Signal Transduction Pathway of Interleukin-4 and Interleukin-13 in Human B Cells Derived from X-linked Severe Combined Immunodeficiency Patients*

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Interleukin-4 (IL-4) and IL-13 are functionally similar cytokines. The functional IL-4 receptor (IL-4R) consists of the IL-4Rα chain (IL-4Rα) and the IL-2Rγ chain (γc), which is shared by the IL-2, IL-7, IL-9, and IL-15 receptors. The functional IL-13R is thought to involve the IL-4Rα but not γc. In this study, we have analyzed activation of members of the γc family and signal transducers and activators of transcription (STAT) 6 induced by IL-4 and IL-13 in Epstein-Barr virus-transformed B cells derived from two patients of X-linked severe combined immunodeficiency, who have mutations of the γc gene in the extracellular or intracellular domains. In these B cells, IL-4 failed to induce tyrosine phosphorylation of Jak3 and activation of STAT6, or activation of these molecules was significantly decreased compared with Epstein-Barr virus-transformed normal B cells. In contrast, IL-13 activated STAT6 in these cells as well as normal B cells. However, Jak3 was not activated by IL-13, even in normal B cells. These results clearly indicated that γc is essential for activation of Jak3 and STAT6 in the signal transduction pathway of IL-4 in human B cells and that IL-13 does not utilize γc but activates STAT6 through an alternative pathway, which is not impaired in B cells of X-linked severe combined immunodeficiency patients.

Both IL-4 and IL-13 are pleiotropic cytokines that are produced by activated T cells and manifest similar functions on hematopoietic cells such as induction of immunoglobulin class switching to IgG4 and IgE, expression of CD23 and major histocompatibility complex class II on human B cells, and anti-inflammatory effects on monocytes (1). These functions are mediated by binding of cytokines to their specific receptors. The functional IL-4 receptor (IL-4R) has been shown to consist of at least two components, the IL-4Rα chain (IL-4Rα) and the IL-2Rγ chain (γc). Although the IL-2Rγ was originally identified as the third chain of the IL-2R (4), it has been shown that the IL-2Rγ is shared by IL-2, IL-4, IL-7, IL-9, and IL-15 receptors, and thus the IL-2Rγ was termed the common γ chain (γc) (2, 3). The initial study of functional IL-13 receptor (IL-13R) suggested that IL-4R and IL-13R share a common component (9), but γc has been proposed to be a common component (2, 3). However, recent studies have revealed that γc may not be a component of functional IL-13, because it has been demonstrated that γc was not chemically cross-linked with radiolabeled IL-13 and that the functional IL-13 was expressed on γc-negative cells (10–13). These reports have also indicated that γc is not absolutely required for functional IL-4R. In contrast, the reports that both IL-4 and IL-13 induce tyrosine phosphorylation of the IL-4Rα and that anti-IL-4Rα antibody inhibits proliferation induced by IL-13 and binding of IL-13 to TF-1 cells suggest that functional IL-13R shares IL-4Rα rather than γc (11, 14, 15).

Recently, involvement of the Janus tyrosine kinase (Jak) family and downstream molecules, signal transducers and activators of transcription (STAT), in many cytokine signal transduction pathways has been extensively investigated (16). IL-4 has been shown to activate members of the Jak family, Jak1 and Jak3 (17, 18), which associate with the IL-4Rα chain and γc, respectively (7, 19, 20). In contrast, IL-13 does not induce tyrosine phosphorylation of Jak3 (21), which is consistent with the idea that γc is not utilized in functional IL-13R. Recently, STAT induced by IL-4 (IL-4 STAT/STAT6), which is able to bind to the consensus sequence in the promoter of IL-4-inducible genes (22–24), has been molecularly cloned (25). IL-13 has also been demonstrated to activate a DNA binding nuclear factor similar to that activated by IL-4 (11, 26).

X-linked severe combined immunodeficiency (XSCID) is an inherited immunodeficiency disease characterized by absent or markedly reduced numbers of T cells and B cells that function abnormally, resulting in recurrent infections in early life and failure to thrive without bone marrow transplantation (27, 28). Recently, it has been demonstrated that XSCID results in mutation of the γc gene (29–31). Various mutations of the γc gene have been reported, such as one point mutation at the extracellular domain or the cytoplasmic domain or the exon/intron junction and deletions mutations at the cytoplasmic domain, the second exon, or the transmembrane domain (32). Cells from XSCID patients provide an excellent assay system for cytokine signal transduction where γc is involved. It has been demonstrated that IL-4R and IL-13R are able to trans-

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* The abbreviations used are: IL, interleukin; R, receptor; γc, IL-2Rγ chain; Jak, Janus tyrosine kinase; STAT, signal transducers and activators of transcription; XSCID, X-linked severe combined immunodeficiency; EMSA, electrophoretic mobility shift assay; EBV, Epstein-Barr virus.
duce signals in XSCID B cells (12). However, the mechanism by which this is done remains unclear. Here, we analyze the Jak-STAT pathway activated by IL-4 and IL-13 in Epstein-Barr Virus (EBV)-transformed B cells derived from two XSCID patients in order to evaluate the role of γc in the signal pathways of IL-4 and IL-13.

**EXPERIMENTAL PROCEDURES**

Materials—Anti-human Jak1 antibody and monoclonal anti-phyosphotyrosine antibody (4G10) were purchased from UBI (Lake Placid, NY). Anti-human Jak3 antibody and anti-human IL-4 STAT antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The antibody against γc, TUGh4, was kindly provided by Dr. Kazuo Sugamura (Tohoku University, Japan). IL-13 was kindly given by Dr. Hiroshi Wakao (Tokyo University, Japan). Double-stranded oligonucleotide (5'-GTCACATCTCCAGAAGACAGA-3') was kindly provided by Dr. Hiroshi Wakao (Tokyo University, Japan).

Analysis of EBV-transformed B Cells Derived from XSCID Patients—The diagnosis of XSCID was ascertained by immunological investigations such as the absence or decreased number of T lymphocytes and the presence of B cells. EBV-transformed B cells were generated from B cells in the peripheries from patients 1 and 2 and a normal donor. Sequencing of DNA in B cells from XSCID patients revealed that patient 1 had a GATT deletion at nucleotide position 816, causing a frameshift in the transmembrane domain following the appearance of a stop codon at 84 nucleotides ahead of the breakpoint, and that patient 2 had a T to C point mutation at nucleotide position 455, causing one amino acid substitution of valine to alanine in the extracellular domain.

Immunofluorescence staining of cells proved that neither patient's B cells showed an appreciable fluorescent intensity, whereas EBV-transformed B cells derived from a normal donor gave a significant intensity by using antibody against γc, TUGh4.

Immunoprecipitation and Western Blotting—Procedures of immunoprecipitation and Western blotting were carried out as described previously (33). Briefly, EBV-transformed B cells were stimulated by 10 ng/ml IL-4 or 10 ng/ml IL-13 for 5 min and then lysed in a lysis buffer containing 1% Triton X-100. The proteins were immunoprecipitated from clarified cell lysates by adding 30 μl of appropriate antibody-preconjugated Sepharose beads and incubated for at least 2 h at 4°C. After Sepharose beads were washed 4 times with the same lysis buffer, immune complexes were eluted from Sepharose beads by boiling with SDS-polyacrylamide gel electrophoresis sample buffer, applied to SDS-polyacrylamide gel electrophoresis, and transferred electrophoretically to nitrocellulose filters. Proteins were probed with monoclonal anti-phyosphotyrosine antibody (4G10) or anti-Jak3 antibody and visualized by enhanced chemiluminescence (ECL, Amersham Corp.).

Extraction of Nuclear Proteins and Electrophoretic Mobility Shift Assay (EMSA)—Procedures of extraction of nuclear proteins and EMSA were carried out as described previously (34). Nuclear extract was mixed with binding buffer (20 mM Hepes-NaOH, pH 7.9, 2 mM EDTA, 100 mM NaCl, 10% glycerol, 0.2% Nonidet P-40), poly(dI-dC), and labeled oligonucleotide probe. The mixtures were incubated at room temperature for 30 min. To detect supershifted bands, anti-IL-4 STAT antibody was added to the assay mixtures. The reaction mixtures were loaded on a 4% polyacrylamide gel and run with a running buffer of 0.25 X TBE. The gel was dried onto Whatman No. 3MM paper. The DNA-protein complexes were visualized by autoradiography.

RESULTS

In order to investigate involvement of γc in activation of Jak1 and Jak3 in the B cells of XSCID, we analyzed tyrosine phosphorylation of Jak1 and Jak3 induced by IL-4 and IL-13 in two patients' B cells. As shown in Fig. 1A, tyrosine phosphorylation of Jak1 induced by IL-4 in normal B cells was much less than that in TF-1 cells. This observation is consistent with the previous report in which levels of tyrosine phosphorylation of Jak1 upon IL-4 stimulation were varied and generally weak among different cell types (35, 36). Thus, we focused on analyzing tyrosine phosphorylation of Jak3 by IL-4 in patient's B cells. Jak3 was tyrosine-phosphorylated in normal B cells upon IL-4 stimulation, whereas in B cells from patient 1, no tyrosine phosphorylation of Jak3 was detected upon IL-4 stimulation, and tyrosine phosphorylation of Jak3 was remarkably decreased in B cells from patient 2 compared with that in normal B cells (Fig. 1B). The loaded amounts of Jak3 were not significantly different in each sample (Fig. 1C), indicating that tyrosine phosphorylation of Jak3 in the patients' B cells is impaired. We assume that, although expression of γc on B cells from patient 2 was not detected, mutated γc, which anti-γc antibody used in staining of B cells was not able to recognize, is expressed on the cell surface and transduces a reduced signal from the ligand-engaged receptors. These results suggest that activation of Jak3 by IL-4 requires expression of γc on the cell surface. In contrast to IL-4, IL-13 did not induce tyrosine phosphorylation of Jak3 (Fig. 2A), which is consistent with the previous report (21). This could not be explained by the low concentration of IL-13, because the concentration of IL-13 used in this experiment was enough to activate STAT6 and induce synthesis of IgE in human peripheral blood mononuclear cells (data not shown). Thus, these results indicate that Jak3 is not involved in the signal transduction pathway of IL-13.

It has already been shown that IL-4-responsive genes, such as Il4, FceRIα, and FcγRI, have the consensus sequence TTC-

![Figure 1. Tyrosine phosphorylation of Jak1 and Jak3 by IL-4.](image-url)
Nonsense (NGAA) at the promoter region and that certain DNA binding factors that associate with this portion are activated by IL-4 (22–24). A member of the STAT family activated by IL-4, which binds to this region, was molecularly cloned and designated as IL-4 STAT (STAT6) (25) and IL-13 has been shown to activate a STAT-like protein, which appeared to be identical to the IL-4-induced STAT. However, it has not been confirmed as STAT6 by anti-STAT6 antibody (11, 26). In order to assess the role of γc in activation of the STAT-like protein induced by IL-4 and IL-13, which was predicted to be STAT6, we analyzed activation of the STAT-like protein using anti-STAT6 antibody in the patients’ B cells. Activation of the DNA binding factor induced by IL-4 and IL-13 was detected in normal B cells, and addition of anti-STAT6 antibody to the DNA-protein complexes induced by IL-4 and IL-13 supershifted the DNA-protein complexes (Fig. 3A), indicating that the DNA binding factor induced by IL-4 and IL-13 is STAT6. Activation of STAT6 by IL-13 appears to be less than that by IL-4, and an even higher concentration of IL-13 did not augment activation of STAT6 (data not shown). These results suggest that in human B cells, the mechanism for activation of STAT6 by IL-4 is distinct from that by IL-13.

In B cells from patient 1, activation of STAT6 by IL-4 was completely abolished, whereas IL-4 induced significantly reduced activation of STAT6 in B cells from patient 2, compared with normal B cells (Fig. 3B). Activation levels of STAT6 correlated well with the intensities of tyrosine phosphorylation of Jak3, suggesting that activation of Jak3 is required for activation of STAT6 in the IL-4 signal transduction pathway. In contrast to IL-4, IL-13 activated STAT6 in one patient’s B cells to the same level as normal B cells, and higher activation of STAT6 was observed in another patient’s B cells (Fig. 3C). Thus far, we don’t know the reason why activation of STAT6 by IL-13 is higher in patient 2 than in normal B cells. These results suggest that the signal transduction pathway arising from γc via Jak3 is essential for IL-4-induced activation of STAT6 and that IL-13 utilizes an alternative pathway to activate STAT6.

DISCUSSION

In this study, we demonstrated that activation of Jak3 and STAT6 by IL-4 was impaired in EBV-transformed B cells established from XSCID patients who have mutations in the gene encoding γc, while activation of STAT6 induced by IL-13 was intact without activation of Jak3 in the same cells. These results clearly show that IL-13 does not require γc for activation of STAT6. Although both Jak1 and Jak3 are tyrosine-phosphorylated by IL-4, recent reports have demonstrated that the degree of tyrosine phosphorylation of Jak1 is much less than that of Jak3 in TF-1 cells and that Jak1 is not tyrosine-phosphorylated by IL-4 in human B cells (35, 36). We also found that tyrosine phosphorylation of Jak1 in EBV-transformed B cells was much less than that in TF-1 cells. These results suggest that Jak1 plays different roles in IL-4-mediated signal transduction in different cell types.

Matthews et al. (12) have shown that B cells derived from a γc-negative XSCID patient, which were costimulated with IL-4 and soluble CD40 ligand, were able to generate IgE synthesis to the same level as normal B cells. Furthermore, germ-line e transcript is also found to be induced by IL-4 in the patients’ B cells used in this study. However, our results demonstrated that the existence of γc is essential for activation of STAT6 by IL-4 in EBV-transformed B cells. These observations suggest that the Jak-STAT pathway is not necessary for IL-4-induced immunoglobulin class switching. However, the study of γc-deficient mice demonstrated that the sera from γc-deficient mice do not contain detectable levels of IgE (37). Although the reasons for these discrepancies are unknown, it is possible that human B cells, but not mouse B cells, have an alternative IL-4-mediated signal pathway for IgE synthesis, which does not require activation of the known Jak-STAT pathway. In addition, Lin et al. (11) have demonstrated that IL-4 activates a STAT protein in COS7 cells expressing IL-4Rα without expression of γc, providing evidence that γc is not essential for activation of STAT6 by IL-4. Taken together, these findings raise the possibility that there are three distinct signal transduction pathways of IL-4. The first one is the Jak-STAT pathway, which is transduced through IL-4Rα and γc as seen in normal human B cells. The second one is the Jak-STAT pathway, which does not require γc, as seen in COS7 cells. The third one is the signal pathway, which induces IgE synthesis without activating the known Jak-STAT pathway, as seen in human XSCID B cells. The third signal pathway seems to be defective in mouse B cells. Molecular cloning of a IL-13 receptor will clarify this possibility.

Hou et al. (25) have suggested that phosphorylated tyrosine residues of IL-4Rα at 578 and 606 amino acids may be impor-
tant for activation and dimerization of STAT6. Recently, Quelle et al. (38) reported that activation of STAT6 by IL-4 in a mouse myeloid cell line 32D requires the distal portion of the intracellular domain of IL-4Rα. In our studies, we have demonstrated that the portion in the intracellular domain of IL-4Rα between amino acids 353 and 393, numbering from the methionine start of the signal peptide, is essential for the synthesis of germ-line c transcript induced by IL-4 (39), which is also essential for IL-4-mediated mitogenic signal (40). These findings also suggest that the Jak-STAT pathway in IL-4-mediated signal transduction is not essential for induction of IgE synthesis (41). Further investigation is awaited to establish the physiological role of the Jak-STAT pathway in the signal transduction of IL-4 and IL-13.

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