Interleukin-13 is a potent activator of JAK3 and STAT6 in cells expressing interleukin-2 receptor- γ and interleukin-4 receptor- α

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The lymphocyte growth factors interleukin-2 (IL2), IL4, IL7, IL9 and IL15 use the common IL2 receptor- γ (IL2R γ) and activate the IL2R γ -associated tyrosine kinase JAK3 (Janus kinase 3). IL13 is structurally related to IL4, competes with IL4 for binding to cell surface receptors and exhibits many similar biological effects. The molecular basis for this functional overlap between IL4 and IL13 has been attributed mainly to a shared use of the 140 kDa IL4R α , since these cytokines appear to be uniquely different in that, according to several recent reports, IL13 does not recruit the IL2R γ or JAK3. This notion has been supported by the identification of a novel 70 kDa IL13 receptor in certain IL13-responsive cell lines that lack IL2R γ . The present study sheds new light on the issue of functional overlap between IL13 and IL4, by demonstrating for the first time that, in cells that express both IL2R γ and IL4R α , IL13 can mimic

IL4-induced heterodimerization of IL2R γ and IL4R α , with consequent marked activation of JAK3 and the transcription factor STAT6 (IL4-STAT). Reconstitution experiments in BA/F3 cells showed that both cytokines require the simultaneous presence of IL4R α and IL2R γ to mediate JAK3 and proliferative responses, and analysis of 12 IL4R α variants showed that IL4 and IL13 signals were equally affected by mutations of the cytoplasmic domain. We conclude that IL13 activates the IL2R γ -associated JAK3 tyrosine kinase in appropriate cell types, and propose that IL13 is capable of interacting with multiple receptor subunits in a cell-dependent and combinatorial manner. Consequently, we predict that partial disruption of IL13 signal transduction also contributes to the severe combined immunodeficiency syndromes associated with inactivation of the IL2R γ or JAK3 genes.

INTRODUCTION

Interleukin-13 (IL13) is a cytokine involved in the regulation of inflammatory and immune responses in many different cell types [1]. Among known cytokines, IL13 is structurally most closely related to IL4, with 25 % amino acid identity [1,2]. IL4 and IL13 are secreted by activated Th2 cells and exert similar biological effects on their target cells, which are primarily B cells and monocytes [3,4]. In monocytes, both IL4 and IL13 inhibit the production of inflammatory cytokines and up-regulate the expression of MHC class II and numerous members of the integrin family [1,2]. In B cells, IL4 and IL13 induce expression of MHC class II and CD23, and play a pivotal role in anti-CD40-dependent IgE class switch, as well as stimulating the synthesis of immunoglobulins G and M [3,5].

The composition of the IL13 receptor complex has remained obscure, but several lines of evidence indicate that IL4 and IL13, at least to some extent, bind to common receptor components. First, IL4 and IL13 cross-compete for binding to surface receptors on human renal cell carcinoma (RCC) cells and human TF-1 erythroleukaemia cells [2,6], and IL4 variants can serve as antagonists of IL13-mediated responses [7]. Secondly, consistent with a shared use of receptor components, IL13 and IL4 do not elicit additive or synergistic effects at saturating concentrations [5]. Furthermore, size separation by SDS/PAGE analysis revealed corresponding ¹²⁵I-ligand–receptor complexes after biochemical cross-linking of radioiodinated IL4 and IL13 to their receptors in COS-7, COS-3, TF-1 and A431 cell lines [8,9].

The components of IL4 receptor complexes and many of the immediate signalling events induced by IL4 have been established. IL4 binds to the 140 kDa IL4 receptor- α (IL4R α) and co-aggregates the 64 kDa IL2 receptor- γ (IL2R γ) [10,11]. This leads to activation of the IL2R γ -associated tyrosine kinase JAK3 (Janus kinase 3), and to a lesser extent to activation of JAK1 [12,13], as well as to activation of the transcription factor STAT6 [14]. Several lines of evidence suggest that IL4R α is also used by IL13. Specifically, the effects of IL13 on TF-1 cells, monocytes and human B lymphocytes are inhibited by preincubation of the cells with anti-IL4R α antibodies [7,8], and IL13 has been reported to induce tyrosine phosphorylation of IL4R α [15]. Whether IL13 can also utilize IL2R γ has remained controversial. This protein is an established common receptor chain for IL2, IL4, IL7, IL9 and IL15 [10,11,16–19], and initial work suggested that IL13 interacts with IL2R γ [20]. However, more recent reports have argued that IL2R γ is not involved in IL13 signalling [21–23]. Instead, the existence of an alternative IL13 receptor subunit has been proposed, based upon studies of IL13-responsive cells that do not express IL2R γ [6,8,24]. Indeed, a novel IL13 binding protein only distantly related to IL2R γ has been cloned recently [25]. These observations, coupled with the reported inability of IL13 to activate the IL2R γ -associated JAK3 tyrosine kinase [23,26,27], have supported the notion that IL13 does not signal via IL2R γ .

However, in contrast with this view, we now demonstrate for the first time that, in cells expressing the IL4R α and IL2R γ subunits, IL13 is clearly capable of activating JAK3 as well as

Abbreviations used: IL, interleukin; ILR α , IL4 receptor- α ; IL2R γ , IL2 receptor- γ ; JAK, Janus kinase; STAT, signal transducers and activators of transcription; mAb, monoclonal antibody; FCS, fetal calf serum; SCID, severe combined immunodeficiency; RCC cells, renal carcinoma cells; BS³, bis(sulphosuccinimidyl)suberate.

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STAT6 by mimicking IL4-induced heterodimerization of IL4R α and IL2R γ . Furthermore, stable introduction of human IL4R α into murine BA/F3 cells, which express IL2R γ constitutively, conferred IL4- and IL13-induced proliferation and JAK3 responses. Mutations in the cytoplasmic domain of IL4R α that abolished IL4-induced proliferation and JAK3 activation also abrogated the same responses to IL13. These results provide important new insight into the molecular basis for the overlapping biological effects of IL13 and IL4, and suggest that partial abrogation of IL13 signals also contributes to the severe combined immunodeficiency (SCID) syndromes associated with genetic inactivation of IL2R γ or JAK3 [28,29].

EXPERIMENTAL

Materials

Monoclonal antibodies (mAbs) against human IL13 (clone 32107.11), the extracellular domain of human IL2R γ (clone 311 40.111) and the extracellular domain of human IL4R α (clone 25433.1), and a polyclonal antibody that recognizes soluble human IL4R α (cat. no. AB-230-NA), were generously provided by Dr. Harvey Gaylord (R&D Systems, Inc., Minneapolis, MN, U.S.A.). Polyclonal anti-IL4 (cat. no. 500-p24) was purchased from PeproTech (Rock Hill, NJ, U.S.A.). Polyclonal rabbit antisera to synthetic peptides derived from human/mouse JAK1, mouse JAK2, mouse JAK3 and human TYK2 sequences were purchased from UBI (cat. nos. 06-272, 06-255, 06-342 and 06-275 respectively). These antibodies recognize both human and mouse forms, with the exception of the anti-(mouse JAK3) serum, and each could be used for immunoprecipitation and immunoblotting independent of the phosphorylation state of the JAK enzymes. We generated polyclonal rabbit antiserum specific to human JAK3, directed to a peptide sequence corresponding to the 21 C-terminal amino acids (amino acids 1104–1124) that are unique to human JAK3, and a second antiserum against an eight-residue peptide corresponding to the C-terminus of human IL2R γ (NH₂-CYTLKPET-COH) [13]. Monoclonal mouse anti-phosphotyrosine antibodies were purchased from UBI (4G10; cat. no. 05-321), and rabbit polyclonal anti-STAT6 antibody (S-20; cat. no. sc-621) was purchased from Santa Cruz Biotechnology.

Cell culture and treatment

The human erythroleukaemic cell line TF-1 [30] was grown in RPMI-1640 medium containing 10% fetal calf serum (FCS; Sigma; cat. no. F2442), 2 mM L-glutamine, 5 mM Hepes buffer, pH 7.3, and penicillin/streptomycin (50 units/ml and 50 mg/ml respectively), supplemented with 5 ng/ml granulocyte/macrophage colony-stimulating factor. TF-1 cells were made quiescent in RPMI-1640 medium with 5% gelded-horse serum (Sigma; cat. no. H1885). The IL3-dependent murine BA/F3 cell line containing IL4R α mutants was grown in RPMI-1640 medium with 10% FCS supplemented with 0.8 mg/ml geneticin sulphate (G-418; Gibco BRL; cat. no. 118111-031), 10 mM Hepes and 2% WEHI-3B supernatant as a source of IL3 [31,32]. Cells were normally stimulated with 100 nM recombinant human IL4 or 100 nM recombinant human IL13 (PeproTech; cat. no. 200-13 or R&D System, cat. no. 213-IL) at 37 °C, as indicated in the corresponding Figure legends. Cell pellets were frozen at –70 °C.

Generation of human IL4R mutants and stable cell lines

The details of this work have been published elsewhere [31,32]. The structures of the individual mutants are reviewed in Figure 7(A).

Solubilization of membrane proteins and immunoprecipitation

Frozen cells were thawed on ice and solubilized in lysis buffer (10⁸ cells/ml) containing 10 mM Tris/HCl, pH 7.6, 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM NaF, 200 mM sodium orthovanadate, 1% Triton X-100, 1 mM PMSF, 5 μ g/ml aprotinin, 1 μ g/ml pepstatin A and 2 μ g/ml leupeptin. Cell lysates were rotated end-over-end at 4 °C for 60 min, and insoluble material was pelleted at 12000 *g* for 20 min. Depending on the experiment, supernatants were incubated rotating end-over-end for 2 h at 4 °C with anti-phosphotyrosine mAb (4G10; 4 μ g/ml), anti-IL4R α (25433.1; 5 μ g/ml), anti-IL4 (PeproTech; 5 μ g/ml), anti-IL13 (R&D Systems, Inc.; 5 μ g/ml), normal rabbit serum or polyclonal sera to individual JAK enzymes (5 μ l/ml). Antibodies were captured by incubation for 30 min with Protein A–Sepharose or Protein G beads. Precipitated material was eluted by boiling in SDS-sample buffer for 4 min, subjected to SDS/7.5%-PAGE under reducing conditions and transferred to a polyvinylidene difluoride membrane (Immobilon; Millipore; cat. no. 1PVH 00010). Immunoblot analysis was performed as previously described [33]. mAbs to phosphotyrosine, IL2R γ and IL4R α were used at 1 μ g/ml, while each of the anti-JAK sera were used at a dilution of 1:1000.

Affinity cross-linking

Covalent cross-linking of ¹²⁵I-labelled human IL4 (NEN) or IL13 (iodinated according to the chloramine T protocol at the Laboratory Animal Sciences Program, NCI Frederick) was carried out as described previously [34], except that 0.5 mM bis(sulphosuccinimidyl)suberate cross-linker (BS³) (Pierce Chemical Co., cat. no. 21579) was used. Cells were then lysed and immunoprecipitated as described above.

Tyrosine kinase assays

JAK3 immune-complex tyrosine kinase assays were carried out by incubating the individually immunoprecipitated tyrosine kinases from lysates of unstimulated and IL4- or IL13-stimulated cells in the presence and absence of ATP, and visualizing incorporated phosphate on tyrosine residues by immunoblotting. Immobilized proteins were washed three times with lysis buffer followed by a single wash with kinase buffer containing 25 mM Hepes, pH 7.3, 0.1% Triton X-100, 100 mM NaCl, 10 mM MgCl₂, 3 mM MnCl₂ and 200 mM sodium orthovanadate. Isotope-free tyrosine kinase reactions were initiated by the addition of 15 μ M unlabelled ATP and allowed to incubate at 37 °C for 15 min. The reactions were quenched by washing the Protein A–Sepharose beads with lysis buffer and eluting bound material by boiling in SDS-sample buffer for 4 min.

Proliferation assay

Quiescent BA/F3 cells (2.5 $\times$ 10⁴/well) were plated in flat-bottom 96-well microtitre plates in 100 μ l of starvation medium (RPMI-1640 with 5% horse serum) in the presence of murine IL3 (50 nM), human IL4 (50 nM) or human IL13 (100 nM). Cells were pulsed for 4 h with [³H]thymidine (0.5 μ Ci/100 μ l) after 48 h of stimulation and harvested on to glass-fibre filters. [³H]Thymidine uptake was analysed by liquid scintillation counting.

RESULTS

IL13 activates JAK3 tyrosine kinase in human TF-1 cells

For the initial comparison of IL13 and IL4 receptor complexes, we used the human erythroleukaemic cell line TF-1, which

Figure 1 IL4 and IL13 induce marked tyrosine phosphorylation of JAK3 but not JAK1, JAK2 or TYK2 in human TF-1 cells

(A) Anti-phosphotyrosine (α PY) immunoblot of proteins immunoprecipitated (IP) with anti-phosphotyrosine antibodies from lysates of TF-1 cells that had been incubated with medium (C; lane a), IL4 (lane b) or IL13 (lane c) for 10 min at 37 °C. Arrows denote cytokine-induced tyrosine-phosphorylated proteins (p180, p140 and p116). Molecular mass markers are shown on the left (kDa). (B) Upper panel: anti-phosphotyrosine (α PY) immunoblot of parallel samples that had been immunoprecipitated with anti-JAK1 (α JAK1; lanes a-c), anti-JAK2 (α JAK2; lanes d-f), anti-JAK3 (α JAK3; lanes g-i) or anti-TYK2 (α TYK2; lanes j-l) antibodies. Lower panel: the same immunoblot reprobed with antibodies to JAK1, JAK2, JAK3 or TYK2. In both panels, arrows denote the positions of JAK enzymes. Molecular size markers are indicated on the left (kDa).

responds to both IL4 and IL13 [3]. Consistent with the notion that IL13 mimics IL4-induced proliferation of TF-1 cells, we demonstrated that the two cytokines induced similar signalling events in these cells. Cells exposed to IL4 or IL13 displayed highly corresponding patterns of protein tyrosine phosphorylation, as revealed by anti-phosphotyrosine immunoprecipitation and subsequent anti-phosphotyrosine immunoblotting (Figure 1A). Specifically, proteins with apparent molecular masses of 180, 140 and 116 kDa were rapidly tyrosine-phosphorylated by either cytokine. We had previously identified an IL2-, IL4- and IL7-activated tyrosine kinase of apparent molecular mass 116 kDa, as judged by SDS/PAGE, to be a leucocyte-specific Janus kinase, JAK3 (previously termed L-JAK) [35]. Anti-phosphotyrosine immunoblotting of immunoprecipitates of currently known forms of Janus tyrosine kinases, including JAK1, JAK2, JAK3 and TYK2, showed that IL13 specifically mimicked IL4-induced tyrosine phosphorylation of JAK3 (Figure 1B, top panel). Only upon prolonged overexposure of the blots did we occasionally observe a low degree of inducible tyrosine phosphorylation of JAK1 with either cytokine. TF-1 cells do express each form of JAK, as demonstrated by reprobing of the same blot with individual anti-JAK antibodies (Figure 1B, bottom panel). In separate experiments we observed similar predominant tyrosine phosphorylation of JAK3 following IL4 or IL13 stimulation in activated human T cells and HUT-102 cells (R. A. Kirken, M. G. Malabarba and W. L. Farrar, unpublished work), indicating that JAK3 activation by IL13 is a widespread phenomenon and not unique to TF-1 cells.

Several studies have revealed a close correlation between cytokine-induced tyrosine phosphorylation and catalytic activation of JAK kinases [12,36–40]. We have previously demonstrated that IL2 or IL4 treatment of cells markedly stimulates the catalytic activity of JAK3 [13,40]. JAK3 immunoprecipitation from lysates of TF-1 cells exposed to control medium, IL4 or IL13 verified that the enzyme activity of JAK3 was indeed

Figure 2 IL4 and IL13 activate JAK3, as measured by an *in vitro* autophosphorylation tyrosine kinase assay

Upper panel: anti-phosphotyrosine immunoblot of anti-JAK3-immunoprecipitated lysates of TF-1 cells that had been incubated with medium (C), IL4 or IL13 for 2 min at 37 °C. Immunoprecipitates using anti-JAK3 serum (lanes a–f) or normal rabbit serum (lanes g–l) were washed and subsequently incubated for 15 min at 37 °C in the absence (–) or presence (+) of 15 μ M unlabelled ATP. Lower panel: anti-JAK3 (α JAK3) immunoblot of blot shown in upper panel to verify equal loading of JAK3. In both panels, arrows denote JAK3 and 116 kDa markers are indicated on the left.

stimulated by either cytokine, as measured by an *in vitro* kinase assay based upon anti-phosphotyrosine immunoblotting and unlabelled ATP (Figure 2, lanes 1–f). No activity was associated with parallel control immunoprecipitates (Figure 2, lanes g–l).

Figure 3 IL4 and IL13 induce tyrosine phosphorylation of STAT6 in TF-1 cells

Anti-phosphotyrosine (α PY) immunoblot of proteins from TF-1 cells that had been stimulated with medium (C), IL4 or IL13 for 10 min at 37 °C, lysed and immunoprecipitated with polyclonal anti-STAT6 serum (α STAT6; lanes a–c) or normal rabbit serum (NRS; lanes d–f). The arrow denotes STAT6. Molecular mass markers are indicated on the left (kDa).

Reprobing of the same blot with anti-JAK3 antibodies verified the presence of equal amounts of JAK3 in each lane.

IL13 induces tyrosine phosphorylation of STAT6

Individual signals induced by different cytokines may be specifically transmitted by particular receptor subunits. Tyrosine phosphorylation of receptor chains creates unique docking sites for phosphotyrosine binding proteins with src homology 2 (SH2) domains or phosphotyrosine binding (PTB) domains [41]. One group of such secondary effector molecules is a family of proteins known as signal transducers and activators of transcription (STAT) [42]. IL4 has been shown previously to selectively activate STAT6 via tyrosine phosphorylation [14]. Consistent with their shared receptor components, we show here that IL13 mimics IL4-induced tyrosine phosphorylation of STAT6 (Figure 3, lanes b and c). Neither cytokine induced detectable tyrosine phosphorylation of STAT1, STAT2, STAT3, STAT4 or STAT5 in TF-1 cells (results not shown). Thus, in addition to having a predominant JAK3 activation pattern in common, IL13 and IL4 both selectively use STAT6.

125 I-labelled IL13 and IL4 receptor complexes are similar in TF-1 cells

Our next approach was to evaluate the quaternary structure of IL4 and IL13 receptors by analysing covalently cross-linked ligand–receptor complexes using 125 I-labelled IL4 and IL13. Direct comparison was facilitated by the similar molecular sizes of IL4 and IL13 (14 kDa and 13 kDa respectively). Radiolabelled IL4 and IL13 receptor complexes from TF-1 cells were of similar sizes, as revealed by SDS/PAGE (Figure 4). Regardless of whether antibodies to either human IL4R α or human IL2R γ were used, the two receptor complexes displayed corresponding bands of approx. 70, 90, 160 and 220 kDa (Figure 4, lanes a, c, e and g). Radiolabelled ligand in each of these complexes was specifically competed by a 250-fold molar excess of unlabelled

Figure 4 125 I-IL4 and 125 I-IL13 receptor complexes in human TF-1 are qualitatively similar

Receptor complexes were immunoprecipitated from lysates of biochemically cross-linked TF-1 cells that had been incubated with radioiodinated human IL4 or IL13 in the presence (+) or absence (–) of a 250-fold molar excess of unlabelled ('cold') ligand for 1 h at 4 °C. Receptor cross-linking was performed by incubation of washed cells with 0.5 mM BS³, and receptor complexes immunoprecipitated (IP) with antibodies to human IL4R α (α IL4R α ; lanes a–d) or to human IL2R γ (α IL2R γ ; lanes e–h) were separated on SDS/7.5%-PAGE. Arrows point to receptor complexes of various sizes, as indicated by their apparent molecular masses (70, 90, 160 and 220 kDa). Molecular mass markers are indicated on the left (kDa).

ligand (Figure 4, lanes b, d, f and h). Furthermore, IL4 and IL13 receptor complexes that were affinity-purified using appropriate anti-ligand antibodies also showed the same size patterns, whereas non-specific control antibodies were negative (results not shown). Although this experimental approach does not identify any of the receptor subunits associated with IL13, the largest complex of 220 kDa could consist of a heterotrimeric complex of IL13 (13 kDa), IL4R α (140 kDa) and IL2R γ (64 kDa). The distinct complex migrating at 160 kDa corresponds to the sum of one molecule of IL13 and IL4R α . Similarly, the weaker complex of 70–90 kDa is consistent with a complex between IL13 and glycosylated forms of IL2R γ (64 kDa).

IL13 induces tyrosine phosphorylation of IL4R α and causes heterodimerization of IL4R α with IL2R γ

Since tyrosine phosphorylation of cytokine receptor subunits is one of the initial events after ligand binding, we compared the abilities of IL4 and IL13 to induce tyrosine phosphorylation of IL4R α . As shown in Figure 5 (lanes 1–c), anti-phosphotyrosine immunoblotting of IL4R α immunoprecipitates showed that both IL4 and IL13 markedly induced tyrosine phosphorylation of the 140 kDa IL4R α . Separate experiments established that the two cytokines induced tyrosine phosphorylation of IL4R α with similar rapid kinetics, reaching peak levels at 10 min and declining towards background levels over the next 30 min (results not shown). Furthermore, IL4R α was detected in both IL4 and IL13 receptor complexes that had been immunoprecipitated with anti-ligand antibodies (Figure 5, lanes d–f). Although the anti-IL13 antiserum appeared to immunoprecipitate IL4R α less efficiently, these data provide additional support for a direct interaction of IL4R α with either cytokine.

Parallel experiments failed to detect tyrosine phosphorylation

Figure 5 IL4 and IL13 bind to and induce tyrosine phosphorylation of IL4R α in human TF-1 cells

Anti-phosphotyrosine (α PY; lanes a–c) or anti-IL4R α (α IL4R α ; lanes d–g) immunoblots of proteins from TF-1 cells that had been stimulated with medium (C), IL4 or IL13 for 10 min at 37 °C, lysed and immunoprecipitated (IP) with anti-IL4R α (α IL4R α ; lanes a–c), anti-IL4 (α IL4; lanes d and e) or anti-IL13 (α IL13; lanes f and g) antibodies. The arrow denotes IL4R α and the bracket indicates immunoglobulin heavy chains (IgG_{HC}). Molecular mass markers are indicated on the left (kDa).

Figure 6 IL4 and IL13 induce heterodimerization of IL4R α and IL2R γ

Anti-IL2R γ (α IL2R γ ; lanes a–d) and anti-IL4R α (α IL4R α ; lanes e–h) immunoblots of proteins from TF-1 cells stimulated with medium (C), IL4 or IL13 for 45 min at 37 °C, lysed and immunoprecipitated (IP) with anti-IL2R γ (α IL2R γ ; lanes a and f–h) or anti-IL4R α (α IL4R α ; lanes b–e). Arrows denote IL2R γ and IL4R α ; the bracket indicates immunoglobulin heavy chains (IgG_{HC}). Molecular mass markers are shown on the left (kDa).

of IL2R γ induced by either cytokine (results not shown). However, both IL4 and IL13 induced the co-association of IL2R γ with IL4R α , as demonstrated by anti-IL2R γ immunoblotting of receptor complexes that had been affinity purified with anti-IL4R α antibodies from lysates of cells treated with or without either cytokine (Figure 6, lanes b–d). Correspondingly, receptor complexes from IL4- or IL13-treated cells that had been

affinity purified using anti-IL2R γ antibodies contained significant amounts of IL4R α , as demonstrated by immunoblotting (Figure 6, lanes f–h). Positive controls identifying the migratory locations of IL4R α and IL2R γ on SDS/PAGE are provided (Figure 6, lanes e and a respectively). Immunoprecipitation with control antibodies was negative for both IL2R γ and IL4R α (results not shown). It should be noted that two unique anti-IL2R γ antibody preparations with distinct epitopes were used to identify IL2R γ . One is a polyclonal antiserum raised against a peptide corresponding to the seven most C-terminal amino acids of this receptor [13]. The second is a monoclonal antibody raised against the extracellular domain of IL2R γ (Clone 311 40.111; R&D System, Inc.). Collectively, these experiments established that IL13 can mimic IL4-induced co-aggregation of IL4R α and IL2R γ in TF-1 cells, as determined by immunorecognition and molecular size comparisons. Furthermore, both IL4 and IL13 induced rapid tyrosine phosphorylation of IL4R α .

IL4R α cytoplasmic mutations that abolish IL4-induced activities abrogate the same responses to IL13

At this point we had established that IL13 and IL4 bind to common receptor components in TF-1 cells and recruit the same JAK and STAT proteins. We therefore postulated that introduction of IL4R α into naive BA/F3 cells, which confers IL4 responsiveness to this IL2R γ -expressing cell line [31,32], would also confer IL13 responsiveness. Expression of wild-type human IL4R α in BA/F3 cells did indeed allow IL13 to induce tyrosine phosphorylation of JAK3 in these cells, and the response was comparable with that caused by IL4 (Figure 7B, wt). Furthermore, a series of mutations in IL4R α that had a deleterious effects on IL4 signalling also abrogated IL13 signals (Figure 7).

The structures of wild-type IL4R α (wt) and mutant receptors Δ SPR1, Δ R1, Δ PRM, Δ ER1, MPS, Δ R2, CT3, Δ R3, MYF, Δ R4 and Δ cyt are reviewed in Figure 7(A). Specifically, mutants Δ R1, Δ PRM, Δ ER1, Δ MPS and Δ cyt were unable to mediate either IL4- or IL13-induced JAK3 activation (Figure 7B), or IL4- or IL13-induced cell proliferation as measured by [³H]thymidine incorporation (indicated in Figure 7A). In contrast, wild-type and mutants Δ SPR1, Δ R2, CT3, Δ R3, MYF and Δ R4 each mediated both IL4- and IL13-induced JAK3 activation (Figure 7B) and cell proliferation. It should be noted that the growth response observed for the Δ R3 mutant was significantly diminished, in agreement with data reported previously [13]. In each case, reprobing of the blots with anti-JAK3 antibody verified equal loading of proteins (results not shown). The specific implications of the functional activity of each individual IL4R α variant are reviewed in the Discussion section. The highly comparable levels of JAK3 tyrosine phosphorylation induced by IL13 and IL4 in each of the BA/F3 cell lines containing functional receptor complexes (Figure 7B) provides further evidence that these two cytokines can signal via the same receptor components. Collectively, we conclude from these experiments that IL13, like IL4, is able to heterodimerize IL4R α and IL2R γ and activate their associated effector proteins in appropriate target cells.

DISCUSSION

Identification of the receptor subunits used by individual cytokines for transmembrane signal transduction is critical for understanding how cytokines exert their biological effects and influence disease processes. Previous work has shown that STAT6 and JAK3 specifically associate with IL4R α and IL2R γ respectively [14,43,44]. The present study demonstrates that IL13 can mimic IL4-induced co-aggregation of IL4R α and IL2R γ , and

Figure 7 Introduction of wild-type human IL4R α into naive murine BA/F3 cells confers responsiveness to IL4 and IL13, whereas cytoplasmic mutations that abrogate IL4-induced proliferation and JAK3 activation abolish the same IL13-induced effects

(A) Structural review of the various human IL4R α mutants stably introduced into the murine IL3-dependent BA/F3 cell line. The wild type (wt) receptor was not modified, Δ cyt lacks the entire cytoplasmic domain, and CT3 was truncated at Ser⁴⁷⁶. Two receptor variants have single amino acid substitutions: MPS contains a Pro²⁴² to Ser substitution, and MYF contains a Tyr⁴⁷² to Phe substitution. The internal deletion mutants are defined by the following excluded segments as indicated in the sketch: Lys²⁶⁴-Ser³³⁰ (Δ SPRI), Iso²³³-Ser³⁶⁵ (Δ RI), Iso²⁴¹-Lys²⁶⁴ (Δ PRM), Ser³³⁰-Ser³⁶⁵ (Δ ERI), Ser³⁶⁵-Thr⁴⁶² (Δ R3) and Ala⁵⁸⁰-Thr⁷¹¹ (Δ R4). The diagram also depicts the relative position of homology box 1 (Box 1), acidic motifs 1 (Acid1) and 2 (Acid2), as well as conserved tyrosine residues. The ability (+) or inability (-) of the various IL4R α variants to mediate IL4- or IL13-induced proliferation is indicated. (B) Anti-phosphotyrosine immunoblot of JAK3 immunoprecipitated from BA/F3 cells stably expressing variants of human IL4R α . Cells had been treated with medium (C), IL4 or IL13 for 10 min at 37 °C, lysed and immunoprecipitated with anti-JAK3 antibodies and blotted with anti-phosphotyrosine antibodies after separation on SDS/7.5%-PAGE. Individual IL4R α variants are labelled below each panel. Arrows denote JAK3; molecular mass markers are indicated on the left (kDa).

that the two cytokines transduce indistinguishable signals by selectively recruiting the transcriptional activator STAT6 and the tyrosine kinase JAK3 via these two receptor components. Based upon receptor complex size analyses and the use of antibodies directed to two distinct and unique epitopes of human IL2R γ , the present study also places IL13 with IL2, IL4, IL7, IL9 and IL15 in a category of cytokines that are capable of sharing the IL2R γ subunit and activating its associated tyrosine kinase JAK3. Consequently, we propose that the interference with IL13 signal transduction also contributes to SCID syndromes caused by inactivation of the IL2R γ or JAK3 genes.

Although a significant portion of the results that we report here have been obtained using human erythroleukaemic TF-1 cells, our results suggest that IL13-induced heterodimerization of IL4R α and IL2R γ is a general phenomenon in cells expressing both receptors. Consistent with this notion, we found that IL13 mimicked IL4-induced JAK3 activation in other cells that also express both IL4R α and IL2R γ , including activated human T lymphocytes and the HUT-102 cell lines (R. A. Kirken, M. G. Malabarba and W. L. Farrar, unpublished work). Moreover, murine BA/F3 cells which express IL2R γ [45], but do not respond to human IL4 or IL13, became responsive to both

cytokines upon stable transfection with human IL4R α . A series of mutations in the cytoplasmic domain of IL4R α that abolished IL4-induced growth and JAK3 activation also abrogated these same IL13-induced effects. Collectively, these results provide important new insight into the molecular basis for the similar biological effects of IL13 and IL4.

Several groups have reported an inability of IL13 to activate JAK3 in a number of cell lines. Many of these results may be explained by the lack of JAK3 and IL2R γ expression in the cell lines tested, e.g. RCC [46]. It is perhaps more difficult to explain why previous reports failed to observe IL13-induced activation of JAK3 in TF-1 cells (seen in Figures 1 and 2), even when IL4 was used as a positive control [26,27]. It is possible that these discrepancies could be attributed to differences in TF-1 cell subtypes. To verify our results we have tested several lots of human IL13 from two different manufacturers (see the Experimental section), and compared the immunoprecipitation efficiency of several anti-JAK3 antisera. In general, we obtained similar results in all of these confirmatory experiments.

The receptor partners interacting with a particular cytokine may vary between cell types and between species. The present study is not in conflict with recent studies that have proposed the existence of a novel 70 kDa IL13 receptor in RCC cells [6], or with the recent cloning of a novel IL13 receptor protein [25]. We have also co-precipitated a similar protein with IL13 from monocytic THP-1 cells, which are devoid of IL2R γ . In these cells IL4 and IL13 do not activate JAK3; instead, both cytokines induced moderate tyrosine phosphorylation of JAK2 and TYK2 (R. A. Kirken, M. G. Malabarba and W. L. Farrar, unpublished work). Consistent with these results is the recent report that IL4 activates JAK2 via an IL2R γ -independent mechanism in a human colon carcinoma cell line [47]. Because of this apparently cell-dependent, combinatorial, use of receptor components, a portion of IL4 and IL13 effects may escape the impact of IL2R γ or JAK3 gene inactivation and remain intact in SCID patients [48,49]. Moreover, the interaction of IL4 and IL13 with specific receptors expressed in some cell types might explain certain cell-specific differences in the effects of the two cytokines, such as the reported inability of IL13 to induce T cell proliferation, in contrast to IL4 [4]. Combinatorial reconstitution of IL4 and IL13 receptor complexes within a defined cell model will be mandatory to address the relative involvement of these receptor chains. These studies should provide valuable insight into how cytokines preferentially procure unique sets of mediator molecules (i.e. JAKs, STATs and src enzymes) to ultimately gain signal specificity.

This study also provides new specific information about the role of the cytoplasmic domain of IL4R α for IL4- and IL13-induced signal transduction. We have previously shown, using an IL4R α mutant designated Δ R1, that a membrane-proximal region of 132 amino acids in the cytoplasmic domain of IL4R α is critical for IL4-induced cell proliferation and activation of JAK3 [13,31]. Here we extend these findings to IL13. This membrane-proximal region of IL4R α contains the proline-rich motif designated Box 1 or PRM that is common to haematopoietin receptors [50,51], and has been proposed to be critical for activation of JAK tyrosine kinase [13]. More selective disruption of Box 1 as obtained in mutant Δ PRM, which lacks only 28 amino acids including Box 1, also abrogated the ability of IL4R α to mediate JAK3 phosphorylation and the proliferation response. Furthermore, the same effect was obtained by specifically converting the conserved Pro-242 of Box 1 to Ser, as achieved in mutant MPS. These observations therefore establish the critical nature of Box 1 of IL4R α for both IL4- and IL13-induced signals. However, independent deletion of the glutamic acid-rich

motif Acid1 of IL4R α , leaving Box 1 intact, also eliminated the JAK3 and proliferation responses. In contrast, selective deletion of a segment between Box 1 and Acid1 did not impede the JAK3 phosphorylation or proliferation responses, as seen in mutant Δ SPR1. Collectively, analysis of mutants Δ R1, Δ SPR1, MPS and Δ ER1 demonstrated that the non-overlapping motifs of Box 1 and Acid1 of IL4R α are essential for JAK3 activation by both IL4 and IL13. Interestingly, placement of Box 1 and Acid1 in close juxtaposition, as obtained in Δ SPR1, showed that the spatial integrity between these motifs is not critical.

The prevailing model of cytokine receptor function holds JAK enzymes as the primary tyrosine kinases that initiate the signalling process, and that additional secondary effector molecules, including other classes of tyrosine kinases, are recruited by binding to specific phosphotyrosine residues of activated receptor-JAK complexes [52,53]. This is consistent with reports of preformed complexes between JAKs and cytokine receptors [36,37,39,54]. However, detailed kinetic studies indicate that the amount of JAK3 in activated IL2 receptor complexes continued to increase well after IL2R β and IL2R γ heterodimerization had reached a plateau [40], thus complicating the model of simple pre-association. Furthermore, results obtained with mutant MPS raise the possibility that IL4R α is capable of mediating certain signals without JAK activation. Intriguingly, mutant MPS, which contains a Pro-242-to-Ser substitution and does not signal JAK3 activation or proliferation in BA/F3 cells, was shown to retain the capacity to mediate IL4-induced c-myc expression in a study complementary to the present one [32]. MPS furthermore conferred the ability of IL4 to retard the onset of apoptosis in BA/F3 cells, suggesting that signalling pathways independent of JAK3 may mediate these effects [32].

Modification of regions of IL4R α located C-terminal to the Acid1 motif did not affect JAK3 activation by IL4 or IL13. STAT6 has been proposed to interact with Tyr-578 and Tyr-606 of IL4R α , based upon affinity studies of phosphopeptide analogues [14]. In the absence of efficient antibodies to murine STAT6, we have not yet been able to test the STAT6 response in BA/F3 cells. However, the currently available repertoire of IL4R α mutants should enable us to verify this proposed location of STAT6 interaction.

In conclusion, the present study demonstrates that IL13 can mimic IL4-induced heterodimerization of IL4R α and IL2R γ , and induces the same signals as IL4 by selectively recruiting the tyrosine kinase JAK3 and the transcriptional activator STAT6 in cells expressing these proteins. We also showed that a series of mutations within the cytoplasmic domain of IL4R α had equivalent effects on signalling by both cytokines. Collectively, these results provide new insight into the molecular basis for the many overlapping biological functions of IL4 and IL13. Furthermore, this work conclusively places IL13 in the group of cytokines that are capable of utilizing the common IL2R γ chain and of activating JAK3. We therefore propose that impaired IL13 signal transduction may contribute to human SCID syndromes caused by inactivation of the IL2R γ and JAK3 genes.

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