Homodimerization of Interleukin-4 Receptor α Chain Can Induce Intracellular Signaling*

(Received for publication, April 26, 1996, and in revised form, August 5, 1996)

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†The abbreviations used are: IL, interleukin; hIL-4, human interleukin-4; IL-4R, human interleukin-4 receptor; hIL-4Ra, human interleukin-4 receptor α chain; γc, common receptor γ chain; Jak, janus kinase; Stat, signal transducer and activator of transcription; α, anti-

The possible role of homodimerization events in intracellular signal transduction triggered by the bipartite human interleukin-4 receptor was addressed. We generated cell lines functionally expressing derivatives of the two receptor subunits α and γc, which allow for a specific and background-free experimental induction of intracellular homo- and heterodimers. A heterodimer of α and γc released an intracellular signal, whereas a γcγc homodimer did not. Unexpectedly, we found the intracellular domain of interleukin-4 receptor α chain to evoke cell proliferation and activation of tyrosine kinase Jak1 as well as of transcription factor Stat6 upon homodimerization. Both recruitment of the common γ chain and activation of kinase Jak3 were shown to be dispensable for these processes.

Interleukin-4 (IL-4) is a pleiotropic immune regulator with a pivotal role in certain allergic processes (1). The bipartite IL-4 receptor comprises the interleukin-4 receptor α chain (IL-4Ra) (2) and the common γ chain (γc) (3, 4). Both receptor subunits belong to the cytokine receptor superfamily (5) and are shared by other cytokines; γc is also part of the receptors for IL-2, IL-7, IL-9, and IL-15 (6), and IL-4Ra contributes to the IL-13 receptor (7, 8).

Ligand-induced juxtaposition of the cytoplasmic domains of IL-4Ra and γc is believed to be a mandatory step in intracellular signaling which involves recruitment and activation of kinases Jak1 and Jak3 (9, 10), transcription factor Stat6 (11), and the adaptor molecule IRS-2 (12). However, the architecture of the IL-4R complex as well as the molecular mechanisms underlying the specificity of IL-4-induced signal transduction are to date poorly understood.

Making use of the strictly species-specific interaction of interleukin-4 with IL-4Ra chain, factor-dependent murine cells were rendered responsive to hIL-4 by expressing human IL-4Ra (2, 13–16). An implication of these results is the ability of human IL-4 to activate IL-4 receptor complexes containing either human or murine common γ chain, thus complicating an analysis of the composition of the signaling competent receptor subunit assembly.

In order to study the role of receptor chain dimerization events in signal release by the hIL-4R complex, we generated an expression system for receptor subunits that allowed us to experimentally induce specific and background-free intracellular hetero- and homodimerization.

Our results show that the juxtaposition of two intracellular domains of IL-4Ra can act as the trigger of specific signaling, including the activation of Jak1 and Stat6 and the induction of cell proliferation. Surprisingly, a hitherto assumed participation of the cytoplasmic portion of common γ chain and of γc-associated kinase Jak3 is not required.

MATERIALS AND METHODS

DNA Manipulations, Stable Transfection of Mouse Cells, and Detection and Quantification of Receptor Expression—Recombinant DNA work was performed according to standard procedures (17). The murine pre-B cell line Ba/F3 (18) has been described. BAF-4α-γc, a Ba/F3 derivative expressing both subunits of the human IL-4R, is identical to BAF-4γc (16).

Hybrid receptor genes were generated by polymerase chain reaction amplification of gene fragments from pKCR-γc (16) encoding the epitope-tagged extracellular domain and transmembrane/intracellular domain of human γc and exchanging them for the corresponding fragments (BanHI/ZhoI or XhoI/HindIII) in pKCR-α (16). The resulting expression plasmids pKCR-αγc and pKCR-α-γc were cotransfected into Ba/F3 cells as described (16).

Surface expression of receptor constructs was assayed by reacting intact cells with antibodies X 14/38 (16, 19) or P5D4 (20) specific for the extracellular portions of recombinant hIL-4Ra or epitope-tagged human γc, respectively, and subsequent detection of bound antibodies by peroxidase-coupled secondary antibodies as detailed elsewhere (21). Briefly, 106 cells in a microtiter well were incubated on ice for 30 min with 5 μg of antibody in a volume of 50 μl of phosphate-buffered saline/3% bovine serum albumin. After washing twice, cells were resuspended in 100 μl of a 100 μg/ml solution of peroxidase-conjugated goat anti-mouse IgG (Dianova) and kept on ice for 30 min. Cell-bound secondary antibody was detected by transferring the cells to 50 μl of a solution containing 0.1% Triton HCl, pH 5.5, 2.5 mM 3-aminomethylindole (Fluka), 400 μM p-chloroacetic acid (Sigma), 5.4 mM H2O2, and measuring elicited chemiluminescence using a MicroLumat LB 96P.

Quantitation of surface-bound antibody molecules was achieved by relating the determined intensity of chemiluminescence to a calibration series of samples containing known concentrations of peroxidase.

Cell Culture, Cytokines, and Proliferation Assay—Cell maintenance and preparation of hIL-4 and mutant Y124D has been described previously (16). Recombinant murine IL-4 was purchased from Sigma. Cytokine-induced proliferation of cell lines was measured by [3H] thymidine incorporation into de novo synthesized DNA as described (16).

Immunoprecipitation, Immunoblotting, and Chemical Cross-linking—Samples of 3 × 106 cells were incubated at 37 °C for 10 min in 1 ml of RPMI containing no cytokine, 7 nM of IL-4, or 50 nM of antibody P5D4 and subsequently lysed as described (16). Cleared lysates were incubated with 1–5 μg of specific antibody. Antibodies used for immunoprecipitations were 4G10 (anti-phosphotyrosine, Upstate Biotechnology),

*This work was supported by Deutsche Forschungsgemeinschaft through SFB 176. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The intracellular domain of hIL-4R tyrosine-phosphorylated proteins in the two cell lines (Fig. 2B) by precipitation (data not shown). Moreover, the modified hIL-4 receptor was found to recapitulate hIL-4-specific activation of E34–1 (22) and anti-Jak1 rabbit serum (23). Immunocomplexes were precipitated by lysates with 50 μl of anti-mouse IgG-agarose or protein A-Sepharose (Sigma) and assayed as described (16) using peroxidase-conjugated antibody RC20 (Transduction Laboratories) at a final concentration of 0.1 μg/ml. Iodination of hIL-4, cross-linking of radioligand to cell-surface receptors, and analysis of immunoprecipitated complexes by electrophoresis was carried out as described (19).

**Analysis of Stat Activation by Electrophoretic Mobility Shift Assay—** Whole cell extracts were prepared from cells stimulated with IL-4 or antibody as described above by suspension of cell pellets in a buffer containing 20 mM Hepes, pH 7.9, 400 mM NaCl, 1 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 5 μg/ml, and 100 μM ortho-vanadate followed by three freeze-thaw cycles and centrifugation at 4°C and 14,000 rpm for 15 min. Supernatants equivalent to 10⁶ cells were used for bandshift assays performed as described (24). As a probe, the Stat6-binding sequence 5′-GTCAACTTCCCAAGAACAGAA-3′ derived from the human Ie-promoter (25) end-labeled with polynucleotide kinase to a specific activity of 8,000 cpm/fmol was applied. Supershifting of Stat6 containing complexes was achieved by adding to the binding reactions before electrophoretic mobility shift assay 1 μg of a chicken antibody directed to amino acids 637–847 of murine Stat6.²

**RESULTS AND DISCUSSION**

We intended to reconstitute in murine cells a functional interleukin-4 receptor complex activable exclusively by human IL-4, which would not evoke any background signaling due to interference with the endogenous murine IL-4 receptor. To this end, we generated a pair of expression constructs encoding hybrid receptor chains derived from hIL-4Rα and hγc with mutually exchanged intracellular domains (Fig. 1A) and introduced it into the murine pre-B cell line Ba/F3.

One clone expressing both 4a/γ and py/4α chimeras was termed BAF-4a/γ-py/4α. The number of surface-expressed receptor molecules per cell was determined in comparison with cell line BAF-4a-py bearing both subunits of the authentic human IL-4R (Fig. 1B). As measured by the binding of specific antibodies recognizing the extracellular receptor domains, in both cell lines surface expression of the receptor chain comprising the intracellular domain of γc was considerably higher than that of the subunit bearing the intracellular part of hIL-4Rα. Irrespective of the “authentic” or “cross-over” composition of the heterologous subunits, similar hIL-4 binding receptor complexes could be formed in both cell lines as revealed by immunoprecipitation of receptor chains cross-linked to radiolabeled hIL-4 (Fig. 1C).

To test if the bipartite human IL-4R with exchanged cytoplasmic domains was capable of transmitting specific signals to the cell interior, we measured IL-4-induced cell proliferation. When stimulated with hIL-4, BAF-4a/γ-py/4α cells expressing the combination of hybrid receptors, like BAF-4a-py/4α, showed a proliferative response (Fig. 2A).

In BAF-4a-py cells, hIL-4 mutant Y124D evoked 60% of the DNA synthesis induced by wild type IL-4. We have previously shown that this degree of reactivity is due to preferential interaction of Y124D with murine γc (16). When assaying BAF-4a/γ-py/4α cells, we found, as earlier observed with human IL-4 reactive cells (19), only 30% of wild type activity for hIL-4 variant Y124D. This result indicates that hIL-4 cross-over receptor, as anticipated and unlike its authentic counterpart, precludes the formation of productive receptor complexes involving endogenous murine common γ chain.

Stimulation with hIL-4 resulted in equivalent patterns of tyrosine-phosphorylated proteins in the two cell lines (Fig. 2B). The intracellular domain of hIL-4Rα is a major substrate of ligand-induced phosphorylation as revealed by specific immunoprecipitation (data not shown). Moreover, the modified hIL-4 receptor was found to recapitulate hIL-4-specific activation of janus kinases Jak1 and Jak3.

We next employed the model receptor system to address the individual roles of the IL-4 receptor subunits in signaling. The ligand and antibody binding properties of the functionally expressed receptor constructs enabled us to specifically induce all three possible intracellular receptor dimers (Fig. 2A). In BAF-4a/γ-py/4α cells, not only hIL-4-induced heterodimerization of the two intracellular receptor domains but surprisingly also antibody-mediated cytoplasmic homodimerization of hIL-4Rα via the extracellular P5D4 epitope tag lead to cell proliferation.

² M. Heim and R. Moriggl, unpublished data.

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**Fig. 1. Generation and characterization of cell lines expressing hIL-4 receptor constructs. A, schematic representation of bipartite authentic hIL-4 receptor expressed in BAF-4a-py cell (top pair) in hIL-4 cross-over receptor expressed in BAF-4a/γ-py/4α cells (bottom pair). s, signal peptide; ex, extracellular domain; t, transmembrane domain; in, intracellular domain; p, epitope tag recognized by antibody P5D4. B, analysis of receptor chain surface expression in BAF-4a-py and BAF-4a/γ-py/4α cells. Samples of 10⁵ cells were reacted with antibodies directed to the extracellular domains of hIL-4Rα or epitope-tagged γc, respectively, washed, and stained with peroxidase-conjugated secondary antibody as described under “Materials and Methods.” Numbers of bound enzyme molecules per cell equivalent to receptor chain copies were determined by quantification of elicited chemiluminescence and correlation of the signal intensity with a calibration series obtained by measuring luminescence produced by different known amounts of peroxidase under assay conditions. C, analysis of ligand-receptor complexes formed on BAF-4a-py and BAF-4a/γ-py/4α cells. After chemical cross-linking of 12⁵hIL-4 to the two cell lines, receptor complexes were immunoprecipitated using the indicated antibodies and subsequently resolved and visualized by SDS-polyacrylamide gel electrophoresis and autoradiography. Radioligand cross-linked receptor chains and complexes are marked with arrows.
Antibody activity on BAF-4 induced intracellular homodimerization of hIL-4R, respectively (Fig. 3C), we found that antibody-involved in IL-4 receptor complex function by hetero- or homodimerization at excess concentration and thus underscores our notion of P5D4-of receptor cross-linking by monovalent antibody binding at the domain of IL-4 receptor subunits and resulting signal transduction. A, schematic representation of stimulus-induced dimerization events. I, hIL-4-induced selective intracellular heterodimerization of hIL-4Rα and human γc in BAF-4α/γ-py/4α-cells. I, intracellular homodimerization of hIL-4α via extracellular antibody and epitope tag in BAF-4α/γ-py/4α-cells. III, antibody-induced homodimerization of γc in BAF-4α/γ-py/4α-cells. B, cell proliferation evoked by the stimuli depicted under A. The respective cell lines were incubated with the indicated concentrations of hIL-4 (I) or antibody P5D4 (II and III) for 24 h before [3H]thymidine uptake was measured. C and D, activation of Jak kinases (C) and activation of Stat6 (D) by the stimuli depicted under A. The respective cell lines were stimulated for 10 min with 10 nm hIL-4 (I) or 100 nM P5D4 (II and III). Cells were then lysed and subjected to immunoprecipitations with anti-Jak antibodies and probing with anti-phosphotyrosine antibody (C) or to a band shift assay using a labeled probe derived from the Ie-promoter (D) as described under "Materials and Methods."
function of the IL-4 receptor. The interaction of ligand with the extracellular domains of both IL-4Ra and γc is necessary for IL-4-induced signal transduction, because mutant forms of hIL-4Ra defective in contacting γc fail to stimulate cell proliferation (26, 29). Functional properties of the intracellular domain of γc in the activation of this particular receptor system have not yet been addressed. Our data indicate that it is not involved in the release of intracellular signals specific for IL-4 and support the notion of a more general role for γc in the formation of the signaling competent IL-4R and probably also other cytokine receptor complexes. In ligand-induced IL-4R activation, one function of γc and Jak3 could be the promotion of a transient assembly of two or more copies of hIL-4Ra, a situation which in turn would lead to specific intracellular signal transduction. Alternatively, in the natural receptor complex, γc-mediated recruitment of Jak3 might result in an activation of Jak1, an event that in our model experiment is mimicked by the juxtaposition of two Jak1 molecules and serves as the master trigger for the various activities of hIL-4Ra. A more general version of such an interpretation of exchangeable Jaks in the hIL-4R complex would be the view that ligand-induced intracellular apposition of several combinations of two Jak molecules would suffice to evoke cell proliferation and the other reactions observed. In this scenario, the major function of the specific receptor chain (here: hIL-4Ra) would be to provide recognition sites for Stats and other downstream components that upon Jak-driven activation mediate the particular effects of IL-4. Directed homodimerization of γc does not result in similar activities because of its lack of recognition sites for downstream signaling molecules. Also in line with such an explanation would be the notion of cytokine receptor signal transduction being relatively unselective and flexible in terms of interactions between receptor chains and intracellular binding partners. This would imply that the main event regulating specificity in cytokine signaling is the recognition between receptor and ligand and the thereby cross-linked combination of receptor subunits.

To discriminate between the two principal explanations compatible with our results (involvement of receptor multimers in “natural” hIL-4 receptor activation or low specificity of Jak activity combined with recruitment of signaling molecules by hIL-4Ra via specific recognition sites), careful investigation of the stoichiometric subunit composition of the active hIL-4 receptor complex and a mutational analysis of the cytoplasmic portion of γc in this context are necessary. Also, the molecular details of Jak recognition, activation, and specificity in the hIL-4R assembly have to be addressed.

Acknowledgments—The expert technical assistance of C. Muller is gratefully acknowledged. We thank W. Sebald for generous support and cytokines and T. Kreis for antibody P5D4.

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J. Biol. Chem. 1996, 271:23634-23637.
doi: 10.1074/jbc.271.39.23634

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