IL4 and IL13 receptors share the γc chain and activate STAT6, STAT3 and STAT5 proteins in normal human B cells

Christine Rolling[a], Dominique Treton[a], Sandra Pellegrini[b], Pierre Galanaud[a], Yolande Richard[a,*]

[a]*INSERM U131, Institut Paris-Sud sur les Cytokines, 32 rue des Carnets, 92140 Clamart, France*
[b]*INSERM U 276, Institut Pasteur, Paris, France*

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Abstract IL13 induces the same biological effects as IL4 in normal human B cells. We show that as in the IL4R complex, both IL4Rα and IL2Rγc are components of the IL13R and that both cytokines induced STAT6, STAT3 and STAT5 activation in B cells. In spite of this similar downstream signalling, IL4 and IL13 used a different set of Janus kinases: IL13 is unable to activate JAK1 and JAK3.

Key words: Human B cell; IL13 signalling; IL4Rα; IL2Rγc; STAT protein

1. Introduction

IL13 and IL4 have similar biological effects on monocytes, mast cells and B lymphocytes [1–4]. They both act on a partially shared receptor [5]. A specific murine IL13 binding subunit has recently been characterized and is a new member of the hematopoietin receptor family. This subunit may be a component of the IL4R complex [6]. There is recent evidence for the direct participation of the human IL4Rα chain (the specific IL4 binding subunit) in the IL13R complex [4,7]. The long cytoplasmic tail of IL4Rα anchors several molecules essential for IL4 signalling, including IRS-2/4PS, NF-IL4/STAT6, JAK1 and indirectly JAK3 [8–12]. IL13 induces the activation of different IL4Rα-associated molecules, such as 4PS and JAK1 in the human TF-1 cell line [13,14] or STAT6 in the murine U937 cell line [15]. However, the details of IL13 signalling remain elusive, especially in human B cells. The involvement of the γc chain in IL4 and IL13 receptor complexes is also unclear. Although many reports have shown that γc is required for IL4 signalling [16–19], recent data suggest that there is an alternative pathway [20]. Indeed, neither X-SCID B lymphocytes [21] nor renal carcinoma cells [22], contain a functional γc chain and IL4 signalling is normal.

We investigated the γc chain in IL13R and IL4R complexes and some of the corresponding transduction mechanisms in normal human B cells. As previously reported in other cell types [23], B cell stimulation by IL13 led to the tyrosine phosphorylation of the IL4Rα chain. Surprisingly, the IL2Rγ chain was recruited by the IL4Rα chain after both IL4R and IL13R triggering. This recruitment was required for JAK3 activation in IL4 signalling. However, no phosphorylation of JAK enzymes was detected in IL13 signalling. IL13R triggering activated various STAT proteins including STAT6, STAT5 and STAT3. Thus, the IL13-induced tyrosine phosphorylation of the IL4Rα and the IL13-induced activation of STATs in human B cells appear to be mediated by as yet uncharacterized proteins tyrosine kinases.

2. Materials and methods

2.1. Reagents and antibodies

Recombinant human IL4 was purchased from Genzyme (Cambridge, CA). Recombinant human IL13 was kindly provided by A. Minty (Sanofi, Labège, France). Human recombinant IL2 was purchased from Biogen (Geneva, Switzerland). Two purified mouse mAbs raised against human IL4 Receptor mAb were used: M-57 (IgG1) from Immunex Corp. (Seattle, WA) and S4S6C9 (IgG) from Immunotech (Marseille, France). S4S6C9 is only satisfactory in immunprecipitation experiments. Mouse anti-human phosphotyrosine (4G10) and agarose-conjugated anti-phosphotyrosine 4G10 were from Upstate Biotechnology Inc. (UBI, Lake Placid, NY). Rabbit JAK3 and JAK1 antisera were kindly provided by J.J. O'Shea (NCI, Frederick, MD) and A. Ziemiecki (University of Berne, Berne, Switzerland), respectively. To immunoprecipitate γc chains, we used a rat γc mAb (TUGh4), provided by Dr. K. Sugamura (Tohoku University School of Medecine, Sendai, Japan). A commercial rabbit γc Ab from Santa Cruz Biotechnology (Tebu, Le Perray en Yvelines, France), was used for Western blotting. The mouse T10-2 monoclonal antibody directed against Tyk2 was provided by S. Pellegrini (Institut Pasteur, Paris, France). Rabbit STAT6 and STAT3 antibodies were purchased from Santa Cruz Biotechnology and mouse anti-STAT5 was from Transduction Laboratories (Lexington, KY). Anti-mouse and anti-rabbit Ig horseradish peroxidase-linked whole antibodies were from Amersham Corp., UK.

2.2. B cells

Human mononuclear cells were obtained from tonsils removed from children with chronic tonsillitis. Resting B cells were isolated from the E-fraction by Percoll gradient separation as previously described [24]. The resting B cell suspensions, consistently containing ≥95% CD19+, < 1% CD3+ and ≤4% CD14+ cells, were frozen until use. After thawing, resting B cells (1×10^6 cells/ml) were stimulated in complete medium (RPMI 1640 containing 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, 1 mM sodium pyruvate, 10 mM HEPES and 10% heat-inactivated FCS) for 48 h with 1 μg/ml phorbol 12,13 dibutyrate (PDB, Sigma, St Louis, MO and 800 ng/ml ionomycin (Sigma). Activated B cells were extensively washed before triggering by cytokines.

2.3. Immunoprecipitation

B cells (1×10^7 cells/ml) were stimulated or not for up to 30 min at 37°C with IL4 (40 ng/10^6 cells), IL13 (200 ng/10^6 cells) or IL2 (50 ng/10^6 cells). The reaction was stopped by addition of cold PBS containing 0.1% azide and 100 μM Na_3V0_4. After centrifugation, the cells were resuspended at 1×10^8 cells/ml in lysis buffer (50 mM HEPES pH 7.5, 0.5% Brij 97, 50 mM NaCl, 50 mM NaF, 10 mM $NaPP_i$, 5 mM EDTA, 1 mM Na_3VO_4, 1 mM Pefabloc, 10 μg/ml aprotinin, 10 μg/ml leupeptin) incubated for 30 min on ice, and centrifuged for 30 min at $13\,000 \times g$ at 4°C. Supernatants (equivalent of 1×10^7cells) were incubated overnight at 4°C with 15 μl of packed agarose-conjugated 4G10 beads (αPY), 5 μg of human IL4R Ab (αIL4R), or 3 μl of γc Ab (αγc). Immune complexes were then collected on 20 μl packed protein G beads by 1 h incubation at 4°C.

*Corresponding author. Fax: (33) (1) 46-32-79-93.

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2.4. Western blotting

Immunoprecipitates were washed three times in cold lysis buffer, boiled in Laemmli buffer and resolved by 8% SDS-PAGE. Proteins were then electrophoretically transferred to nitrocellulose filters (Hybond-ECL, Amersham Corp., UK). The filters were blocked for at least 3 h in TBS (20 mM Tris-HCl pH 7.6, 137 mM NaCl) containing 3% skimmed milk and 0.1% Tween 20 (blocking buffer). Proteins were probed by overnight incubation at 4°C with different antibodies (as indicated in the figure legends). After washing in TBS-0.1% Tween 20, blots were incubated in blocking buffer with horseradish peroxidase-labelled anti-mouse (1/3000) or anti-rabbit Ig (1/10 000) Ab for 45 min at 20°C, washed again and exposed to an enhanced chemiluminescence detection system (ECL, Amersham Corp., UK). ECL detection was recorded on ECL Hyperfilms (Amersham). The exposed films were scanned on an Imstar densitometer.

Before reprobing, membranes were stripped in 30 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl buffer pH 6.7, for 30 min at 50°C.

3. Results

3.1. IL13 induces time-dependent tyrosine phosphorylation of IL4Rα chain and increases its association with IL2Rγc

Human B cells were stimulated with IL13 and IL4 for 1–30 min, and lysates were immunoprecipitated with αPY-4G10 Ab and blotted with anti-IL4Rα Abs (Fig. 1). IL13 strongly increased the degree of tyrosine phosphorylation of IL4Rα as early as 1 min after cell stimulation. Phosphorylation increased up to 15 min and plateaued thereafter (Fig. 1A). Under similar experimental conditions, tyrosine phosphorylation of the hIL4Rα was not detectable following IL4 stimulation (Fig. 1B). M57 mAb detected two bands of 97 and 130 kDa in 4G10 immunoprecipitates but detected only the upper band in S4S6C9 (IL4Rα) immunprecipitates. This suggests that the 97 kDa band corresponded to a degraded form of the IL4Rα, previously observed in M57 immunoprecipitates blotted with M57 [24].

The kinetics of the association between γc and IL4Rα after IL13 and IL4 stimulation were next studied to determine whether γc also participated in the IL13R complex. Both IL13 and IL4 induced a 3-fold increase in the amount of IL4Rα detected in IL2Rγc immunoprecipitates after 1 or 5 min of stimulation. However, the amount decreased thereafter (Fig. 2A,B). In anti-IL4Rα immunoprecipitates, the kinetics of recruitment of γc were different after IL4 or IL13 stimulation: recruitment was maximal between 1 and 5 min with IL4 but increased up to 15 min with IL13 (Fig. 2C,D). Thus, these data suggest that in normal human B cells, both IL4Rα and γc are involved in IL13 signalling.

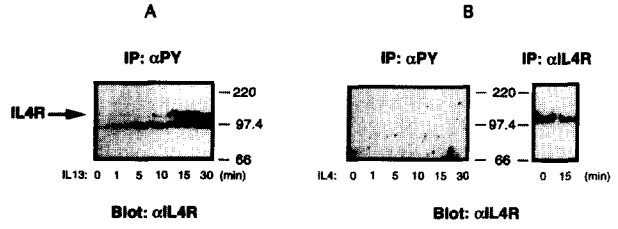

Fig. 1. Time course of IL4Rα tyrosine phosphorylation after IL13 stimulation. Human B cells were not stimulated or stimulated at 37°C with IL13 (200 ng/ml) (A) or IL4 (40 ng/ml) (B) for various times. 4G10 and IL4Rα (clone S4S6C9) immunoprecipitates were blotted with IL4Rα (10 μg/ml) antibody. Molecular mass standards (kDa) are indicated in the right margin. The position of IL4Rα is shown in the left margin (arrow).

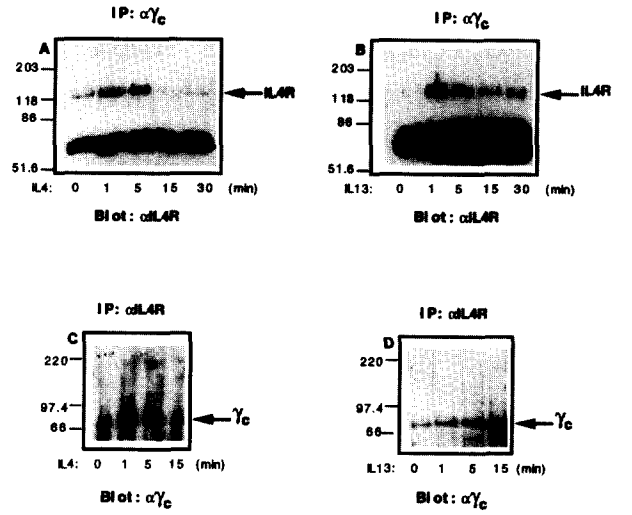

Fig. 2. Association between IL4Rα and γc upon IL13 and IL4 stimulation. Human B cells were not stimulated or stimulated at 37°C with IL4 (40 ng/ml) (A,C) or IL13 (200 ng/ml) (B,D) for various times. γc and IL4Rα immunoprecipitates were blotted with IL4Rα (10 μg/ml) (A,B) and with γc (1 μg/ml) antibodies (C,D), respectively. Molecular mass standards (kDa) are indicated in the right margin. The position of the IL4Rα or the γc is shown in the left margin (arrow).

3.2. Neither JAK1 nor JAK3 is activated after IL13R triggering

We next investigated whether JAK3 and JAK1 are stimulated after IL13R triggering. The kinetics of JAK tyrosine phosphorylation were studied in activated human B cells after stimulation by IL4 or IL13 (Fig. 3). JAK1 and JAK3 were tyrosine phosphorylated after IL4R triggering from 1 to 15 min, in all experiments performed. In contrast, neither JAK3 nor JAK1 was activated by IL13R triggering. In 5 of 9 experiments, both IL4 and IL13 induced the tyrosine phosphorylation of Tyk2, another member of the JAK family (Fig. 3). No tyrosine phosphorylation of JAK2 was observed in B cells after IL4 or after IL13 stimulation (data not shown).

3.3. A similar pattern of STAT proteins is activated by IL4 and IL13 in human B cells

We compared the pattern of signal transducer and activator of transcription (STAT) proteins activated by IL4, IL13 and IL2 in human activated B cells. IL4 but not IL2 induced strong tyrosine phosphorylation of STAT6 in human B cells. STAT6 activation was clearly detected at 5 min of IL4 stimulation and remained steady for 30 min (Fig. 4A). IL13 induced STAT6 tyrosine phosphorylation was maximum at 5 min and decreased slowly thereafter (Fig. 4A).

In human activated B cells, IL4, like IL2 in activated T cells [25,26], induced the tyrosine phosphorylation of STAT5 and STAT3 proteins. IL4 induced tyrosine phosphorylation within 5 min and continued until 30 min for both STAT proteins (Fig. 4B,C). STAT5 exhibited a sharp peak of tyrosine phosphorylation 5 min after IL13R triggering. IL13 also strongly induced activated STAT3 at 1 and 5 min, but activation decreased within 15 min. In all experiments, phosphorylation of STAT6, STAT5 and STAT3 proteins was weaker after stimulation by IL13 than by IL4 (Fig. 4A–C).

4. Discussion

We compared the structure of IL13R and IL4R, and their signalling mechanisms in human B cells. Although one of the early events in IL4R signalling is tyrosine phosphorylation of IL4Rα chain, variations of its basal level of phosphorylation in human B cells were hardly detectable in IL4Rα immunoprecipitates [24]. We therefore analyzed IL4Rα phosphorylation induced by IL13 in 4G10 immunoprecipitates. Tyrosine phosphorylation of the IL4Rα was detectable from 1 to 30 min following IL13 stimulation. In contrast, IL4-induced IL4Rα phosphorylation remained undetectable after IL4R triggering in 4G10 immunoprecipitates. These results, suggesting the involvement of the IL4Rα in the IL13R as well as in the IL4R complex, extend those of Zurawski et al. [4] and entirely agree with data from Smertz-Berling and Duschl [23] showing IL13 induced-IL4Rα phosphorylation in the erythroleukemic cell line TF1. The different phosphorylation pattern of the IL4Rα chain after IL4R and IL13R triggering might indicate that the stochiometry of the IL4Rα is different in each of these receptors.

Stimulation by IL13 enhanced the association between IL4Rα and IL2Rγ in human B cells. This contrasts with previous studies suggesting that γc is not essential for the IL13R complex [20,22]. Although part of the IL13R complex in human B cells, γc did not function in this complex as a docking protein for JAK3 as it does in the IL4R [16]: we observed no phosphorylation of JAK3 upon IL13 stimulation. There are two possible explanations: (i) γc makes a greater contribution to conformation than for function of the IL13R; (ii) γc associates with JAK another than JAK3. Murata et al. [27] reported JAK2 activation after IL13R and IL4R triggering in human colon carcinoma cells. However, we were unable to detect JAK2 phosphorylation in human B cells (data not shown). Thus, in our model, IL13 did not induce JAK1, JAK3 or JAK2 phosphorylation, and activated Tyk2 in only half of the experiments, suggesting that an unkown PTK is responsible for IL13 induced-IL4Rα phosphorylation. Lai et al. [28] recently identified JAK3 isoforms which differ from that of classic JAK3 in their carboxylic parts, although all have similar NH_2 regions. Thus, they compete with JAK3 for complexing with the γc chain without being recognized by antibodies specific for the classic JAK3 protein. Possibly,

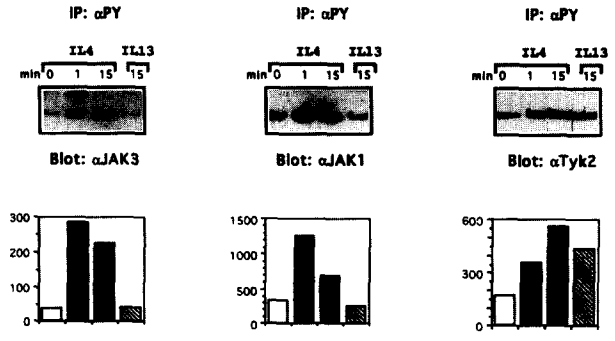

Fig. 3. Activation of JAK kinases by IL4 and IL13. Human activated B cells were not stimulated or stimulated at 37°C with IL4 (40 ng/ml) or IL13 (200 ng/ml) for the indicated times. The 4G10 immunoprecipitates were blotted with JAK1 (1/500), JAK3 (1/100) or Tyk2 (1/3000) antibodies. Each film was scanned on a densitometer. The relative intensities of the bands are shown as a diagramm and expressed in arbitrary units (peak area).

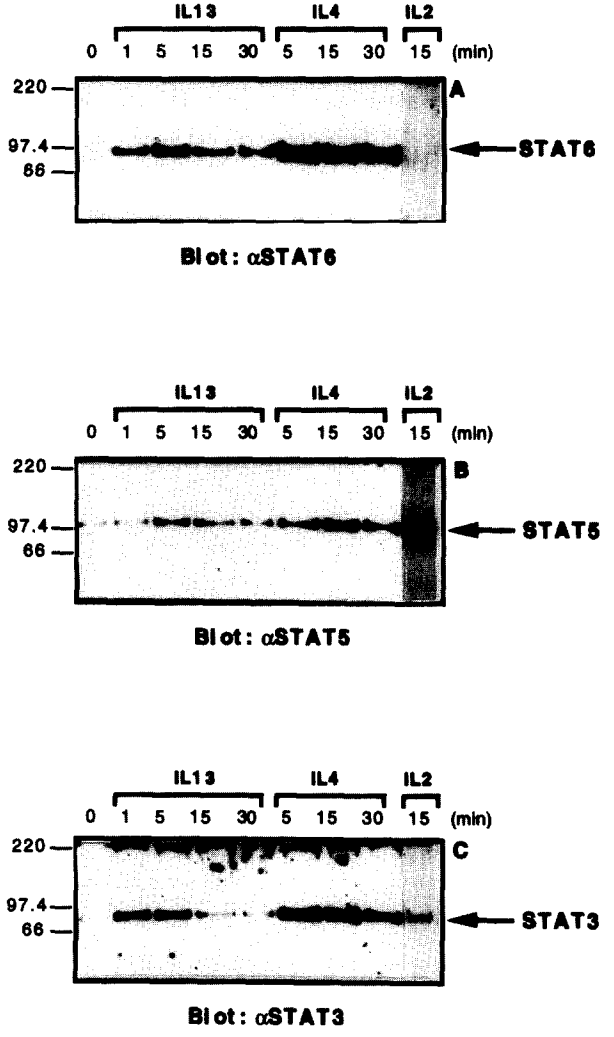

Fig. 4. Activation of the STATs proteins by IL4 and IL13. Human activated B cells were not stimulated or stimulated at 37°C with IL4 (40 ng/ml), IL13 (200 ng/ml), or IL2 (50 ng/ml) for the indicated times. 4G10 immunoprecipitates were blotted with STAT6 (1/100) (A), STAT5 (1/200) (B) STAT3 (1/100) (C) antibodies.

γc/JAK3 isoform complexes are involved in IL13 signalling in normal human B cells.

STAT6 was activated upon IL13 stimulation. This is consistent with the data of Izuhara et al. [29] showing that IL13, but not IL4, activates STAT6 in EBV-transformed B cells of XSCID patients. These results suggest that STAT6 activation requires, in addition to IL4Rα working as a STAT6 anchor chain, the expression of complex including a γ'c chain and an unidentified JAK. The human equivalent of the mouse IL13 binding chain [6] (also called the IL13Rα chain) seems a good candidate for γ'c. We also detected the phosphorylation of STAT3 and STAT5 after IL4R and IL13R triggering. As for STAT6, the maximum level of phosphorylation of STAT 3 and 5 induced by IL13 was weaker than that induced by IL4. The activation of these STATs was steady for 30 min during IL4 stimulation, but decreased quickly (within 15 min) upon IL13 stimulation. As previously reported by two groups [25,26], IL4, IL2 and IL15 all induce STAT3 tyrosine phosphorylation in T cells, suggesting that its activation depends on γc expression. In contrast, STAT5 activation induced by IL4 and IL13, detected in B cells, might be associated with

IL13Rα chain expression. The nature of the biological effects of these STAT and the mechanism by which they are activated, since there are no specific anchors for STAT3 and STAT5 on IL4Rα chain, are unknown. In view of a recent report that STAT3 associates with c-jun, it seems likely that there are various mechanisms for activating STAT proteins [30].

In conclusion, we have shown that γc is recruited by IL4Rα after either IL4 or IL13 stimulation. Although IL13 induces tyrosine phosphorylation of IL4Rα and of STAT6 in human B cells, it does not activate classic JAKs. We conclude therefore that unlike the IL4R complex, IL13R includes three chains: IL13Rα, IL4Rα and γc in normal human B cells. However, in B cells from XSCID patients, IL4 and IL13 act in the absence of a functional γc chain [21,29]. This suggests that the γc (normal B cells) or a γ'c (X-SCID B cells and carcinoma cells) are used in the IL13R complex. Thus, IL4R and IL13R appear to be highly flexible receptors with different structures in different cell types and using different sets of PTKs. In addition to STAT6, they are also able to activate STAT5 and STAT3 proteins in B cells.

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