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The Type I and Type II Receptor Complexes for IL-4 and IL-13 Differentially Regulate Allergic Lung Inflammation

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1. Introduction

Approximately 300 million (M) people worldwide currently suffer from asthma; this number is projected to reach 400M by 2025 (Bahadori, et al., 2009). During the allergic immune response, inhaled allergens first stimulate epithelial cells, basophils, mast cells, and macrophages. This priming leads to the generation of allergen specific T-cells. Atopic asthma is strongly correlated with a robust CD4+ Th2 effector response, which results in elevated levels of the cytokines IL-4, 5, and 13. These cytokines act on multiple cell types to initiate and propagate the hallmark features of asthma such as pulmonary inflammation, periodic narrowing of airways, and mucus hypersecretion. Two of these cytokines, IL-4 and IL-13, share receptor chains and signaling proteins. In this chapter we discuss the cells that produce IL-4 and IL-13 including CD4+ T-cells and cells of the innate immune system, the structure of their receptors, their binding potency and kinetics, and their signal transduction. Furthermore, we present evidence for the differential effects of IL-4 and IL-13 acting via these receptor complexes on features of allergic lung inflammation. Finally, we discuss their contribution to the control of negative regulatory mechanisms that act to suppress allergic inflammation.

2. Cells that produce IL-4 and IL-13

Interleukin-4 and Interleukin-13 are closely related cytokines (Chomarat & Banchereau, 1998) critical to the development of T cell-mediated humoral immune responses, which are associated with allergy and asthma. Both cytokines display many overlapping functions (Chomarat & Banchereau, 1998). However, studies in cytokine-knockout mice and the use of blocking antibodies in vivo have shown that IL-4 is a critical cytokine for Th2 development whereas IL-13 plays critical roles in allergic asthmatic response (Grunig, et al., 1998, Wills-Karp, et al., 1998). Both cytokines can be secreted by many cell types but Th2 cells are considered to be the major producers. In this section, we will focus on cells capable of producing either IL-4, or IL-13, or both.

2.1 T cells

Upon antigen receptor stimulation, naive CD4+ T cells can differentiate into several different types, including T helper type 2 cells (Th2) depending on the cytokine milieu.
present during the priming. IL-4 itself is important for the differentiation of naïve CD4+ T cells into Th2 cells capable of producing large amounts of IL-4, IL-13, and IL-5 (Zhu & Paul, 2008). Recent evidence points to basophils (van Panhuys, et al., 2011), mast cells (Plaut, et al., 1989), natural killer (NK) T cells (Akbari, et al., 2003), and γδ T cells (Ferrick, et al., 1995) as early producers of IL-4 in the innate immune response necessary for the optimal priming of the Th2 adaptive response. Therefore, in addition to CD4+ T cells, other T cell subpopulations such as NKT cells and γδ T cells are capable of making IL-4.

IL-4 and IL-13 producing NKT cells have been shown to be essential for the development and progression of allergic airway inflammation (Akbari, et al., 2003). NKT cell-deficient (Cd1d1-/-) mice showed reduced Th2 responses after allergen challenge including allergic airway inflammation and airway hyperreactivity. The abrogated Th2 response in these mice could be restored by the adoptive transfer of purified NKT cells producing IL-4 and IL-13, but not by IL-4-deficient and IL-13-deficient NKT cells. IL-13 instillation to the mice could restore allergic airway responses. This led the authors to conclude that IL-4 and IL-13 produced by NKT cells potentiate the development of Th2 response in the lung. However, other studies suggest that NKT cells do not play an important role in allergic lung inflammation models (Das, et al., 2006).

Gamma/delta T cells also can differentiate into cells producing Th2 cytokines (Wen, et al., 1998). When WT mice were infected with *Nippostrongylus brasiliensis*, an extracellular parasite known to induce Th2 responses, γδ T cells from these mice produced IL-4 (Ferrick, et al., 1995). The ability of human γδ T cells to differentiate into Th2 cytokine producing cells was tested using stimulation of peripheral blood-derived γδ T cells with phosphoantigen isoprenyl pyrophosphate (Wesch, et al., 2001). When these cells were stimulated with Ag under Th2 priming conditions, they developed into cells producing IL-4. CD8+ T cells have been shown to produce IL-4 under specific in vitro stimulation (Seder, et al., 1992). When mouse CD8+ T cells were stimulated with immobilized CD3 in the presence of IL-2 and IL-4, they became high IL-4 producers. Moreover, both CD4+ T cells and CD8+ T cells separated from bulk lymph node T cell cultures with anti-CD3 plus IL-2 and IL-4 where equally effective in secretion of IL-4 after restimulation with anti-CD3 plus IL-2. The authors suggested that in certain in vivo conditions, CD8+ T cells could be major IL-4 producers. Human CD8+ T cell clones capable of IL-4 secretion were identified previously (Paliard, et al., 1988).

### 2.2 Granulocytes (Mast cells, basophils, and eosinophils)

Mouse non-T, non-B cells derived from spleen produce IL-4 and IL-13 in response to FcR crosslinkage (Ben-Sasson, et al., 1990). The same phenomenon was described for human bone marrow non-B and non-T cells in response to stimulation through either Fce or Fcγ receptors (Piccinni, et al., 1991). It has been suggested then that those IL-4-secreting cells could be either mast cells or basophils, or both. Indeed, the ability of mast cells to secrete both cytokines has been reported utilizing human cord blood derived mast cells (Toru, et al., 1998). These cells can generate both cytokines after stimulation with PMA or FccR crosslinking. However, these cells do not produce Th2 cytokines spontaneously. Similarly, the ability of mouse mast cells to secrete IL-4 and IL-13 in response to specific immunological stimulation has been reported for fetal liver derived mast cell lines, bone marrow derived mast cells, and the mast cell line C1.MC/C57.1 (Brown, et al., 1987, Burd, et al., 1995). Of note, mast cells secrete relatively high IL-13 but low IL-4 levels.
As noted above, basophils were among three major cell populations of IL-4 producers in the lung under inflammatory conditions (Voehringer, et al., 2004). The ability of basophils to secrete both cytokines and other mediators of inflammatory response was extensively reviewed by Min and Paul in 2008 (Min & Paul, 2008). Importantly, it has been shown that peripheral blood basophils in asthmatic patients are the main producers of IL-4 and IL-13 (Schroeder, et al., 1995), suggesting a role in asthma exacerbation. IL-4 was detected in cultures of human basophils treated with diesel exhaust particles (Devouassoux, et al., 2002) suggesting that environmental exposure can predispose basophils to initiate Th2 responses. Eosinophils can also make IL-4 under certain circumstances. When mice were injected with Schistosoma mansoni eggs intraperitoneally, there were high levels of IL-4 in the peritoneal exudate cell cultures (Sabin, et al., 1996). IL-5 and eosinophils were necessary for the observed IL-4 production as suggested from similar experiments using egg-immunized IL-5−/− mice or anti-IL-5 treated mice (Kopf, et al., 1996). Interestingly, these eosinophils were found to produce IL-4 early after immunization. The authors suggested that Schistosoma mansoni induced an early IL-5 production by mast cells that attracted eosinophils which, in their turn, produced IL-4 thus stimulating the development of antigen-specific Th2 cells. The ability of eosinophils to make Th2 cytokines was also tested in a more recent study (Voehringer, et al., 2004). The authors characterized IL-4 producing cells in the inflamed lungs by immunohistochemistry, flow cytometry, and microarray in mice with a bicistronic knock-in IL-4 gene linked via internal ribosomal entry site (IRES) with enhanced green fluorescent protein (eGFP). Eosinophils, basophils, and Th2 cells were reported as three cell populations producing IL-4 in these mice.

2.3 Myeloid cells
Macrophages have been shown to produce IL-4 or IL-13 in response to certain stimuli. A strong expression of IL-13 in the lung was observed in the experimental model of particle inhalation-induced inflammation (Kang, et al., 2005). Immunostaining of lung tissues of TiO2-exposed mice demonstrated that alveolar macrophages are major producers of IL-13 and IL-25 in the inflamed lungs (Kang, et al., 2005). It has also been shown that human alveolar macrophages can produce IL-4 (Pouliot, et al., 2005). It has been shown that infectious pathogens including Francisella tularensis and respiratory syncitial virus (RSV) induce lung and peritoneal macrophages to produce IL-4 and IL-13 (Shirey, et al. 2008, Shirey, et al., 2010).

3. Receptor structure, ligand binding properties, and signal transduction
3.1 The structure of the IL-4 and IL-13 receptors
IL-4 and IL-13 elicit a wide variety of cellular responses by binding to high affinity receptor complexes expressed on the surface of cells. The IL-4/IL-13 receptor system is complex (Figure 1). The IL-4 specific receptor is composed of the IL-4Rα chain paired with the common γ chain, or γC, forming the Type I IL-4 receptor complex. The IL-4Rα chain can also pair with the IL-13Rα1 chain, forming the Type II receptor (Figure 1). Type I receptors are activated by the binding of IL-4 to the ligand-binding IL-4Rα chain and Type II receptors can be activated by either IL-4 or IL-13, with the IL-4Rα or IL-13Rα1 acting as the initial ligand-binding chain, respectively. IL-13 can also engage another kind of IL-13 receptor, the IL-13Rα2 chain.
The IL-4 and IL-13 Receptor System. A functional IL-4 receptor is composed of two transmembrane proteins. IL-4Rα chain binds IL-4 with high affinity, leading to dimerization with either common gamma chain (γC) or IL-13Rα1, forming the Type I or Type II receptor complex, respectively. IL-13 binds to IL-13Rα1 with lower affinity, followed by heterodimerization with IL-4Rα to form a high affinity complex. IL-13 also binds to IL-13Rα2 (the so-called “decoy receptor”) at the cell surface, or in soluble form, but this interaction fails to activate the JAK/STAT pathway and generally it is thought to be inhibitory. Soluble forms of IL-4Rα (sIL-4Rα), IL-13Rα1 and IL-13Rα2 exist that can also bind ligand. Following ligand binding and heterodimerization, receptor-associated Janus Kinases (JAKs) are activated.

3.1.1 IL-4Rα
The IL-4Rα chain (CD124) is a 140 kDa protein. Human and mouse IL-4 receptors show a broad distribution on hematopoietic and non-hematopoietic cells (Park, et al., 1987, Lowenthal, et al., 1988), generally expressed at low levels (20-4000 receptors per cell). The IL-4Rα cDNA was cloned from the mouse cytotoxic T-lymphocytic (CTLL-2) cell line (Mosley, et al., 1989) and from the human myeloid cell line, TF-1 (Galizzi, et al., 1990). Sequence analysis demonstrated that the IL-4Rα belongs to the hematopoietin receptor superfamily. There are two extracellular structural features that characterize this family: type III fibronectin (FN) repeats and a membrane-proximal WSXWS motif. The 2.3 Å resolution crystal structure of human IL-4 complexed to the extracellular domain of the human IL-4Rα determined the overall shape of the two, linked type III FN-like domains, D1 and D2 (Hage, et al., 1999). Five IL-4Rα peptide loops protrude from these two D domains and interact with IL-4. Binding of IL-4 to IL-4Rα occurs through interaction of IL-4 with two clusters or sites (I and II) within the receptor, with Y183 and D72 at the center, surrounded by a shell of hydrophobic residues.

3.1.2 γC
The involvement of the IL-2Rγ subunit, or common γ chain, in forming heterodimeric IL-4 receptors was recognized by three groups in the early 90’s (Kondo, et al., 1993, Leonard, et
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al., 1994, Russell, et al., 1993). The γC chain (CD132), which dimerizes with the IL-4Rα chain to form a functional type I IL-4 receptor, is a 60 kDa protein. The extracellular domain of γC possesses the tandem FN-III domains with four Cys residues and membrane-proximal WSXWS motif creating the classical CHR. Mouse γC chain was cloned in 1993 (Kumaki, et al., 1993) and human γC in 1992 (Takeshita, et al., 1992). Both human and mouse γC genes map to the X-chromosome in humans (Noguchi, et al., 1993) and mouse (Cao, et al., 1993). The γC subunit participates in the formation of many other cytokine receptor complexes including the IL-2, IL-7, IL-9, IL-15, and IL-21 receptors. Thus, mutations that either diminish or eliminate γC expression or prevent association of the JAK3 kinase with γC impair activity of these cytokines important for the development and proliferation of many different cells of the immune system, resulting in X-linked severe combined immunodeficiency (XSCID) in humans (Leonard, et al., 1994). γC-deficient mice have no NK cells and severely diminished T- and B-cells, virtually absent lymph nodes and spontaneously develop inflammatory bowel lesions (Cao, et al., 1995).

3.1.3 IL-13 receptor α1 (IL-13Rα1)

The IL-13Rα1 chain (CD213a1) is a 65-70 kDa glycosylated protein, encoded on the X chromosome in mice and humans. The mouse IL-13Rα1 cDNA was cloned in 1996 (Hilton, et al., 1996), followed by characterization of the human gene (Aman, et al., 1996), revealing the characteristic WSXWS motif and four conserved Cys residues. IL-13Rα1 can act either as a ligand-binding chain for IL-13 or as a dimerization partner to the type II receptor’s IL-4Rα ternary complex (Zurawski, et al., 1993). The IL-13Rα1 chain is widely expressed on the surface of many hematopoietic and non-hematopoietic cells. It is through this Type II receptor complex that IL-4 and IL-13 mediate their effects on non-hematopoietic cells, which generally lack γC, and therefore Type I receptor expression. IL-13Rα1 surface expression is absent on resting mouse and human T-cells and on mouse B-cells (Ogata, et al., 1998, Umeshita-Suyama, et al., 2000), although recent studies suggested inducible expression on mouse and human CD4+ T-cells (Newcomb, et al., 2009, Newcomb, et al., 2011). Related evolutionarily to γC, IL-13Rα1 has acquired a third extra Ig-like domain, D1, allowing extra contacts with IL-13 in Type II receptor complexes (LaPorte, et al., 2008). This D1 domain is required for IL-13 binding and the formation of a functional Type II receptor, while it is not required for IL-4 binding or its activation of the Type II receptor (Ito, et al., 2009).

3.1.4 IL-13 receptor α2 (IL-13α2)

IL-13 binds to a second IL-13 receptor, IL-13Rα2 (CD213a2, IL13BP) that was cloned in humans (Caput, et al., 1996) and from mice (Donaldson, et al., 1998). The gene is also found on the X-chromosome. IL-13Rα2 (~65 kDa) is inducibly expressed on fibroblasts, keratinocytes, epithelial cells, macrophages, and certain tumor cells and requires STAT6 for its expression (David, et al., 2003). The soluble form can be generated by proteolytic cleavage of the membrane-bound form by matrix metalloproteinases (MMPS) (Matsumura, et al., 2007) or by alternative splicing (Tabata, et al., 2006). Interestingly, sIL-13Rα2 is detected in serum from mice but not humans (Chen, et al., 2009). Treatment of cells with IL-4 or IL-13 in combination with TNF-α upregulated IL-13Rα2 cell surface expression (Zheng, et al., 2003). In contrast to IL-13Rα1, IL-13Rα2 binds to IL-13 with very high affinity 10^{-11} M (Andrews, et al., 2002) one of the highest measured protein-protein interactions, possibly
due to an interlocking IL-13-binding interface (Lupardus, et al., 2010). The IL-13Rα2 is proposed to act as a “decoy receptor” for IL-13 (Yoshikawa, et al., 2003) and, more recently, for IL-4 signaling (Rahaman, et al., 2002,). Consistent with this model, mice deficient in IL-13Rα2 have exaggerated IL-13 responses, such as severe liver fibrosis following S. mansonii infection (Chiaramonte, et al., 2003; Mentink-Kane, et al., 2004), reversible by soluble IL-13Rα2-Fc. A signaling role was hypothesized, however, in TNBS-induced colitis, tumor surveillance, and cancer (Strober, et al., 2009) and in monocytic cell lines through AP-1 (Fichtner-Feigl, et al., 2006).

3.2 Ligand binding properties
IL-4 binds to the IL-4 receptor α chain with high affinity with a Kd ranging between 20 - 300 pM (Lowenthal, et al., 1988), allowing ligand binding at low IL-4 concentrations, as would be present in the initiating phase of an allergic inflammatory response. There is species specificity for the IL-4:IL-4Rα interaction (Park, et al., 1987), yet dimerization of the binary IL-4:IL-4Rα complex with the γC chain is not species specific (Idzerda, et al., 1990). Dimerization with the γC chain forming Type I IL-4 receptors increases the affinity of IL-4 binding approximately three-fold (Russell, et al., 1993). The affinity of interaction of the binary IL-4:IL-4Rα complex with either of its dimerization partners, γC or IL-13Rα1, is low (559 nM for γC with IL-4:IL-4Rα (Andrews, et al., 2006; LaPorte, et al., 2008; Zhang, et al., 2002) and 487 nM for IL-13Rα1 (LaPorte, et al., 2008)). In contrast, IL-13 binding to IL-13Rα1 is a relatively low affinity interaction (Kd ~ 30 nM (Andrews, et al., 2002; LaPorte, et al., 2008)) that is not species-specific (Andrews, et al., 2001). Dimerization of IL-13:IL-13Rα1 with IL-4Rα to form the ternary Type II complex is a high affinity, species-specific interaction (Andrews, et al., 2001; LaPorte, et al., 2008).

The crystal structures of the three ternary complexes were solved in 2008 (LaPorte, et al., 2008) revealing that the IL-4/IL-13 receptor system was unique in that when forming a Type II receptor the “binder” (i.e. ligand-binding) and “trigger” (i.e. dimerizing partner) chains were switched depending on whether IL-4 or IL-13 is the ligand. Furthermore, there was a ~8° angle difference in the position of the IL-4Rα chain relative to the IL-13Rα1 chain between the two Type II structures. The impact of these subtle differences in initial binding affinities, order of chain assembly, and 3-dimensional structure of the extracellular domains of the ternary complexes on responsiveness to IL-4 and IL-13 is not clear. A recent study suggests that the relative abundance of the two receptor types and ratio of the ligand-binding chain to the trigger chain can fine-tune sensitivity to these cytokines (Junttila, et al., 2008).

3.3 Signal transduction pathways activated by Type I and Type II receptor engagement

3.3.1 Janus kinases
Ligand-induced dimerization of the Type I or Type II IL-4 receptor activates receptor-associated kinases of the Janus kinase (JAK) family. JAK1 was activated by IL-4 and IL-13 (Welham, et al., 1995). IL-13 did not induce JAK3 activation, as the IL-13Rα1 does not recruit JAK3 (Keegan, et al., 1995, Welham, et al., 1995). JAK2 appeared to be constitutively associated with the IL-4Rα chain in human monocytes and stimulation of the Type II receptor complex with IL-13 enhanced the interaction (Roy, et al., 2002). IL-13 predominantly activates TYK2 (Murata & Puri, 1997), as well as JAK2 (Murata, et al., 1996). JAK1 is a substrate for other non-JAK kinases that can affect the activation of IL-4-induced
signaling. PKCζ is required for full IL-4-induced JAK1 activation (Martin, et al., 2005). Mutational studies on cytoplasmic domain of IL-4Rα have revealed the presence of a membrane-proximal, proline-rich “box 1” motif (aa262 – 267 for hIL-4Rα and 263 – 271 for mIL-4Rα) to which JAK1 can bind (Fujiwara, et al., 1997, Russell, et al., 1994). The cytoplasmic domain of the γC also has a “box 1” motif :Pro-X-Pro and a preceding cluster of hydrophobic amino acids (aa286 – 294 that binds JAK3, Murakami, et al., 1991).

The proline-rich region in the IL-13Rα1 (aa 373 – 378 in mouse and aa 376 – 381 in human) and the next six amino acids downstream mediate the interaction between IL-13Rα1 and the associated JAKs (TYK2, JAK1 in a transfected FDCP-1 cell line (Orchansky, et al., 1999)). The cytoplasmic domain of the IL-13Rα1 chain is shorter than that of the IL-4Rα or γC. It contains the box 1 motif and two tyrosines, Y402 and Y405, which act as STAT3 binding motifs (Orchansky, et al., 1999, Umeshita-Suyama, et al., 2000). Engagement of the receptor chain by IL-13 activates JAK1 and TYK2 and triggers a variety of signaling cascades which will be discussed below.

Tyrosine residues within the cytoplasmic domains of the Type I and Type II receptor subunits are targets for rapid phosphorylation by the JAKs (Figure 2). Both IL-4 and IL-13 induce tyrosine phosphorylation of IL-4Rα (Wang, et al., 1992). Tyrosine phosphorylation of IL-13Rα1 was not detected in immunoprecipitated lysates from IL-13-stimulated FD-5 cells transfected with the IL-13Rα1 subunit (Orchansky, et al., 1999) although mutational studies suggested that IL-13Rα1 tyrosines would indeed become phosphorylated (Umeshita-Suyama, et al., 2000). Studies using deletion, Y-to-F mutants, and chimeric forms of the IL-4Rα (Deutsch, et al., 1995, Keegan, et al., 1994, Koettritz & Kalthoff, 1993, Seldin & Leder, 1994) have characterized distinct regions of the IL-4Rα cytoplasmic domain containing five essential tyrosine residues (Y1-Y5). These phosphorylases become docking sites for SH2- and PTB-containing proteins: Y1 (Y497) recruits IRS proteins, Y2-Y4 (Y575, Y603, Y631) recruit STAT6 and Y5 (Y713) is bound by the phosphatases, SHP-1, -2 and SHIP (Hanson, et al., 2003, Kashiwada, et al, 2001).

3.3.2 IRS proteins

IL-4 strongly induced the tyrosine phosphorylation of a ~170 kDa protein in the mouse IL-3-dependent hematopoietic cell line, FDCP-2 (Wang, et al., 1992), named IL-4-induced phosphotyrosine substrate (4PS) and it associated with PI3-K and PI3-K activity. This protein was identical to that tyrosine phosphorylated in response insulin and IGF-I in FDC cells (Wang, et al., 1993). 4PS was cloned from myeloid progenitor cells and renamed IRS-2 due to similarity to IRS-1 (Sun, et al., 1995). IRS-1 is also tyrosine phosphorylated in response to IL-4 stimulation and whether IRS-1 or IRS-2 or both are tyrosine phosphorylated after IL-4 stimulation depends on cellular expression of each protein and the surface expression of Type I or II receptors (Sun, et al., 1997). IRS-2 is predominantly found in hematopoietic cells and IRS-1 in non-hematopoietic cells: studies in 32D cells, which express neither IRS protein, revealed the role of both IRS-1 and IRS-2 in IL-4-induced cellular proliferation (Wang, et al., 1993).

The sequence of amino acids important for IRS binding to the IL-4 receptor was determined by truncation mutational analysis (between aa437 and 557). Within this interval, there is a homologous sequence that binds IRS proteins in the insulin and IGF-I receptor, known as the insulin and IL-4 receptor (I4R) motif, whose sequence is 488PL-(X)4-NPXYSXSD902. The central tyrosine when phosphorylated is critical for association of the PTB domain of IRS proteins with the I4R motif of the IL-4Rα (Keegan, et al., 1994, Zhou, et al., 1996).
The IRS proteins are tyrosine phosphorylated in response to engagement of the IL-4 receptors. JAK1 is required for this to occur (Wang, et al., 1997). Once phosphorylated, tyrosines that are part of typical SH2-binding motifs (Sun, et al., 1991) provide docking sites for a variety of different SH2-domain-containing downstream molecules, such as the p85 subunit of PI3-K and Grb2 (Pruett, et al., 1995). There are three tyrosines, part of classical YXXM motifs, that act as p85 binding sites in IRS-1 and two in IRS-2 (White, 2002). Binding of p85 can activate PI3-K thus allowing IL-4 to initiate a large number of downstream signaling cascades. The IRS proteins can also bind SHP-2 following IL-4 stimulation and IRS-2 can recruit PLC-γ in response to IL-13 (Sozzani, et al., 1998). The IRS proteins also interact with the SOCS proteins that are negative regulators of IL-4 signaling and will be discussed in more detail in a later section.

The contribution of IRS-2 to allergy and asthma is not well understood. Transgenic overexpression of IRS-2 enhanced IgE production in vivo, and increased IL-5 secretion from in vitro differentiated CD4+ Th2 cells (Kelly-Welch, et al., 2004). Early studies of T-cells isolated from IRS2−/− mice found reduced T-cell proliferation and IL-5 production by Th2 cells compared to wildtype T-cells (Wurster, et al., 2002). Surprisingly, mice with a mutation in the IRS-2 docking site of the IL-4Ra (Y500F) demonstrated enhanced allergic inflammation, suggesting a significant contribution of this region of the IL-4Ra to inflammation control in vivo (Blaeser, et al., 2003). Activation of the IRS-2 pathway was abrogated but this Y500 region of the IL-4Ra also recruits a number of other signaling molecules including Shc, FRIP1, p62DOK, and p85β (Nelms, et al., 1998) that may negatively regulate the pathway (described below).
3.3.3 Activation of PI3-K
The p85 or regulatory subunit of PI3-K binds phosphotyrosines on the IRS protein via SH2-domains and the resulting conformational change releases inhibition of the enzymatic activity of the p110 (catalytic) subunit and allows it to translocate to the plasma membrane. Activation of PI3K activity in response to IL-4 was first demonstrated in hematopoietic FDCP cells inducing mitogenic signals (Wang, et al., 1992). The kinase transfers a phosphate group from ATP to phosphoinositol (PI) to rapidly form PIP3, activating a myriad of downstream pathways. PIP3 has the potential to activate protein kinase C (PKC) and protein kinase B (PKB)/Akt. Activation of Akt in response to IL-4 has been shown in human eosinophils (Coffer, et al., 1998), although we found no induction of phosphorylation on Akt^{Ser473} in mouse eosinophils by IL-4 or IL-13 (Heller et al, under review).

3.3.4 Signal Transducers and Activators of Transcription (STATs)
IL-4 receptor engagement can activate a number of members of the STAT family. STAT6 is the predominantly activated STAT but other members of the STAT family can also be activated to a lesser degree. The human STAT6 gene was cloned in 1994 and the same group determined that STAT6 (IL-4 Stat) directly interacted with the IL-4 receptor cytoplasmic domain, homodimerized via its SH2-domain and characterized the DNA binding motif recognized by STAT6 (Hou, et al., 1994). STAT6 docks via its highly conserved SH2-domain to the “gene regulation domain” (aa 557 – 657) encompassing three of the five conserved tyrosines of human IL-4Rα (Ryan, et al., 1996). STAT6 becomes tyrosine phosphorylated on Y641 and forms homodimers through pY641-SH2 interactions (Mikita et al., 1996). The C-terminus of STAT6 contains the transcriptional activation domain (Goenka, et al., 1999). In addition to tyrosine phosphorylation, the STAT6 protein can be post-translationally modified in other ways to affect its function: methylation (Chen, et al., 2003), serine phosphorylation (Pesu, et al., 2000) on S756 (Wang, et al., 2004) and S707 (Shirakawa, et al., 2011) and acetylation (Shankaranarayanan, et al., 2001). Once in the nucleus, STAT6 homodimers bind to consensus DNA motifs in STAT6-responsive genes (reviewed in Goenka & Kaplan, 2011). The preferred DNA binding motif recognized by STAT6 is a dyad symmetric recognition element TTC-GAA separated by four nucleotides although STAT6 can also bind the dyad element separated by three nucleotides. STAT6 often co-operates with other transcription factors and co-activators to activate transcription including NK-κB, CEBPβ, CBP and p300 and p160 steroid receptor nuclear coactivator (NCoA-1).

STAT6 deficiency (Takeda, et al., 1996) is protective in many different in vivo models of allergy including allergic airway disease, food allergy, eosinophilic esophagitis and atopic dermatitis. In contrast, mice expressing constitutively active STAT6 are predisposed to an allergic phenotype (Sehra, et al., 2008, Sehra, et al., 2010). Hyperactive STAT6 can lead to cellular transformation and various cancers, due to dysregulated p27Kip1/cell cycle progression (Bruns, et al., 2003). Mice deficient in STAT6 have compromised expulsion of helminth parasites: they cannot produce Th2-cells, mount an effective IgE response, produce mucus or chemokines (Kaplan, et al., 1998).

While STAT6 is clearly the dominant STAT family member activated by IL-4 and IL-13, there are reports of activation of several other STATs to varying degrees. Some STAT1a activation was documented in the mouse T-helper cell line, HT-2, in response to IL-4 (Brunn, et al., 1995) and by IL-4 and IL-13 in five primary human cell types generally to a lesser
Primary human monocytes respond to IL-13 with tyrosine phosphorylation of STAT1a (Roy, et al., 2002). Furthermore, STAT5 phosphorylation was detected in human B-cells in response to IL-4 and IL-13 (Rolling, et al., 1996) and in primary human monocytes by IL-13 (Roy, et al., 2002). The role of STAT1 and STAT5 in IL-4- or IL-13-induced responses is unknown.

The cytoplasmic domain of IL-13Rα1 contains two STAT3 binding motifs (Y402 and Y405, (Orchansky, et al., 1999)). STAT3 activation by IL-4 and IL-13 is dependent upon expression of the IL-13Rα1 (Orchansky, et al., 1999) and occurs in response to IL-4 and IL-13 in human B-cells (Rolling, et al., 1996) and weakly in HMVEC-L and NHLF cells (Wang, et al., 2004). STAT3 phosphorylation was induced by IL-13 in primary human monocytes (Roy, et al., 2002) and by IL-4 in keratinocytes (Wery-Zennaro, et al., 1999). We have observed the relatively weak induction of STAT3 phosphorylation by IL-4 and IL-13 in murine bone-marrow-derived macrophages and the human lung adenocarcinoma cell line A549 (LaPorte, et al., 2008). The specific function of STAT3 in mediating responses to IL-4 and IL-13 is unclear. A recent report demonstrated that STAT3 played a role in Th2 differentiation, however the cytokines responsible for STAT3 activation in that setting were thought to be IL-6 or IL-21 (Stritesky, et al., 2011).

### 3.3.5 Other pathways activated by IL-4Rα (Ras/MAPK, Shc, Dok)

Activation of the Ras/MAPK pathway is not generally observed in response to IL-4 despite IRS activation leading to interaction with the adapter molecule, Grb2 (Pruett, et al., 1995, Wang, et al., 1995). Since Grb2 is constitutively associated with the guanine nucleotide exchange protein, SOS, that catalyzes exchange of GDP bound to Ras for GTP, it is often assumed that IL-4 will trigger the phosphorylation cascade of Raf/MEK/ERK-1/-2. However, IL-4 does not activate p21ras (Duronio, et al., 1992, Satoh, et al., 1991, Welham, et al., 1994), Raf1 or ERK1/2 (Welham, et al., 1992, Welham, et al., 1994). We too have been unable to detect ERK-1/2 activation in response to IL-4 or IL-13 in primary mouse bone marrow-derived macrophages (Heller, et al., 2008) or peripheral blood eosinophils. Activation of the Ras/MAPK pathway was demonstrated to enhance IL-4 signaling, possibly through MEK phosphorylation of JAK1 and STAT6 (Yamashita, et al., 1999). Activation of Shc can be linked to Ras activation, via the Grb2 adapter-Sos interaction. There are three widely-expressed Shc proteins (~46, 52 and 66 kDa) containing a C-terminal SH2 domain, regions homologous to the α1 chain of collagen (Pelicci, et al., 1992) and an N-terminal PTB domain, similar in structure to IRS protein PTB domain (Zhou, et al., 1996). Shc moves to the plasma membrane and docks to the phosphorylated I4R motifs on activated IL-4 receptors via its SH2 and PTB domains (Wolf, et al., 1995). Phospho-Shc is then bound by the SH2/3 motifs of the Grb2 adaptor and bound Sos is activated. Shc activation in response to IL-4 appears to be dependent on cell type (Crowley, et al., 1996, Wery, et al., 1996, Welham, et al., 1994).

Two hematopoietically-expressed members of the Dok proteins play a role in IL-4R-induced responses. The N-terminal PH and PTB domains of the Dok proteins suggest that they could bind phosphotyrosines in membrane-localized receptors (Mashima, et al., 2009). Indeed, Dok-2 (also known as FRIP, Dok-R or p56(dok)) has been shown to interact with the I4R motif of the IL-4Rα (Nelms, et al., 1998). T-cells from Dok-2-deficient mice (the hairless allele, hr/hr) have an increased proliferative response to IL-2 and IL-4 (Nelms, et al., 1998). Studies of Dok-1 (p62(dok))-deficient cells suggest this protein plays a positive role in sustaining IL-4
signaling responses (IL-4-induced T-cell proliferation and CD23 expression and IgE class switching in B-cells (Inoue, et al., 2007)). The Dok proteins have multiple docking sites for SH2-containing proteins in their C-terminus and thus act as adaptor proteins. Because both Dok proteins bind RasGAP, which inactivates Ras by hydrolysis of GTP, it is thought that they might be negative regulators of the Ras/MAPK pathway. Recruitment of the Dok proteins to the IL-4Rα may explain the lack of Ras activation by IL-4 in some cell types.

3.4 Negative regulation of receptor signaling

3.4.1 SHP-1 and SHP-2

Protein tyrosine phosphatases (PTP) that remove phosphate groups from phosphotyrosine residues that are activating signals can downregulate the signaling cascades initiated by IL-4. SHP-1 and SHP-2 are PTP molecules containing two N-terminal SH2-domains, a single central phosphatase domain and C-termini with two potential tyrosine phosphorylation sites that affect activity. The SH2-domain targets the phosphatase to a particular cellular location and binds to and inhibits the catalytic domain when the phosphatase is not bound to substrate. The expression of SHP-1 is restricted to hematopoietic cells (Yi, et al., 1992) and low expression is also found in epithelial cells. SHP-2, on the other hand, is widely expressed including in cells that express SHP-1.

SHP-1 may positively (White, et al., 2001) or negatively (Imani, et al., 1997) regulate IL-4 responses, depending upon the cell type (Huang, et al., 2005). Interestingly, SHP-1 constitutively associates with the IL-4Rα chain even in resting lymphocytes (Huang & Paul, 2000). Subsequent studies have indicated that SHP-1 also negatively regulates signaling responses to IL-13 by downregulating the phosphorylation of STAT6 (Haque, et al., 1998).

SHP SH2-domains bind to ITIM sequences [I/V/L]xY(p)x[I/V/L] in activated receptors (Ravetch & Lanier, 2000). The cytoplasmic domain of the IL-4Rα possesses a putative ITIM surrounding the fifth tyrosine, Y713 (Figure 2) and a variety of studies have indicated this motif may interact with SHP-1, SHP-2, Shc and SHIP (Kashiwada, et al., 2001, Hershey, et al., 1997, Kruse, et al., 2002). Our group showed that Y713 in human IL-4Rα mediated recruitment of SHP-1 (Hanson, et al., 2003) and SHIP (Zamorano & Keegan, 1998) to the IL-4 receptor complex. Knock-in of a Y713F mutant of IL-4Rα in mice resulted in enhanced STAT6 phosphorylation, IgE production, and allergic lung inflammation (Tachdjian, et al., 2010). Loss of Y713 had a more dramatic effect on the magnitude of responses to IL-13 vs. IL-4. Taken together these results suggest that IL-4 and IL-13 signaling is modulated by SHP1 or other phosphatases capable of binding to Y713, and that this modulation may be more profound for the Type II receptor complex (Figure 2).

While tyrosine phosphorylation of SHP-1 increases its phosphatase activity, tyrosine phosphorylation of SHP-2 has been proposed to allow this molecule to function as an adaptor protein by providing docking sites for other SH2-domain-containing proteins (Lorenz, 2009). Conflicting data describe that SHP-2 was (Kruse, et al., 2002, Wang, et al., 1999) or was not (Gadina, et al., 1999) tyrosine phosphorylated in response to IL-4 stimulation. IL-13 stimulation of PBMC also induced tyrosine phosphorylation of SHP-2 (Kruse, et al., 2002). SHP-2 interacts with IRS-1 (Xiao, et al., 2002), JAK1, JAK3 and coprecipitates with Grb-2 and p85 after cytokine stimulation (Gadina, et al., 1998, Kuhne, et al., 1993). Peptides derived from the IL-4Rα (aa 545–558) were able to pull down SHP-2 from lysates of IL-13-stimulated PBMC (Kruse, et al., 2002).
Hematopoietically-expressed SH2-domain-containing inositol 5’-phosphatase (SHIP) dephosphorylates PIP3, the product of the PI3-K enzyme, to form PIP2. IL-4 stimulation can induce tyrosine phosphorylation of SHIP, suggesting that SHIP can dock to multiple sites in the IL-4Rα (Zamorano & Keegan, 1998). SHIP-1-deficient mice spontaneously developed allergic lung inflammation, have increased mast cells that spontaneously released histamine indicating a potential homeostatic role for SHIP-1 in regulating Th2-responses in vivo (Oh, et al., 2007). Recent exciting data have defined a role for SHIP-1 in the skewing of macrophage phenotype (Rauh, et al., 2005).

3.4.2 Suppressors of Cytokine Signaling (SOCS)
The suppressor of cytokine signaling (SOCS) proteins are a family of cytokine-induced negative regulators of cytokine signaling (Starr, et al., 1997, Yoshimura, et al., 2003). The general structure of the SOCS protein includes a central SH2-domain, critical for binding to their tyrosine phosphorylated substrates, and a C-terminal SOCS box that mediates ubiquitin-dependent proteolysis. SOCS-1, -3 and CIS are induced by IL-4 and SOCS-1 and -3 were shown to inhibit IL-4 signaling transduction (Haque, et al., 2000, Losman, et al., 1999). SOCS-1 and SOCS-3, both of which are induced by IL-4, have an additional kinase inhibitory region that functions as a pseudosubstrate to inhibit JAK activity (Yasukawa, et al., 1999). Another mechanism of action is by SOCS interaction with the phosphorylated tyrosines within the cytoplasmic domains of the receptor. SOCS-3 directly interacted with IL-4α (O’Connor, et al., 2007). SOCS proteins can also target activated signaling intermediates to the proteasome. SOCS-1 is able to regulate the half-life of JAKs and insulin/IGF-I-induced IRS-2 (Rui, et al., 2001) in this manner.

4. Differential roles for IL-4 and IL-13 acting via the Type I or Type II receptors on features of allergic lung inflammation
Recent evidence suggests that even though IL-4 and IL-13 share receptor components and signaling proteins, and elicit overlapping responses in vitro, they can elicit different functional responses in vivo. IL-4 is primarily responsible for regulating Th2 development and inflammation while IL-13 is responsible for effector activities such as airway hypersensitivity, collagen production, and mucus hypersecretion (Gavett, et al., 1994, Pernis & Rothman, 2002, Wills-Karp, et al., 1998). The molecular basis for this variation is not understood clearly, since both IL-4 and IL-13 use the Type II receptor complex. It has been postulated that differences in the relative abundance of the Type I or Type II receptor subunits in different cell types may be responsible for the differences in responses elicited by IL-4 versus IL-13. Certainly the presence or absence of individual receptor subunits and appropriate Janus kinases in each cell determines whether a cell can respond to IL-4 or IL-13. However, many of the cell types involved in the effector activities express the Type II receptor that is activated by both IL-4 and IL-13.

4.1 Different receptor signaling pathways utilized by IL-4 and IL-13 via the Type I and Type II receptors
One reason proposed for differential functions of these cytokines is the observed differences in the amounts of IL-4 and IL-13 produced in tissues during Type II inflammation. Various reports have shown that IL-13 is secreted by large number of cell types and in much greater quantities than IL-4 during Th2 responses in both asthma patients (Huang, et al., 1995) as
well as mouse models of this disease (Munitz, et al., 2008). However, analysis of the binding affinities of the Type I and Type II receptors with their respective ligands have shown that the relative amounts of each cytokine does not necessarily explain the functional differences between IL-4 and IL-13.

As discussed above, LaPorte and colleagues have shown that although IL-4 binding to the IL-4Rα chain occurs with high affinity, complex formation of IL-4: IL-4Rα with γC or IL-13Rα1 is quite unstable and inefficient (LaPorte, et al., 2008). On the other hand, IL-13 binds to IL-13Rα1 with low affinity, but the interaction of IL-13: IL-13Rα1 with IL-4Rα is more favorable and stable. As a result even at very low concentrations, IL-4 is able to mediate efficient and rapid STAT6 phosphorylation via Type I and Type II receptors, while cells have to be stimulated with much higher concentrations of IL-13 and for a longer time to obtain similar responses via the Type II receptor. Since the IL-4 bound complexes are less stable, LaPorte et. al. proposed that when expression of receptor chains in cells become limiting, IL-4 responses would be limited, while IL-13 responses would take over.

However, experiments using transgenic overexpression of large quantities of IL-4 or IL-13 still showed differences in the pathophysiology elicited by these two cytokines (Rankin, et al., 1996, Zhu, et al., 1999). These results suggest that there are real signaling differences between IL-4 and IL-13. To analyze potential signaling differences, we undertook a careful, side-by-side comparison of primary cells and cell lines that expressed either both Type I and II receptors or Type II receptors only. IL-4 stimulated tyrosine phosphorylation of STAT6 in the human airway epithelial cell line, A549, and the human B-cell line, Ramos, at significantly lower doses than IL-13. We demonstrated that IL-4 signaling through the Type I receptor induced robust tyrosine phosphorylation of the downstream adaptor protein IRS-2 and greater expression of the mRNAs for a subset of alternatively activated macrophage genes in primary mouse bone marrow-derived macrophages (BMM) (Heller, et al., 2008). This was in contrast to IL-4/IL-13 signaling through the Type II receptor which resulted in weaker tyrosine phosphorylation of IRS-2 and less mRNA for the AAM genes studied. This marked difference in IRS-2 phosphorylation and AAM gene expression induced by IL-4 was dependent upon expression of the γC subunit.

4.2 Differential functions of IL-4 and IL-13 in allergic lung inflammation

In both humans and mice, IL-4 and IL-13 signaling through the Type I and Type II receptors play a critical role in inducing asthma. The hallmark features of this disease include excessive pulmonary inflammation, periodic narrowing of airways, airway hyperresponsiveness (AHR) and enhanced mucus secretion. IL-4 and IL-13 have differential roles in asthma pathogenesis. Studies using IL-4Rα−/− and STAT6−/− mice in our lab and by other investigators have suggested that many of the asthma symptoms mentioned above are regulated by IL-4Rα and STAT6 (Cohn, et al., 1997, Corry, et al., 1998, Grunig, et al., 1998, Kelly-Welch, et al., 2004, Kuperman, et al., 1998, Mathew, et al., 2001, Wills-Karp, et al., 1998). However, since IL-4/IL-13 binding to either the Type I or Type II receptor activates STAT6, the contributions of these individual pathways in inducing the pathophysiology associated with this disease was unclear, until recently.

It is known that IL-4 is predominantly required for Th2 cell differentiation and proliferation (Kaplan, et al., 1996). Since most naive T cells lack the Type II receptor, they are unresponsive to IL-13. IL-4 signaling through the Type I IL-4R/STAT6 axis upregulates GATA3, the Th2 master transcription factor (reviewed in (Zhu & Paul, 2008)). STAT6-deficient T cells cannot differentiate into Th2 cells in vitro (Kaplan, et al., 1996). Recent
studies have shown that STAT6 is not required for in vivo differentiation, although it is required for stabilization of Th2 cells and generating memory responses (reviewed in (Chapoval, et al., 2010)). Activated Th2 cells then secrete large quantities of IL-4, IL-5 and IL-13 which can act on many different cell types. IL-4 induces expression of MHC Class II in resting B cells and also causes antibody class switching from IgM to IgE and IgG1 (reviewed in Nelms, et al., 1999). Treatment with anti-IL-4 antibody blocks both primary and secondary IgE responses in vivo, when administered at the time of antigenic challenge (Finkelman, et al., 1988). IL-13 on the other hand is thought to be responsible for causing AHR, excessive mucus production and lung fibrosis. Neutralization of IL-13 was able to completely reverse allergen induced airway resistance and abolished mucus production by airway epithelial cells seen in control mice (Grunig, et al., 1998, Wills-Karp, et al., 1998).

Apart from its action on lymphocytes, IL-4 also activates mast cells. This cytokine enhances surface expression of FcεRI, the high affinity receptor for IgE (Toru, et al., 1996). Binding of IgE to FcεRI causes crosslinking of the cytoplasmic Fc domain of this receptor and triggers degranulation (release of mast cell granules). This process causes rapid release of many inflammatory mediators such as histamine, leukotrienes and prostaglandins (reviewed in (Weller, et al., 2011)). Histamine increases blood circulation and permeability of blood vessels, causing increased recruitment of inflammatory cells, including eosinophils, T cells, dendritic cells and monocytes. Leukotrienes and prostaglandins promote bronchoconstriction and stimulate epithelial cell induced mucus production. The importance of FcεRI in allergic responses has been demonstrated in studies using a soluble form of FcεRI and mice lacking the α chain of this receptor (Dombrowicz, et al., 1993, Ra, et al., 1993). In both cases IgE-mediated allergic responses were abrogated.

In addition to mast cells, eosinophils are closely associated with asthma pathogenesis. Increased numbers of eosinophils in the lung and other tissues in asthmatic patients usually correlate with disease severity and it is thought to be the central effector cell involved in airway inflammation (reviewed in (Hogan, et al., 2008)). IL-5 plays an important role in eosinophil development, proliferation and survival in the bone marrow. It is also required for migration of eosinophils into the blood and subsequently the lung. Recruitment of eosinophils to the peribronchial regions of the lung is thought to be mediated by secretion of various eotaxins (eotaxin 1, 2 and 3) by airway epithelial cells. Moreover, IL-5 and the eotaxins cooperate to induce tissue eosinophilia. Various eosinophilic granule components such as major basic protein (MBP) and eosinophilic cationic protein (ECP) have been implicated in initiating and propagating many features of asthma including pulmonary inflammation, airway hyperresponsiveness and bronchoconstriction. Eosinophils express both the Type I and Type II receptors. We have shown that IL-4, but not IL-13, can enhance chemotaxis of eosinophils to eotaxin 1 in vitro through the Type I receptor (Heller and Keegan, unpublished). Studies using IL-13Rα1−/− mice have shown that while eotaxin production and secretion by epithelial cells was completely dependent on IL-13 signaling through the Type II receptor, recruitment of eosinophils into the lungs was not (Munitz, et al., 2008, Ramalingam, et al., 2008). Therefore, it is possible that the Type I receptor is compensating for the absence of the Type II receptor. Alternatively, IL-5 may be playing a role in this response. Unlike hematopoietic cells, epithelial, endothelial and smooth muscle cells contain only the Type II receptor. Although both IL-4 and IL-13 can bind to this receptor complex, IL-13 is considered to be the main effector cytokine responsible for AHR, excessive mucus production and lung fibrosis.
The unique contributions of the Type II receptor in allergic lung inflammation have been studied using IL-13Rα1/− mice. Mucus secretion, airway resistance, eotaxin production and induction of pro-fibrotic mediators such as TGFβ were completely dependent on the IL-13Rα1 chain, and thus the Type II receptor (Munitz, et al., 2008, Ramalingam, et al., 2008). However, the authors showed that Th2 cell differentiation, IgE secretion in response to T cell dependent antigens (such as ovalbumin) and recruitment of eosinophils and other inflammatory cells into the lungs could occur independently of IL-13Rα1. In addition, DNA microarray analysis of cells isolated from allergen or IL-4 treated WT or IL-13Rα1/− mice indicated that several AAM genes were differentially regulated. Munitz et.al. showed that allergen and IL-4 induced Retnla expression levels were similar in both WT and IL-13Rα1/− mice, but induction of chitinase (Chia) was completely dependent on IL-13Rα1 (Munitz, et al., 2008). Interestingly enough, allergen induced arginase 1 expression required the Type II receptor, but IL-4 induced arginase 1 expression did not. Thus, it appears that IL-4 utilizes both the Type I and Type II receptors to stimulate AAM development in the lung.

Studies conducted by us as well as other groups have shown that IL-4 preferentially induces robust AAM gene expression, while IL-13 does so only weakly (Heller, et al., 2008, Munitz, et al., 2008, Ramalingam, et al., 2008). Intriguingly, mutation of the ITIM motif in the IL-4Ra chain resulted in increased sensitivity of macrophages to IL-13 mediated AAM activation. As demonstrated earlier, the Y709 (WT) BMMs treated with IL-4 led to significantly higher expression of AAM genes (Arginase1, Chi3l3) and also Ccl11 in contrast to IL-13. Mutation of Y709 residue to F709 resulted in dramatic amplification of Arginase1, Chi3l3 and Ccl11 genes in response to IL-13, while leaving the IL-4 induced responses intact or slightly enhanced (Tachdjian, et al., 2010). IL-13 but not IL-4 induced similar responses in primary lung fibroblasts. These results suggest that there is a disproportionate increase in AAM activation induced by IL-13 signaling via the Type II receptor. The authors hinted that differential recruitment of SHP-1 by the Type I and Type II receptors may be the reason behind these observations (Figure 2). Although much progress has been made in understanding the mechanisms by which IL-4 and IL-13 may elicit different responses in different cell types and in the lung during allergic diseases and asthma, many more questions remain unanswered. Future research in this area will shed light on the molecular basis of the separation of functions of IL-4 and IL-13 and their consequences in vivo.

4.3 Single nucleotide polymorphisms leading to amino acid changes are commonly found in the IL-4Ra

Commonly occurring genetic polymorphisms leading to amino acid changes in the IL-4Ra have been linked to susceptibility to asthma and/or to asthma severity (Hershey, et al., 1997, Ober, et al., 2000, Risma, et al., 2002, Shirakawa, et al., 2000). The E400A, Q576R and the S503P polymorphisms reside in the cytoplasmic domain while the I50V resides in the extracellular domain of the IL-4Ra chain. Two of the polymorphisms located in the cytoplasmic domain, S503 to P and the Q576 to R, are frequently linked (Kruse, et al., 1999). This double mutation (S503P/Q576R) was associated with lower total IgE concentrations, similar to the single S503P polymorphism, and an increase in the phosphorylation of IRS2. E400A is especially prevalent in African-American populations and was associated with severe asthma exacerbations (Wenzel, et al., 2007). An I50 in the extracellular domain of the IL-4Ra chain was linked with enhanced signal transduction culminating in an increase in the production of IgE (Mitsuyasu, et al., 1998, Mitsuyasu, et al., 1999). On the other hand, several other studies reported no correlation of this polymorphism with enhanced IgE levels.
in patients (Khoo, et al., 2006, Noguchi, et al., 1999). Furthermore, the V<sup>50</sup> polymorphism has been linked with enhanced CD23 expression, an increase in atopic asthma, and an increase in allergic bronchopulmonary aspergillosis (Knutsen, et al., 2006, Risma, et al., 2002). More recently the V<sup>50</sup> polymorphism reduced the ability of IL-4 to suppress IL-17 production by human peripheral mononuclear cells (Wallis, et al., 2011). Because many of these polymorphisms are located in the cytoplasmic domain of the IL-4Rα, many investigators have hypothesized that they modulate signal transduction. However, experiments designed to analyze the direct effects of these polymorphisms on receptor signaling have lead to contradictory reports (Franjkovic, et al., 2005, Kruse, et al., 1999, Mitsuyasu, et al., 1998, Mitsuyasu, et al., 1999, Prots, et al., 2006, Risma, et al., 2002, Stephenson, et al., 2004).

We analyzed the impact of the Q576R, S503P, and I50V polymorphisms on signal transduction by the Type I receptor complex in vitro. While the R<sup>576</sup> and P<sup>503</sup> polymorphisms had no effect on STAT6 or IRS2 activation induced by IL-4 (Wang, et al., 1999), we found that the V<sup>50</sup> polymorphism located in the extracellular domain of the IL-4Rα mediated a prolonged STAT6 signaling induced by IL-4 through the Type I receptor (Ford, et al., 2009). This was associated with a prolonged expression of the SOCS family member Cis. The effect of this polymorphism on signaling by the Type II receptor is unknown.

Using mouse knock-in strategies, the murine IL-4Rα-Q<sup>576</sup> was replaced with IL-4Rα expressing the R<sup>576</sup> polymorphism (Tachdjian, et al., 2009). This change was shown to enhance allergic asthma in vivo. The IL-4Rα-R<sup>576</sup> enhanced Th2 differentiation and IgE production; both of these responses are Type I receptor dependent in the mouse model. Furthermore, it enhanced the production of CCL11 by BMM, fibroblasts, and tracheal epithelial cells (TEC) in response to IL-4 or IL-13. These results suggest the R<sup>576</sup> polymorphism affects STAT6-dependent responses down stream of both the Type I and Type II receptors. However, there was no apparent effect of the R<sup>576</sup> on the tyrosine phosphorylation of STAT6 (Tachdjian, et al., 2009), consistent with our study in cell lines (Wang, et al., 1999). Furthermore, there was no effect on the tyrosine phosphorylation of Shc. The effect of the R<sup>576</sup> on IRS2 phosphorylation was not reported. Interestingly, the longevity of Erk1,2 phosphorylation in TEC was dramatically enhanced by R<sup>576</sup>, however the mechanism by which R<sup>576</sup> leads to Erk phosphorylation is still unclear. It will be important to understand the effects of the polymorphisms and their interactions on both IL-4 and IL-13 signaling since they are linked to different clinical phenotypes in human populations (Wenzel, et al., 2007).

5. Contribution of Type I and Type II receptor complexes to the control of regulatory mechanisms that act to modulate allergic inflammation

During Th2-driven allergic lung inflammation, a number of effector and regulatory mechanisms are orchestrated by IL-4 and IL-13 through Type I and Type II receptors. Several of these regulatory mechanisms allow for the amplification of Th2 differentiation and function, while others function as part of a negative feed-back loop to limit Th2-driven inflammation (Figure 3). Just as IL-4 and IL-13 are differentially involved in promoting various features of allergic lung disease, these cytokines utilize separate mechanisms to negatively regulate the signaling pathways activated by each other. These regulatory mechanisms will need to be considered in the design of inhibitors of the IL-4/IL-13 system.
Fig. 3. Differential control of regulatory mechanisms by the Type I and II Receptors. IL-4 signaling through the Type I receptor on Th2 cell surfaces renders them resistant to control by T regulatory cells. Furthermore, IL-4 signaling STAT6 activation through the Type I receptor on naive CD4+ T-cells inhibits the differentiation of iTregs and Th1 cells. These responses lead to enhanced allergic inflammation. On the other hand, IL-4 signals through the Type I receptor on NK cell surfaces to induce IFNγ production. IFNγ in turn suppresses Th2 differentiation and inhibits signaling by both Type I and Type II receptors by inducing SOCS family members. IL-13 signals through the Type II receptor that is induced on the surface of Th17 cells and decreases IL-17 production. This suppression of IL-17 production by Th17 cells would limit the influx of neutrophils that are present in steroid resistant asthma.

5.1 Type I IL-4 receptor modulation of regulatory T-cells

Signaling through the Type I IL-4 receptor antagonizes the differentiation and function of regulatory T cells (Tregs). Tregs are a subset of T lymphocytes that regulate immune responses and prevent excessive immune system activation (Sakaguchi, et al., 2008, Shevach, 2009). The most studied Tregs are CD4+CD25+FoxP3+, which have been found to play a role in allergic disease. FoxP3 is a transcription factor important for Treg development and function. Mice lacking FoxP3 expression (Scurfy mice) or humans with mutations in their foxP3 gene (X-linked autoimmunity-allergic dysregulation syndrome) develop widespread autoimmune disease with a Th2-mediated allergic component (Khattri, et al., 2003). There are many mechanisms elicited by Tregs to suppress effector T cells, such as: (1) cell-mediated presentation of TGF-β or galectin-1 (Shevach, 2009), (2) secretion of the immunosuppressive cytokines IL-10 or TGF-β (Bettini & Vignali, 2009), or (3) consumption of IL-2, a limiting growth factor for T cells (Scheffold, et al., 2007). Th2 cells utilize several
methods to inhibit immunosuppression by Tregs. Gata3 is the master transcriptional regulator of Th2 cells and is activated by signal transduction through the Type I IL-4 receptor. Using co-immunoprecipitation assays, Dardalhon and colleagues have shown physical interaction between Gata3 and FoxP3 in transiently transfected human embryo kidney 293 cells. The authors also observed IL-4 inhibition of FoxP3 induction in Ag-specific adaptive (inducible) Tregs, which was dependent on STAT6 expression (Dardalhon, et al., 2008). Our recent findings are in support of this antagonistic relationship between STAT6 and Tregs and IL-4-induced, STAT6-dependent inhibition of FoxP3. We have found that STAT6⁻/⁻ mice have higher numbers of Tregs than wildtype mice (Chapoval, et al., 2011). This observation has also been confirmed by Takaki and colleagues who identified a STAT6-binding site in the silencer region in the FoxP3 mRNA transcript. STAT6 binding to this site reduced TGF-β1 induction of FoxP3 transcriptional activation (Takaki, et al., 2008).

Additionally, IL-4 was found to serve as a survival factor for CD4⁺CD25⁻ T helper (Th) cells and to aid in their protection from immunosuppression by CD4⁺CD25⁺ Tregs (Pace, et al., 2005). IL-13 failed to have this same effect. Therefore, this phenomenon was found to be dependent on Th cell surface expression of IL-4Rα chain and a functional Type I IL-4 receptor complex, as IL-4Rα⁻/⁻ Th cells were not protected by IL-4 from Treg immunosuppression. IL-4 activation of the Type I IL-4 receptor, but not the Type II receptor, maintains anti-apoptotic and pro-proliferative processes in Th cells and protects them from Treg-induced perturbed cell growth and proliferation. Surprisingly, CSFE-labeled cocultures of IL-4Rα⁻/⁻ Th cells and IL-4Rα⁺/+ Tregs revealed that although IL-4 has on effect on Treg immunosuppression of IL-4Rα⁻/⁻ Th cells, it promoted proliferation of Tregs in-vitro (Pace, et al., 2006). This could be a direct effect of IL-4 that would further complicate the role of this Th2 cytokine in modulating Treg immunosuppression.

5.2 Role of Type I receptors in control of Th1 responses that act to suppress allergic inflammation

Th1 cell programming antagonizes Th2 cell differentiation and could serve as a regulatory mechanism to suppress Th2-mediated allergic response. Gavett and colleagues showed that IL-12 can inhibit antigen induced-airway hyperreactivity and inflammation and to also reduce Th2 cytokine production (Gavett, et al., 1995). Th2 cells inhibit Th1 cell induction during the allergic response. Gata3 is activated downstream of STAT6 phosphorylation, which is induced by signaling through the Type I receptor (Kurata, et al., 1999, Nelms, et al., 1999). Not only does it serve as the master regulator transcription factor (TF) for Th2 cells, but Gata3 has also been shown to inhibit Th1 cell-specific factors. Gata3-deficient cell clones produced high levels of the Th1 cytokine, IFNγ, and had enhanced expression of T-bet (the Th1 cell master regulator TF) (Zhu, et al., 2006). Therefore, activation of the Type I IL-4 receptor could lead to activation of Gata3, which in turn inhibits Th1-inducing factors during the Th2-mediated allergic response. Th1 cells have also been shown to play a potentially stimulatory role in airway inflammation. Hansen et al. found that Th1 cells decreased airway eosinophilia, but failed to reduce airway hyperreactivity in ovalbumin-immunized Balb/c mice (Hansen, et al., 1999). But this may be a late phenomenon in airway inflammation during which additional non-Th2 inflammatory cells aid in amplifying chronic lung inflammation. This would be in direct contrast to the initiation of allergic airway inflammation which is characterized by strong Th2 immune responses and can be inhibited by non-Th2-promoting immune cells.
5.3 Modulation of NK cells
Although IL-4 and IFN\(_\gamma\) are antagonistic towards each other during T cell differentiation, IL-4 can increase IL-2 and IL-12 induced IFN\(_\gamma\) secretion by Natural Killer (NK) cells. Bream and co-authors also found that the increase in IFN\(_\gamma\) production caused by IL-4 in conjunction with IL-2 was STAT6 dependent, while IL-4 synergy with IL-12 was independent of STAT6 activation (Bream, et al., 2003, Morris, et al., 2006). Further studies have shown that IL-13 was unable to cause a similar increase in NK cell derived IFN\(_\gamma\) release (Morris, et al., 2006). This result is in agreement with the fact that IL-13 signals through the Type II receptor, and this receptor complex is absent in NK cells. IL-4 stimulated IFN\(_\gamma\) production by NK cells has significant implications in the context for allergic lung inflammation. One group has shown that Sendai virus infection of mice suppressed NK cell derived IFN\(_\gamma\) secretion. This led to enhanced Th2 responses and subsequent development of exacerbated allergic lung disease (Kaiko, et al., 2010). IFN\(_\gamma\) production would suppress Th2 differentiation and inhibit signaling by both Type I and Type II receptors by inducing SOCS family members.

The role of NK cells in human allergic disease has been extensively examined; because of their ability to produce cytokines, NK cells have the potential to heavily influence the adaptive allergic immune response. Based on cytokine production, NK cells can be divided into 2 classes: (1) NK1 cells produce Th1 cytokines, such as IFN\(_\gamma\) (Romagnani, 1992) and (2) NK2 cells produce Th2 cytokines IL-4, 5, and 13 (Hoshino, et al., 1999, Peritt, et al., 1998, Warren, et al., 1995). In a recent study analyzing NK cell populations in healthy and allergic patients, the authors found a predominance of NK2 cells in the peripheral blood of allergic patients. These NK2 cells produced high amounts of Th2 cytokines that could promote allergic inflammation (Timonen & Stenius-Aarniala, 1985, Wei, et al., 2005).

5.4 IL-13 inhibition of Th17 cells via the Type II receptor in severe asthma
It has long been thought that naïve T cells do not express the IL-13R\(_\alpha\)1 chain of the Type II receptor complex and therefore cannot be regulated by IL-13. Due to restricted expression of IL-13R\(_\alpha\)1 on non-hematopoetic cells, Type II receptor signaling has been limited to those cells and not seen in T cells. But this central dogma has been recently challenged by observations that Th17 cells are able to induce surface expression of IL-13R\(_\alpha\)1 chain (Newcomb, et al., 2009, Newcomb, et al., 2011). Th17 cells are a distinct population of CD4+ T cells, whose differentiation is induced by IL-6 or IL-21 and TGF\(\beta\) (McGeachy, et al., 2007). Th17 cells produce IL-17, IL-6, and tumor necrosis factor. They have been shown to play a role in autoimmune diseases including the experimental autoimmune encephalitis (EAE) model of multiple sclerosis (Langrish, et al., 2005). Th17 cells also provide protection from some extracellular pathogens, such as Klebsiella pneumoniae infection of the lung (Happel, et al., 2005).

A statistically higher number of IL-17 + cells can be found in the sputum and BAL of asthmatic patients compared to controls and the greater expression of IL-17A in the lungs was associated with increased asthma severity (Jatakanon, et al., 1999, Molet, et al., 2001). IL-17A induces neutrophil recruitment to the airway and augments the pathogenesis of steroid-resistant, severe asthma (McKinley, et al., 2008). Th17 cells alone cannot induce eosinophilic infiltration into the airway following immunization and challenge, but in the presence of Th2 cells, antigen-specific Th17 cells can enhance the eosinophil-activating properties of Th2 cells (Wakashin, et al., 2008).
Th17 polarized cells from mouse spleens were shown to have increased mRNA and protein levels of IL-13Rα1 after stimulation in vitro. When added to Th17 cell cultures, IL-13 reduced IL-17A production by Th17 cells and decreased the percentage of CD4+ Th17 cells. Additionally, IL-13 caused a reduction in the expression of RORγt, the master regulator transcription factor for Th17 cells (Newcomb, et al., 2009). This phenomenon of IL-13 suppression of IL-17A production by Th17 cells was also observed in vitro using human CD4+ T cells (Newcomb, et al., 2011). Therefore, activated Th17 cells upregulate their surface expression of IL-13Rα1 chain and this allows for IL-13 to signal through a functional Type II receptor complex to decrease IL-17 production by Th17 cells. Thus, paradoxically, IL-13, a major effector cytokine of atopic asthma, inhibits the Th17 component of severe asthma. This concept could also explain the observation that IL-25-induced production of IL-13 inhibited Th17-mediated EAE disease progression (Kleinschek, et al., 2007). This is a unique mechanism whereby a Th2 immune-mediated illness is prevented from becoming more severe by IL-13, a Th2 cytokine. Consequently, efforts to suppress IL-13 function to treat allergic asthma may lead to Th17 induction and severe and persistent asthma in susceptible individuals.

6. Conclusion

The importance of the Type I IL-4 receptor in regulating T cells to become Th2 cells has been well documented. Furthermore, numerous studies have indicated the IL-4Rα expressed on lung epithelium is necessary for goblet cell differentiation and mucus hypersecretion. In addition, the IL-4Rα is expressed on many cell types that could contribute to the overall pathology and severity of asthma. The relative role of the Type I and Type II receptors on these cells has not yet been fully delineated. Using mice lacking one or the other complex (i.e. γC-/- or IL-13Rα1-/-) several groups have recently delineated interesting differences in their contributions to lung pathology. The Type I receptor is the major regulator of eosinophilic inflammation and the alternative activation of macrophages while the Type II receptor controls mucus hypersecretion and airway hyperresponsiveness. These receptors differentially regulate potential regulatory pathways including the control of T regulatory cells and the production of cytokines by NK cells; both of these responses are controlled by the Type I receptor complex. The Type II receptor complex can be induced on Th17 cells and allows IL-13 to down regulate Th17 differentiation. This could be of clinical importance for severe, steroid resistant forms of human asthma that may be mediated by Th17 cells. In this scenario, inhibiting IL-13 could be detrimental to the patient and illustrates the need to stratify patients prior to treatment.

As mentioned above, the intricate differences between the Type I and Type II receptor complexes could impact the therapeutic effectiveness of agents designed to target these receptors in asthma. Indeed initial trials to inhibit IL-4 using the soluble IL-4Rα in asthmatic patients were largely unsuccessful and possibly detrimental (Borish, et al., 2001, Wenzel, et al., 2007). The ability of IL-4 to suppress TNFα production, a highly pro-inflammatory cytokine, was suggested to be part of a negative regulatory mechanism that was unintentionally blocked by the therapy (Borish, 2010). Studies using soluble IL-13Rα2 to inhibit IL-13 signaling are ongoing. However, blocking one cytokine at a time may not prove beneficial. Since both cytokines can elicit effector functions, blocking only one could actually exacerbate disease because of the loss of a negative regulatory pathway. The most promising approach thus far has been to use a mutant IL-4 (Pitrakinra) that binds to the IL-4Rα, and blocks dimerization with either the γC or the IL-13Rα1 (Wenzel, et al., 2007). Thus, this single agent can prevent the formation of both the Type I and the Type II receptor
complexes. Further understanding of the complex IL-4/IL-13 receptor system and its contribution to various features of allergic asthma will be essential to fine-tune therapeutic strategies for the treatment of asthma.

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