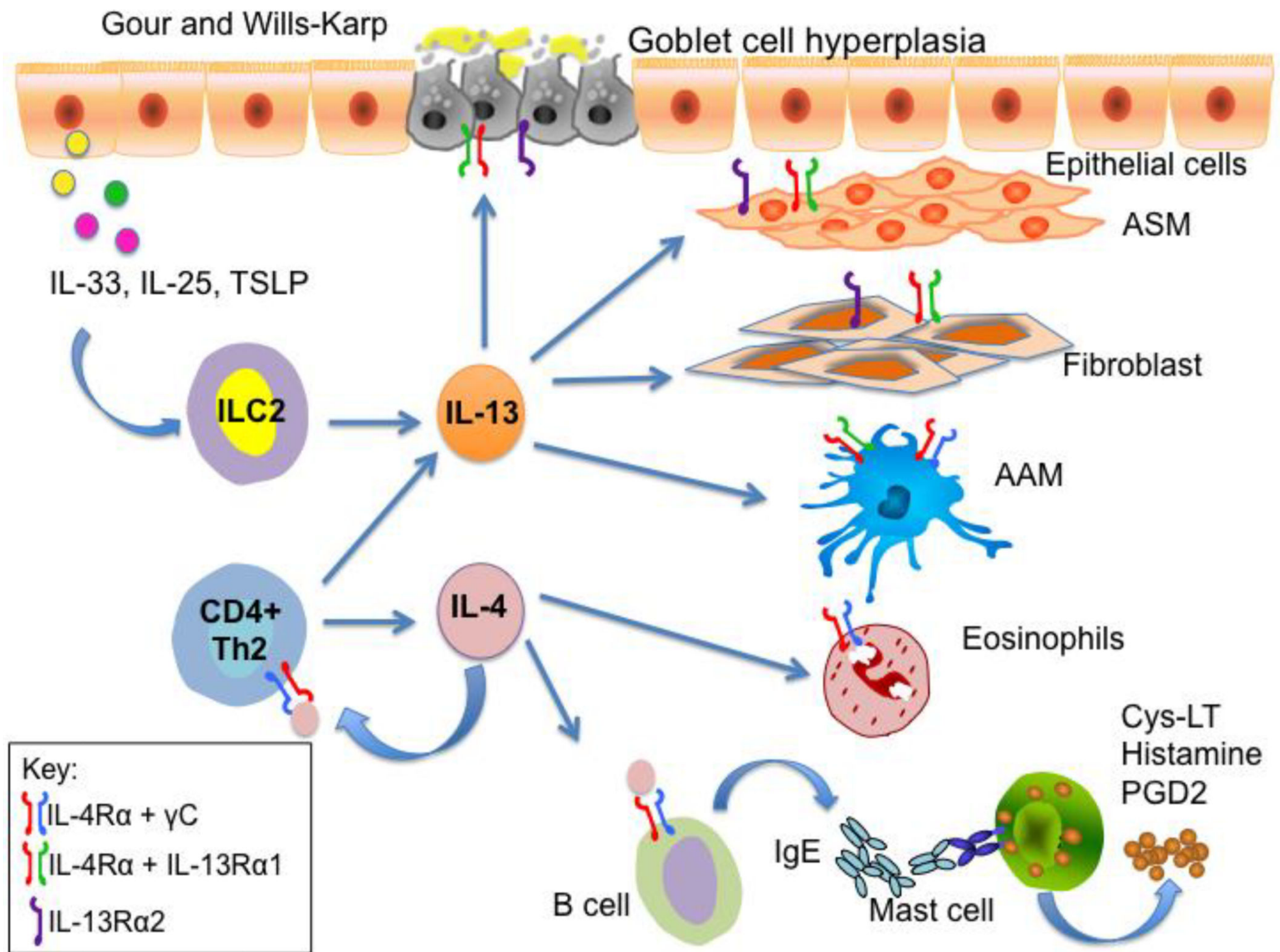

Figure 1. Differential Roles of IL-4 and IL-13 in Allergic Inflammation

The type 2 cytokines, IL-4 and IL-13 are both produced by CD4+ T cells, whereas only IL-13 is produced by innate lymphoid cells (ILC2s). Through ligation of the type I IL-4R (IL-14Rα/γc) on T cells and mouse B cells, IL-4 preferentially regulates Th2 cell function and IgE synthesis. Although both IL-4 and IL-13 can activate the type II IL-4R (IL-4Rα/IL-13Ra1) on a variety of cells including epithelial cells, airway smooth muscle cells, and fibroblasts, IL-13, not IL-4, mediates the main physiological features of asthma including mucus hypersecretion, subepithelial fibrosis, and airway hyperresponsiveness. IL-13-induced subepithelial fibrosis has been postulated to be mediated via activation of the high affinity IL-13 receptor, IL-13Rα2 on fibroblasts. Cells such as alternatively activated macrophages express both the type I and type II IL-4 receptors.

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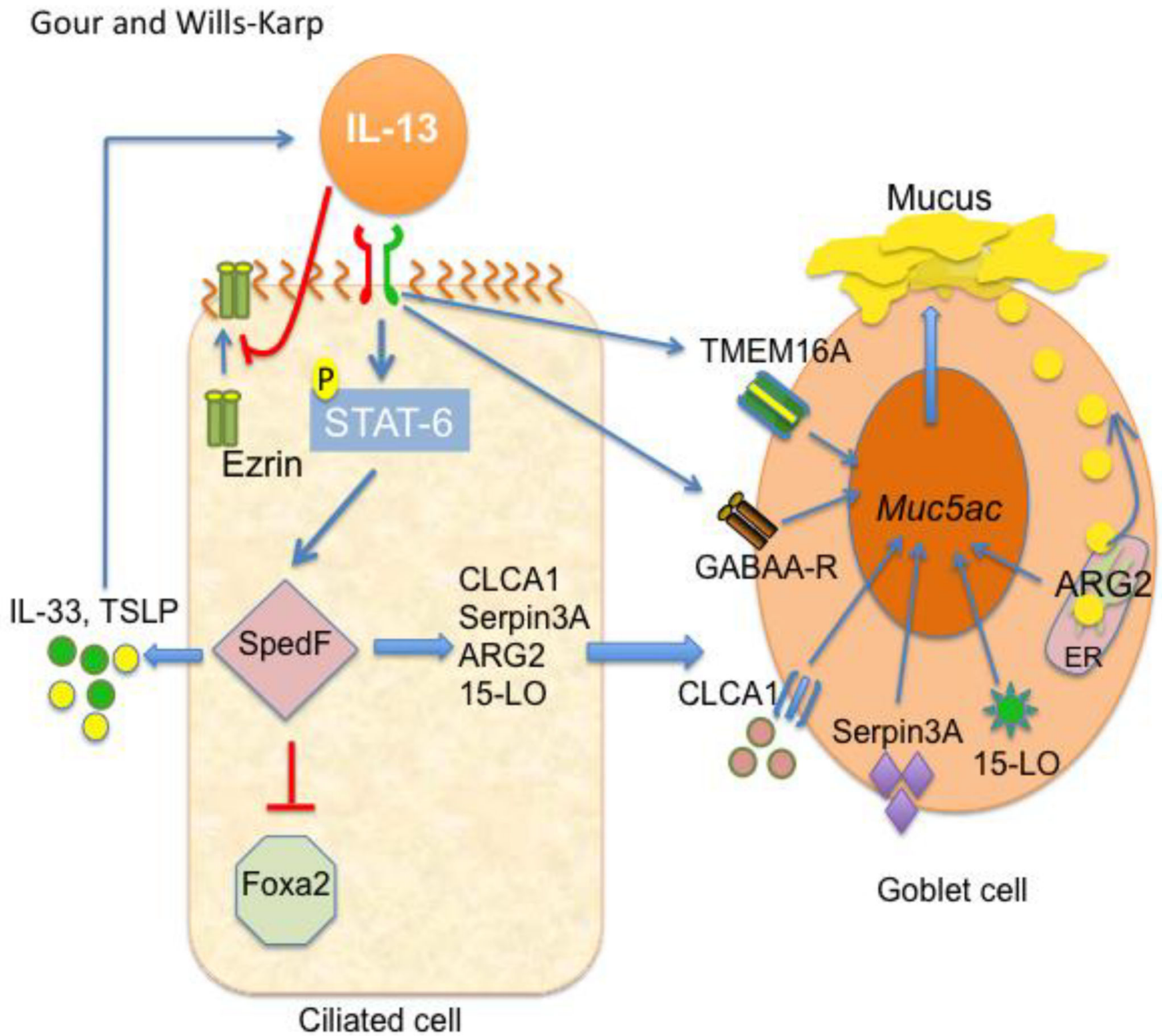

Figure 2. Potential Mechanisms of IL-13-induced Mucus Cell Metaplasia

Overzealous mucus production is a hallmark of asthma. In the allergic lung, mucus production is predominantly an IL-13-mediated process. IL-13 has been shown to play a pivotal role in several processes important in mucus hypersecretion including: mucus cell metaplasia, mucin gene induction, mucus packaging, and mucus release. The first step is believed to involve the transition of Clara (club) cells to a goblet cell phenotype through the coordinate actions of several transcription factors including Spdef, and Foxa2. IL-13 induces the expression of the Sam pointed domain-containing ETS transcription factor (Spdef) through Type II receptor activation of STAT6-dependent. Spdef, in turn, inhibits the transcription factor, Foxa2 that is required for maintenance of normal differentiation of the airway epithelium. Inhibition of Foxa2 allows induction of a number of genes important in mucus cell metaplasia including: CLCA1, Serpin3A, and 15-lipoxygenase. These genes are thought to directly induce MUC5AC gene expression. On the other hand, anterior gradient

2, Arg2, is thought to play a direct role in mucin folding or another function necessary for maintaining normal mucin production and packaging in the mucus cell endoplasmic reticulum. Other IL-13-inducible genes that play an important role in mucus hypersecretion include: TMEM16A, GABAA-R, and ezrin. Transmembrane protein 16A (TMEM16A), which is a calcium-activated chloride channel, that plays a role in muc5ac expression and mucus release. The γ-aminobutyric acid receptor (GABAAR), which is a pentameric chloride channel, is also thought to regulate mucin gene induction and mucus secretion. Lastly, IL-13 decreases ciliary beat frequency by interfering with the apical localization of the actin binding protein ezrin. This cycle of events is further perpetuated by the ability of Spdef to induce the production of IL-13-promoting cytokines such as IL-33, and TSLP in the epithelium.

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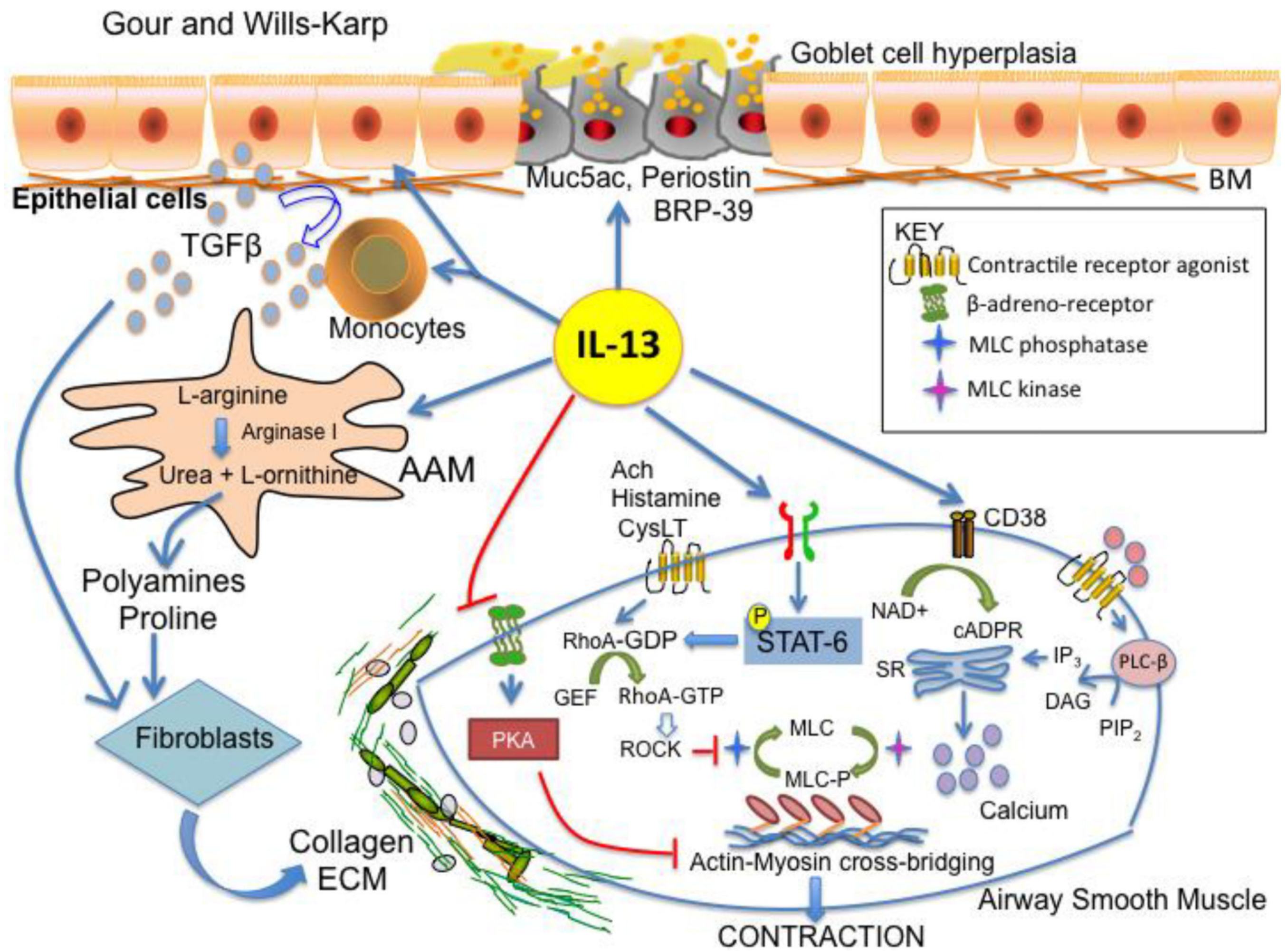

Figure 3. Potential Mechanisms of IL-13-induced Airway Hyperresponsiveness

Interleukin 13 induces several cellular changes in the airway wall that may contribute to the main physiological manifestation of asthma, airway hyperresponsiveness. These include: 1) mucus cell metaplasia and mucus production; 2) airway smooth muscle contraction; and 3) collagen deposition and fibrosis. Through STAT6-mediated processes, IL-13 induces a number of epithelial genes including MUC5AC and periostin, which have been shown to result in mucus plugging of the airways and airway hyperresponsiveness. IL-13 also enhances airway smooth muscle sensitivity to a number of contractile agonists (acetylcholine (Ach), histamine, and leukotrienes) through a series of alterations in ASM signaling including: increasing sensitivity to contractile agonist stimulation through enhancing RhoA-ROCK activation and increased myosin light chain phosphorylation, increases in CD38-mediated calcium release, which culminate in actin-myosin cross-bridging and contraction. Lastly, IL-13-induces collagen deposition, through inducing the production of arginase I in alternatively activated macrophages (AAM) and inducing epithelial cell and monocyte production of TGF-β, which induces collagen production from fibroblasts leading to stiffening of the airway wall. RhoA, small GTP binding protein A; ROCK, Rho-associated kinase; GEF, RhoA guanine nucleotide exchange factor.