

## Shared gamma(c) subunit within the human interleukin-7 receptor complex. A molecular basis for the pathogenesis of X-linked severe combined immunodeficiency.

S Y Lai, ... , J Molden, M A Goldsmith

*J Clin Invest.* 1997;99(2):169-177. <https://doi.org/10.1172/JCI119144>.

### Research Article

Genetic evidence suggests that mutations in the gamma(c) receptor subunit cause X-linked severe combined immunodeficiency (X-SCID). The gamma(c) subunit can be employed in receptor complexes for IL-2, -4, -7, -9, and -15, and the multiple signaling defects that would result from a defective gamma(c) chain in these receptors are proposed to cause the severe phenotype of X-SCID patients. Interestingly, gene disruption of either IL-7 or the IL-7 receptor (IL-7R) alpha subunit in mice leads to immunological defects that are similar to human X-SCID. These observations suggest the functional importance of gamma(c) in the IL-7R complex. In the present study, structure/function analyses of the IL-7R complex using a chimeric receptor system demonstrated that gamma(c) is indeed critical for IL-7R function. Nonetheless, only a limited portion of the cytoplasmic domain of gamma(c) is necessary for IL-7R signal transduction. Furthermore, replacement of the gamma(c) cytoplasmic domain by a severely truncated erythropoietin receptor does not affect measured IL-7R signaling events. These findings support a model in which gamma(c) serves primarily to activate signal transduction by the IL-7R complex, while IL-7R alpha determines specific signaling events through its association with cytoplasmic signaling molecules. Finally, these studies are consistent with the hypothesis that the molecular pathogenesis of X-SCID is due primarily to gamma(c)-mediated defects in the IL-7/IL-7R system.

**Find the latest version:**

<https://jci.me/119144/pdf>



## Shared $\gamma_c$ Subunit Within the Human Interleukin-7 Receptor Complex

### A Molecular Basis for the Pathogenesis of X-Linked Severe Combined Immunodeficiency

Stephen Y. Lai,\* Jaime Molden,\* and Mark A. Goldsmith\*\*

\*Gladstone Institute of Virology and Immunology, San Francisco, California 94141-9100; and †Department of Medicine, School of Medicine, University of California, San Francisco, California 94122

#### Abstract

Genetic evidence suggests that mutations in the  $\gamma_c$  receptor subunit cause X-linked severe combined immunodeficiency (X-SCID). The  $\gamma_c$  subunit can be employed in receptor complexes for IL-2, -4, -7, -9, and -15, and the multiple signaling defects that would result from a defective  $\gamma_c$  chain in these receptors are proposed to cause the severe phenotype of X-SCID patients. Interestingly, gene disruption of either IL-7 or the IL-7 receptor (IL-7R)  $\alpha$  subunit in mice leads to immunological defects that are similar to human X-SCID. These observations suggest the functional importance of  $\gamma_c$  in the IL-7R complex. In the present study, structure/function analyses of the IL-7R complex using a chimeric receptor system demonstrated that  $\gamma_c$  is indeed critical for IL-7R function. Nonetheless, only a limited portion of the cytoplasmic domain of  $\gamma_c$  is necessary for IL-7R signal transduction. Furthermore, replacement of the  $\gamma_c$  cytoplasmic domain by a severely truncated erythropoietin receptor does not affect measured IL-7R signaling events. These findings support a model in which  $\gamma_c$  serves primarily to activate signal transduction by the IL-7R complex, while IL-7R $\alpha$  determines specific signaling events through its association with cytoplasmic signaling molecules. Finally, these studies are consistent with the hypothesis that the molecular pathogenesis of X-SCID is due primarily to  $\gamma_c$ -mediated defects in the IL-7/IL-7R system. (*J. Clin. Invest.* 1997. 99:169–177.)  
**Key words:** interleukin-7 • receptor • X-linked severe combined immunodeficiency • signal transduction • specificity

#### Introduction

Various cytokines contribute to the development and regulation of lymphocytes. A number of cytokines including IL-2, -4, -7, -9, and -15 engage receptor complexes composed of a specific subunit in conjunction with the shared  $\gamma_c$  chain (1–9). Hu-

man mutations in  $\gamma_c$  have been linked to X-linked severe combined immunodeficiency (X-SCID),<sup>1</sup> a disease characterized by severe lymphopenia and recurring persistent infections in the first months of life (10–12). Additionally, gene deletion studies in mice of  $\gamma_c$  have revealed a similar immunodeficiency (13, 14). The wide array of receptor signaling defects that result from  $\gamma_c$  mutations are hypothesized to lead to the severe immunological defects found in both X-SCID patients and  $\gamma_c$ -deletion murine models.

Recent studies, however, suggest that more selective signaling defects due to  $\gamma_c$  mutations may cause X-SCID and the murine immunodeficiency syndrome. Gene ablation of IL-2 (15) and IL-2 receptor (IL-2R) $\beta$ /IL-15R $\beta$  (16) do not cause early developmental defects of the immune system. Furthermore, IL-4 gene-ablation studies do not detect early lymphopoietic and functional disturbances, suggesting that  $\gamma_c$ -independent forms of the IL-4R are biologically active (17–21). Additionally, the role of  $\gamma_c$  in the IL-9R complex remains undefined. The  $\gamma_c$  subunit does not appear to modulate IL-9R binding affinity for IL-9 (8) and IL-9 may act primarily on mast cells (22). In contrast, gene disruption of either IL-7 (23) or the IL-7R $\alpha$  subunit (24) leads to severe developmental perturbations. Thus, signaling defects in the IL-7/IL-7R system resulting from changes in  $\gamma_c$  may be sufficient to account for the developmental anomalies that lead to X-SCID.

IL-7 was identified as a factor secreted by stromal cells of the bone marrow and thymus that stimulates proliferation of immature B and T lymphocytes (25–31). The biological effects of IL-7 are mediated through a receptor complex containing the IL-7R $\alpha$  subunit (32) and the  $\gamma_c$  chain (3, 6, 9). Both of these receptor subunits are members of the cytokine receptor superfamily (33), sharing a number of structural features including two partially conserved, intracellular regions termed Box1 and Box2 that are involved in Janus kinase (JAK) association (7, 34–36). Engagement of these subunits by IL-7 leads to the activation of the Janus kinases, JAK1 and JAK3, and the subsequent induction of a signal transducer and activator of transcription (STAT) factor, STAT-5 (7, 19, 37). Additionally, activation of the src-like kinase family members p56lck, p59fyn, and p53/p56lyn has been reported in various cellular contexts (38–40). Downstream of the activation of tyrosine kinases is the activation of the insulin receptor substrate-1 (IRS-1)

Address correspondence to Mark A. Goldsmith, Gladstone Institute of Virology and Immunology, P.O. Box 419100, San Francisco, CA 94141-9100. Phone: 415-695-3775; FAX: 415-826-1514; E-mail: mark\_goldsmith.givi@quickmail.ucsf.edu

Received for publication 9 September 1996 and accepted in revised form 6 November 1996.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/97/01/169/09 \$2.00

Volume 99, Number 2, January 1997, 169–177

1. *Abbreviations used in this paper:* CMF-PBS, calcium- and magnesium-free PBS; EMSA, electrophoretic mobility shift assay; EPOR, erythropoietin receptor; IRS-1, insulin receptor substrate-1; JAK, Janus kinase; STAT, signal transducer and activator of transcription; X-SCID, X-linked severe combined immunodeficiency.

(41) and phosphatidylinositol-3-kinase (42, 43). Collectively, these and related specific molecular events culminate in differentiation and proliferation by IL-7.

While many of the specific signaling events activated by the IL-7R complex have been delineated, the functional roles of the receptor subunits themselves in generating these signals are relatively undefined. However, the heterodimeric structure of the IL-7R complex suggests distinct functions for the individual receptor subunits. Thus, the present studies were undertaken to identify the functional roles of IL-7R $\alpha$  and  $\gamma_c$  within the IL-7R complex. These observations define the structural determinants of signaling specificity within the IL-7R complex and further clarify mechanisms associated with the molecular pathogenesis of X-SCID.

## Methods

**Cell lines and reagents.** HT-2, an IL-2-dependent murine helper T cell line (American Type Culture Collection, Rockville, MD) was cultured in RPMI 1640 supplemented with 10% FBS, 55  $\mu$ M  $\beta$ -mercaptoethanol ( $\beta$ -ME), 2 mM L-glutamine, and 200 U/ml recombinant human IL-2 (a generous gift of Chiron Corp, Emeryville, CA). 32D/IRS-1, a pro-myeloid cell line stably expressing the IRS-1, was maintained in 32D medium (RPMI 1640 containing 10% FBS and 5% WEHI 3B-conditioned medium). The SCID-MA cell line was maintained in RPMI 1640 with 10% FBS, 10 mM Hepes, 2 mM L-glutamine, and 5  $\mu$ M  $\beta$ -ME.

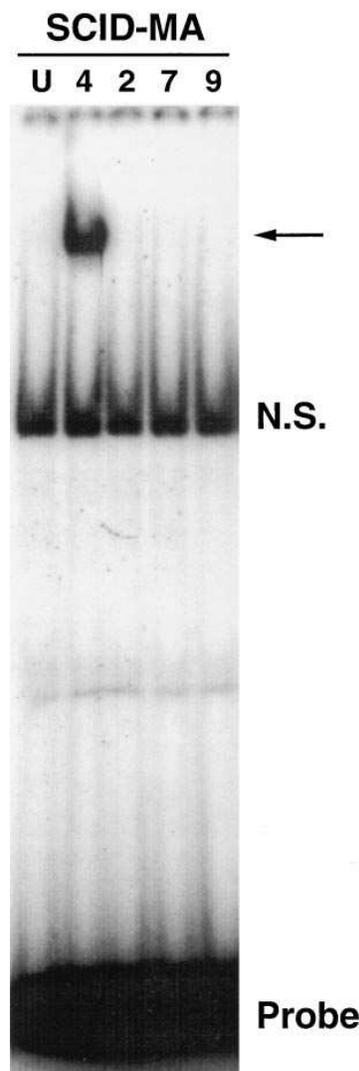
Electroporation of cells was performed as described previously (44), and stable transfectants were obtained by selection in G418 (1 mg/ml Geneticin; GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD). Clones isolated by limiting dilution were screened by Northern blot analysis to identify clones expressing the transfected receptor subunit(s). HT-2 stable cell lines expressing two receptor subunits were derived from cells already expressing either the EPO $\gamma$  or EPO $\gamma$ YF chain. After electroporation, stable transfectants were isolated by selection in G418 (1 mg/ml) and hygromycin B (500  $\mu$ g/ml; Boehringer Mannheim Biochemicals, Indianapolis, IN) and screened by Northern blot analysis. The antiphosphotyrosine monoclonal antibody (4G10), anti-JAK1, -JAK2, and -JAK3 were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). Human IL-4 and IL-9 were obtained from R & D Systems, Inc. (Minneapolis, MN). Recombinant human IL-7 was from Genzyme, Corp. (Cambridge, MA) and recombinant human EPO was the generous gift of Ortho Diagnostic Systems Inc. (Raritan, NJ).

**Plasmid constructs.** All receptor cDNAs were subcloned into the expression vectors pCMV4Neo (44) or pCMV4Hygro, a derivative of pCMV4 (45) containing a hygromycin B-resistance gene as a selectable marker. The cytoplasmic portion of the IL-7R $\alpha$  subunit was isolated by PCR from PBMC cDNA. pEPO7neo was constructed by PCR using a NheI site at the fusion junction. The chimeric receptor subunit contains the extracellular domain of the erythropoietin receptor (EPOR) fused just above the transmembrane segment to the human IL-7R $\alpha$  transmembrane and cytoplasmic domains (resulting sequence: . . . (EPOR-T-A-S)-(I-N-N-IL-7 $\alpha$ ) . . .). The EPO $\gamma$  and the chimeric  $\gamma_c$  receptor mutants were constructed as previously described (46). A stop codon was introduced at the HindIII site present in the coding sequence of EPO7 to create the EPO7<sub>TR</sub> subunit that lacks the distal 100 amino acid residues. For all constructs requiring synthetic oligonucleotides or PCR reactions, sequences were confirmed by DNA sequencing.

**JAK and IRS-1 phosphorylation studies.** 40  $\times$  10<sup>6</sup> cells were washed twice in calcium- and magnesium-free phosphate-buffered saline (CMF-PBS), stripped of cell-bound ligand for 1 min in 10 mM sodium citrate, pH 4.0, and 140 mM NaCl and rested for 4 h or overnight in RPMI 1640 medium containing 1% bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO). After stimulation with the

appropriate factor, cells were washed in CMF-PBS and lysed (1% Nonidet P-40, 20 mM Tris-HCl, pH 8.0, and 150 mM NaCl, 50 mM NaF, 100 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10  $\mu$ g/ml leupeptin, 10  $\mu$ g/ml aprotinin, 1  $\mu$ g/ml pepstatin A). Immunoprecipitations were performed with the indicated antibodies and protein A-Sepharose. Immunoblot analysis was performed with the appropriate antibodies with detection by ECL (Amersham Corp., Arlington Heights, IL) signal development. For JAK analysis, blots were stripped (100 mM 2-mercaptoethanol, 2% sodium dodecyl sulphate, 62.5 mM Tris-HCl, pH 6.7) for 30 min at 55°C and reprobed with anti-JAK1 and -JAK3 antisera to verify equivalent protein loading (data not shown).

**Electrophoretic mobility shift assay.** 40–60  $\times$  10<sup>6</sup> cells were rested and stimulated as described above and washed in CMF-PBS. Nuclear extracts were prepared as described (47) in the presence of 1 mM sodium orthovanadate and the following protease inhibitors (in  $\mu$ g/ml): antipain 0.5, aprotinin 0.5, bestatin 0.75, leupeptin 0.5, pepstatin A 0.05, phosphoramidon 1.4, and soybean trypsin inhibitor 0.5 (Sigma Chemical Co.). The IgG Fc receptor STAT response element probe was end-labeled with [ $\gamma$ -<sup>32</sup>P]dATP (Amersham Corp.) and polynucleotide kinase (New England Biolabs Inc., Beverly, MA). DNA binding studies were performed with 10<sup>5</sup> cpm probe, 3  $\mu$ g poly[d(I-C)] and 10  $\mu$ g nuclear extract as described (47a). Preincubation of nuclear extracts with different antibodies were performed in the absence of

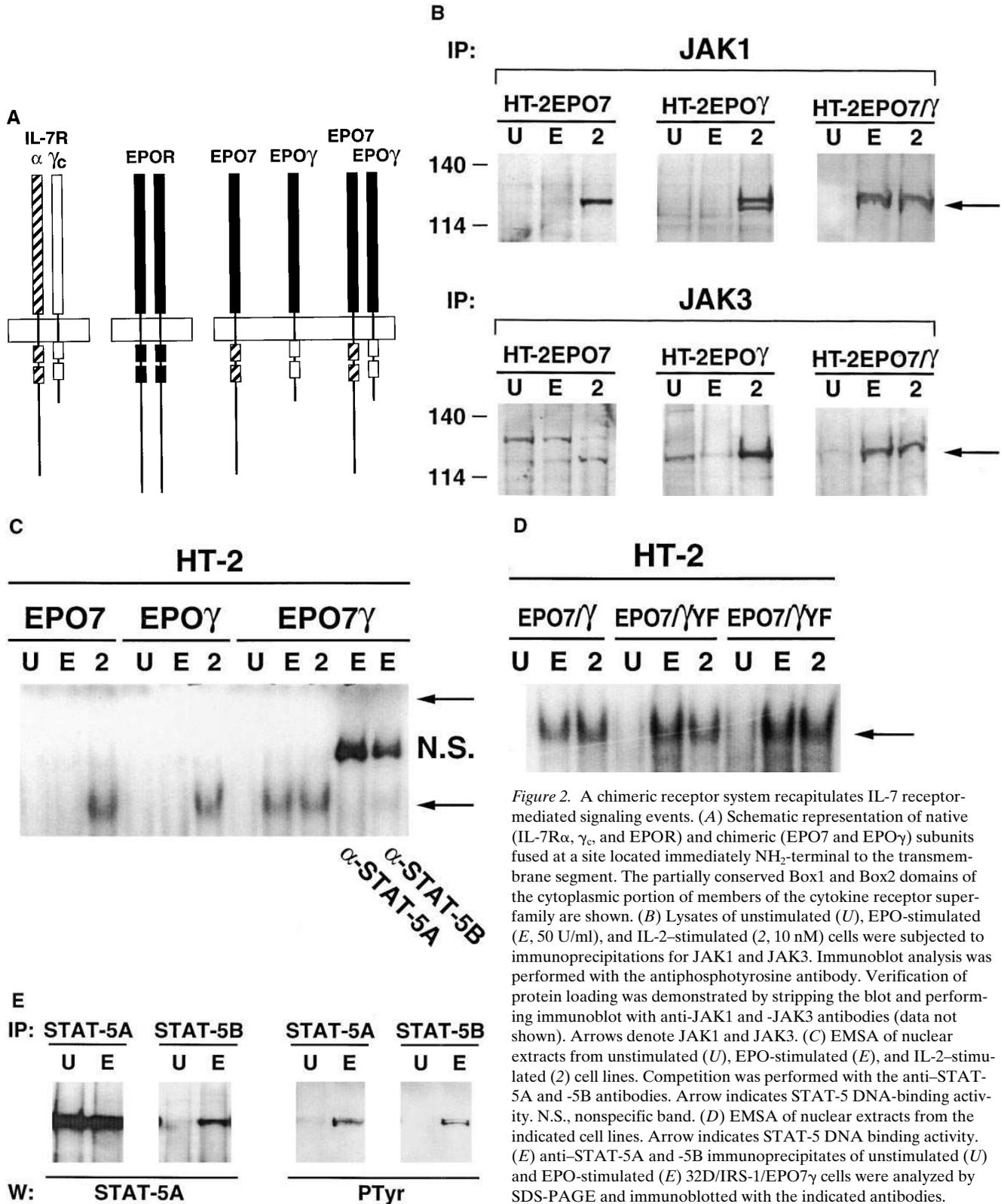


**Figure 1.** The  $\gamma_c$  mutation in an X-SCID patient results in multiple cytokine receptor signaling defects. 40  $\times$  10<sup>6</sup> cells/sample were unstimulated (U) or stimulated for 15 minutes with various cytokines: IL-4 (4, 100 ng/ml), IL-2 (2, 10 nM), IL-7 (7, 100 U/ml), and IL-9 (9, 100 U/ml). EMSA of prepared nuclear extracts are shown. Cytokine bioactivity was verified by stimulation of various responsive cell lines and the integrity of the nuclear extracts was verified by EMSA with the SP-1 probe (data not shown). Arrow denotes IL-4-induced STAT-6 DNA-binding activity.

poly[d(I-C)] and binding buffer for 45 min on ice before initiation of the binding assay by addition of radiolabeled probe.

**Proliferation assays.** Conventional 24-h [<sup>3</sup>H]thymidine (DuPont-NEN, Boston, MA) incorporation assays were performed as previously described (44). Briefly, 32D cells were counted, washed twice in CMF-PBS and resuspended at 10<sup>6</sup> cells/ml of 32D medium without

the WEHI 3B-conditioned medium supplement. 10<sup>5</sup> cells per well were grown in the indicated concentrations of EPO for 24 h with [<sup>3</sup>H]thymidine incorporation measured in the last 4 h. Transient transfection assays of EPO-induced proliferation were performed as described previously (44). [<sup>3</sup>H]Thymidine incorporation was measured on days 8–16 after transfection.



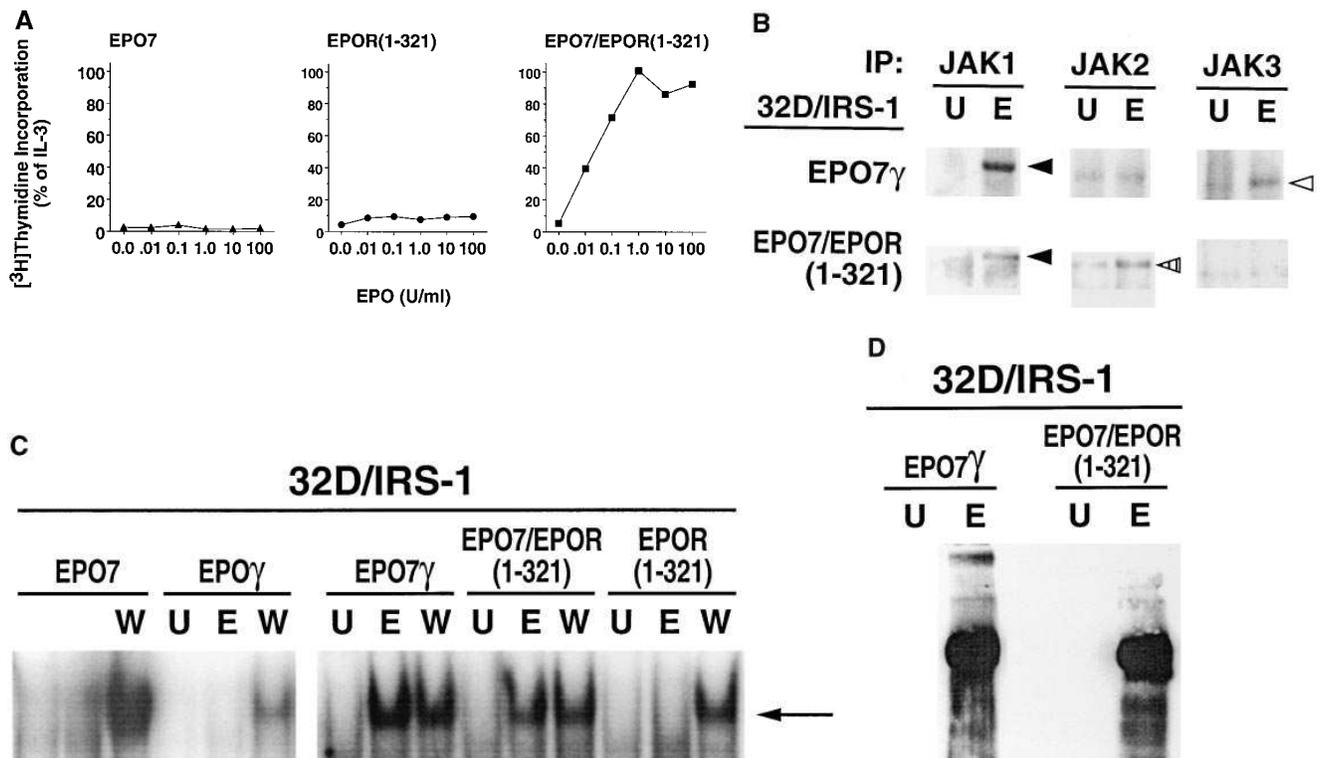
**Figure 2.** A chimeric receptor system recapitulates IL-7 receptor-mediated signaling events. (A) Schematic representation of native (IL-7R $\alpha$ ,  $\gamma_c$ , and EPOR) and chimeric (EPO7 and EPO $\gamma$ ) subunits fused at a site located immediately NH<sub>2</sub>-terminal to the transmembrane segment. The partially conserved Box1 and Box2 domains of the cytoplasmic portion of members of the cytokine receptor superfamily are shown. (B) Lysates of unstimulated (U), EPO-stimulated (E, 50 U/ml), and IL-2-stimulated (2, 10 nM) cells were subjected to immunoprecipitations for JAK1 and JAK3. Immunoblot analysis was performed with the antiphosphotyrosine antibody. Verification of protein loading was demonstrated by stripping the blot and performing immunoblot with anti-JAK1 and -JAK3 antibodies (data not shown). Arrows denote JAK1 and JAK3. (C) EMSA of nuclear extracts from unstimulated (U), EPO-stimulated (E), and IL-2-stimulated (2) cell lines. Competition was performed with the anti-STAT-5A and -5B antibodies. Arrow indicates STAT-5 DNA-binding activity. N.S., nonspecific band. (D) EMSA of nuclear extracts from the indicated cell lines. Arrow indicates STAT-5 DNA binding activity. (E) anti-STAT-5A and -5B immunoprecipitates of unstimulated (U) and EPO-stimulated (E) 32D/IRS-1/EPO7 $\gamma$  cells were analyzed by SDS-PAGE and immunoblotted with the indicated antibodies.

## Results

B cells from an X-SCID patient are unresponsive to multiple cytokines that bind to receptor complexes containing the  $\gamma_c$  subunit. Epstein-Barr virus-transformed B cell lines derived from X-SCID patients have various mutations in the  $\gamma_c$  gene. For example, the SCID-MA cell line contains a mutation that results in the lack of detectable mRNA transcripts for the  $\gamma_c$  subunit (reference 12 and data not shown). To assess the functional consequences of this genetic defect, SCID-MA cells were stimulated with cytokines that employ  $\gamma_c$ -containing receptor complexes (Fig. 1). The activation of STAT factors was evaluated in an electrophoretic mobility gel shift assay (EMSA) using an oligonucleotide probe that contains the IgG Fc receptor promoter element. SCID-MA cells retained responsiveness to IL-4, as demonstrated by the retarded mobility of the radiolabeled probe and anti-STAT-6 antibody competition (Fig. 1 and data not shown). Previous studies are consistent with the hypothesis that IL-4 may engage another receptor complex(es) that does not require the  $\gamma_c$  subunit (17–21). However, in the absence of a functional  $\gamma_c$  chain, SCID-MA cells were unresponsive to IL-2, -7, and -9, raising the possibility that  $\gamma_c$  is critical for signaling by these receptor systems. Since IL-2 and IL-9 do not appear to affect early lymphocyte development, further analysis of the IL-7R complex and its link to X-SCID was undertaken.

*A chimeric receptor system recapitulates JAK-STAT signaling of the IL-7 receptor complex.* To investigate the functional roles of the individual receptor subunits in the IL-7 receptor complex, chimeric receptor subunits were formed by fusing the extracellular domain of the EPOR to the intracellular portions of IL-7R $\alpha$  and  $\gamma_c$  to form EPO7 and EPO $\gamma$ , respectively (Fig. 2 A). These chimeric receptor subunits were evaluated for their abilities to recapitulate IL-7 receptor signaling, specifically through the JAK-STAT pathway. Stable transfectants expressing EPO7 or EPO $\gamma$  were established in HT-2 cells, a murine helper T cell line. Antiphosphotyrosine immunoblot analysis of JAK kinase immunoprecipitates from HT-2 stable transfectants demonstrated the phosphorylation of JAK1 and JAK3, but not JAK2, in response to a positive control ligand, IL-2 (Fig. 2 B and data not shown). Upon EPO stimulation, neither the HT-2EPO7 nor the HT-2EPO $\gamma$  cell lines demonstrated an increase in the phosphorylation of JAK1 and JAK3 when compared with unstimulated cells. In contrast, a stable cell line expressing both EPO7 and EPO $\gamma$  subunits, HT-2EPO7 $\gamma$ , displayed a marked increase in tyrosine phosphorylation of JAK1 and JAK3 in response to EPO (Fig. 2 B). Therefore, activation of the chimeric receptor system leads to the induction of the same JAK kinases as those reportedly linked to the native IL-7 receptor complex (7, 37, 48).

Activation of JAK kinases in cytokine receptor complexes typically leads to the recruitment and activation of STAT fac-



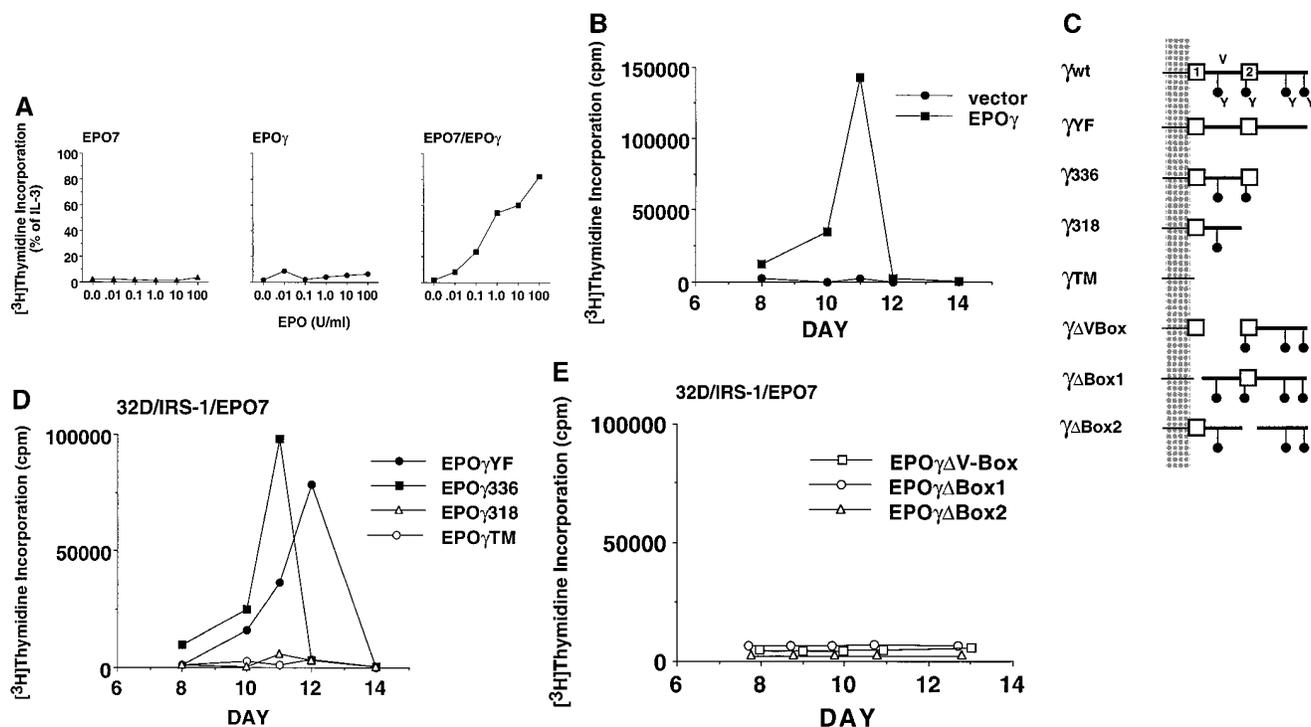
**Figure 3.** The functional replacement of  $\gamma_c$  by a truncated EPOR does not alter specific signaling events. (A) Stable 32D/IRS-1 transfectants were stimulated with EPO in [ $^3$ H]thymidine incorporation experiments. (B) Serial immunoprecipitates of JAK1, JAK2, and JAK3 from lysates of the indicated cell lines. Immunoblot analysis was performed with the antiphosphotyrosine antibody. Verification of protein loading was demonstrated by stripping the blot and performing immunoblot analysis with the anti-JAK1, -JAK2 and -JAK3 antibodies. Arrowheads denote JAK1 (solid), JAK2 (hatched), and JAK3 (open). (C) EMSA of nuclear extracts from the indicated 32D/IRS-1 stable transfectants that were unstimulated (U) or treated with EPO (E) or Wehi 3B-conditioned media (W, 10%). Arrow depicts STAT-5 DNA-binding activity. (D) anti-IRS-1 immunoprecipitates of lysates from the indicated cell lines that were unstimulated (U) or EPO stimulated (E). Immunoblotting was performed with the antiphosphotyrosine antibody.

tors (49). As demonstrated by EMSA using a probe corresponding to the IgG Fc receptor STAT responsive element, EPO stimulation of HT-2EPO7 and HT-2EPO $\gamma$  cell lines did not result in activation of a specific DNA-binding activity (Fig. 2 C). Like the native IL-7 receptor complex (19), engagement of both chimeric receptor subunits by EPO stimulation of the HT-2EPO7 $\gamma$  cell line resulted in the activation of STAT-5 (Fig. 2 C). This DNA-binding complex contained both STAT-5A and -5B, as demonstrated by specific antibody competition that diminished the presence of the retarded nucleoprotein complex.

To examine the IL-7R complex in another cell context, the chimeric receptor system was also established in 32D/IRS-1, a promyeloid cell line that expresses IRS-1 (50). As in the HT-2 cell line, EPO stimulation of 32D/IRS-1 stable transfectants expressing EPO7 or EPO $\gamma$  alone did not affect the phosphorylation of JAK1 or JAK3 (data not shown). EPO stimulation of a 32D/IRS-1 stable cell line expressing both EPO7 and EPO $\gamma$  (32D/IRS-1/EPO7 $\gamma$ ) resulted in a marked increase in the phosphorylation of JAK1 and JAK3 (Fig. 3 B). Additionally, EPO stimulation of 32D/IRS-1/EPO7 and 32D/IRS-1/EPO $\gamma$  cell lines did not result in the activation of DNA-binding activity (Fig. 3 C). However, EPO stimulation of a 32D cell line expressing both EPO7 and EPO $\gamma$ , 32D/IRS-1/EPO7 $\gamma$ , led to the activation of a DNA-binding complex (Fig. 3 C). To determine the composition of the DNA-binding complex, 32D/IRS-1/EPO7 $\gamma$  cellular lysates were subjected to immunoprecipitation with the STAT-5A and -5B antibodies after activation of the

EPO7/EPO $\gamma$  heterodimer. Anti-STAT-5A immunoblot analysis of the immunoprecipitates demonstrated the coimmunoprecipitation of STAT-5B with STAT-5A after EPO stimulation (Fig. 2 E). Similarly, immunoprecipitates of the STAT-5A antibody demonstrated the EPO-induced association of STAT-5B (data not shown). Therefore, as in the IL-2R system (51), signaling through the IL-7R $\alpha/\gamma_c$  heterodimer results in the activation and heterodimerization of STAT-5A and -5B. Thus, these findings demonstrate that signaling by the IL-7 receptor complex through the JAK-STAT pathway is reproduced by the chimeric receptor system in two cytokine-dependent hematopoietic cell lines. Furthermore, heterodimerization of the cytoplasmic tails of IL-7R $\alpha$  and  $\gamma_c$  are required to activate signal transduction by the IL-7R complex.

*The cytoplasmic tyrosines of the  $\gamma_c$  subunit are not required for JAK-STAT signaling by the IL-7R complex.* Previous studies in the IL-2 and IL-4 receptor complexes demonstrated that the four cytoplasmic tyrosine residues of the  $\gamma_c$  subunit were dispensable for signal transduction in response to those cytokines (20, 46, 51). To investigate the functional role of the tyrosine residues of the  $\gamma_c$  subunit in the IL-7R complex, HT-2 stable cell lines were established that expressed EPO7 and EPO $\gamma$ YF, a chimeric  $\gamma_c$  mutant in which all four cytoplasmic tyrosine residues are replaced by phenylalanines. Like wild-type HT-2EPO7 $\gamma$  cells, EPO-stimulation of two distinct HT-2EPO7/ $\gamma$ YF cell lines resulted in the activation of a DNA-binding activity (Fig. 2 D). Additionally, antibody competition experiments confirmed the presence of STAT-5A and -5B in



**Figure 4.** Heterodimerization of the IL-7R $\alpha$  and  $\gamma_c$  cytoplasmic domains results in the activation of IL-7-specific signaling events. (A) Stable 32D/IRS-1 transfectants were stimulated with EPO in [ $^3$ H]thymidine incorporation experiments. (B) Transient transfection assay of 32D/IRS-1/EPO7 cells demonstrates that EPO-induced proliferation requires the presence of both EPO7 and EPO $\gamma$ . (C) Schematic diagram of the  $\gamma_c$  mutants.  $\gamma$ 336,  $\gamma$ 318 and  $\gamma$ TM mutants are truncated immediately after amino acids 336, 318, and 286, respectively.  $\gamma\Delta$ Box1 lacks residues 281–294,  $\gamma\Delta$ V-BOX lacks residues 295–320, and  $\gamma\Delta$ Box2 lacks residues 321–334. (D) Transfection assay of 32D/IRS-1/EPO7 cells with various EPO $\gamma$  mutants demonstrates the critical role of the cytoplasmic membrane proximal region of  $\gamma_c$  for growth signaling. (E) Evaluation of internal deletion mutants of  $\gamma_c$  by the transient transfection assay. Each transfection assay done in 32D/IRS-1/EPO7 cells included EPO $\gamma$  as a positive control.

this DNA-binding complex (data not shown). Thus, as in other  $\gamma_c$ -containing receptor complexes, the specific JAK-STAT signaling events directed by the IL-7 receptor complex are independent of the tyrosine residues of the  $\gamma_c$  subunit.

Only the membrane-proximal portion of the  $\gamma_c$  subunit is necessary for growth signaling by the IL-7R. Growth signaling mediated by the chimeric EPO7/EPO $\gamma$  heterodimer was evaluated in 32D/IRS-1 stable cell lines. In [ $^3$ H]thymidine incorporation assays, EPO stimulation of the 32D/IRS-1/EPO7 and 32D/IRS-1/EPO $\gamma$  cell lines did not induce detectable proliferative responses (Fig. 4 A). In contrast, 32D/IRS-1/EPO $\gamma$  cells demonstrated a strong, dose-dependent proliferation in response to EPO. Additionally, a transient transfection assay originally established to study the IL-2R (44) was employed to evaluate IL-7R complex subunit requirements. In such experiments, expression of the EPO $\gamma$  expression plasmid in the 32D/IRS-1/EPO7 cell line restored EPO-induced proliferation signaling, as measured by [ $^3$ H]thymidine incorporation (Fig. 4 B). Similarly, introduction of the EPO7 subunit into the 32D/IRS-1/EPO $\gamma$  cell line also restored growth signaling in response to EPO (Fig. 5). Thus, heterodimerization of the IL-7R $\alpha$  and  $\gamma_c$  subunits is necessary and sufficient for IL-7-mediated growth signaling.

Establishment of the transfection assay in these cells permitted the rapid assessment of various  $\gamma_c$  mutants in the IL-7R complex. The  $\gamma_c$  mutants included the previously described EPO $\gamma$ YF lacking all four cytoplasmic tyrosine residues, various truncation mutants, and internal deletion mutants lacking specific membrane-proximal regions of the  $\gamma_c$  subunit (Fig. 4 C). Expression of these chimeric  $\gamma_c$  mutants had been previously verified (44). As with JAK-STAT signaling, growth signaling was intact with the EPO $\gamma$ YF mutant (Fig. 4 D). Additionally, truncation of the  $\gamma_c$  subunit to the Box2 region (EPO $\gamma$ 336) did not adversely affect EPO-mediated proliferation. However, more severe truncations of the chimeric  $\gamma_c$  mutant (EPO $\gamma$ TM and EPO $\gamma$ 318) resulted in the abrogation of growth signaling, demonstrating the importance of the membrane-proximal regions of  $\gamma_c$  in IL-7R function (Fig. 4 D). This finding was further verified by internal deletion mutants of the Box1, V-Box, and Box2 regions of the  $\gamma_c$  chimeric receptor, each of which also abolished proliferation signaling (Fig. 4 E). Therefore, only the membrane-proximal regions of the  $\gamma_c$  subunit are necessary and sufficient for growth signaling mediated by the IL-7R $\alpha$ / $\gamma_c$  heterodimer.

The  $\gamma_c$  subunit is functionally replaceable in the IL-7R complex for signal transduction. Previous studies of the IL-2R complex demonstrated that the  $\gamma_c$  subunit serves primarily to activate receptor signaling, rather than to determine specific signaling events (52). Since comparable minimal structural elements of the  $\gamma_c$  subunit are required for IL-7R signaling, it is reasonable to predict that  $\gamma_c$  may perform a similar function in the IL-7R $\alpha$ / $\gamma_c$  heterodimer. To test this hypothesis, the  $\gamma_c$  subunit was replaced by a heterologous receptor subunit in the chimeric receptor system. Specifically, the EPO $\gamma$  subunit was substituted by a truncated EPOR mutant, EPOR(1-321), which retains only the membrane-proximal cytoplasmic region that mediates JAK2 association (53). In [ $^3$ H]thymidine-incorporation assays, 32D/IRS-1 stable cell lines expressing EPO7 or EPOR(1-321) alone did not demonstrate a detectable proliferation signal in response to EPO (Fig. 3 A). However, EPO induced a vigorous growth response in cells that expressed both receptor subunits [32D/IRS-1/EPO7/EPOR(1-321)]. Thus,

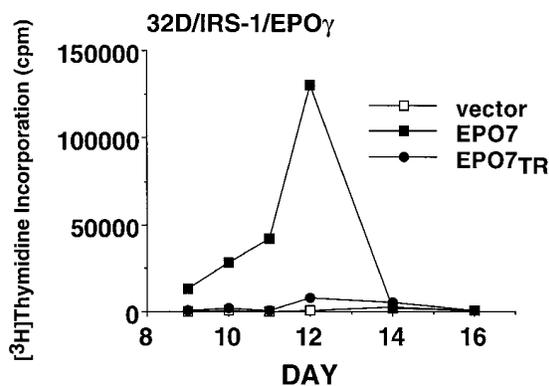


Figure 5. The cytoplasmic domain of the IL-7R $\alpha$  subunit is required for proliferation signaling. Transient transfection assay was performed in 32D/IRS-1/EPO $\gamma$  cells.

downstream signaling events such as proliferation were unaffected by the substitution of the  $\gamma_c$  cytoplasmic domain by the truncated EPOR subunit.

Distinct molecular signaling events were evaluated to examine other potential consequences of this receptor subunit substitution. As described above, the tyrosine phosphorylation of JAK1 and JAK3 was increased upon EPO-stimulation of the 32DEPO7 $\gamma$  cell line (Fig. 3 B). In the 32DEPO7/EPOR(1-321) cell line, EPO stimulation resulted in the increased tyrosine phosphorylation of JAK1 and JAK2, but not of JAK3. Furthermore, this replacement of JAK3 by JAK2 in the IL-7R complex did not alter the specificity of the JAK-STAT pathway. EMSA of nuclear extracts from 32D/IRS-1/EPO7 and 32D/IRS-1/EPOR(1-321) cells demonstrated no detectable DNA-binding activity in response to EPO (Fig. 3 C). In contrast, EPO stimulated the induction of STAT-5 in 32D/IRS-1/EPO7/EPOR(1-321) cells (Fig. 3 C and data not shown). Additionally, signaling through the IL-7R complex results in the activation of IRS-1 (41). Immunoblot analysis with the anti-phosphotyrosine antibody of anti-IRS-1 immunoprecipitates demonstrated no detectable increase in phosphorylation of IRS-1 in EPO-stimulated 32D/IRS-1/EPO7 or 32D/IRS-1/EPO $\gamma$  cells (data not shown). In contrast, IRS-1 demonstrated a marked EPO-dependent increase in tyrosine phosphorylation in 32D/IRS-1/EPO7 $\gamma$  cells (Fig. 3 D). Similarly, IRS-1 was phosphorylated upon EPO-induced heterodimerization of the IL-7R $\alpha$  and truncated EPOR(1-321) cytoplasmic tails. Therefore, a variety of specific signaling events mediated by the IL-7R complex occur independently of the presence of the  $\gamma_c$  subunit. The  $\gamma_c$  subunit therefore appears to serve primarily to activate signaling by the IL-7R, rather than to specify signaling events directed by the receptor complex.

The distal portion of the IL-7R $\alpha$  subunit is required for growth signaling. Because the major role of the  $\gamma_c$  subunit within the IL-7 receptor complex appears to be during initiation of receptor signaling, it was hypothesized that the IL-7R $\alpha$  subunit itself serves to direct distinct signaling events. Specifically, the IL-7R $\alpha$  subunit contains three tyrosine residues in the distal portion of the cytoplasmic tail, which may serve as docking sites for various signaling molecules. To evaluate the functional contribution of IL-7R $\alpha$  in the receptor complex, a chimeric receptor containing the extracellular portion of

EPOR and a truncated cytoplasmic portion of the IL-7R $\alpha$  subunit lacking the three distal tyrosines (EPO7<sub>TR</sub>) was constructed. Expression of the EPO7<sub>TR</sub> subunit was verified by immunoblotting of lysates from transfected COS cells (data not shown). In a transient transfection assay, expression of the wild-type EPO7 subunit in 32D/IRS-1/EPO $\gamma$  cells led to the proliferation of these cells in response to EPO (Fig. 5). Strikingly, introduction of the EPO7<sub>TR</sub> subunit into this same cell line did not restore growth signaling. Thus, the IL-7R $\alpha$  subunit and specifically, its distal cytoplasmic domain, are critical for proliferation signaling in the IL-7 receptor complex.

## Discussion

Genetic analysis indicates that human X-SCID results from mutations in the  $\gamma_c$  subunit (11, 12), a receptor chain shared by several cytokine receptors (1–9). While recent observations suggest that the most critical functional defects in X-SCID are manifested in the IL-7R system (23, 24, 54, 55), the specific role of  $\gamma_c$  in that receptor complex remains poorly defined. The present studies analyzing the functional architecture of the IL-7R complex demonstrate the essential requirement of  $\gamma_c$  for proper IL-7R function. Furthermore, these studies define the role of  $\gamma_c$  in the IL-7R complex, leading to a potential molecular mechanism for the pathogenesis of X-SCID.

Structure/function analysis using a chimeric receptor system demonstrated the absolute requirement for heterodimerization of the IL-7R $\alpha$  and  $\gamma_c$  cytoplasmic domains to activate IL-7-specific signaling events. The EPO7/EPO $\gamma$  heterodimer activated STAT-5A and -5B in both HT-2 and 32D/IRS-1 stable transfectants. However, in the HT-2 cellular context, STAT-5 activation alone was not sufficient to mediate proliferation (data not shown), a finding that is presently being investigated. In the 32D/IRS-1 stable cell line, these molecular events were not affected by mutations in the  $\gamma_c$  subunit that COOH terminally truncated the cytoplasmic domain to the membrane-proximal Box2 region or converted the four cytoplasmic tyrosine residues to phenylalanines. However, more severe truncation mutants or internal deletions of the membrane-proximal region of  $\gamma_c$  that binds JAK3 (7) abolished proliferation signaling. These results complement recent studies in which a severely truncated  $\gamma_c$  subunit acted in a dominant-negative fashion to inhibit IL-7-mediated growth signaling (56) and demonstrate the critical contribution of the membrane-proximal segment of  $\gamma_c$  to IL-7R function. In contrast, a relatively distal truncation of the IL-7R $\alpha$  subunit completely abrogated growth signaling by the IL-7R complex in the present system. Thus, while both receptor subunits were observed to be essential for IL-7R signaling competence, the structural requirements for these subunits were quite distinct.

Collectively, these findings demonstrate an asymmetric structure/function organization in the IL-7R complex that is strikingly similar to the “trigger-driver” arrangement of the IL-2R complex (20). In this configuration, specific receptor signaling events are determined by a distinct profile of signaling intermediates that physically associate with a single “driver” subunit, represented by the IL-7R $\alpha$  chain. For example, these interactions may be mediated by conserved SH2 or PTB domains within signaling molecules that interact with phosphorylated tyrosine residues embedded within specific peptide motifs of such receptor subunits (57, 58). Indeed, in the IL-7R $\alpha$  chain, removal of the three distal tyrosine residues

was sufficient to abolish growth signaling by IL-7R. This finding is consistent with the recent studies of the murine IL-7R $\alpha$  subunit, which demonstrated the importance of a distal tyrosine residue for proliferation in B cell lymphopoiesis (59). Thus, the IL-7R $\alpha$  subunit functions as a “driver” subunit in determining the specific signaling events mediated by the IL-7R complex.

As in another  $\gamma_c$ -containing receptor, the IL-2R system, the  $\gamma_c$  subunit transports JAK3 into the IL-7R complex to activate receptor signaling. The intimate association of  $\gamma_c$  and JAK3 is confirmed by patients with JAK3 mutations who have immunodeficiencies that are very similar to X-SCID (60). Indeed,  $\gamma_c$ - and JAK3-deletion mice share similar immunodeficiency phenotypes (13, 14, 61). Furthermore, the nonfunctional  $\gamma_c$  mutants employed in the present studies affected similar portions of the  $\gamma_c$  subunit as mutations found in X-SCID patients (11, 12), and disrupted the membrane-proximal region of  $\gamma_c$  reported to mediate association with JAK3 (7). However, the requirement for JAK3 itself within this signaling complex is not absolute. Functional replacement of the  $\gamma_c$  cytoplasmic domain by EPOR(1-321) did not alter downstream signaling events measured in the chimeric IL-7R system. Furthermore, signaling via the EPO7/EPOR(1-321) heterodimer depended on the engagement of JAK1 and JAK2, but not JAK3 (data not shown). Importantly, this substitution of JAK2 for JAK3 in this complex did not affect events such as cellular proliferation, phosphorylation of IRS-1, or induction of STAT-5A or -5B. Therefore, various signaling events previously thought to be linked to JAK3 itself may instead be coupled to the IL-7R $\alpha$  “driver” chain (62). Thus, the present studies support the model that  $\gamma_c$  and JAK3 are not required for specific signaling events, but act instead as a relatively generic “trigger” for activation of receptor-mediated signal transduction.

Several experimental approaches have demonstrated the important role of the IL-7/IL-7R system to early lymphocyte development (23, 24, 54, 55). The delineation of the functional role of the  $\gamma_c$  subunit within the IL-7R complex provides a possible molecular mechanism for the pathogenesis of X-SCID.  $\gamma_c$  defects that prevent the transport of JAK3 into the receptor complex would prevent IL-7-mediated signaling and the subsequent development of B and T cells. Based on the present findings, the critical step affected by mutations in the  $\gamma_c$  subunits appears to be the initiation of IL-7R signal transduction. Interestingly, unlike  $\gamma_c$ -deficient mice and X-SCID patients, IL-7- and IL-7R $\alpha$ -deletion mice retain functional natural killer cells (63), raising the possibility that an unrecognized cytokine(s) may also employ  $\gamma_c$ -containing receptor complex(es). Nevertheless, the developmental defects for T and B cells are at least as severe as those for X-SCID individuals and  $\gamma_c$ -deficient mice. Therefore, these findings suggest that the earliest signaling events that would affect lymphocyte development in X-SCID appear to be mediated by the IL-7R complex. While the  $\gamma_c$  subunit is present in other receptor complexes, such as the IL-2R, -4R, -9R, and -15R, these receptors appear to function in the later stages of lymphoid development and regulation. Thus, the early block of lymphocyte development due to defects in IL-7R function would mask potential functional aberrations due to the lack of signaling by these receptors.

The present studies define the role of  $\gamma_c$  in the IL-7R complex and provide a possible molecular mechanism for the pathogenesis of X-SCID. These studies also define further at

least two distinct classes of receptors that employ the  $\gamma_c$  subunit. First, the IL-7R and -2R complexes exhibit a strict requirement for  $\gamma_c$  in the receptor complex. In these receptor systems,  $\gamma_c$  initiates signaling by transporting the associated JAK3 into the receptor complex, while IL-7R $\alpha$  and -2R $\beta$  function in their respective receptor complexes to mediate specific signaling events. Interestingly, a similar functional arrangement of the individual receptor subunits has recently been demonstrated in the interferon- $\gamma$  receptor complex (64). In the second class of receptors, represented by the IL-4R,  $\gamma_c$  is not absolutely essential for receptor function. In SCID-MA cells, the lack of detectable  $\gamma_c$  expression (reference 12 and data not shown) does not affect IL-4 responsiveness (Fig. 1 and reference 20). This observation supports the recent findings that the IL-4R complex may exist in multiple forms (17–21). In certain cell lines derived from X-SCID patients, mutant forms of  $\gamma_c$  may act in a dominant negative fashion to inhibit the IL-4-mediated activation of JAK3 and STAT-6 (65). In the absence of  $\gamma_c$ , another receptor subunit, perhaps the IL-13R $\alpha$  and/or other unidentified partner chains, may perform a similar role as that of the  $\gamma_c$  subunit during initiation of signaling through the receptor complex (17–21, 66). This may account for the responsiveness of some gggccc-negative cells to IL-4. Additionally, circumstantial evidence also exists for a homomeric form of IL-4R that consists of only the IL-4R $\alpha$  subunit (reference 20 and data not shown). Further studies are in progress to determine the functional role of  $\gamma_c$  in the other receptors, such as the IL-9R complex, and to understand the structure/function arrangements of heterodimeric receptor systems.

## Acknowledgments

The authors appreciate the critical comments and input of Dr. W.C. Greene, Dr. S.L. Gaffen, and K.D. Liu. We thank Dr. R. Gelezianus for the PBMC cDNA used as a template in constructing EPO7, Dr. L. Hennighausen for the anti-STAT-5A and anti-STAT-5B antibodies, Dr. S.L. McKnight for the anti-STAT-6 antibody, Dr. M. White for the anti-IRS-1 antibody, and Dr. J. Puck for the SCID-MA cell line. The authors acknowledge the excellent assistance of Mr. Michael Ceniceros, Mr. John Carroll, and Ms. Amy Corder in the preparation of this manuscript. IL-2 was a generous gift of Chiron Corp., and EPO was the generous gift of Ortho Diagnostic Systems, Inc.

Support for this work was provided by the J. David Gladstone Institutes and National Institutes of Health (NIH) grant GM-54351 to M.A. Goldsmith. S.Y. Lai is in the NIH Medical Scientist Training Program and the Biomedical Sciences Program at the University of California, San Francisco.

## References

1. Takeshita, T., H. Asao, K. Ohtani, N. Ishii, S. Kumaki, N. Tanaka, H. Munakata, M. Nakamura, and K. Sugamura. 1992. Cloning of the gamma chain of the human IL-2 receptor. *Science (Wash. DC)*. 257:379–382.
2. Kondo, M., T. Takeshita, N. Ishii, M. Nakamura, S. Watanabe, K.-I. Arai, and K. Sugamura. 1993. Sharing of the interleukin-2 (IL-2) receptor  $\gamma$  chain between receptors for IL-2 and IL-4. *Science (Wash. DC)*. 262:1874–1877.
3. Noguchi, M., Y. Nakamura, S.M. Russell, S.F. Ziegler, M. Tsang, X. Cao, and W.J. Leonard. 1993. Interleukin-2 receptor  $\gamma$  chain: a functional component of the interleukin-7 receptor. *Science (Wash. DC)*. 262:1877–1880.
4. Russell, S.M., A.D. Keegan, N. Harada, Y. Nakamura, M. Noguchi, P. Leland, M.C. Friedmann, A. Miyajima, R.K. Puri, W.E. Paul, and W.J. Leonard. 1993. Interleukin-2 receptor  $\gamma$  chain: a functional component of the interleukin-4 receptor. *Science (Wash. DC)*. 262:1880–1883.
5. Giri, J.G., M. Ahdieh, J. Eisenman, K. Shanebeck, K. Grabstein, S. Kumaki, A. Namen, L.S. Park, D. Cosman, and D. Anderson. 1994. Utilization of the  $\beta$  and  $\gamma$  chains of the IL-2 receptor by the novel cytokine IL-15. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:2822–2830.

6. Kondo, M., T. Takeshita, M. Higuchi, M. Nakamura, T. Sudo, S. Nishikawa, and K. Sugamura. 1994. Functional participation of the IL-2 receptor  $\gamma$  chain in IL-7 receptor complexes. *Science (Wash. DC)*. 263:1453–1454.
7. Russell, S.M., J.A. Johnston, M. Noguchi, M. Kawamura, C.M. Bacon, M. Friedmann, M. Berg, D.W. McVicar, B.A. Witthuhn, O. Silvennoinen, et al. 1994. Interaction of IL-2R $\beta$  and  $\gamma_c$  chains with Jak1 and Jak3: implications for XSCID and XCID. *Science (Wash. DC)*. 266:1042–1045.
8. Kimura, Y., T. Takeshita, M. Kondo, N. Ishii, M. Nakamura, J. Van Snick, and K. Sugamura. 1995. Sharing of the IL-2 receptor gamma chain with the functional IL-9 receptor complex. *Intl. Immunol.* 7:115–120.
9. Ziegler, S.E., K.K. Morella, D. Anderson, N. Kumaki, W.J. Leonard, D. Cosman, and H. Baumann. 1995. Reconstitution of a functional IL-7 receptor demonstrates that the IL-2 receptor gamma chain is required for IL-7 signal transduction. *Eur. J. Immunol.* 25:399–404.
10. Conley, M.E. 1992. Molecular approaches to analysis of X-linked immunodeficiencies. *Ann. Rev. Immunol.* 10:215–238.
11. Noguchi, M., H. Yi, H.M. Rosenblatt, A.H. Filipovich, S. Adelstein, W.S. Modi, O.W. McBride, and W.J. Leonard. 1993. Interleukin-2 receptor  $\gamma$  chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell*. 73:147–157.
12. Puck, J.M., S.M. Deschenes, J.C. Porter, A.S. Dutra, C.J. Brown, H.F. Willard, and P.S. Henthorn. 1993. The interleukin-2 receptor  $\gamma$  chain maps to Xq13.1 and is mutated in X-linked severe combined immunodeficiency, SCIDX1. *Hum. Mol. Genet.* 2:1099–1104.
13. Cao, X., E.W. Shores, J. Hu-Li, M.R. Anver, B.L. Kelsall, S.M. Russell, J. Drago, M. Noguchi, A. Grinberg, E.T. Bloom, et al. 1995. Defective lymphoid development in mice lacking expression of the common cytokine receptor  $\gamma$  chain. *Immunity*. 2:223–238.
14. DiSanto, J.P., W. Muller, D. Guy-Grand, A. Fischer, and K. Rajewsky. 1995. Lymphoid development in mice with a targeted deletion of the interleukin-2 receptor  $\gamma$  chain. *Proc. Natl. Acad. Sci. USA*. 92:377–381.
15. Schorle, H., T. Holschke, T. Hunig, A. Schimpl, and I. Horak. 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature (Lond.)*. 352:621–624.
16. Suzuki, H., T.M. Kundig, C. Furlonger, A. Wakeham, E. Timms, T. Matsuyama, R. Schmits, J.J. Simard, P.S. Ohashi, and H. Griesser. 1995. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science (Wash. DC)*. 268:1472–1476.
17. He, Y.-W., and T.R. Malek. 1995. The IL-2 receptor  $\gamma_c$  chain does not function as a subunit shared by the IL-4 and IL-13 receptors: implication for the structure of the IL-4 receptor. *J. Immunol.* 155:9–12.
18. Keegan, A.D., J.A. Johnston, P.J. Tortolani, L.J. McReynolds, C. Kinzer, J.J. O'Shea, and W.E. Paul. 1995. Similarities and differences in signal transduction by IL-4 and IL-13: analysis of Janus kinase activation. *Proc. Natl. Acad. Sci. USA*. 92:7681–7685.
19. Lin, J.-X., T.-S. Migone, M. Tsang, M. Friedmann, J.A. Weatherbee, L. Zhou, A. Yamauchi, E. T. Bloom, J. Mietz, S. John, and W. J. Leonard. 1995. The role of shared receptor motifs and common stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity*. 2:331–339.
20. Lai, S.Y., J. Molden, K.D. Liu, J.M. Puck, M.D. White, and M.A. Goldsmith. 1996. Interleukin 4-specific signal transduction events are driven by homotypic interactions on the interleukin-4 receptor  $\alpha$  subunit. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:4506–4514.
21. Palmer-Crocker, R.L., C.C.W. Hughes, and J.S. Pober. 1996. IL-4 and IL-13 activate the JAK2 tyrosine kinase and Stat6 in cultured human vascular endothelial cells through a common pathway that does not involve the  $\gamma_c$  chain. *J. Clin. Invest.* 98:604–609.
22. Renaud, J.C., A. Kermouni, A. Vink, J. Louahed, and J. Van Snick. 1995. Interleukin-9 and its receptor: involvement in mast cell differentiation and T cell oncogenesis. *J. Leukocyte Biol.* 57:353–360.
23. von Freeden-Jeffry, U., P. Vieira, L.A. Luciani, T. McNeil, S.E. Burdach, and R. Murray. 1995. Lymphopenia in interleukin (IL-7)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* 181:1519–1526.
24. Peschon, J.J., P.J. Morrissey, K.H. Grabstein, F.J. Ramsdell, E. Maraskovsky, B.C. Gliniak, L.S. Park, S.F. Ziegler, D.E. Williams, C.B. Ware, et al. 1994. Early lymphocyte expansion is severely impaired in interleukin-7 receptor-deficient mice. *J. Exp. Med.* 180:1955–1960.
25. Namen, A.E., A.E. Schmierer, C.J. March, R.W. Overell, L.S. Park, D.L. Urdal, and D.Y. Mochizuki. 1988. B cell precursor growth-promoting activity: purification and characterization of a growth factor active on lymphocyte precursors. *J. Exp. Med.* 167:988–1002.
26. Namen, A.E., S. Lupton, K. Hjerrild, J. Wignall, D.Y. Mochizuki, A. Schmierer, B. Mosley, C.J. March, D. Urdal, and S. Gillis. 1988. Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature (Lond.)*. 333:571–573.
27. Conlon, P.J., P.J. Morrissey, R.P. Nordan, K.H. Grabstein, K.S. Prickett, S.G. Reed, R. Goodwin, D. Cosman, and A.E. Namen. 1989. Murine thymocytes proliferate in direct response to interleukin-7. *Blood*. 74:1368–1373.
28. Goodwin, R.G., S. Lupton, A. Schmierer, K.J. Hjerrild, R. Jerzy, W. Clevenger, S. Gillis, D. Cosman, and A.E. Namen. 1989. Human interleukin 7: Molecular cloning and growth factor activity on human and murine B-lineage

cells. *Proc. Natl. Acad. Sci. USA*. 86:302–306.

29. Takeda, S., S. Gillis, and R. Palacios. 1989. In vitro effects of recombinant interleukin-7 on growth and differentiation of bone marrow pro-B- and pro-T-lymphocyte clones and fetal thymocyte clones. *Proc. Natl. Acad. Sci. USA*. 86:1634–1638.
30. Okazaki, H., M. Ito, T. Sudo, M. Hattori, S. Kano, Y. Katsura, and N. Minato. 1989. IL-7 promotes thymocyte proliferation and maintains immunocompetent thymocytes bearing alpha beta or gamma delta T-cell receptors in vitro: synergism with IL-2. *J. Immunol.* 143:2917–2922.
31. Edington, H., and M.T. Lotze. 1994. Interleukin-7. In *The Cytokine Handbook*. Academic Press Inc., Orlando, FL. 169–184.
32. Goodwin, R.G., D. Friend, S.F. Ziegler, R. Jerzy, B.A. Falk, S. Gimpel, D. Cosman, S.K. Dower, C.J. March, A.E. Namen, and L.S. Park. 1990. Cloning of the human and murine interleukin-7 receptors: demonstration of a soluble form and homology to a new receptor superfamily. *Cell*. 60:941–951.
33. Bazan, J.-F. 1990. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc. Natl. Acad. Sci. USA*. 87:6934–6938.
34. Murakami, M., M. Narazaki, M. Hibi, H. Yawata, K. Yasukawa, M. Hamaguchi, T. Taga, and T. Kishimoto. 1991. Critical cytoplasmic region of the interleukin-6 signal transducer gp130 is conserved in the cytokine receptor family. *Proc. Natl. Acad. Sci. USA*. 88:11349–11353.
35. Miyazaki, T., A. Kawahara, H. Fujii, Y. Nakagawa, Y. Minami, Z.-J. Liu, I. Oishi, O. Silvennoinen, B.A. Witthuhn, J.N. Ihle, and T. Taniguchi. 1994. Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. *Science (Wash. DC)*. 266:1045–1047.
36. Wilks, A.F., and A.G. Harpur. 1994. Cytokine signal transduction and the JAK family of protein tyrosine kinases. *Bioessays*. 16:313–320.
37. Boussiotis, V.A., D.L. Barber, T. Nakarai, G.J. Freeman, J.G. Gribben, G.M. Bernstein, A.D. D'Andrea, J. Ritz, and L.M. Nadler. 1994. Prevention of T cell anergy by signaling through the  $\gamma_c$  chain of the IL-2 receptor. *Science (Wash. DC)*. 266:1039–1042.
38. Venkataraman, A.R., and R.J. Cowling. 1992. Interleukin-7 receptor functions by recruiting the tyrosine kinase p59fyn through a segment of its cytoplasmic tail. *Proc. Natl. Acad. Sci. USA*. 89:12083–12087.
39. Seckinger, P., and M. Fougereau. 1994. Activation of src family kinases in human pre-B cells by IL-7. *J. Immunol.* 153:97–109.
40. Page, T.H., F. V. Lali, and B.M. Foxwell. 1995. Interleukin-7 activates p56lck and p59fyn, two tyrosine kinases associated with the p90 interleukin-7 receptor in primary human T cells. *Eur. J. Immunol.* 25:2956–2960.
41. Johnston, J.A., L.M. Wang, E.P. Hanson, X.J. Sun, M.F. White, S.A. Oakes, J.H. Pierce, and J.J. O'Shea. 1995. Interleukins 2, 4, 7, and 15 stimulate tyrosine phosphorylation of insulin receptor substrates 1 and 2 in T cells: potential role of JAK kinases. *J. Biol. Chem.* 270:28527–28530.
42. Dadi, H.K., and C.M. Roifman. 1993. Activation of phosphatidylinositol-3 kinase by ligation of the interleukin-7 receptor on human thymocytes. *J. Clin. Invest.* 92:1559–1563.
43. Dadi, H.K., S. Ke, and C.M. Roifman. 1993. Interleukin-7 receptor mediates the activation of phosphatidylinositol-3 kinase in human B-cell precursors. *Biochem. Biophys. Res. Commun.* 192:459–464.
44. Goldsmith, M.A., W. Xu, M.C. Amaral, E.S. Kuczek, and W.C. Greene. 1994. The cytoplasmic domain of the IL-2 receptor  $\beta$  chain contains both unique and functionally redundant signal transduction elements. *J. Biol. Chem.* 269:14698–14704.
45. Andersson, S., D.L. Davis, H. Dahlback, H. Jornvall, and D.W. Russell. 1989. Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. *J. Biol. Chem.* 264:8222–8229.
46. Goldsmith, M.A., S.Y. Lai, W. Xu, M.C. Amaral, E.S. Kuczek, L.J. Parent, G.B. Mills, K.L. Tarr, G.D. Longmore, and W.C. Greene. 1995. Growth signal transduction by the human IL-2 receptor requires cytoplasmic tyrosines of the  $\beta$  chain and non-tyrosine residues of the  $\gamma_c$  chain. *J. Biol. Chem.* 270:21729–21737.
47. Schreiber, E., P. Matthias, M.M. Muller, and W. Schaffer. 1989. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res.* 17:6419.
- 47a. Latchman, D.S. 1993. *Transcription Factors: A Practical Approach*. IRL Press at Oxford University Press, Oxford, UK. 1–26.
48. Musso, T., J.A. Johnston, D. Linnekin, L. Varesio, T.K. Rowe, J.J. O'Shea, and D.W. McVicar. 1995. Regulation of JAK3 expression in human monocytes: phosphorylation on response to interleukins 2, 4, and 7. *J. Exp. Med.* 181:1425–1431.
49. Darnell, J.E., I.M. Kerr, and G.R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science (Wash. DC)*. 264:1415–1421.
50. Wang, L.-M., M.G. Myers, X.-J. Sun, S.A. Aaronson, M. White, and J.H. Pierce. 1993. IRS-1: essential for insulin- and IL-4-stimulated mitogenesis in hematopoietic cells. *Science (Wash. DC)*. 261:1591–1594.
51. Gaffen, S.L., S.Y. Lai, M. Ha, X. Liu, L. Hennighausen, W.C. Greene, and M.A. Goldsmith. 1996. Distinct tyrosine residues within the IL-2 receptor  $\beta$  chain drive signal transduction specificity, redundancy and diversity. *J. Biol. Chem.* 271:21381–21390.
52. Lai, S.-Y., W. Xu, S.L. Gaffen, G.D. Longmore, W.C. Greene, and M.A. Goldsmith. 1996. The molecular role of the common  $\gamma_c$  subunit in signal transduction reveals functional asymmetry within multimeric cytokine receptor complexes. *Proc. Natl. Acad. Sci. USA*. 93:231–235.
53. Witthuhn, B.A., F.W. Quelle, O. Silvennoinen, T. Yi, B. Tang, O. Miura, and J.N. Ihle. 1993. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. *Cell*. 74:227–236.
54. Grabstein, K.H., T.J. Waldschmidt, F.D. Finkelman, B.W. Hess, A.R. Alpert, N.E. Boiani, A.E. Namen, and P.J. Morrissey. 1993. Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. *J. Exp. Med.* 178:257–264.
55. Sudo, T., S. Nishikawa, N. Ohno, N. Akiyama, M. Tamakoshi, H. Yoshida, and S.-I. Nishikawa. 1993. Expression and function of the interleukin 7 receptor in murine lymphocytes. *Proc. Natl. Acad. Sci. USA*. 90:9125–9129.
56. Kawahara, A., Y. Minami, and T. Taniguchi. 1994. Evidence for a critical role for the cytoplasmic region of the interleukin 2 (IL-2) receptor gamma chain in IL-2, IL-4, and IL-7 signaling. *Mol. Cell. Biol.* 14:5433–5440.
57. Songyang, Z., S.E. Shoelson, M. Chaudhuri, G. Gish, T. Pawson, W.G. Haser, F. King, T. Roberts, S. Ratnoffsky, and R.J. Lechleider. 1993. SH2 domains recognize specific phosphopeptide sequences. *Cell*. 72:767–778.
58. Kavanaugh, W.M., and L.T. Williams. 1994. An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. *Science (Wash. DC)*. 266:1862–1865.
59. Corcoran, A.E., F.M. Smart, R.J. Cowling, T. Crompton, M.J. Owen, and A.R. Venkataraman. 1996. The interleukin 7 receptor  $\alpha$  chain transmits distinct signals for proliferation and differentiation during B lymphopoiesis. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:1924–1932.
60. Russell, S.M., N. Tayebi, H. Nakajima, M.C. Riedy, J.L. Roberts, M.J. Aman, T.S. Migone, M. Noguchi, M.L. Markert, and R.H. Buckley. 1995. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. *Science (Wash. DC)*. 270:797–800.
61. Nosaka, T., J.M. van Deursen, R.A. Tripp, W.E. Theifelder, B.A. Witthuhn, A.P. McMickle, P.C. Doherty, G.C. Grosveld, and J.N. Ihle. 1995. Defective lymphoid development in mice lacking Jak3. *Science (Wash. DC)*. 270:800–802.
62. Sharfe, N., H. Dadi, and C.M. Roifman. 1995. JAK3 protein tyrosine kinase mediates interleukin 7-induced activation of phosphatidylinositol-3' kinase. *Blood*. 86:2077–2085.
63. Maki, K., S. Sunaga, Y. Komagata, Y. Kodaira, A. Mabuchi, H. Karasuyama, K. Yokomuro, J.-I. Miyazaki, and K. Ikuta. 1996. Interleukin 7 receptor-deficient mice lack  $\gamma\delta$  T cells. *Proc. Natl. Acad. Sci. USA*. 93:7172–7177.
64. Bach, E.A., J.W. Tanner, S. Marsters, A. Ashkenazi, M. Aguet, A.S. Shaw, and R.D. Schreiber. 1996. Ligand-induced assembly and activation of the gamma interferon receptor in intact cells. *Mol. Cell. Biol.* 16:3214–3221.
65. Izuhara, K., T. Heike, T. Otsuka, K. Yamaoka, M. Mayumi, T. Imamura, Y. Niho, and N. Harada. 1996. Signal transduction pathway of interleukin-4 and interleukin-13 in human B cells derived from X-linked severe combined immunodeficiency patients. *J. Biol. Chem.* 271:619–622.
66. Hilton, D.J., J.-G. Zhang, D. Metcalf, W.S. Alexander, N.A. Nicola, and T.A. Willson. 1996. Cloning and characterization of a binding subunit of the IL-13 receptor that is also a component of the IL-4 receptor. *Proc. Natl. Acad. Sci. USA*. 93:497–501.