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► **To cite this version:**

N. Taylor, F. Candotti, S. Smith, S. A. Oakes, T. Jahn, et al.. Interleukin-4 signaling in B lymphocytes from patients with X-linked severe combined immunodeficiency. *Journal of Biological Chemistry*, 1997, 272 (11), pp.7314–7319. 10.1074/jbc.272.11.7314 . hal-02195790

HAL Id: hal-02195790

<https://hal.science/hal-02195790v1>

Submitted on 27 May 2021

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Interleukin-4 Signaling in B Lymphocytes from Patients with X-linked Severe Combined Immunodeficiency*

(Received for publication, October 29, 1996, and in revised form, December 17, 1996)

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Interleukin-4 (IL-4) is an important cytokine for B and T lymphocyte function and mediates its effects via a receptor that contains γ_c . B cells derived from patients with X-linked severe combined immunodeficiency (X-SCID) are deficient in γ_c and provide a useful model in which to dissect the role of this subunit in IL-4-mediated signaling. We found that although IL-4 stimulation of X-SCID B cells did not result in Janus tyrosine kinase-3 (JAK3) phosphorylation, other IL-4 substrates including JAK1 and IRS-1 were phosphorylated. Additionally, we detected signal transducers and activators of transcription 6 (STAT6) tyrosine phosphorylation and DNA binding activity in X-SCID B cells with a wide range of γ_c mutations. However, reconstitution of these X-SCID B cells with γ_c enhanced IL-4-mediated responses including STAT6 phosphorylation and DNA binding activity and resulted in increased CD23 expression. Thus, γ_c is not necessary to trigger IL-4-mediated responses in B cells, but its presence is important for optimal IL-4-signaling. These results suggest that two distinct IL-4 signaling pathways exist.

wide range of mutations in the common gamma chain (γ_c) gene that result in either a lack of γ_c message, unstable γ_c proteins that are poorly expressed, or defective γ_c receptor subunits that are expressed but nonfunctional (2–4). γ_c was originally identified as a component of the IL-2 cytokine receptor (IL-2R γ), but as it has been shown to be shared by receptors for IL-2, IL-4, IL-7, IL-9, and IL-15, it is now designated γ_c (5–9).

IL-4 is thought to be important for mature B cell functions including immunoglobulin class switching to IgG4 and IgE as well as expression of CD23 and major histocompatibility complex class II genes (10). Because IL-4 regulates B lymphocyte function, it is important to determine the response of X-SCID B cells to this cytokine. The functional IL-4 receptor (IL-4R) consists of at least two components, IL-4R α and γ_c subunits (6, 11). Signal transduction through the IL-4R, as well as through other hematopoietic receptors, is initiated by activation of Janus family tyrosine kinases (JAKs) (12, 13). IL-4 elicits tyrosine phosphorylation of the JAK family members JAK1 and JAK3, which interact with the IL-4R α and γ_c subunits, respectively (14–17). The current model of cytokine signaling proposes that upon cytokine binding, members of the JAK family are rapidly activated and subsequently tyrosine-phosphorylate the receptor, forming a docking site for signal transducers and activators of transcription (STATs) that are also phosphorylated by JAKs (12, 13). The STAT proteins then dimerize and translocate to the nucleus where they bind DNA sequences on target genes. One important STAT that is activated in response to IL-4, STAT6 (IL-4 STAT), has been shown to bind to promoter sequences of IL-4-inducible genes (18–21). As STAT6 knockout animals parallel the IL-4 null phenotype and exhibit defects in Th2 helper T cell differentiation and immunoglobulin class switching (22–24), STAT6 appears to be essential for many IL-4-mediated effects.

It might be predicted that in the absence of a functional γ_c chain, X-SCID B cells would not be able to respond to IL-4. Indeed, two groups have reported that neither JAK1 phosphorylation nor STAT6 DNA binding activity is induced upon IL-4 stimulation of Epstein-Barr virus-transformed B cells (B-LCL) derived from X-SCID patients (25, 26). However, Matthews and colleagues (27) have recently demonstrated that although X-SCID B cells cannot undergo immunoglobulin class switching, they can proliferate *in vitro* in response to IL-4 when co-stimulated with CD40 ligand or anti-IgM. Although the underlying biochemical mechanisms are not clear, these data suggest that IL-4-mediated signaling in X-SCID B cells can occur.

It was therefore important to analyze IL-4-signaling in a panel of B-LCL derived from X-SCID patients with a wide range of γ_c mutations in order to clarify these discrepancies.

Patients with X-linked severe combined immunodeficiency (X-SCID)¹ present with very few T cells and absent mitogenic responses (1). Although B cells are present, immunoglobulin levels are low, and specific antibody production is lacking. The combination of these defects is fatal by 1–2 years of age unless the immune system is reconstituted by allogeneic bone marrow transplantation. These clinical manifestations are due to a

* This work was supported in part by a Howard Hughes Medical Institute Postdoctoral Physician Grant (to N. T.), the Howard Hughes Medical Institute-National Institutes of Health Research Scholars Program (to S. A. O.), the Fondation pour la Recherche Médicale, Philippe Foundation, and National Institutes of Health Grant AI25071. 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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¹ The abbreviations used are: X-SCID, X-linked severe combined immunodeficiency; IL, interleukin; JAK, Janus tyrosine kinase; STAT, signal transducers and activators of transcription; B-LCL, B cell lymphoblastoid cell lines; GAS, interferon- γ activation site; R, receptor; IRS, insulin receptor substrate.

IL-4 failed to stimulate JAK3 tyrosine phosphorylation in X-SCID B cells, but we found that JAK1 and IRS-1 were phosphorylated. IL-4 also induced STAT6 phosphorylation as well as DNA binding activity in these cells. However, STAT6 activation and CD23 expression were significantly enhanced in X-SCID B-LCL reconstituted with wild type γ_c . Thus, we have demonstrated a γ_c -JAK3-independent pathway through which IL-4 activates JAK1 and STAT6 in B cells derived from X-SCID patients. These results have important implications for the understanding of IL-4 signal transduction and the lack of mature B cell function in patients with X-SCID.

EXPERIMENTAL PROCEDURES

Cytokines and Antibodies—Recombinant human IL-2 and IL-13 were the generous gifts of C. Paradise (Chiron Corp., Emeryville, CA) and C. Reynolds (BRMP, Frederick, MD), respectively. Polyclonal rabbit anti-sera against STAT6 was kindly provided by R. LaRoche (National Cancer Institute, Bethesda) or was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-JAK1 antibodies were purchased from Transduction Laboratories (Lexington, KY) and Santa Cruz Biotechnology Inc. Anti-IRS-1 antibodies were obtained from J. Pierce (National Institutes of Health, Bethesda). The 4G10 monoclonal anti-phosphotyrosine antibody was purchased from Upstate Biotechnology Inc. (Lake Placid, NY), and the pY72 anti-phosphotyrosine was the gift of B. Sefton (Salk Institute, La Jolla, CA).

Cell Lines—Male patients with SCID were initially diagnosed with the X-linked form based on maternal X chromosome inactivation patterns (3). Peripheral blood mononuclear cells from X-SCID patients and normal donors were obtained upon informed consent, and B cell lymphoblastoid cell lines (B-LCL) were established by Epstein-Barr virus immortalization. The mutations in the γ_c gene in each X-SCID B-LCL have been previously described (3, 28, 29). Each X-SCID B-LCL is named by the position and identity of the base pair or amino acid substitution within the γ_c cDNA or protein, respectively. M11 has an M to I substitution at amino acid 1, and other substitutions include R224W, R226C, R226H, and F227C. R289* prematurely terminates the γ_c protein at R289, dup235-7 has a duplication of amino acids 235-237, 924delC has a C deleted at cDNA position 924 resulting in a missense amino acid after amino acid 308. The X-SCID B-LCL identified as cDNA468+1 has a mutation which disrupts the splice donor site after exon 3 and results in no detectable γ_c mRNA (3). The X-SCID B-LCL cDNA468+1 (boy 5) into which the wild type γ_c gene was introduced by retroviral mediated transduction has previously been reported (30) and is noted as cDNA468+1/ γ_c . Expression of γ_c protein on the cell surface of control and X-SCID B-LCL was assessed using a γ_c -specific rat monoclonal antibody, TUGH4 (Pharmigen, San Diego, CA). Immunofluorescence analyses revealed either normal, trace, or absent levels of γ_c protein on the cell surface (data not shown). The HUT78 T cell line was obtained from the ATCC (Rockville, MD). All cell lines were cultured in RPMI 1640 with 10% fetal calf serum, 2 mM glutamine, 50 units/ml penicillin, and 50 μ g/ml streptomycin.

Immunoprecipitation and Western Blotting Analysis—Cells were stimulated essentially as described previously (15). Briefly, cells were resuspended at 1×10^7 cells/ml and incubated with either 10^3 units/ml IL-2, 100 ng/ml IL-4, or 250 ng/ml IL-13. After 10 min, cells were lysed in a 1% Nonidet P-40 lysis buffer. Supernatants were immunoprecipitated with the specified antibody at 4 °C, and immunoprecipitates were collected on protein A/G agarose (Santa Cruz Biotechnology Inc.) and separated on 7.5% SDS-polyacrylamide gels. Membranes were blotted with the 4G10 and pY72 anti-phosphotyrosine mAbs as described previously (31). Blots probed with polyclonal JAK1, JAK3, STAT6, and IRS-1 antibodies were blocked in TBS (150 mM NaCl, 20 mM Tris (pH 7.5)) containing 5% bovine serum albumin and 0.1% Tween 20. Blots were incubated sequentially with the primary antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG (Amersham Corp.) and visualized using the enhanced chemiluminescence detection system (Amersham Corp.). For reblotting, filters were stripped as reported (31).

Electrophoretic Mobility Shift Assay—B-LCLs were cultured without serum for 4 h prior to a 15-min stimulation with IL-4 or IL-13 (1000 units/ml). Cells were lysed in a buffer containing 0.5% Nonidet P-40, 50 mM Tris-HCl (pH 8.0), 10% glycerol, 100 mM EDTA (pH 8.0), 50 mM NaF, 150 mM NaCl, 100 mM Na_3VO_4 , 1 mM dithiothreitol, 400 mM phenylmethylsulfonyl fluoride, and 1 mg/ml leupeptin and aprotinin. Whole cell extracts were prepared by centrifugation, and electrophoretic mobility shift assays were performed essentially as reported (32), using a ^{32}P random prime-labeled double-stranded oligonucleo-

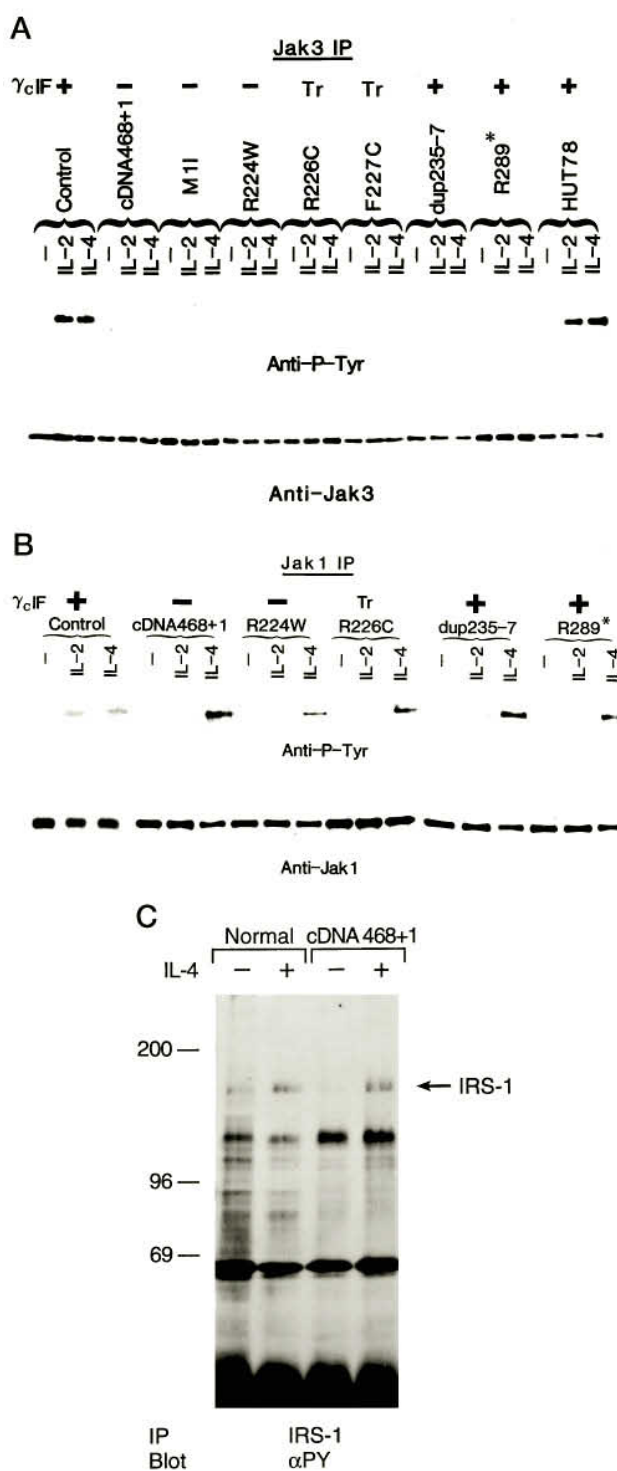


Fig. 1. Tyrosine phosphorylation of JAK3, JAK1, and IRS-1 following IL-2 and IL-4 stimulation. Normal (control) B-LCL, B-LCL derived from X-SCID patients with various γ_c mutations, and the HUT-78 T cell line were stimulated with IL-2 (1000 units/ml) or IL-4 (100 ng/ml) for 10 min at 37 °C. X-SCID B-LCL are labeled by the amino acid position of their mutation within the γ_c protein, except for the B-LCL cDNA468+1 where there is a 1-base pair insertion at position 468 of the γ_c cDNA. The ability to detect γ_c protein in each cell line was assessed by immunofluorescence with an anti- γ_c monoclonal antibody (γ_c IF) and is indicated as either normal (+), absent (-), or trace (Tr) levels. Lysates were immunoprecipitated (IP) with either a rabbit polyclonal anti-JAK3 antibody (A), rabbit polyclonal anti-JAK1 antibody (B), or a rabbit polyclonal anti-IRS-1 antibody (C), resolved on polyacrylamide gels, and immunoblotted with an anti-phosphotyrosine monoclonal antibody (α PY) (upper panels). Blots were then stripped and reblotted with either anti-JAK3 or anti-JAK1 antibodies to verify equivalent levels of protein in each lane (lower panels).

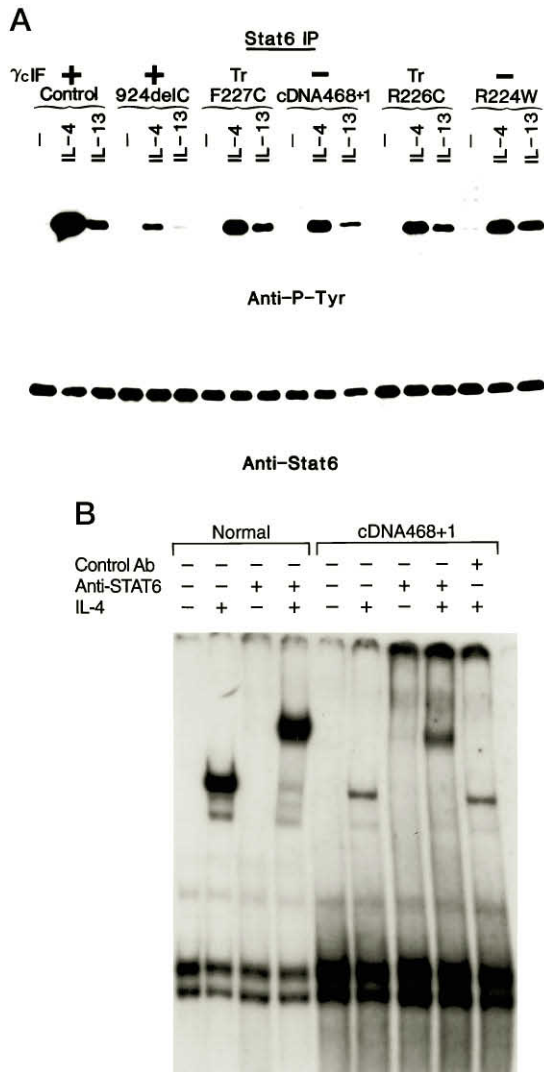


FIG. 2. Analysis of STAT6 tyrosine phosphorylation and DNA binding activity upon IL-4 and IL-13 stimulation of X-SCID B-LCL. A, immunoprecipitation with a polyclonal anti-STAT6 antibody was performed on lysates from control and X-SCID B-LCL following either no stimulation (–) or stimulation with IL-4 (100 ng/ml) or IL-13 (250 ng/ml) for 10 min at 37 °C and immunoblotted with anti-phosphotyrosine monoclonal antibodies (*upper panel*). Blots were then stripped and reprobed with the anti-STAT6 antibody to monitor the level of STAT6 (*lower panel*). B, whole cell extracts were incubated with ³²P-labeled GAS oligonucleotide and then subjected to polyacrylamide gel electrophoresis. For supershift reactions, cells were preincubated with either an anti-STAT6 antibody or a control antibody prior to addition of probe and were either untreated (–) or stimulated with IL-4 (+).

tide corresponding to the GAS-like element present in the CD23 promoter (5'-AAGACATTTCTAAGAAATCTATC-3') (33). Briefly, whole cell extracts were incubated with labeled probe in binding buffer for 15 min at 4 °C prior to electrophoresis on 6% polyacrylamide gels and autoradiography. When used, anti-STAT6 antibodies were incubated with cell extracts for 15 min following the addition of probe.

Analysis of CD23 Cell Surface Expression—For flow cytometric analysis of surface markers, serum was removed from the cells, and cells were cultured in RPMI 1640 with or without IL-4 for 48 h at a density of 5×10^5 per well in a 6-well plate. Cells were then incubated for 30 min at 4 °C in the presence of 25 ng of phycoerythrin-conjugated-EBVCS-5 (anti-human CD23, IgG1) or an isotype control. CD23 surface staining was measured using a FACscan flow cytometer, and the data were analyzed with Cellquest software (Becton-Dickinson, San Jose, CA). The mean fluorescence intensities were calculated by deducting the corresponding isotype control.

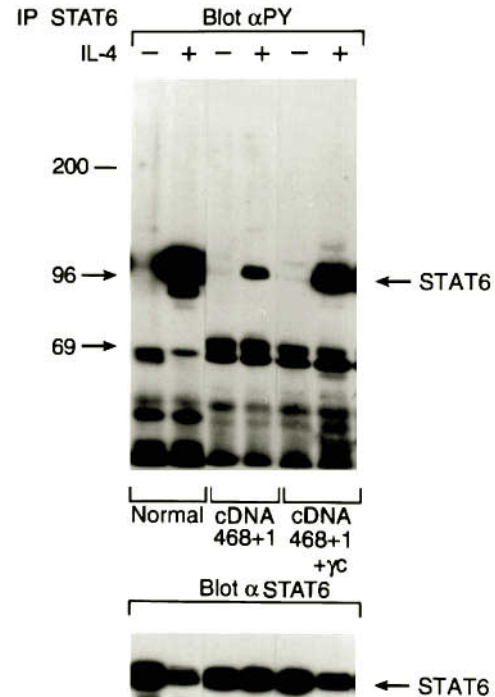


FIG. 3. STAT6 phosphorylation is enhanced following γ_c reconstitution of an X-SCID B-LCL. Immunoprecipitation with a polyclonal anti-STAT6 antibody was performed on lysates from a control (*normal*), X-SCID B-LCL (*cDNA468+1*), and the same X-SCID B-LCL reconstituted with γ_c (*XSCID+ γ_c*) following either no stimulation (–) or stimulation with IL-4 (+). Immunoprecipitates were blotted with anti-phosphotyrosine monoclonal antibodies (*upper panel*) and reprobed with an anti-STAT6 antibody (*lower panel*).

RESULTS

JAK1 and IRS-1, but Not JAK3, Are Phosphorylated following IL-4 Stimulation of X-SCID B-LCL—In order to investigate whether there is any γ_c -independent cytokine-mediated signal transduction in X-SCID B cells, we assessed the phosphorylation of the major intermediates determined to be involved in IL-4 signaling.

JAK3 associates with the shared γ_c subunit of the IL-2 and IL-4 receptors and is tyrosine-phosphorylated upon addition of either cytokine (14–17). We therefore analyzed JAK3 tyrosine phosphorylation in response to IL-2 and IL-4 stimulation in Epstein-Barr virus-transformed X-SCID B-LCL with heterogeneous γ_c mutations. Although JAK3 was tyrosine-phosphorylated upon both IL-2 and IL-4 stimulation of a control B-LCL and the HUT78 T cell line, we did not detect tyrosine phosphorylation of JAK3 following IL-2 or IL-4 stimulation of any of the X-SCID B-LCLs (Fig. 1A, *upper panel*). Equivalent levels of immunoprecipitated JAK3 could be demonstrated in each lane by immunoblotting the same filter with a polyclonal anti-JAK3 antibody (Fig. 1A, *lower panel*). These data indicate that JAK3 is not phosphorylated following IL-2 or IL-4 stimulation of X-SCID B-LCL with a diversity of γ_c mutations (28, 29, Fig. 1).

JAK1 constitutively associates with the IL-2R β and IL-4R α subunits of the IL-2 and IL-4 receptors, respectively, and is phosphorylated upon receptor stimulation (14, 16, 34). It might be predicted that in the absence of JAK3 phosphorylation, JAK1 would not be phosphorylated in response to IL-2 or IL-4 in X-SCID B-LCL. In fact, JAK1 was not phosphorylated in any of the X-SCID B-LCLs tested following the addition of IL-2 (Fig. 1B, *upper panel*). However, treatment with IL-4 resulted in JAK1 phosphorylation in all X-SCID B-LCLs. It is of interest to note that the phosphorylation of JAK1 was observed irrespective of the presence of a γ_c subunit on the surface of the

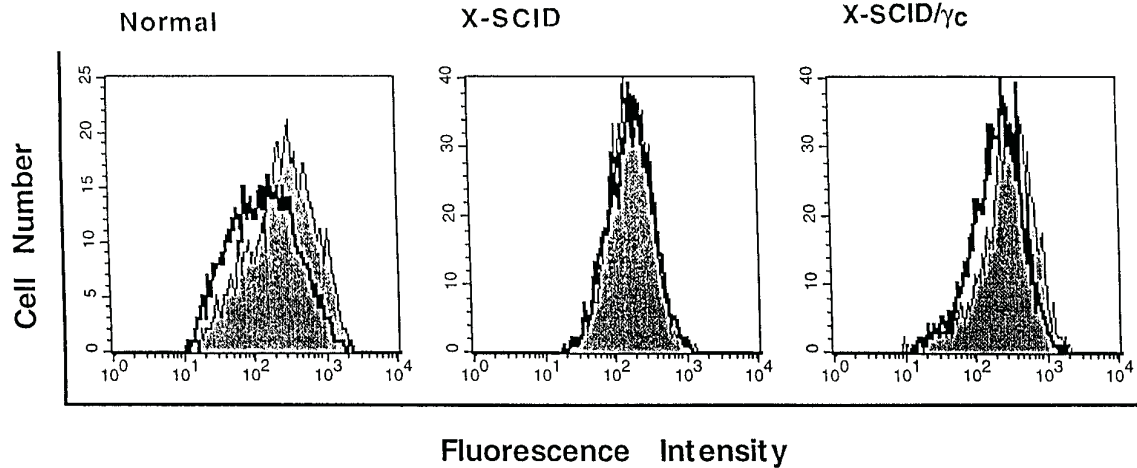


FIG. 4. **Modulation of CD23 cell surface expression by IL-4.** Normal, X-SCID, and γ_c -reconstituted B cells were treated with or without IL-4 in serum-free conditions for 48 h. CD23 expression was measured by fluorescence-activated cell sorting as described. Expression in untreated and IL-4-stimulated cells are shown as open and closed histograms, respectively. The mean fluorescence intensity of CD23 on the cell surface, with or without IL-4 stimulation, was analyzed with Cellquest software (Becton-Dickinson). The mean fluorescence intensity in the normal, X-SCID, and γ_c reconstituted X-SCID B-LCL increased by 1.93 (170.36 to 330.09), 1.00 (183.45 to 181.76), and 1.48 (232.56 to 344.91)-fold, respectively. Results are representative of data from four independent experiments.

X-SCID B-LCL. These results demonstrate that JAK1 is activated by an IL-4-responsive pathway in X-SCID B-LCL which is independent of γ_c and JAK3.

The large cytosolic docking molecule IRS-1 is also tyrosine-phosphorylated in response to IL-4 and has been hypothesized to be important for an IL-4-mediated proliferative response (35, 36). We therefore examined the effects of IL-4 on IRS-1 phosphorylation in X-SCID B cells. Following immunoprecipitation with specific antibodies, we detected IL-4-induced tyrosine phosphorylation of IRS-1 in both B-LCL from a normal donor and a patient with X-SCID (Fig. 1C). Thus, γ_c expression is not required for IL-4-mediated IRS-1 phosphorylation.

IL-4 and IL-13 Induce STAT6 Phosphorylation and Activation in X-SCID B-LCL—Stimulation of many cell types with IL-4 leads to the phosphorylation of STAT6 (37). As JAK3 was not phosphorylated in X-SCID B cells, it was important to determine whether IL-4-mediated STAT6 phosphorylation would also be affected by the loss of γ_c . Previous data indicated that IL-13, which shares many structural and functional characteristics with IL-4 (38–40), but not IL-4 itself, induced STAT6 activation in X-SCID B-LCL (25). However, we found that STAT6 was tyrosine-phosphorylated upon both IL-4 and IL-13 stimulation in every X-SCID B-LCL (Fig. 2A). Nevertheless, the IL-4-stimulated phosphorylation of STAT6 was significantly greater in control B-LCL than in all X-SCID B-LCL tested (Figs. 2A and 3). In contrast, the IL-13-mediated stimulation of STAT6 was roughly equivalent among the control and X-SCID B-LCLs.

We also found that IL-4 stimulated DNA binding activity in control and X-SCID B-LCL as assessed by an electrophoretic mobility shift assay (Fig. 2B). Since IL-4 is known to induce the expression of CD23 in B cells (41), we assessed DNA binding activity with a probe corresponding to the interferon- γ activation sequence (GAS) element in the CD23 promoter (42). As shown in Fig. 2B, gel-retarded CD23 GAS element-binding complexes were formed following IL-4 stimulation of both a control B-LCL as well as an X-SCID B-LCL (cDNA468+1). However, as might be expected from our finding that STAT6 was phosphorylated at lower levels in IL-4-stimulated X-SCID B-LCL as compared with control B-LCL (Fig. 2A), IL-4-induced DNA binding activity was significantly lower in all X-SCID B-LCL than in control B-LCL (Fig. 2B and data not shown). To ascertain whether the DNA binding complexes contained

STAT6, we performed supershift analyses using STAT6 antisera. In each of the IL-4-induced DNA binding complexes, a supershift was detected using STAT6 antisera but not with a control rabbit antisera (Fig. 2B). This demonstration of IL-4-stimulated STAT6 DNA binding activity in cells that do not express γ_c further supports the presence of an IL-4 signaling pathway independent of γ_c and JAK3.

STAT6 Phosphorylation and DNA Binding Activity Are Enhanced following Reconstitution of an X-SCID B-LCL with Wild Type γ_c —In order to determine the role of γ_c in IL-4-mediated signaling, we assessed STAT6 phosphorylation and DNA binding activity in an X-SCID B-LCL in which γ_c expression was reconstituted using retroviral mediated gene transduction (30). The data presented in Fig. 3 demonstrate that IL-4-mediated STAT6 phosphorylation was significantly enhanced in the γ_c reconstituted X-SCID B-LCL, approaching the level observed in the control B cell line. Moreover, anti-STAT6 supershifted DNA binding complexes induced by IL-4 were more abundant in γ_c reconstituted X-SCID B-LCL than in the X-SCID B-LCL (data not shown). Therefore, wild type levels of STAT6 phosphorylation and DNA binding activity in control B-LCL are likely due to significant transduction through the γ_c -dependent IL-4 pathway.

IL-4-induced Regulation of CD23 Expression in X-SCID and γ_c Reconstituted B-LCL—Our data indicate that JAK1, STAT6, and IRS-1 can be phosphorylated by IL-4 in a γ_c -independent fashion. However, it was not clear whether these IL-4-mediated biochemical responses in B cells derived from X-SCID patients would result in biological outcomes, such as changes in gene expression in X-SCID B cells. It has been shown that IL-4 induces CD23 (Fc ϵ R2) antigen expression in normal mature B cells (10). In order to determine whether γ_c expression had a significant effect on IL-4 functional responses, IL-4-induced regulation of CD23 in normal, X-SCID, and γ_c reconstituted X-SCID B-LCL was examined. A 48-h incubation of control B cells with IL-4 resulted in an almost 2-fold increase in CD23 levels (Fig. 4), whereas no changes in CD23 expression were detected in B-LCL derived from a number of patients. However, in X-SCID cells reconstituted with γ_c , there was an almost 2-fold increase of CD23 levels when compared with the same cells incubated without IL-4. This was a consistent finding in four replicate experiments. These results suggest that γ_c is important for transducing some of the functional responses to IL-4.

DISCUSSION

Stimulation of B cells with IL-4 results in proliferation, immunoglobulin class switching, and regulation of cell surface proteins such as major histocompatibility complex class II molecules and the CD23 receptor. Previous reports have concluded that the JAK-STAT pathway is not activated by IL-4 in the absence of γ_c (25, 26). However, IL-4 can induce functional, although suboptimal, responses in X-SCID B cells (27) suggesting that the IL-4 signal transduction pathway is conserved in these cells. X-SCID B cells, therefore, provide an important model in which to examine the generation of γ_c -independent IL-4 responses. In this study, we determined that JAK1, IRS-1, and STAT6 were tyrosine-phosphorylated in response to IL-4 in B cell lines derived from X-SCID patients with a wide diversity of γ_c mutations and that IL-4 induced detectable levels of STAT6 DNA binding activity in these cells.

The finding that JAK3 was not phosphorylated in X-SCID B-LCL stimulated with either IL-2 or IL-4 was not surprising as a number of recent studies have demonstrated a physical association between γ_c and JAK3 (14, 16). This interaction is thought to be critical for IL-2, IL-4, IL-7, IL-9, and IL-15 signaling in lymphocytes (14, 16, 43). The data described here show that IL-4-induced JAK1 and IRS-1 phosphorylation as well as STAT6 activation can occur via mechanisms that are independent of γ_c and JAK3. IRS-1 is thought to be important for mediating IL-4 proliferative effects, but the mechanism by which IL-4 stimulates IRS-1 phosphorylation is unclear (44). Janus kinases likely play an important role (45), and our data suggest that in the absence of JAK3, JAK1 may mediate IRS-1 tyrosine phosphorylation in response to IL-4. It remains to be determined whether other tyrosine kinases known to be induced by IL-4, including JAK2, Tyk2, and Fes (46–48), play a role in IL-4-mediated responses in X-SCID B cells.

Matthews *et al.* (27) showed that functional responses such as proliferation, IgE secretion, and CD23 expression could occur in X-SCID B cells when co-stimulated *in vitro* by IL-4 or IL-13 together with CD40L or IgM. However, in our studies, we did not detect an increase in CD23 expression in X-SCID B-LCL even though CD23 expression did increase in normal and γ_c -reconstituted X-SCID B-LCL following stimulation with IL-4. Because of the already high level of CD23 expression on Epstein-Barr virus-transformed B cells, slight increases in CD23 expression may be difficult to detect. Nevertheless, even in the studies performed by Matthews *et al.* (27) in primary B cells, the increase in CD23 in X-SCID B cells was significantly lower than that detected in control B cells (27). Suboptimal CD23 activation in response to IL-4 suggests that the observed humoral deficiency in X-SCID patients may result, at least in part, from the inability to transduce the full set of IL-4-induced signals through non- γ_c containing receptors. Our finding that IL-4-induced STAT6 phosphorylation and CD23 expression were restored to wild type levels following introduction of γ_c into these cells indicates that γ_c potentiates the IL-4 signal transduction pathway. The importance of JAK3 in this γ_c -dependent pathway is further supported by the recent observation that IL-4-induced CD23 expression and STAT6 activation are also suboptimal in B cells that express γ_c but are deficient in JAK3 (49).

Although the precise mechanism by which this γ_c -independent signaling pathway is initiated is not clear, several groups have previously shown IL-4-induced proliferation and protein phosphorylation in endothelial, colon carcinoma, and plasmocytoma cell lines that lack γ_c (27, 46, 47, 50–52). The γ_c -independent pathway in these cells has been suggested to function through a receptor that is shared by IL-4 and IL-13 since antibodies to the IL-4R α chain can block the binding and

function of IL-4 as well as IL-13 (53–57). Accordingly, IL-4 and IL-13 stimulate the phosphorylation of many of the same downstream substrates (47, 51, 55, 58–60). Moreover, IL-13 receptor subunits that have varied capacities to bind IL-4 have recently been identified (61, 62). Our data support common signaling mechanisms as IL-4-activated JAK1 and STAT6 but not JAK3 in X-SCID B cells, and this concurs with what has been reported for IL-13 (47, 51, 58, 60). Thus, an IL-4-mediated signal transduction pathway in γ_c -deficient X-SCID cells may occur via the same mechanisms utilized by IL-13.

In contrast to other forms of SCID that present with a complete absence of both T and B lymphocytes, X-SCID patients have normal to elevated numbers of nonfunctional B cells. However, it is clear that the IL-4 signaling that we have detected is not sufficient for normal B cell function. The findings presented here provide insight into IL-4 signal transduction pathways and B cell function in X-SCID patients. The physiological role of the described γ_c /JAK3 independent IL-4R pathway in normal individuals as well as in patients with X-SCID awaits further investigation.

Acknowledgments—We are grateful to Drs. R. Callard, C. Rooney, W. Leonard, J. DiSanto, G. de Saint Basile, and A. Fischer for their valuable suggestions. We thank M. Sibon for his support and critical comments on the manuscript. We appreciate the assistance of M. Dao.

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