The Journal of Immunology

IL-4 Receptor α Is an Important Modulator of IL-4 and IL-13 Receptor Binding: Implications for the Development of Therapeutic Targets

Allison-Lynn Andrews, John W. Holloway, Stephen T. Holgate and Donna E. Davies

---

References This article **cites 48 articles**, 16 of which you can access for free at:
http://www.jimmunol.org/content/176/12/7456.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

IL-4 Receptor α Is an Important Modulator of IL-4 and IL-13 Receptor Binding: Implications for the Development of Therapeutic Targets[1]

Allison-Lynn Andrews,[2]* John W. Holloway,[†] Stephen T. Holgate,* and Donna E. Davies*

IL-4 is a key cytokine associated with allergy and asthma. Induction of cell signaling by IL-4 involves interaction with its cognate receptors, a complex of IL-4Rα with either the common γ-chain or the IL-13R chain α1 (IL-13Rα1). We found that IL-4 bound to the extracellular domain of IL-4Rα (soluble human (sh)IL-4Rα) with high affinity and specificity. In contrast with the sequential mechanism of binding and stabilization afforded by IL-4Rα to the binding of IL-13 to IL-13Rα1, neither common γ-chain nor IL-13Rα1 contributed significantly to the stabilization of the IL-4:IL-4Rα complex. Based on the different mechanisms of binding and stabilization of the IL-4R and IL-13R complexes, we compared the effects of shIL-4Rα and an IL-4 double mutein (R121D/Y124D, IL-4R antagonist) on IL-4- and IL-13-mediated responses. Whereas IL-4R antagonist blocked responses to both cytokines, shIL-4Rα only blocked IL-4. However, shIL-4Rα stabilized and augmented IL-13-mediated STAT6 activation and eotaxin production by primary human bronchial fibroblasts at suboptimal doses of IL-13. These data demonstrate that IL-4Rα plays a key role in the binding affinity of both IL-13R and IL-4R complexes. Under certain conditions, shIL-4Rα has the potential to stabilize binding IL-13 to its receptor to augment IL-13-mediated responses. Thus, complete understanding of the binding interactions between IL-4 and IL-13 and their cognate receptors may facilitate development of novel treatments for asthma that selectively target these cytokines without unpredicted or detrimental side effects. *The Journal of Immunology,* 2006, 176: 7456–7461.

Interleukin-4 is a key cytokine in the development of allergic inflammation through its ability to drive the differentiation of naive Th cells into Th2 lymphocytes, leading to the production of effector cytokines such as IL-4, IL-5, IL-9, and IL-13, but not IFN-γ (1, 2). It is associated with the induction of the ε isotype switch and secretion of IgE by B lymphocytes (3, 4) and enhances IgE-mediated responses by up-regulating IgE receptors on B lymphocytes, mast cells, and basophils (5, 6). IL-4 also induces VCAM-1 on vascular endothelium and thus directs the migration of T lymphocytes, monocytes, basophils, and eosinophils to the inflammation site. In asthma, IL-4 contributes both to inflammation and to airway obstruction through the induction of mucin gene expression and the hypersecretion of mucus (7). It also inhibits eosinophil apoptosis and promotes eosinophilic inflammation by inducing chemotaxis and activation through the increased expression of eotaxin (8, 9). Some of these effects may be mediated by bronchial fibroblasts that respond to IL-4 with increased expression of eotaxin and other inflammatory cytokines (10). Thus, the inhibition of IL-4-mediated effects may have clinical benefits in the treatment of allergic disease.

IL-4 exerts its activities by interacting with specific type I and type II receptors on the cell surface. The type I receptor comprises a 140-kDa binding chain, IL-4Rα and the common γ-chain (γc)[3] of the IL-2R, which is shared by multiple cytokine receptors. In the absence of γc, IL-4 can use the type II IL-4R, comprising IL-4Rα and an IL-13 binding chain, IL-13Rα1 (11–17). This receptor complex is also a functional receptor for IL-13, and this largely explains the overlap of the biological effects of IL-4 and IL-13. Type I receptor complexes can only be formed by IL-4 and appear to be responsible for mediating IL-4 responses in T cells, which do not express IL-13Rα1 (18). Type II receptor complexes can be formed by either IL-4 or IL-13, and they are activated by either IL-4 or IL-13. We have previously described a sequential binding sequence for this receptor complex where IL-13 first binds to IL-13Rα1 and then this complex recruits IL-4Rα to form a high-affinity binding site (19). In the case of IL-4, this sequence of events is reversed, where IL-4 first binds to IL-4Rα with high affinity before associating with the second subunit.

Soluble IL-4Rs (sIL-4Rs) are present in biological fluids. sIL-4R contains only the extracellular portion of IL-4R and lacks the transmembrane and intracellular domains. It is not known whether human sIL-4R is actively produced during an immune response. In vitro, sIL-4R blocks B cell binding of IL-4, B cell proliferation, and IgE and IgG1 secretion (20). In vivo, sIL-4R inhibits IgE production by up to 85% in anti-IgD-treated mice (21) and reduces airway inflammation, suggesting that sIL-4R may be useful in the treatment of IgE-mediated inflammatory diseases such as asthma. To this end, nebulized recombinant soluble human (sh)IL-4R has been evaluated as a therapy for asthma (22, 23). One potential disadvantage of shIL-4R as a treatment is that it does not block the effects of IL-13, because, in asthma, the effects of allergic inflammation are mediated by both IL-4 and IL-13. Furthermore, IL-13 appears to have a distinct role in the inflammatory and remodeling process observed in the asthmatic lung.

[3] Abbreviations used in this paper: γc, common γ-chain; s, soluble; h, human; IL-4RA, IL-4R antagonist; SPR, surface plasmon resonance.

An alternative approach to block IL-4-mediated effects has been the use of IL-4R antagonists (IL-4RA). An IL-4RA, a double mutein of IL-4 (R121D/Y124D), has been developed by Bayer (BAY 16-9996) for s.c. application for the therapy of asthma and is currently in phase II clinical trials. Within the four helix bundle of IL-4, tyrosine (Y124), on the D helix, forms an important contact with the γ_c. When Y124 is mutated to aspartic acid (Y124D), the resulting molecule is an IL-4RA that binds to the IL-4R with the same affinity as the wild-type IL-4 but has only 0.5% of the agonistic activity (24). Similarly, the arginine at position 121 of IL-4 interacts with IL-13Rα1; thus, the double mutein R121D/Y124D is able to antagonize the activity of IL-4 as well as that of IL-13 (25, 26).

In this report, we characterize the binding interactions that occur during the formation of liganded IL-4R complexes using surface plasmon resonance (SPR). These results demonstrate that IL-4Rα makes an important contribution to the overall binding affinity of both IL-4R and IL-13R complexes. Based on the different mechanisms of binding and stabilization observed for the IL-4R and IL-13R complexes, we compared the effects of shIL-4Rα and IL-4RA on IL-4- and IL-13-mediated responses and report a novel agonist activity for shIL-4Rα in IL-13-mediated responses.

Materials and Methods

Reagents

CM5 sensor chips, HBS buffer (10 mM HEPES with 0.15 M NaCl, 3.4 mM EDTA, and 0.005% surfactant P20), amine coupling kit, and regeneration agents were supplied by BIAcore, unless otherwise stated. IL-8 ELISA kits were obtained from BioSource International. SDS-PAGE reagents were obtained from Amersham Biosciences. Eotaxin ELISA kits, recombinant shIL-13Rα1.Fc, shIL-2γ (γ_c), and shIL-4Rα.Fc were obtained from R&D Systems. rIL-13 and IL-4 were purchased from PeproTech. The IL-4RA (IL-4 R121D Y124D, BAY 16-9996) was a gift from Bayer.

Primary human cell culture

Fibroblasts were grown from endobronchial biopsies obtained from volunteers over the age of 18 years, as previously described (27). Written informed consent was obtained from all volunteers and ethical approval was obtained from the Joint Ethics Committee of Southampton University and General Hospital. Biopsies were obtained from the third to fifth subcarinae, placed in chilled DMEM (Invitrogen Life Technologies) with 10% FBS and finely cut using a scalpel. The pieces were then placed into 25-cm³ flasks and incubated at 37°C/5% CO₂ for 5–7 days until fibroblasts migrated out from the tissue and proliferated. The tissue was then removed, and fibroblasts were harvested and passaged; all studies were performed on cells between the third and sixth passages.

Sensor chip preparation

The molecular interactions between shIL-13Rα1.Fc, shIL-4Rα.Fc, and IL-4 or IL-4RA were determined by SPR measurements using a BIAcore 2000 biosensor as described in detail elsewhere (28, 29). shIL-4Rα.Fc was diluted in HBS running buffer (10 mM HEPES (pH 7.4), 150 mM NaCl,

3.4 mM EDTA, and 0.005% surfactant P20) and coupled to a CM5 sensor chip surface using the manufacturer's protocol.

SPR measurements

Both IL-4 and IL-4RA were diluted from stock to the desired concentration in HBS buffer. To determine kinetic constants, sensograms were collected at 25°C, with a flow rate of 50 μl/min and data collection rate of 1 Hz. Sensograms were recorded and normalized to a baseline of 0 resonance units. Equivalent concentrations of each protein were injected over an untreated surface to serve as blank sensograms for subtraction of bulk refractive index background. The sensor chip surface was regenerated between runs with a 1-min injection of 10 mM HCl, at 10 μl/min. The resultant sensograms were evaluated using the BIAevaluation 2.0 software to provide kinetic data.

Enzyme immunoassay for eotaxin-1

Fibroblasts were plated into 24-well dishes and allowed to grow to confluence before being serum starved in Ultraculture (BioWhittaker) for 24 h. The stimuli were then added in the described concentrations and culture supernatants harvested after 24 h for analysis of eotaxin concentration by ELISA following the manufacturer's protocol. Cell culture supernatants were assayed without any purification or concentration. Cell number was determined by uptake of methylene blue (30), and cytokine release was expressed as picograms/milliliter/10⁶ cells.

STAT6 phosphorylation

Primary human fibroblasts were grown to confluence before being serum starved for 24 h. Cells were treated with IL-13 (8 and 800 pM) in the absence or presence of shIL-4Rα (1 μg/ml) for 60 min. The cells were then solubilized in boiling SDS sample buffer before being subjected to SDS-PAGE and Western blotting with a phospho-STAT6 Ab (BD Biosciences), as described previously (31).

Results

Kinetic analysis of IL-4 and IL-4RA

The binding kinetics of IL-4 were evaluated using a BIAcore 2000 with shIL-4Rα.Fc immobilized to a CM5 sensor chip surface. The calculated dissociation constant ($K_D = k_{off}/k_{on}$) was found to be 382 ± 115 pM, confirming our previous observations (32). In similar experiments using IL-4RA, the dissociation constant (K_D) for IL-4RA was found to be 724 ± 120 pM. The association rate for the IL-4RA was significantly slower than IL-4 ($2.32 \pm 0.74 \times 10^6$ $M^{-1}s^{-1}$) but the dissociation rate was found to be similar ($1.68 \pm 0.97 \times 10^{-3}$ s^{-1}) (Table I). In control experiments, no binding was observed when IL-4 or IL-4RA were replaced with IL-13. Similarly, no direct binding of IL-4 to either IL-13Rα1 or γ_c was observed even at micromolar concentrations.

Formation of ternary complexes between shIL-4Rα/IL-4 and γ_c or IL-13Rα1

We have previously shown that IL-13 first binds to IL-13Rα1 before recruiting IL-4Rα to form a high-affinity complex (19). Therefore, we next used the SPR technology to evaluate the ability of γ_c

Table I. *Association (K_{on}) and dissociation (K_{off}) rate constants of IL-4 and IL-4RA with IL-4Rα or in complex with γ_c or shII-13Rα1*[a]

	K_{on} (M^{-1} s^{-1})	K_{off} (s^{-1})	K_D
IL-4	$4.37 \pm 0.51 \times 10^6$	$1.67 \pm 0.86 \times 10^{-3}$	382 ± 115 pM
IL-4RA	$2.32 \pm 0.74 \times 10^6$	$1.68 \pm 0.97 \times 10^{-3}$	724 ± 120 pM
IL-4Rα/IL-4/γ_c	$1.00 \pm 0.81 \times 10^5$	0.11 μM*	1.1 μM*
IL-4Rα/IL-4RA/γ_c	ND	ND	ND
IlL-4Rα/IL-4/IL-13Rα1	ND	ND	ND
IL-4Rα/IL-4RA/Il-13Rα	ND	ND	ND

[a] Association and dissociation rates for the binding of IL-4 and IL-RA to shIL-4Rα and the formation of IL-4R complexes. Constants were determined as described in *Materials and Methods*. Data are mean $\pm$ SD; $n = 10$.

* Represent approximate values due to the limits of software.

or IL-13Rα1 to stabilize the IL-4:IL-4Rα interaction. Thus, shIL-4Rα.Fc was immobilized on a CM5 sensor chip before being saturated with IL-4. Sequential injections of γc resulted in a concentration-dependent binding to the IL-4-saturated surface. The association rate was found to be $1.0 \pm 0.81 \times 10^5$ $M^{-1}s^{-1}$ while the rapid decay of the complex was measured at 0.11 s^{-1}. From these data, a dissociation constant, K_D, of 1.1 μM was calculated. In the absence of IL-4 or when IL-4 was replaced with IL-4RA, no significant binding was observed at any concentration. In similar experiments where γc was replaced by IL-13Rα1, we were unable to determine the affinity of shIL-13Rα1.Fc for the same complex (Table I). From these data, we concluded that neither γc nor IL-13Rα1 contributed significantly to the stabilization of the IL-4/IL-4Rα complex.

To complement our evaluation of the effects of IL-4RA on IL-4-dependent binding interactions, we also investigated the effect of IL-4RA on the ability of IL-4Rα to stabilize the IL-13:IL-13Rα1 complex. In this case, shIL-13Rα1.Fc and shIL-4Rα.Fc were immobilized on a CM5 sensor chip as previously described (19). When increasing concentrations of IL-13 were injected over the sensor surface, a sequential binding sequence was observed leading to the formation of a high-affinity receptor complex, confirming the ability of IL-4Rα to stabilize the IL-13R complex. However, when the surface was pretreated with 200 nM IL-4RA, this sequential mechanism was inhibited and the only binding interaction observed was the low-affinity binding of IL-13 to IL-13Rα1 (Fig. 1).

Comparison of the effects of IL-4RA or shIL-4Rα on IL-4 and IL-13 function

Two distinct methods of blocking IL-4 responses have been developed: sIL-4Rα, which binds to IL-4 with high affinity, thus preventing it from binding to receptor on the cell surface, and a receptor antagonist (IL-4RA), which binds to cell-bound IL-4Rα and inhibits the formation of the signaling receptor complex. We proceeded to evaluate the effect of both antagonists on functional responses to IL-4 and IL-13. In particular, we wanted to determine whether the stabilizing effect of sIL-4Rα on IL-13 binding that was observed in the SPR experiments had any functional consequences in cells. Thus, experiments were undertaken with primary bronchial fibroblasts that express both IL-4Rα and IL-13Rα1, as well as γc. The cells were cultured for 24 h in the presence of IL-4 or
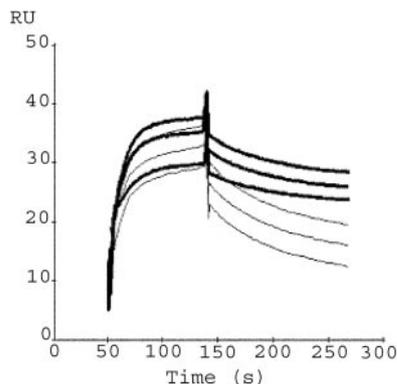

FIGURE 2. Inhibitory effect of IL-4RA on IL-13- and IL-4-mediated responses. Primary human fibroblasts were grown to confluence before being serum starved and treated with IL-4 or IL-13 (800 pM) in the absence or presence of IL-4RA (800 pM). After 24 h, eotaxin release into the culture supernatants was measured by ELISA. Data are from 10 individual fibroblast donors and are expressed as median, interquartile range, and 95% confidence intervals; statistical analysis was performed using the Wilcoxon rank sum test.

IL-13 (800 pM), alone or in combination with IL-4RA or shIL-4Rα. Both cytokines produced a significant increase in eotaxin release (Fig. 2), which was suppressed in the presence of IL-4RA consistent with its ability to block recruitment of either γc or IL-13Rα1 to IL-4Rα. We also examined the induction of IL-6 mRNA in fibroblasts when treated with IL-4 or IL-13 in the presence or absence of IL-4RA. An 8- to 10-fold induction of IL-6 mRNA was observed when fibroblasts were treated with IL-4, which was completely abolished by the presence of IL-4RA. The fold induction of IL-6 mRNA when cells were treated with IL-13 was more modest (2- to 4-fold), but this was also inhibited by the presence of IL-4RA (data not shown). When cells were treated with IL-4 in the presence of shIL-4Rα, the release of eotaxin was also significantly reduced. However, the release of eotaxin from cells treated with a maximally stimulating dose of IL-13 was unaffected by the presence of sIL-4Rα (Fig. 3).

Given that shIL-4Rα can stabilize the binding of IL-13 to IL-13Rα1, we postulated that this might augment responses to lower doses of IL-13. Therefore, we investigated its effects on eotaxin

FIGURE 1. Effect of IL-4RA on the IL-13Rα1:IL-4Rα complex. shIL-13Rα1 and shIL-4Rα were immobilized on a CM5 sensor chip as previously described. Increasing concentrations of IL-13 (bold lines) demonstrated binding to a high-affinity complex. Pretreatment of the surface with 200 nM IL-4RA abolished this high-affinity interaction (thin lines), revealing only a weak interaction similar to that observed for IL-13 and IL-13Rα1.
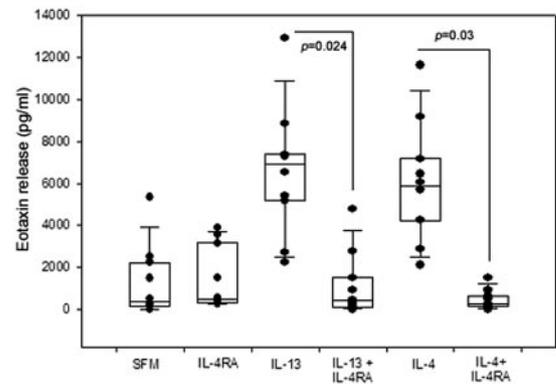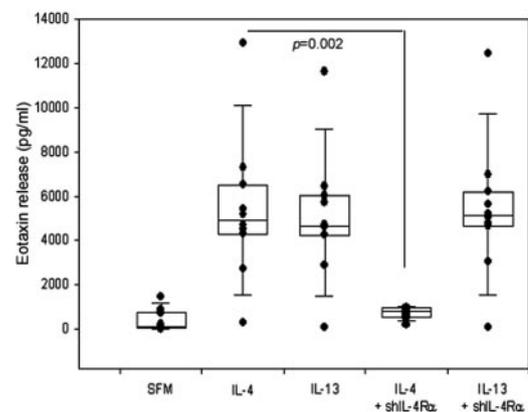

FIGURE 3. shIL-4Rα inhibits IL-4- but not IL-13-mediated responses. Primary human fibroblasts were grown to confluence before being serum starved and treated with IL-4 or IL-13 in the absence or presence of shIL-4Rα (1 μg/ml). After 24 h, eotaxin release into the culture supernatants was measured by ELISA. Data are from 10 individual fibroblast donors and are expressed as median, interquartile range, and 95% confidence intervals; statistical analysis was performed using the Wilcoxon rank sum test.
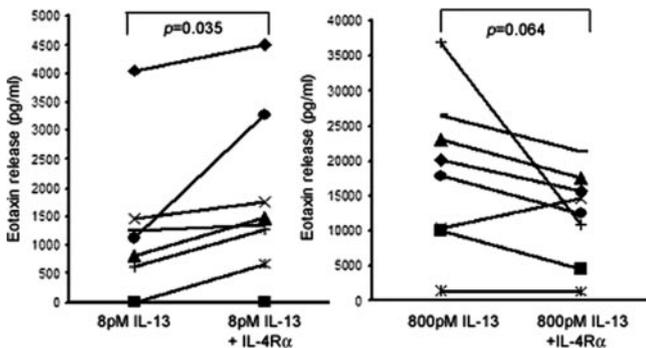
FIGURE 4. Stabilization of suboptimal dose of IL-13 by shIL-4Rα. Confluent and serum-starved primary human fibroblasts were treated with 8 or 800 pM IL-13 in the absence or presence of shIL-4R (1 μg/ml). After 24 h, eotaxin release into the culture supernatants was measured by ELISA. Data are from 10 individual fibroblast donors; statistical analysis was performed using the Wilcoxon rank sum test.

production in response to increasing doses of IL-13 (8–800 pM). Under these conditions, eotaxin release appeared to be affected by the presence of shIL-4Rα. This was particularly evident at suboptimal doses (8 pM) of IL-13 where the presence of shIL-4Rα produced a modest, but significant, increase in eotaxin release. At higher doses of IL-13 (800 pM), the presence of the shIL-4Rα had a slight inhibitory effect, but this failed to reach statistical significance (Fig. 4).

To further explore the ability of shIL-4Rα to augment IL-13-mediated responses, the level of STAT6 phosphorylation was examined. As shown in Fig. 5, the presence of shIL-4Rα enhanced STAT6 phosphorylation in response to a suboptimal dose of IL-13.

Discussion

IL-4Rα is known to play a key role in both IL-4 and IL-13 responses, because it forms an important functional signaling component of both cytokine receptor complexes. In this study, we have demonstrated that IL-4Rα plays a critical role in the ligand binding properties of both receptors but that the mechanism of formation and stabilization of liganded IL-4R and IL-13R differs. As a consequence, we have shown that this has functional implications for agents that have been developed to antagonize these receptors. In particular, we reveal that, whereas shIL-4Rα can inhibit IL-4-induced responses, the same soluble receptor is able to act as a weak agonist through its ability to stabilize suboptimal doses of IL-13 to augment its activity.

In this study, we used SPR to confirm that IL-4 binds to the IL-4Rα receptor chain with high affinity (19), obtaining results that compare favorably with values obtained in whole-cell assays (350
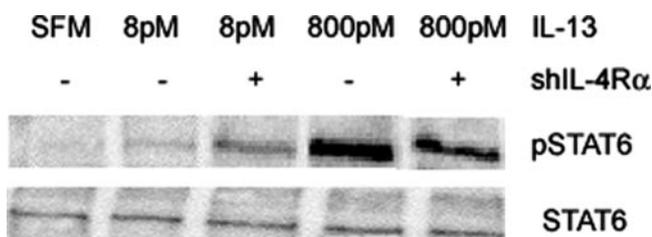

FIGURE 5. Effect of soluble IL-4Rα on STAT6 phosphorylation. Confluent cultures of primary human fibroblasts were serum starved and treated with IL-13 (8 and 800 pM) as indicated in the presence or absence of sIL-4Rα (1 μg/ml) for 60 min. Cells were then solubilized in sample buffer before Western blotting and probing with a phospho-STAT6 Ab. Bands were normalized for loading using a pan-STAT6 Ab ($n = 3$).

and 250 pM, respectively) (33). A very weak affinity of K_D of 1.1 μM was measured for the interaction of γ_c with a complex of IL-4Rα:IL-4, and we were unable to determine the affinity of IL-13Rα1 for the same complex. An explanation for the low affinity of γ_c is that it needs to interact with IL-4Rα only transiently to initiate signal transduction. Although γ_c has no intrinsic tyrosine kinase domain, it has been shown to be constitutively associated with the cytoplasmic tyrosine kinase, Jak3. Thus, its low affinity enables it to shuttle rapidly between IL-4:IL-4Rα complexes, so that its role is more that of a catalytic activator than a stoichiometric binding protein. This proposal is consistent with the fact that the biological potency of IL-4 reflects the binding properties of IL-4Rα (34). That almost all of the binding affinity is generated by the interaction between IL-4 and IL-4Rα may be an important regulatory mechanism in determining the hierarchy of IL-4- and IL-13-mediated responses, because IL-4Rα is a shared component of both the IL-4R and IL-13R complexes.

We also used SPR to examine the inhibitory mechanism of action of the IL-4 R121D/Y124D double mutein, IL-4RA. Its rate of association with IL-4Rα was found to be significantly lower than that of IL-4, but the dissociation rate was the same. However, no interaction between IL-4RA:IL-4Rα and γ_c could be detected even at high micromolar concentrations. This suggests that the mutant protein is able to bind to IL-4Rα but is unable to recruit the second receptor chain, either γ_c or IL-13α1, and is therefore unable to initiate a signaling receptor complex.

Previous studies have shown that mutations of IL-4, particularly substitution of the main determinant, Y124, result in pronounced antagonistic activity with a 100-fold reduction in R_{max} during T cell proliferation assays (35). Similarly, mutations of R121 or S125 result in mutants that can bind to IL-4Rα but are antagonists of both IL-4 and IL-13-induced responses (36). A murine double mutant, Q116D/Y119D, has been shown to inhibit IL-4-induced STAT6 phosphorylation in BA/F3 cells (37). In addition to IL-4 muteins, others have investigated the inhibition of IL-4 responses by IL-13 muteins such as IL-13E13K, a powerful IL-13 antagonist (38). These studies showed that IL-13E13K inhibited not only IL-13 responses but partially those of IL-4 as well. The results indicated that Glu[13] in IL-13 associated with IL-4Rα and a mutation to lysine decreases its binding to IL-4Rα. Although IL-13E13K prevents recruitment of IL-4Rα to IL-13Rα1, full inhibition of IL-4 responses could not be achieved due to the availability of the alternative IL-4R complex, IL-4Rα:γ_c. This contrasts with the IL-4 double mutein (IL-4RA), which blocks the IL-4Rα, thus eliminating possibilities of both IL-4Rs signaling.

The inhibitory effect of IL-4RA toward both IL-4- and IL-13-mediated responses contrasts with that of shIL-4Rα, which was only able to inhibit the IL-4-induced response, as predicted by its specificity. However, based on our previous studies where IL-13Rα1 had a low affinity for IL-13 and required IL-4Rα to stabilize this interaction to form a high-affinity complex, we postulated that the presence of shIL-4Rα would assist in these binding interactions in a cell-based system. When tested in bronchial fibroblasts, we observed that the presence of the shIL-4Rα enhanced IL-13-stimulated eotaxin production in response to suboptimal doses of IL-13. Further investigation revealed that this was associated with increased STAT6 phosphorylation. These results may have biological significance, because this suboptimal concentration (8 pM) of IL-13 is similar to the levels of IL-13 detected in vivo (39, 40). Furthermore, although the levels of eotaxin produced by the fibroblasts were only modest at low doses of IL-13 and sIL-4Rα, these are also likely to be biologically relevant. Thus, in previous studies by Dent et al. (41) examining the chemotatic activity of asthmatic eosinophils in the presence of

eotaxin, comparable levels of eotaxin are on the rising part of the dose-response curve. This suggests that the small but significant increase in eotaxin may increase eosiniphilic infiltration and lead to a prolonged inflammatory response.

The ability of a soluble receptor to act as an agonist by stabilizing interactions at the cell surface has also been reported in the IL-6R system (42). shIL-4Rα has been shown to some agonistic properties for IL-4 at low doses. However, in this case, it was suggested that shIL-4Rα acts more as a carrier protein from which IL-4 can disassociate over time rather than any form on binding activity at the cell surface (43). Similar observations were also made when an anti-IL-4 Ab was used in cell-based assays (44). It was found that binding to IL-4 was not irreversible and that the Ab may have offered some form of protection against proteolytic digestion.

Given that full-length IL-4Rα plays an important role in transducing intracellular signals via STAT6 and IRS1/2, it is unlikely that the shIL-4Rα stabilizes the IL-13R complex to initiate a signal. Instead, we suggest that shIL-4Rα functions to maintain higher local concentrations of IL-13 bound to IL-13Rα1, thereby facilitating transfer of IL-13Rα1 from the soluble receptor to the full-length native IL-4Rα (Fig. 6). This would explain why shIL-4α is a relatively weak agonist by comparison with sIL-6R, which transduces intracellular signals by binding to gp130. At the higher concentration of IL-13 tested, we propose that a similar mechanism is taking place and that there may even be residual IL-13-bound IL-13Rα1 receptors complexed with sIL-4Rα as illustrated in Fig. 6. However, this would not increase the overall response because the signaling receptors are already fully saturated. In support of this mechanism, in experiments using three fibroblast cultures, we found that the presence of sIL-4Rα increased the binding of IL-13 at all doses tested (8–800 pM) resulting in a $20 \pm 4\%$ increase in B_{max}. This increase in binding would be highly dependent on IL-4Rα levels, which we have found to vary between subjects (A.-L. Andrews, unpublished data). This may be one reason why the stabilization effect is more evident in some subjects than others.
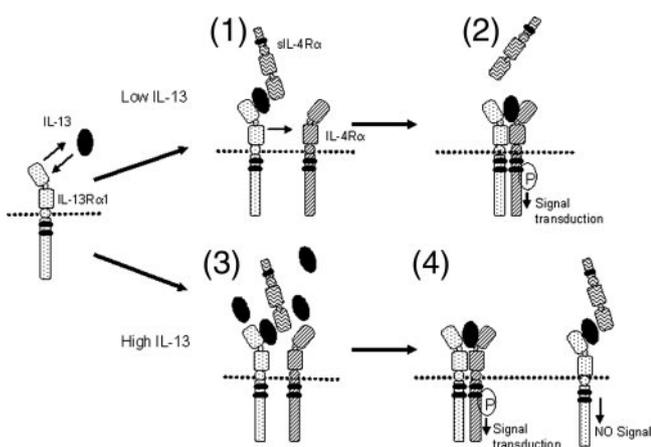
The concept of using a soluble receptor to block cytokine activity could represent new therapeutic opportunities. Treatment with soluble rIL-4Rα has been reported to moderately improve severe atopic asthma in initial placebo-controlled trial (23). These studies showed that administration of sIL-4Rα was associated with significant differences in forced expiratory volume in 1 s, although it did not affect serum markers such as VCAM-1 or CD23. However, a subsequent large-scale trial indicated that this reagent had no clinical efficacy in asthma, and the development of this project has been halted (45). Alternatively, our observations that sIL-4Rα stabilizes IL-13 binding might provide some insight into this poor response. It is likely that, for successful inhibition of both IL-4 and IL-13, all subunits must be blocked as in the case of the IL-4 double mutein. An alternative approach has been taken by Economides et al. (46) who created soluble receptors incorporating both receptor components (IL-13Rα1 and IL-4Rα). These cytokine "traps" have been shown to be potent blockers cytokine responses with an exceptional high affinity for both IL-13 and IL-4.

In conclusion, IL-4Rα plays a key role in the binding of IL-4 and IL-13 to their cognate receptors. In the case of IL-4, IL-4Rα contributes most of the binding affinity ensuring that IL-4 binds rapidly before recruiting a second receptor chain. This contrasts with its role in IL-13 binding where it is recruited by IL-13:IL-13Rα1 complex and stabilizes this interaction to form a high-affinity complex. Given the sequential nature of the IL-13R system, it is not unreasonable to find that a sIL-4R could form a complex with IL-13Rα1 on the cell surface. This weak agonist activity of shIL-4Rα for IL-13 was previously unrecognized. The fact that this stabilization effect occurs at suboptimal doses of IL-13 that are likely to be comparable with physiological levels may provide a partial explanation for the poor efficacy of shIL-4Rα in clinical trials (45, 47). Furthermore, IL-13 is produced for a longer period of time than IL-4 and is particularly important for allergic diseases such as asthma (48). Thus, complete understanding of the binding interactions between IL-4 and IL-13 and their cognate receptors may facilitate the development of novel treatments for asthma that selectively target these cytokines without unpredicted or detrimental side effects.

Disclosures

The authors have no financial conflict of interest.

References

1. Hsieh, C. S., A. B. Heimberger, J. S. Gold, A. O'Garra, and K. M. Murphy. 1992. Differential regulation of T helper phenotype development by interleukins 4 and 10 in an αβ T-cell-receptor transgenic system. *Proc. Natl. Acad. Sci. USA* 89: 6065–6069.
2. Seder, R. A., W. E. Paul, M. M. Davis, S. T. De, and B. F. Groth. 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. *J. Exp. Med.* 176: 1091–1098.
3. Coffman, R. L., J. Ohara, M. W. Bond, J. Carty, A. Zlotnik, and W. E. Paul. 1986. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* 136: 4583–4541.
4. de Vries, J., J. M. Carballido, and G. Aversa. 1999. Receptors and cytokines involved in allergic TH2 cell responses. *J. Allergy Clin. Immunol.* 103: S492–S496.
5. Pawankar, R., M. Okuda, H. Yssel, K. Okumura, and C. Ra. 1997. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J. Clin. Invest.* 99: 1492–1499.
6. Gascan, H., J. F. Gauchat, M. G. Roncarolo, H. Yssel, H. Spits, and J. de Vries. 1991. Human B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4+ T cell clones. *J. Exp. Med.* 73: 747–750.
7. Dabbagh, K., K. Takeyama, H. M. Lee, I. F. Ueki, J. A. Lausier, and J. A. Nadel. 1999. IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. *J. Immunol.* 162: 6233–6237.
8. Fukuda, T., Y. Fukushima, T. Numao, N. Ando, M. Arima, H. Nakajima, H. Sagara, T. Adachi, S. Motojima, and S. Makino. 1996. Role of interleukin-4 and vascular cell adhesion molecule-1 in selective eosinophil migration into the airways in allergic asthma. *Am. J. Respir. Cell Mol. Biol.* 14: 84–92.

FIGURE 6. Schematic diagram of proposed shIL-4Rα stabilization. IL-13 interacts weakly with IL-13Rα1. At low concentrations of IL-13 (*1*), shIL-4Rα stabilizes IL-13:IL-13Rα1 interaction, thus increasing the probability of recruiting membrane-bound IL-4Rα to form a high-affinity signaling complex (*2*). At higher concentrations of IL-13 (*3*), localization of IL-13 is still taking place due to the presence of sIL-4Rα; however, this does not increase response levels, because all of the signaling receptors are fully saturated. This then may lead to a competition between IL-13-bound IL-13Rα1 and the full receptor complex consisting of IL-13Rα1:IL-4Rα (*4*), resulting in reduced signal transduction.

9. Moser, R., J. Fehr, and P. L. Bruijnzeel. 1992. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J. Immunol.* 89: 6065–6069.

10. Doucet, C., D. Brouty-Boye, C. Pottin-Clemenceau, C. Jasmin, G. W. Canonica, and B. Azzarone. 1998. IL-4 and IL-13 specifically increase adhesion molecule and inflammatory cytokine expression in human lung fibroblasts. *Int. Immunol.* 10: 1421–1433.

11. Idzerda, R. L., C. J. March, B. Mosley, S. D. Lyman, T. Vanden Bos, S. D. Gimpel, W. S. Din, K. H. Grabstein, M. B. Widmer, L. S. Park, et al. 1990. Human interleukin 4 receptor confers biological responsiveness and defines a novel receptor superfamily. *J. Exp. Med.* 171: 861–873.

12. Galizzi, J. P., C. E. Zuber, N. Harada, D. M. Gorman, O. Djossou, R. Kastelein, J. Banchereau, M. Howard, and A. Miyajima. 1990. Molecular cloning of a cDNA encoding the human interleukin 4 receptor. *Int. Immunol.* 2: 669–678.

13. Kondo, M., T. Takeshita, N. Ishii, M. Nakamura, S. Watanabe, K. Arai, and K. Sugamura. 1993. Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. *Science* 262: 1874–1877.

14. Russell, S. M., A. Keegan, D. Harada, N. Y. Nakamura, M. Noguchi, P. Leland, M. C. Friedmann, A. Miyajima, R. K. Puri, W. E. Paul, and W. J. Leland. 1993. Interleukin-2 receptor γ chain: a functional component of the interleukin-4 receptor. *Science* 262: 1880–1883.

15. Obiri, N. I., W. Debinski, W. J. Leonard, and R. K. Puri. 1995. Receptor for interleukin 13. Interaction with interleukin 4 by a mechanism that does not involve the common γ chain shared by receptors for interleukins 2, 4, 7, 9, and 15. *J. Biol. Chem.* 270: 8797–8804.

16. Miloux, B., P. Laurent, O. Bonnin, J. Lupker, D. Caput, N. Vita, and P. Ferrar. 1997. Cloning of the human IL-13R alpha1 chain and reconstitution with the IL4Rα of a functional IL-4/IL-13 receptor complex. *FEBS Lett.* 401: 163–166.

17. Nelms, K., A. D. Keegan, J. Zamorano, J. J. Ryan, and W. E. Paul. 1999. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu. Rev. Immunol.* 17: 701–738.

18. Kopf, M., G. Le Gros, M. Bachman, M. C. Lamers, H. Bluemann, and G. Kohler. 1993. Disruption of the murine *IL-4* gene blocks Th2 cytokine responses. *Nature* 362: 245–248.

19. Andrews, A. L., J. W. Holloway, S. M. Puddicombe, S. T. Holgate, and D. E. Davies. 2002. Kinetic analysis of the interleukin-13 receptor complex. *J. Biol. Chem.* 277: 46073–46078.

20. Maliszewski, C. R., T. A. Sato, T. Vanden Bos, S. Waugh, S. K. Dower, J. Slack, M. P. Beckmann, and K. H. Grabstein. 1990. Cytokine receptors and B cell functions. I. Recombinant soluble receptors specifically inhibit IL-1- and IL-4-induced B cell activities in vitro. *J. Immunol.* 144: 3028–3035.

21. Sato, T., A. M. B. Widmer, F. D. Finkelman, H. Madani, C. A. Jacobs, K. H. Grabstein, and C. R. Maliszewski. 1993. Recombinant soluble murine IL-4 receptor can inhibit or enhance IgE responses in vivo. *J. Immunol.* 150: 2717–2725.

22. Borish, L. C., H. S. Nelson, J. Corren, G. Bensch, W. W. Busse, J. B. Whitmore, and J. M. Agosti. 2001. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J. Allergy Clin. Immunol.* 107: 963–970.

23. Borish, L. C., H. S. Nelson, M. J. Lanz, L. Claussen, J. B. Whitmore, J. M. Agosti, and L. Garrison. 1999. Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* 160: 1816–1823.

24. Letzelter, F., Y. Wang, and W. Sebald. 1998. The interleukin-4 site-2 epitope determining binding of the common receptor γ chain. *Eur. J. Biochem.* 257: 11–20.

25. Grunewald, S. M., A. Werthmann, B. Schnarr, C. E. Klein, E. B. Brocker, M. Mohrs, F. Brombacher, W. Sebald, and A. Duschl. 1998. An antagonistic IL-4 mutant prevents type I allergy in the mouse: inhibition of the IL-4/IL-13 receptor system completely abrogates humoral immune response to allergen and development of allergic symptoms in vivo. *J. Immunol.* 160: 4004–4009.

26. Tony, H. P., B. J. Shen, P. Reusch, and W. Sebald. 1994. Design of human interleukin-4 antagonists inhibiting interleukin-4-dependent and interleukin-13-dependent responses in T-cells and B-cells with high efficiency. *Eur. J. Biochem.* 225: 659–665.

27. Lordan, J. L., F. Bucchieri, A. Ritcher, A. Konstantindis, J. W. Holloway, M. Thornber, S. M. Puddicombe, D. Buchanan, S. J. Wilson, R. Djukanovic, et al. 2002. Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J. Immunol.* 169: 407–414.

28. Karlsson, R., A. Michaelsson, and L. Mattsson. 1991. Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J. Immunol. Methods* 145: 229–240.

29. Jonsson, U., L. Fagerstam, B. Ivarsson, B. Johnsson, R. Karlsson, K. Lundh, S. Lofas, B. Persson, H. Roos, and I. Ronnberg. 1991. Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. *BioTechniques* 11: 620–627.

30. Oliver, M. H., N. K. Harrison, J. E. Bishop, J. Cole, and G. J. Laurent. 1989. A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors. *J. Cell Sci.* 92: 513–518.

31. Mullings, R. E., S. J. Wilson, S. M. Puddicombe, J. L. Lordan, F. Bucchieri, R. Djukanovic, P. H. Howarth, S. Harper, S. T. Holgate, and D. E. Davies. 2001. Signal transducer and activator of transcription 6 (STAT-6) expression and function in asthmatic bronchial epithelium. *J. Allergy Clin. Immunol.* 108: 832–838.

32. Andrews, A. L., J. W. Holloway, S. M. Puddicombe, S. T. Holgate, and D. E. Davies. 2003. Kinetic analysis of the contribution of IL-4 receptor to the IL-4 and IL-13 receptor complexes. *Am. J. Respir. Crit. Care Med.* 167: A727.

33. Zurawski, S. M., F. Vega, Jr., B. Huyghe, and G. Zurawski. 1993. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J.* 12: 2663–2670.

34. Mueller, T. D., J.-L. Zhang, W. Sebald, and A. Duschl. 2002. Structure, binding, and antagonists in the IL-4/IL-13 receptor system. *Biochem. Biophys. Acta* 1592: 237–250.

35. Kruse, N., H. P. Tony, and W. Sebald. 1992. Conversion of human interleukin-4 into a high affinity antagonist by a single amino acid replacement. *EMBO J.* 11: 3237–3244.

36. Kruse, N., B. J. Shen, S. Arnold, H. P. Tony, T. Muller, and W. Sebald. 1993. Two distinct functional sites of human interleukin 4 are identified by variants impaired in either receptor binding or receptor activation. *EMBO J.* 12: 5121–5129.

37. Grunewald, S. M., S. Kunzmann, B. Schnarr, J. Ezernieks, W. Sebald, and A. Duschl. 1997. A murine interleukin-4 antagonistic mutant protein completely inhibits interleukin-4-induced cell proliferation, differentiation, and signal transduction. *J. Biol. Chem.* 272: 1480–1483.

38. Kioi, M., K. Kawakami, and R. K. Puri. 2004. Mechanism of action of interleukin-13 antagonist (IL-13E13K) in cells expressing various types of IL-4R. *Cell. Immunol.* 229: 41–51.

39. Berry, M. A., D. Parker, N. Neale, L. Woodman, A. Morgan, P. Monk, P. Bradding. A. J. Wardlaw, I. D. Pavord, and C. E. Brightling. 2004. Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J. Allergy Clin. Immunol.* 114: 1106–1109.

40. Arima, K., R. Umeshita-Suyama, Y. Sakata, M. Akaiwa, X. Q. Mao, T. Enomoto, Y. Dake, S. Shimazu, T. Yamashita, N. Sugawara, et al. 2002. Upregulation of IL-13 concentration in vivo by the IL13 variant associated with bronchial asthma. *J. Allergy Clin. Immunol.* 109: 980–987.

41. Dent, G., C. Hadjicharalambous, T. Yoshikawa, R. L. Handy, J. Powell, I. K. Anderson, R. Louis, D. E. Davies, and R. Djukanovic. 2004. Contribution of eotaxin-1 to eosinophil chemotactic activity of moderate and severe asthmatic sputum. *Am. J. Respir. Crit. Care Med.* 169: 1110–1117.

42. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto. 1989. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* 58: 573–581.

43. Jung, T., K. Wagner, C. Neumann, and C. H. Heusser. 1999. Enhancement of human IL-4 activity by soluble IL-4 receptors in vitro. *Eur. J. Immunol.* 29: 864–871.

44. Finkelman, F. D., K. B. Madden, S. C. Morris, J. M. Holmes, N. Boiani, I. M. Katona, and C. R. Maliszewski. 1993. Anti-cytokine antibodies as carrier proteins: prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J. Immunol.* 151: 1235–1244.

45. Holtzman, M. J. 2003. Drug development for asthma. *Am. J. Respir. Cell Mol. Biol.* 29: 163–171.

46. Economides, A. N., L. R. Carpenter, J. S. Rudge, V. Wong, E. M. Koehler-Stec, C. Hartnett, E. A. Pyles, X. Xu, T. J. Daly, M. R. Young, et al. 2004. Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nat. Med.* 9: 47–52.

47. Djukanovic, R., S. J. Wilson, M. Kraft, N. N. Jarjour, M. Steel, K. F. Chung, W. Bao, A. Fowler-Taylor, J. Matthews, W. W. Busse, et al. 2004. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am. J. Respir. Crit. Care Med.* 15: 583–593.

48. Kroegel, C., P. Julius, H. Matthys, J. C. Virchow, and W. Luttmann. 1996. Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. *Eur. Respir. J.* 9: 899–904.