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IL-13 and IL-4 share signal transduction elements as well as receptor components in TF-1 cells

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Abstract IL-13 and IL-4 are growth factors for the human erythroleukemia cell line TF-1. In these cells both cytokines share overlapping binding sites, but the number of sites for IL-13 is half of that for IL-4. Two monoclonal antibodies against the extracellular domain of the IL-4Rα chain completely abolish the binding of IL-13, although IL-13 does not bind to this chain. Following receptor triggering, IL-13 and IL-4 induce the phosphorylation of a 170 kDa protein, probably the IL-4-induced phosphotyrosine substrate. In addition the phosphorylation of the 170 kDa protein results in its tight association with phosphatidylinositol-3-kinase.

Key words: IL-13 binding; IL-13 signal transduction; IL-4-induced phosphotyrosine substrate; Phosphatidylinositol-3-kinase

1. Introduction

Interleukin-13 (IL-13) is a cytokine secreted by activated T lymphocytes which regulates inflammatory and immune responses [1,2]. It shares several biological activities with IL-4, another T-cell derived cytokine, in a variety of cell types such as B cells, monocytes, fibroblasts and endothelial cells [3]. The functional redundancy of IL-4 and IL-13 could result from the utilization of the same receptor complex, but the differences observed between the biological effects of these cytokines on, for example, T cells, suggested an overlapping but not identical population of receptors. In fact, Zurawski et al. [4] recently showed that the receptors for IL-4 and IL-13 are structurally related. Furthermore, it has been shown that a mutated IL-4 [5] that blocks the biological activity of IL-4 also antagonises IL-13 activity [4,6], adding support for a shared component(s) important for signal transduction between both receptors. Two proteins have been described as components of the high affinity IL-4 receptor, a glycoprotein of ≈130 kDa (IL-4Ra) [7,8] that when expressed in COS-7 cells binds IL-4 with a K_d of 50–100 pM [4], and the γ chain of the IL-2 receptor (γ_c) [9,10] that when associated to the IL-4R results in a 2–3-fold increase in affinity for IL-4 [9] and also participates in some of the IL-4 mediated signal transduction events [10]. We recently showed that neither of these two chains was responsible for the binding cross-competition of IL-4 and IL-13, and we proposed that the IL-13 receptor may be constituted by the IL-4 receptor complex associated with another component, probably a protein of 55–70 kDa [11].

Here we describe the characterization of the receptors for IL-13 and IL-4 and signal transduction events triggered by these cytokines in the human premyeloid erythroleukemia cell line TF-1, a cell line that proliferates in response to both proteins. Taken together, our results show that the IL-13 binding site is closely associated with the IL-4Rα chain which, by itself, does not bind IL-13; and that following receptor triggering both cytokines elicit the phosphorylation of a 170 kDa protein, probably the IL-4-induced phosphotyrosine substrate (4PS), and the association of this protein with phosphatidylinositol-3-kinase (PI-3K).

2. Materials and methods

2.1. Growth factors, antibodies and cells

Recombinant IL-13 was produced and purified in our laboratory as previously described [1], human IL-4 was obtained from Tebu (Le Perray en Yvelines, France) and GM-CSF from Genzyme (Cambridge, MA). The anti-phosphotyrosine (P-Tyr) antibody, the agarose conjugated 4G10 anti-P-Tyr, the anti-rat PI-3K (p85), the anti-mouse JAK-1, JAK-2 and JAK-3 were from UBI (Lake Placid, NY). The anti-rabbit IgG and anti-mouse IgG peroxidase conjugates were purchased from Sigma (St. Louis, MO) and the protein G-plus/protein A-Agarose from Oncogene Science (Uniondale, NY). The anti-IL-4 receptor antibodies X2/45 and S456C9 were provided by Dr. Sebald (Würzburg) or purchased from Immunotech (Marseille, France).

TF-1 cells, kindly provided by Dr. P. Manoni (Marseille, France), were cultured in RPMI 1640, 10% fetal calf serum (FCS), 2 mM glutamine, penicillin/streptamycin (100 units/ml) and 1 ng/ml GM-CSF, at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. Cell proliferation and binding assay

TF-1 cells were washed twice with GM-CSF-depleted medium and incubated with appropriate amounts of IL-4 or IL-13 for 72 h. To test the inhibition of proliferation, the cells were preincubated for 1 h at 37°C with appropriate amounts of anti-IL-4 receptor antibodies. The biological activity was measured by an in vitro colorimetric assay previously described [12].

Binding experiments were carried out as described [11] with ^{125}I-[F_{43}]IL-13-GYGY (360 pM) as labeled ligand. For the inhibition of binding, the cells were incubated for 1 h at 37°C with the appropriate amounts of the different antibodies.

2.3. Western blotting

TF-1 cells were washed twice in RPMI 1640 containing 2 mM glutamine (starvation medium) and incubated overnight in the same medium at 37°C. The cells were collected, resuspended at 10^6 cells/ml in starvation medium containing 50 μM Na_3VO_4, and stimulated with either IL-4, IL-13 or GM-CSF for 10 min at 37°C. The cells were washed at 4°C with 10 mM Tris-HCl, pH 7.4, 150 mM NaCl (TBS) containing 100 μM Na_3VO_4, pelleted and lysed in 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP40, 1 mM EDTA, 1 mM Na_3VO_4, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and 1 μg/ml pepstatin (lysis buffer). Cell lysates were cleared by centrifugation for 15 min at 15,000 × g, analyzed in 7.5% SDS-PAGE and transferred to nitrocellulose membranes (BioRad, Richmond, CA). The membranes were blocked with TBS containing 6% BSA, and probed with antibodies in TBS containing 0.1% Tween 20 and 0.5% BSA for 1–2 h and visualised by an ECL system (Amersham Corp., Buckinghamshire, UK).

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2.4. Immunoprecipitation

Proteins (1 mg) were immunoprecipitated from clarified cell lysates by addition of 70 μl agarose conjugated 4G10 anti P-Tyr or with 5 μl of other antisera plus 50 μl of protein G/A-Agarose and incubated overnight at 4°C. The immunoprecipitates were washed three times with lysis buffer, solubilized with Laemmli buffer and analyzed on 7.5% SDS-PAGE. Western blotting was carried out as described above.

3. Results

3.1. Monoclonal antibodies anti IL-4 receptor inhibit IL-13 binding to TF-1 cells

Both IL-13 and IL-4 are growth factors for the premyeloid erythroleukemia cell line TF-1. In these cells the binding of iodinated IL-13 is fully displaced by IL-4 (Fig. 1A) but the binding of IL-4 is only partially displaced by IL-13 [11]. The Scatchard plot generated from saturation experiments carried out with labeled IL-13 and IL-4 showed that the number of binding sites for IL-13 is half of that for IL-4 (data not shown and [11]) suggesting that not all of the IL-4 binding sites are shared by IL-13. The high affinity IL-13 binding (K_d 330 ± 20 pM) was displaced by two monoclonal antibodies, X2/45 and S456C9, raised against the extracellular domain of the IL-4Rα chain. As shown in Fig. 1B, both antibodies are able to fully compete with labeled IL-13 on TF-1 cells with IC_{50} of 4 ± 1 nM and 2 ± 0.5 nM, respectively. Similar displacement were obtained when iodinated IL-4 was used as labeled ligand in binding experiments (data not shown).

These results are in agreement with the observation that these antibodies inhibited the IL-13 and IL-4 induced proliferation of TF-1 cells at concentrations similar to those necessary to inhibit the binding (Fig. 1C).

3.2. IL-13 and IL-4 induce similar tyrosine phosphorylation patterns in TF-1 cells

Since IL-13 receptors overlap with those for IL-4 in TF-1 cells we decided to investigate whether these cytokines had similar effects on the events following receptor triggering. We analyzed protein tyrosine phosphorylation after stimulation by IL-4 and IL-13. The phosphorylation induced by GM-CSF, a well-known growth factor for these cells, was also investigated. The cells, washed and deprived of GM-CSF and FCS overnight, were stimulated with IL-4 or IL-13 (100 ng/ml), or with GM-CSF (2 ng/ml). Tyrosine phosphorylation was analyzed as described in section 2. As shown in Fig. 2, IL-4 and IL-13 induced a prominent tyrosine-phosphorylated band of 170 kDa. GM-CSF stimulation resulted in a different pattern of phosphoproteins, three bands of 97, 80 and 60 kDa were observed, whereas no protein corresponding in size to the phosphorylated 170 kDa band induced by IL-13 and IL-4 was detected. Weak phosphorylation of an 80 kDa protein was observed in some experiments in response to IL-4 and IL-13.

The 170 kDa protein was tyrosine-phosphorylated in a dose-dependent manner by IL-13 (Fig. 3A). Weak tyrosine phosphorylation of the 170 kDa was observed in response to a low concentration of IL-13 (0.028 nM), and was maximal at 2.8 nM. Similar results were observed with IL-4 (Fig. 3B). The protein tyrosine phosphorylation induced by IL-13 was in a dose range similar to that necessary to induce proliferation, suggesting that the phosphorylation may correlate with IL-13-dependent growth signal transduction.

The tyrosine phosphorylation of the 170 kDa protein induced by IL-13 was rapid. Two min after stimulation the phosphorylation was already maximal, the effect remained stable for 30 min and decreased after 2 h (Fig. 3C).

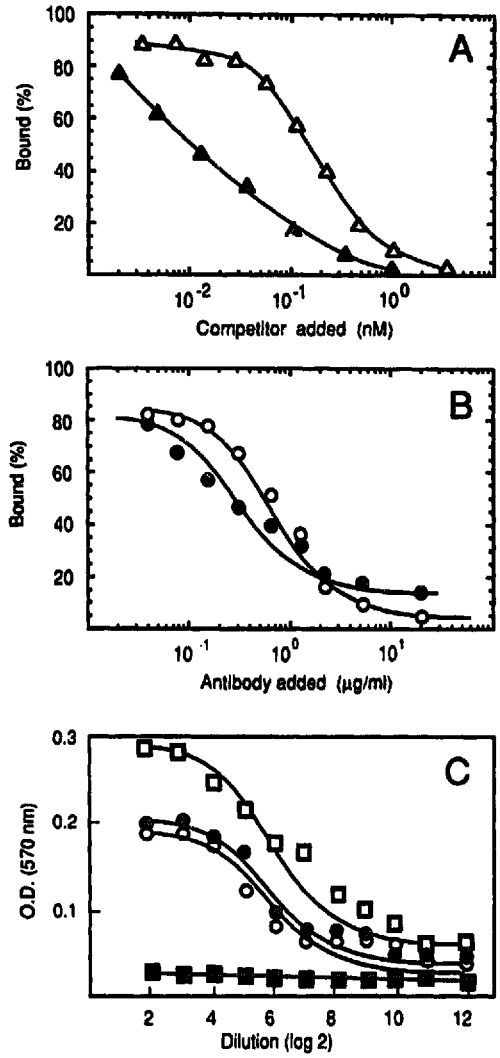

Fig. 1. IL-13 binding and growth promoting activity inhibition by antibodies anti-IL-4Rα chain. (A) Competitive displacement of ^{125}I-[F_{43}]-IL-13-GYGY binding by IL-13 (△) and IL-4 (▲). (B) Competitive displacement of ^{125}I-[F_{43}]-IL-13-GYGY binding by monoclonal antibodies S456C9 (●) and X2/45 (○), raised against the extracellular domain of the IL-4Rα chain. Each point represents the mean of triplicates. (C) IL-13 (0.5 μg/ml) growth promoting activity in the absence (□) and in the presence of monoclonal antibodies S456C9, 16 μg/ml, (●), and X2/45, 10 μg/ml (○), and 20 μg/ml (■). Each point represents the mean of triplicates.

The phosphorylation of Janus kinases in the different samples was analyzed by blotting with anti P-Tyr either directly, or after immunoprecipitation with anti JAK-1 and JAK-3 antibodies. Both techniques failed to reveal IL-13- or IL-4-induced phosphorylation of these kinases (data not shown).

3.3. IL-13 induced phosphorylation of the 170 kDa protein results in its association with PI-3K in TF-1 cells

PI-3K has been shown to associate, through the 4PS, with the IL-4 receptor complex in response to IL-4 [13]. Since we

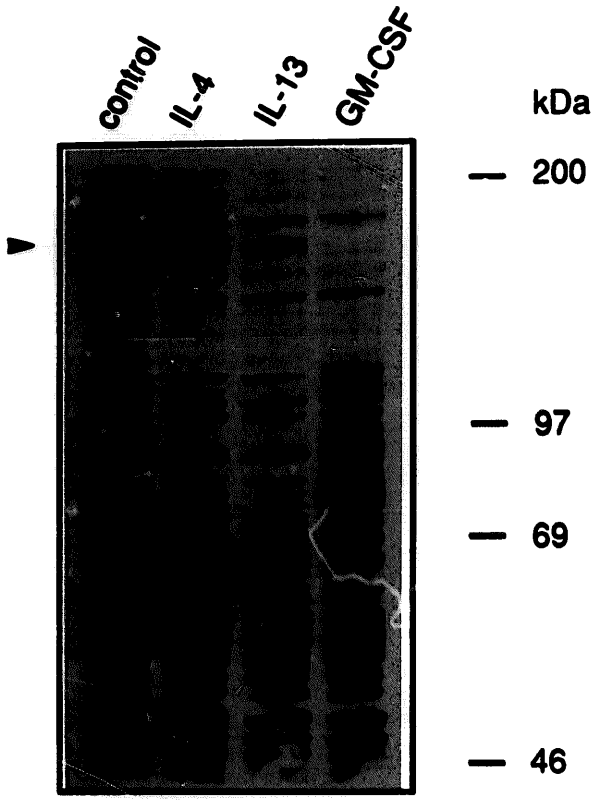

Fig. 2. Protein tyrosine phosphorylation in TF-1 cells. The cells were starved and stimulated with cytokines as described in section 2. Cell lysates were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with anti-P-Tyr antibodies.

observed the same tyrosine phosphorylation pattern induced by IL-13 or IL-4, we investigated whether the IL-13 also induces the association of PI-3K to the receptor complex. Fig. 4A shows the detection of the 170 kDa protein phosphorylated in response to IL-13 and IL-4 as detected with antibodies anti P-Tyr on whole cell lysates. When the samples were first immunoprecipitated with an antibody directed against the 85 kDa subunit of PI-3K and subsequently immunoblotted with anti P-Tyr antibodies, the phosphorylated 170 kDa protein was also clearly detected (Fig. 4B). If the same samples were immunoblotted with antibodies directed against the 85 kDa subunit of PI-3K, this protein was observed at the expected position in the gel (Fig. 4C). These results show that the 170 kDa protein associates with the PI-3K after receptor triggering by IL-13 and IL-4. They also show that neither IL-13 nor IL-4 stimulates the phosphorylation of the 85 kDa subunit of PI-3K. GM-CSF-treated lysates immunoprecipitated with anti-p85 serum and immunoblotted with anti P-Tyr did not show any specific phosphorylated protein.

4. Discussion

The purpose of this study was to characterize the receptors for IL-13 and IL-4 and the signal transduction events trigger by both cytokines in the premyeloid erythroleukemia cell line TF-1. These cells are dependent on GM-CSF but they can proliferate in response to IL-13 and IL-4 and thus provide an interesting model for the study of the IL-4/IL-13 receptor system. We recently proposed that the IL-13 receptor consist of the IL-4 receptor complex associated with at least one additional protein [11]. We show here that, in line with this model, two monoclonal antibodies against the extracellular domain of the IL-4Ra chain completely abolish the binding of labeled IL-13. The IL-4Ra chain by itself does not bind IL-13 [4,11], thus the inhibition of the binding by these two antibodies is probably due to steric competition and suggests a close association of the IL-13 binding molecule with the IL-4Ra.

Our results also show that, in TF-1 cells, both IL-13 and IL-4 induce rapid tyrosine phosphorylation of a 170 kDa protein. This protein is most probably 4PS previously described [13]. 4PS is a protein functionally and structurally related to IRS-1 [14], which following insulin interaction with its receptor, is phosphorylated and associates with signaling molecules presenting the SH-2 (Src homology) domain (for review see [15]). The IL-4 induced phosphorylation of 4PS results in its associa-

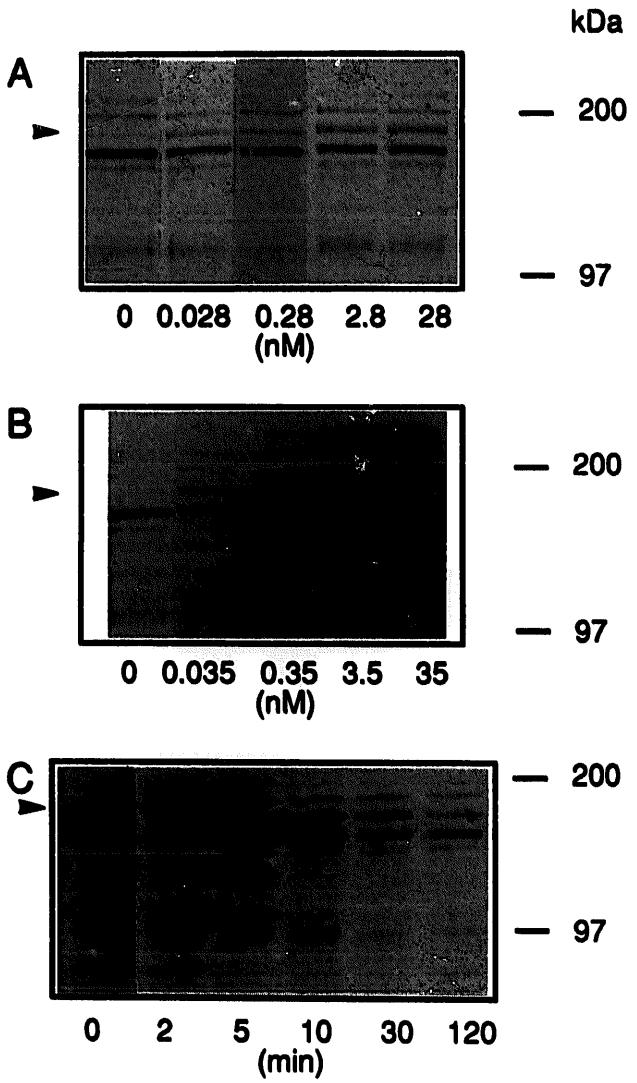

Fig. 3. Protein tyrosine phosphorylation in TF-1 cells. (A) IL-13 and (B) IL-4 dose dependent tyrosine phosphorylation. (C) Time course phosphorylation following IL-13 treatment. Cells were lysed and phosphoproteins analyzed as in Fig. 2.

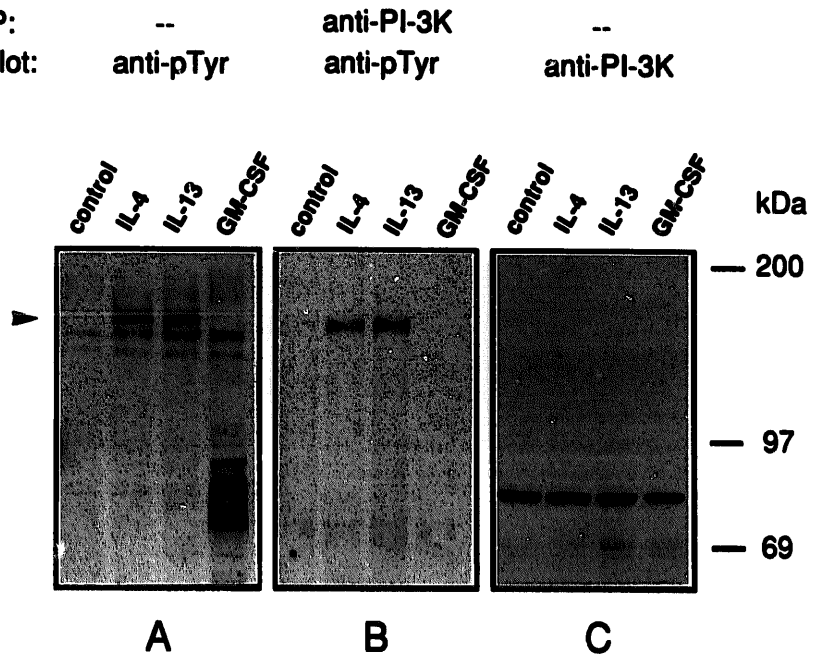

Fig. 4. Association of the phosphorylated 170 kDa/4PS protein with PI-3K in TF-1 cells. (A) Protein tyrosine phosphorylation in cell lysates immunoblotted with anti-P-Tyr antibodies. (B) Protein tyrosine phosphorylation in cell lysates immunoprecipitated with anti-PI-3K antibodies and immunoblotted with anti-P-Tyr antibodies. (C) Cell lysates immunoblotted with anti-PI-3K antibodies. Samples were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted as described in section 2.

tion with, among other proteins, the p85 subunit of PI-3K, thereby inducing PI-3K activity [13]. Our results show that in TF-1 cells, IL-13, as well as IL-4 activation, results in the association of PI-3K to the phosphorylated 170 kDa protein. The response of the TF-1 cells to GM-CSF, which also promotes proliferation of these cells, was clearly different, since among several proteins that were phosphorylated the 170 kDa one was absent. Thus, these results support the hypothesis of common signal transduction proteins for the IL-13/IL-4 receptor complex and are in line with the observation that both cytokines activate the same recently identified transcription factor NF-IL-4 which binds to the specific responsive element IL-4RE [16].

We failed to observe the previously described phosphorylation [13,17] of the IL-4Rα chain by either IL-13 or IL-4 by Western blot with the anti-P-Tyr antibody on total TF-1 lysates and on IL-4Rα immunoprecipitates, probably because of the low number of receptors. However, phosphorylation of this chain appears to be a pre-requisite for phosphorylation of the 170 kDa protein [13]. It should also be noted that under our conditions we did not see phosphorylation of the Janus kinases JAK-1 and JAK-3 following receptor triggering by IL-13 or IL-4. IL-4-induced JAK-1 and JAK-3 phosphorylation has been described in natural killer and T cells [18,19]. Interestingly, some Janus kinases are associated to the receptor through the IL-2 receptor γ_c, but the participation of the γ_c may be cell dependent, because recently it has been shown that in X-linked severe combined immunodeficient B cells, in which the γ_c gene is disrupted, the response to IL-4 and IL-13 is not impaired [20]. Further work is in progress to assess whether this observation is associated with the number of receptors or with the type of receptor complex present in TF-1 cells.

In summary, our results show that the IL-13 binding site is closely associated to the IL-4Rα chain which, by itself, does not bind IL-13; and that both cytokines elicit similar intracellular events following receptor triggering. These findings are in line with the hypothesis that the IL-13 receptor may constitute a subset of IL-4 receptors containing the IL-4 binding subunit associated with at least one additional protein.

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