Interleukin 4 and interleukin 13: same response, different receptors
R. E. Callard, D. J. Matthews and L. M. Hibbert
Immunobiology Unit, Institute of Child Health, 30 Guilford St., London WC1N 1EH, U.K.

Introduction
Interleukin (IL)-4 and IL-13 are two related cytokines with overlapping but not identical biological properties. Both inhibit production of proinflammatory cytokines and chemokines by monocytes [1,2] and both induce the same morphological changes and increase expression of vascular cell adhesion molecule 1 on endothelial cells [3,4]. Human B-cell responses to IL-4 and IL-13 are also very similar. Activation by either cytokine results in increased surface expression of CD23 and IgM whereas co-stimulation with anti-IgM or CD40 monoclonal antibody induces B-cell proliferation [5–7]. In addition, IgE secretion is obtained by co-stimulation with CD40 monoclonal antibodies or recombinant CD40L [6]. Although the responses of human B-cells to IL-4 and IL-13 are qualitatively the same, the responses to IL-13 are generally smaller than those to IL-4. In addition, IL-4 is active on T-cells and murine B-cells whereas IL-13 is not [2].

Receptors for IL-4 and IL-13
The very similar biological activities of IL-4 and IL-13 on many cell types suggests that these two cytokines share a common receptor or receptor component. The IL-4 receptor (IL-4R) is a heterodimeric complex consisting of the IL-4Ra-chain (p140), which binds IL-4, and the common y-chain (yc-chain), which is also shared by the receptors for IL-2, IL-7, IL-9 and IL-15 [8,9]. IL-13 does not bind to the IL-4Ra-chain or to the IL-4Ra/yc complex [10], but there is now good evidence that the IL-4Ra-chain is a non-binding component of the IL-13 receptor (IL-13R) [11]. This conclusion is based on experiments with the IL-4 mutant protein IL-4Y124D in which the tyrosine residue at position 124 is replaced with aspartic acid, and with blocking antibodies specific for the IL-4Ra-chain. The mutant IL-4Y124D binds to the IL-4Ra-chain with approximately the same affinity as...
wild-type IL-4 but is unable to signal because of the amino acid substitution in the site required for interaction with the γc-chain [12–14]. The fact that IL-4T12D inhibits responses to both IL-4 and IL-13 implicates the IL-4Ra-chain as a component of the receptors for both cytokines. This conclusion is supported by experiments showing that monoclonal antibody to IL-4Ra-chain also inhibits responses to IL-4 and IL-13 [15,16].

In the past year, human and murine IL-13Rs have been cloned [17,18]. The human IL-13R is a 354-amino acid type-I membrane glycoprotein belonging to the cytokine receptor superfamily [17]. It has an extracellular segment consisting of a distal immunoglobulin-like domain followed by a cytokine receptor domain with four conserved cysteine residues and a proximal fibronectin type-III (FNIII) domain with the conserved WSXWS motif characteristic of these receptors (Figure 1). The transmembrane domain is followed by a short intracellular segment of only 17 amino acids. The mouse IL-13R is similar, with an extracellular immunoglobulin-like domain followed by a cytokine receptor domain and an FNIII domain. The cytoplasmic segment is longer than in the human receptor, however, with 60 amino acids [18]. Both chains have been shown to associate with the IL-4Ra-chain to form a receptor that binds both IL-4 and IL-13. CTLL2 cells expressing the IL-13R–IL-4Ra complex respond weakly to IL-13, but Ba/F3 B-cells transfected with both chains are unable to respond to IL-13, suggesting further complexity. Interestingly, there is little sequence similarity between the human and mouse IL-13R and it is possible that the receptor complex may exist in different forms.

A minimal model showing the relationship between the receptors for IL-4 and IL-13 is shown in Figure 2. The possibility that there are two IL-13R proteins and the evidence for another low-affinity receptor for IL-4 that is not related to any of these structures [19] adds further complexity to this model that has not yet been elucidated.

**Signalling**

IL-4 seems to activate two signalling pathways consistent with coupling to two IL-4Rs. High concentrations of IL-4 activate a unique signal-transduction pathway in human B-cells characterized by rapid and transitory hydrolysis of phosphatidylinositol bisphosphate and Ca²⁺ mobilization followed after a short lag period by an increase in cytoplasmic cAMP [20]. This pathway is also activated in human monocytes by IL-13 [21], and we have found that IL-13 stimulates inositol trisphosphate production and a delayed increase in intracellular cAMP in human B-cells (L. M. Hibbert and R. E. Callard, unpublished work). On the other hand, IL-4 binding to the high-affinity IL-4Ra/γc activates protein tyrosine kinases including Jak1 and Jak3 with phos-
phorylation of 4PS which has recently been identified as IRS-2 [22–26]. Jak1 forms complexes with the IL-4Rα-chain and the 4PS protein [23] whereas Jak3 is associated with the γc-chain [26–28]. The recent finding that IL-13 also activates Jak1 but not Jak3 [22] and that both IL-4 and IL-13 induce phosphorylation of the IL-4Rα-chain and activation of STAT6 [22,29–31] also implicates IL-4Rα but not the γc-chain in the IL-13R.

**X-linked severe combined immunodeficiency (X-SCID)**

X-SCID is a genetic disease caused by mutations in the γc-chain [32–34]. Affected males typically have profoundly defective cell-mediated and humoral immunity with very low numbers of or absent T-cells but normal numbers of B-cells. It is fatal by 1 or 2 years of age unless treated by bone marrow transplantation. B-cells from patients with X-SCID can secrete immunoglobulin on stimulation with pokeweed mitogen in the presence of normal T-cells, but some responses to mitogens and cytokines have been shown to be defective. Mothers carrying these deleterious mutations in the γc-chain gene show unilateral X-inactivation in their T-cells, B-cells and other cells of the haemopoietic lineage, demonstrating the importance of the γc-chain for propagation and/or survival of a wide range of haemopoietic cells [35,36].

In a recent study from this laboratory, B-cells from patients with X-SCID were used as a naturally occurring gene-inactivation model to examine the biological role of the γc-chain in B-cell responses to IL-2, IL-4, IL-13 and IL-15 [37]. As expected, these B-cells were unable to respond to IL-2 or IL-15, consistent with the γc-chain being a functional component of the receptors for both these cytokines [38,39]. In contrast, IL-4 responses by these B-cells were normal in assays for B-cell activation (surface CD23 and IgM expression), proliferation on co-stimulation with anti-IgM or CD40 antibodies, and IgE production on co-stimulation with anti-IgM or CD40 antibodies. Detailed examination of X-SCID B-cell responses to IL-4 showed that the absence of the γc-chain did not reduce their sensitivity to IL-4 [40]. X-SCID B-cell responses to IL-13 were also unaffected [37,40]. These results show that the γc-chain is not required for B-cell responses to IL-4 and suggested that IL-4 could activate human B-cells through an alternative receptor other than the IL-4Rα/γc complex. One possibility is that IL-4 acts through the IL-13R consisting of the IL-4Rα/γc complexed with the recently identified IL-13-binding proteins. X-SCID B-cell responses to IL-4 and IL-13 were inhibited by both IL-4Rα/CD124 and a blocking IL-4Rα specific monoclonal antibody, showing that the IL-4Rα-chain is a component of the alternative IL-4R expressed on X-SCID B-cells. Formal proof that IL-4 activates B-cells through the IL-13R complex, however, awaits blocking experiments with IL-13 mutant proteins and/or IL-13R-specific monoclonal antibodies.

**γc-chain cytokine receptors preferentially co-stimulate with B-cell antigen receptor whereas non-γc-chain receptors preferentially co-stimulate with CD40**

We have recently found that co-stimulation of human tonsillar or peripheral blood B-cells with CD40 antibody and IL-13 results in a significantly bigger proliferative response than co-stimulation with anti-IgM and IL-13 (D. J. Matthews and R. E. Callard, unpublished work). Exactly the opposite is observed with IL-2, where co-stimulation with anti-IgM gives a bigger response than co-stimulation with CD40 antibody. This reciprocal effect suggests that the B-cell receptor signalling (anti-IgM) preferentially co-stimulates with cytokine receptors that include the γc-chain, whereas CD40 preferentially co-stimulates with receptors that do not include the γc-chain, for example the IL-13R complex. The comparable proliferative responses obtained by IL-4 co-stimulation with anti-IgM and CD40 are consistent with IL-4 activation of B-cells through both IL-4Rα/γc and IL-4Rα–IL-13R complexes. In contrast, responses of X-SCID B-cells to IL-4 and CD40 are greater than to IL-4 and anti-IgM because, in this model, IL-4 acts only through the IL-13R complex.

**Functional implications of two IL-4Rs on B-cells**

The expression of two IL-4Rs on normal B-cells has significant functional implications. High- and low-affinity IL-4Rs on human lymphocytes were shown some time ago by Scatchard plots from conventional binding studies [41]. Evidence for two IL-4Rs was also obtained from experiments
showing that 50 times higher concentrations of IL-4 are required for optimal CD23 expression than soluble IgM expression by human B-cells [42]. These differences are unlikely to be due to IL-4 interaction with IL-4Rα/γc and IL-4Rα-IL-13R complexes for two reasons. First, expression of both CD23 and soluble IgM is increased by IL-13 and IL-4, and second the γc-chain is responsible for only a small increase in affinity of the IL-4Rα-chain for IL-4, and the affinity of the IL-4Rα-IL-13R complex is similar to the affinity of the IL-4Rα/γc complex for IL-4 [9,43]. The difference may be due instead to the existence of a low-affinity IL-4R, which has been described previously but not cloned [19].

In other experiments, low doses of IL-4 were found to enhance secretion of IgM, IgG and IgA but not IgE by Epstein-Barr virus-activated B-cells, whereas high doses of IL-4 induced secretion of IgE and IgG4 but not the other isotypes [44]. In similar experiments with IL-13, only IgE secretion was obtained. IL-13 was unable to increase secretion of IgM, IgG or IgA at any concentration (K. Kotowicz and R. E. Callard, unpublished work). These experiments suggest that the effect of IL-4 on IgM, IgG and IgA was mediated by the IL-4Rα/γc complex whereas the production of IgE depended on IL-4 or IL-13 activation of the IL-4Rα-IL-13R complex. The possibility that IgE production depends on signalling through the IL-13R complex has major implications for our understanding of the role of IL-4 in allergy and for the development of mutant IL-4 (or IL-13) proteins as receptor antagonists for the treatment of diseases associated with type-2 immunity.

26 Musso, T., Johnston, J. A., Linnekin, D., Varesio,

Received 7 November 1996