REFERENCES AND NOTES

1. M. K. Jenkins, D. M. Pardoll, J. Mizuguchi, T. M. Chused, R. H. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5409 (1987); M. K. Jenkins, J. D. Ashwell, R. H. Schwartz, *J. Immunol.* **140**, 3324 (1988); M. J. Dallman, O. Shiho, T. H. Page, K. J. Wood, P. J. Morris, *J. Exp. Med.* **173**, 79 (1991); C. Go and J. Miller, *ibid.* **175**, 1327 (1992); S. M. Kang *et al.*, *Science* **257**, 1134 (1992); G. Lombardi, S. Sidhu, R. Batchelor, R. Lechler, *ibid.* **264**, 1587 (1994).
2. F. A. Harding, J. G. McArthur, J. A. Gross, D. H. Raulet, J. P. Allison, *Nature* **356**, 607 (1992).
3. C. D. Gimmi, G. J. Freeman, J. G. Gribben, G. Gray, L. M. Nadler, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6586 (1993).
4. D. J. Lenschow *et al.*, *Science* **257**, 789 (1992); L. A. Turka *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11102 (1992); P. Tan *et al.*, *J. Exp. Med.* **177**, 165 (1993); H. Lin *et al.*, *ibid.* **178**, 1801 (1993).
5. P. S. Linsley *et al.*, *J. Exp. Med.* **173**, 721 (1991); C. D. Gimmi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6575 (1991); G. J. Freeman *et al.*, *Science* **262**, 909 (1993); G. J. Freeman *et al.*, *ibid.*, p. 907; G. J. Freeman *et al.*, *J. Exp. Med.* **178**, 2185 (1993).
6. B. Beverly, S. Kang, M. Lenardo, R. Schwartz, *Int. Immunol.* **4**, 661 (1992).
7. G. Essery, M. Feldmann, J. Lamp, *Immunology* **64**, 413 (1988).
8. V. Boussiotis, G. Freeman, G. Gray, J. Gribben, L. Nadler, *J. Exp. Med.* **178**, 1753 (1993).
9. H. Schorle, T. Holtschke, T. Hunig, A. Schimpl, I. Horak, *Nature* **352**, 621 (1991); A. Shahinian *et al.*, *Science* **261**, 609 (1993).
10. V. Boussiotis, G. Freeman, J. Gribben, L. Nadler, unpublished results.
11. V. A. Boussiotis *et al.*, *J. Exp. Med.*, in press.
12. M. Kondo *et al.*, *Science* **262**, 1874 (1993); S. M. Russell *et al.*, *ibid.*, p. 1880; M. Noguchi *et al.*, *ibid.*, p. 1877.
13. M. Noguchi *et al.*, *Cell* **73**, 147 (1993); J. M. Puck *et al.*, *Hum. Mol. Genet.* **2**, 1099 (1993); J. P. DiSanto, A. Dautry-Varsat, S. Certain, A. Fischer, G. de Saint Basile, *Eur. J. Immunol.* **24**, 475 (1994).
14. Expression of IL-2Rα, -β, and -γ was equivalent as determined by fluorescence-activated cell sorting (FACS) analysis in the T cell clones studied. The amount of IL-4 and IL-7 receptor private chain expression was below the limit of detection by FACS analysis, but equivalent amounts of IL-4 and IL-7 private chain receptors were detected on the cell surface by immunoprecipitation.
15. A. F. Wilks *et al.*, *Mol. Cell. Biol.* **11**, 2057 (1991); A. G. Harpur, A. C. Andres, A. Ziemiecki, R. R. Astrom, A. F. Wilks, *Oncogene* **7**, 1347 (1991); I. Firmbach-Kraft, M. Byers, T. Shows, R. Dalla-Favera, J. J. Krolewski, *ibid.* **5**, 1329 (1990).
16. J. N. Ihle *et al.*, *Trends Biochem. Sci.* **19**, 222 (1994).
17. M. Kawamura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6374 (1994); J. A. Johnston *et al.*, *Nature* **370**, 151 (1994).
18. B. A. Whitthuhn *et al.*, *Nature* **370**, 153 (1994).
19. D. L. Barber and A. D. D'Andrea, *Mol. Cell. Biol.* **14**, 6506 (1994).
20. Y. Nakamura *et al.*, *Nature* **369**, 330 (1994); B. H. Nelson, J. D. Lord, P. D. Greenberg, *ibid.*, p. 333.
21. R. Kuhn, K. Rajewsky, W. Muller, *Science* **254**, 707 (1991).
22. S. M. Zuraski, F. Vega Jr., G. Huyghe, G. Zurawski, *EMBO J.* **12**, 2663 (1993).
23. K. H. Grabstein *et al.*, *Science* **264**, 965 (1994); J. G. Giri *et al.*, *EMBO J.* **13**, 2822 (1994).
24. A. Bhandoola *et al.*, *J. Immunol.* **151**, 2355 (1993); T. F. Gajewski, D. Qian, P. Fields, F. W. Fitch, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 38 (1994).
25. IL-2Rα (IgG2a; from Coulter, Hialeah, FL) (*26*), IL-2Rβ (IgG; from Coulter) (*27*), IL-4R (IgG1; from Genzyme, Cambridge, MA) (*28*), IL-7R (IgG1; from Genzyme) (*29*) or γ_c (IgG1) (*30*), and R–anti-M IgG (Jackson ImmunoResearch, West Grove, PA) were all used at the saturating concentration of 10 μg/ml. Identical results were obtained when biotinylated γ_c antibody was used and cross-linking was done with streptavidin (10 μg/ml), in biotin-free culture medium.
26. D. A. Fox *et al.*, *J. Immunol.* **133**, 1250 (1984).
27. M. Kamio *et al.*, *Int. Immunol.* **2**, 521 (1990).
28. W. C. Fanslow *et al.*, *Blood* **81**, 2998 (1993).
29. R. G. Goodwin *et al.*, *Cell* **60**, 941 (1990).
30. T. Nakarai *et al.*, *J. Exp. Med.* **180**, 241 (1994).
31. HLA-DR7 alloantigen-specific T cell clones were generated according to standard methodology (*11*). Before each experiment, T cell clones were rested for 10 to 14 days in IL-2 without alloantigen restimulation. Before use, cells were cultured overnight in media. LBL-DR7 cells and NIH 3T3 transfectants were treated with mitomycin-C (*8*). T cell clones were cultured in a primary culture for 24 hours, separated from LBL-DR7 cells by Ficoll gradient centrifugation and from NIH 3T3 transfectants by Percoll gradient centrifugation, and cultured in media without IL-2 for 12 hours. Each population was subsequently rechallenged with LBL-DR7 stimulators in secondary culture.
32. Cells were lysed with lysis buffer containing 10 mM tris-HCl (pH 7.6), 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM NaFl, 1 mM sodium orthovanadate, aprotinin (5 μg/ml), pepstatin (1 μg/ml), soybean trypsin inhibitor (2 μg/ml), 1 mM phenylmethylsulfonyl fluoride, 0.5% NP-40, and 0.5% Brij 96 (Sigma). After immunoprecipitations, immune complexes were isolated on protein A–Sepharose, washed three times with lysis buffer, and analyzed on 6 to 12% gradient gels in SDS–polyacrylamide gel electrophoresis (PAGE). Proteins were transferred to nitrocellulose, and membranes were blocked for 1 hour at room temperature by being shaken in tris-buffered saline with Tween-20 (TBST) [20 mM tris-HCl (pH 7.6), 137 mM NaCl, and 0.1% Tween-20] containing 10% bovine serum albumin (BSA). For detection of phosphotyrosine proteins, the blots were incubated with antiphosphotyrosine (mAb 4G10 at 1:2000) for 60 min at room temperature. The blots were washed three times with wash buffer (TBST), then incubated for 60 min with horseradish peroxidase–conjugated antibody to mouse IgG (at 1:5000) (Amersham, Arlington Heights, IL). The blots were washed three times with wash buffer then incubated with the enhanced chemiluminescence substrate (Amersham), exposed to x-ray film, and developed.
33. Stripping of the immunoblot was done by incubation in 62.5 mM tris-HCl (pH 6.8), 3% w/v SDS, and 100 mM β-mercaptoethanol at 50°C for 1 hour. Subsequently, immunoblot was blocked for 1 hour at room temperature by being shaken in TBST containing 10% BSA, and it was reprobed with a different antibody.

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Interaction of IL-2Rβ and γ_c Chains with Jak1 and Jak3: Implications for XSCID and XCID

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Interleukin-2 (IL-2) signaling requires the dimerization of the IL-2 receptor β (IL-2Rβ) and common γ (γ_c) chains. Mutations of γ_c can result in X-linked severe combined immunodeficiency (XSCID). IL-2, IL-4, IL-7 (whose receptors are known to contain γ_c), and IL-9 (whose receptor is shown here to contain γ_c) induced the tyrosine phosphorylation and activation of the Janus family tyrosine kinases Jak1 and Jak3. Jak1 and Jak3 associated with IL-2Rβ and γ_c, respectively; IL-2 induced Jak3–IL-2Rβ and increased Jak3-γ_c associations. Truncations of γ_c, and a γ_c, point mutation causing moderate X-linked combined immunodeficiency (XCID), decreased γ_c-Jak3 association. Thus, γ_c mutations in at least some XSCID and XCID patients prevent normal Jak3 activation, suggesting that mutations of Jak3 may result in an XSCID-like phenotype.

The interaction of IL-2 with high-affinity IL-2 receptors regulates the magnitude and duration of the T cell immune response (*1*).

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High-affinity IL-2 receptors contain the IL-2 receptor α (*2*), β (*3*), and γ_c (*4*) chains. Intermediate-affinity receptors contain IL-2Rβ and γ_c and mediate IL-2 signals on natural killer (NK) and some resting T cells. Both IL-2Rβ and γ_c are members of the cytokine receptor superfamily (*5*), whereas IL-2Rα is not. The heterodimerization of β and γ_c chains is induced by IL-2 binding and is required for IL-2 signaling (*6*). Mutations of the γ_c gene can result in XSCID in humans (*7*); the severity of XSCID results from γ_c being a component of multiple cytokine receptors (*1*, *8*–*11*).

Interleukin-2 induces the tyrosine phosphorylation of multiple cellular substrates (*12*), including the Janus family kinases Jak1 and Jak3 (*13*, *14*) (Fig. 1, A and B),

but not Tyk2 (Fig. 1C) or Jak2 (Fig. 1D). Immunoprecipitation with antibodies to Jak2 (anti-Jak2) yielded a tyrosine-phosphorylated band in response to IL-2 (Fig. 1D); this band migrated faster than Jak2 and is human Jak3, immunoprecipitated through cross-reactivity with the Jak2 antiserum, as shown by its removal by preclearing with a Jak3-specific antiserum (Fig. 1D). We also tested IL-4 and IL-7, two cytokines whose receptors contain γ_c, and IL-9 for their abilities to induce the tyrosine phosphorylation of Jak1 and Jak3. The IL-9 receptor also contains γ_c, because an antibody to γ_c inhibited IL-9–induced proliferation (*15*) and γ_c could be affinity-labeled by ^{125}I–IL-9, yielding a band of appropriate size for IL-9 cross-linked to γ_c (Fig. 1E). Each of these cytokines induced tyrosine phosphorylation (Fig. 1, A and B) and in vitro kinase activity (Fig. 1F) of Jak1 and Jak3.

Given the essential roles of IL-2Rβ and γ_c in IL-2 signaling, we investigated their abilities to associate with Jak1 and Jak3 (Fig. 2). We immunoprecipitated lysates of the NK-like YT cells with Mikβ1 (anti–IL-2Rβ) monoclonal antibody (mAb) or R878 (anti-γ_c) antiserum, immunoblotted with Jak3 and reblotted with Jak1. Jak1 constitutively associated with IL-2Rβ, and the association did not increase after IL-2 stimulation (Fig. 2). Jak1 did not constitutively associate with γ_c, but after IL-2 stimulation and consequent IL-2Rβ–γ_c dimerization (*16*), some Jak1 coprecipitated with γ_c. Jak3 weakly associated with γ_c in the absence of IL-2, and its association increased after IL-2 treatment (Fig. 2) (*17*). After IL-2 stimulation, Jak3 was coprecipitated with both anti–IL-2Rβ mAbs Mikβ1 (Fig. 2) and TU11 (*15*). This was expected for TU11, which coprecipitates γ_c in the presence of IL-2 (*4*, *18*), but was unexpected for Mikβ1, which competes for IL-2 binding (*19*) and cannot coprecipitate γ_c. These data suggest that IL-2Rβ primarily associates with Jak1, and γ_c with Jak3, but that IL-2Rβ–Jak3 interactions also occur. Although the basis for the IL-2–induced association of IL-2Rβ with Jak3 is unknown, IL-2–induced dimerization of IL-2Rβ and γ_c may juxtapose Jak3 to IL-2Rβ, thereby facilitating their interaction. It is possible that distinct regions of Jak3 interact with IL-2Rβ and γ_c.

To further evaluate the association of IL-2Rβ and γ_c with Jak1, we transiently transfected COS-7 cells with complementary DNAs (cDNAs) encoding Jak1 and either IL-2Rβ or γ_c (Fig. 3). Jak1 and IL-2Rβ were coprecipitated with antibodies to either protein (Fig. 3A), but γ_c and Jak1 did not associate (Fig. 3B). We confirmed the specificity of Jak1 for IL-2Rβ using IL-2R αaβ and αγγ chimeric receptor constructs containing the IL-2Rα extracellular domain and the IL-2Rβ or γ_c cytoplasmic domains (*6*). The anti–IL-2Rα mAb coprecipitated Jak1 only when αaβ was cotransfected (Fig. 3C).

We evaluated the association of Jak3 with γ_c mutants using antibodies to the extracellular (3B5) (*20*) (Fig. 4A) and intracellular (R878) (Fig. 4B) domains of γ_c. Jak3 efficiently interacted with γ_c, but not with two truncated forms of γ_c (γ_c-ΔCT and γ_c-ΔSH2, in which 80 and 48

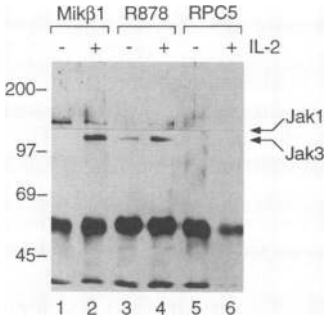

Fig. 2. Association of IL-2Rβ and γ_c with Jak1 and Jak3. YT cells were stimulated or not stimulated with IL-2 and then lysed with 10 mM tris (pH 7.5) containing 2 mM EDTA, 0.15 M NaCl, 0.875% Brij 96, 0.125% Nonidet P40, 0.4 mM sodium vanadate, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (ICN), 2.5 mM leupeptin, 2.5 mM aprotinin. Immunoprecipitations were done with Mikβ1 (anti–IL-2Rβ) mAb (lanes 1 and 2), R878 antiserum (*8*) to γ_c (lanes 3 and 4), or RPC5 (control mAb, lanes 5 and 6). The gel was immunoblotted sequentially with antisera to Jak3 (bottom) and then Jak1 (top) with the use of ECL. Molecular sizes are indicated on the left (in kilodaltons).

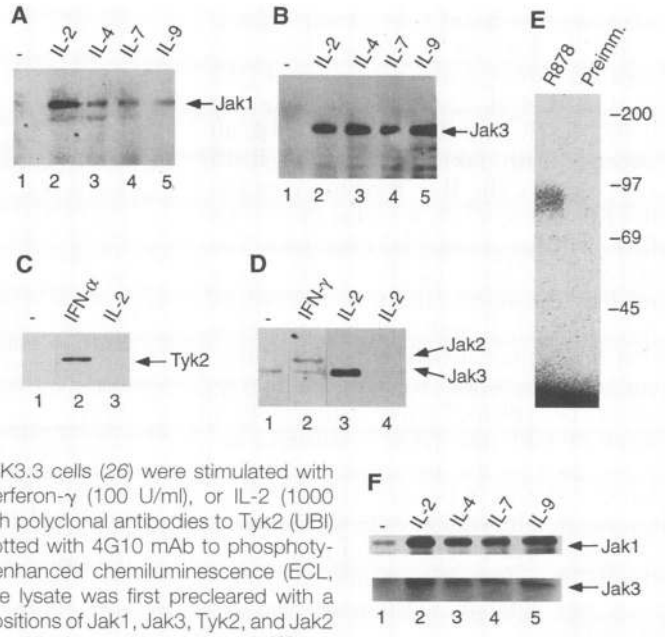

Fig. 1. Activation and phosphorylation of Jak1 and Jak3, but not Jak2 or Tyk2, by interleukins whose receptors contain γ_c. (**A** and **B**) Peripheral blood lymphocytes (PBLs) were induced for 15 min with IL-2 (1000 U/ml), IL-4 (100 U/ml), IL-7 (100 ng/ml), or IL-9 (100 ng/ml) after PHA stimulation (*25*). Cells were lysed and immunoprecipitated with polyclonal antibodies to Jak1 (UBI) (A) or Jak3 (*14*) (B), and phosphotyrosine-containing proteins were detected by immunoblotting with 4G10 (UBI). (**C** and **D**) NK3.3 cells (*26*) were stimulated with interferon-α (1000 U/ml), interferon-γ (100 U/ml), or IL-2 (1000 U/ml), immunoprecipitated with polyclonal antibodies to Tyk2 (UBI) or Jak2 (UBI), and immunoblotted with 4G10 mAb to phosphotyrosine (UBI) with the use of enhanced chemiluminescence (ECL, Amersham). In (D), lane 4, the lysate was first precleared with a polyclonal antibody to Jak3. Positions of Jak1, Jak3, Tyk2, and Jak2 are indicated. (**E**) Affinity labeling (*8*) of γ_c on MO7E cells by ^{125}I–IL-9. Lysates of cells were immunoprecipitated with either preimmune serum or R878 antiserum to γ_c. Molecular sizes are indicated on the right (in kilodaltons). (**F**) Activation by IL-2, IL-4, IL-7, and IL-9 of Jak1 (upper panel) and Jak3 (lower panel). PHA-activated T cells were treated as in (A) and (B) and then immunoprecipitated with antibodies to Jak1 and Jak3. In vitro kinase assays were done with ^{32}P-labeled γ-ATP (adenosine triphosphate) by standard methods (*14*).

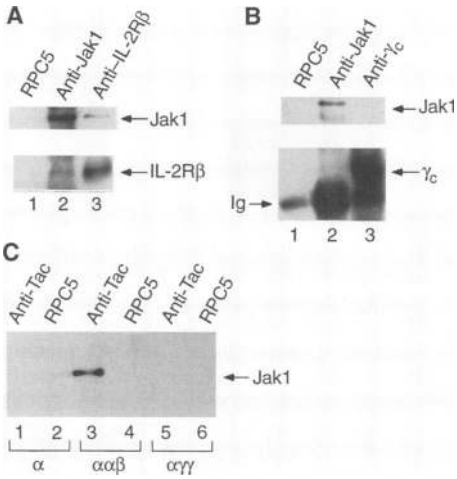

Fig. 3. Association of Jak1 with IL-2Rβ but not γ_c in cotransfected COS-7 cells. COS-7 cells were cotransfected with IL-2Rβ (**A**) or γ_c (**B**) and Jak1, lysed (*27*), immunoprecipitated with the indicated antibodies, and immunoblotted with antiserum to Jak1 (A and B, upper panels), ErdA antiserum to IL-2Rβ (A, lower panel), or R878 antiserum to γ_c (B, lower panel). (**C**) COS-7 cells were cotransfected with Jak1 and constructs encoding chimeras of the IL-2R α, β, and γ chains, αaβ or αγγ (*6*), lysed as described in Fig. 2, immunoprecipitated with RPC5 control mAb or anti-Tac mAb to IL-2Rα, and immunoblotted with antiserum to Jak1.

amino acids, respectively, are deleted from the COOH-terminus) (8) (Fig. 4A). The 48–amino acid truncation is smaller than the truncation (62 amino acids) in the XSCID patient with the smallest known naturally occurring γ_c truncation (7). Thus, defective γ_c-Jak3 association is predicted to be found in many XSCID patients. Affected individuals in a family with a more moderate X-linked combined immunodeficiency (XCID) (21) have a single nucleotide change that results in replacement of Leu^{271} with Gln within the γ_c cytoplasmic domain (22). This mutation substantially diminished, but did not abrogate, γ_c association with Jak3 (Fig. 4, A and B), consistent with the disease phenotype in this pedigree being less severe than typical XSCID. Nevertheless, affected males have diminished development of

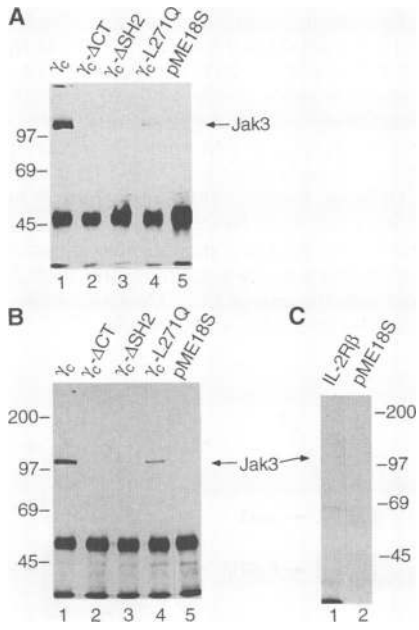

Fig. 4. Association of Jak3 with IL-2 receptor chains: partial delineation of the site or sites of interaction between Jak3 and γ_c. COS-7 cells were transfected with Jak3 plus wild-type γ_c, or γ_c-ΔCT (8), γ_c-ΔSH2 (8), or γ_c-L271Q (prepared with the pAlter-1 Mutagenesis Vector system, Promega), or with the vector control (pME18S), lysed as described in Fig. 2, immunoprecipitated with anti-γ_c antibodies 3B5 (**A**) or R878 (**B**), and immunoblotted with Jak3 antiserum. In the transfectants, levels of expression of wild type and mutant γ_c constructs were similar as determined by flow cytometry (15). Note that whereas 3B5 mAb binds to an extracellular epitope and thereby binds all the mutant forms of γ_c tested, R878 cannot bind γ_c-ΔCT or γ_c-ΔSH2 truncation mutants that lack its epitope. Thus, lanes 2 and 3 in (B) represent the background signal seen with R878. (**C**) IL-2Rβ or pME18S were cotransfected with Jak3, cells were lysed as described in Fig. 2, and immunoprecipitated with anti–IL-2Rβ. The immunoprecipitations in (B) and (C) were performed simultaneously but were run on separate gels.

$CD4^+$ and $CD8^+$ T cells, decreased T cell responses to mitogens and IL-2, and a skewed T cell repertoire (21). Leu^{271} is not contained within the region deleted in the γ_c-ΔSH2 mutant, suggesting that Jak3 may contact residues both proximal and distal to the γ_c-ΔSH2 deletion point. Because Jak3 was coprecipitated with Mikβ1 from YT cells that had been stimulated with IL-2, we cotransfected COS-7 cells with IL-2Rβ and Jak3 and found that Mikβ1 weakly but reproducibly coprecipitated Jak3, indicating that IL-2Rβ–Jak3 interactions can occur even in the absence of γ_c (Fig. 4C).

Our data show that Jak1 and Jak3 have differential associations with IL-2Rβ and γ_c. It has been reported that IL-2Rβ and γ_c associate with Jak1 and a 114-kD Jak2 kinase, respectively (23). No activation of Jak1 was observed in MOLT4-β leukemia cells (23), but Jak1 was activated in normal T cells (Fig. 1A). Because of its molecular size, the 114-kD Jak2 kinase was speculated to be a new member of the Janus family of tyrosine kinases, although no supportive data were presented (23). We now show that Jak3 but not Jak2 is activated and tyrosine-phosphorylated in response to IL-2. The reported activation of Jak2 was probably due to a cross-reactivity of the Jak2 antiserum with human Jak3 (Fig. 1D), because the same Jak2 antiserum can immunoblot both Jak2 and human Jak3 when transfected into COS-7 cells, whereas our antiserum to Jak3 will only react with Jak3 (15).

The activation of Jak3 by all cytokine receptors that have γ_c as a component is logical, because Jak3 physically associates with γ_c. However, the activation of Jak1 by IL-2, IL-4, IL-7, and IL-9 suggests that IL-4R, IL-7R, and IL-9R, like IL-2Rβ, all associate with Jak1. Because the IL-15 receptor, like the IL-2 receptor, contains IL-2Rβ and γ_c (11), it seems likely that IL-15 will also activate Jak1 and Jak3. The activation of Jak1 and Jak3 is presumably vital to the signals that are induced by IL-2, IL-4, IL-7, and IL-9, but it is clear that the distinct signals induced by different cytokines whose receptors contain γ_c cannot be explained solely by the involvement of Jak1 and Jak3. Distinctive actions, such as the induction by IL-4 of tyrosine phosphorylation of insulin receptor substrate (24), may help to determine cytokine-specific actions and may reflect the abilities of specific receptor complexes to recruit different substrates for Jaks or other kinases.

The differential association of Jak1 and Jak3 with IL-2Rβ and γ_c clarifies why heterodimerization of IL-2Rβ and γ_c is required to transduce an IL-2 signal. The phenotype in XSCID emphasizes the critical role of γ_c. Our results are consistent with the hypothesis that Jak3 activation is essential in the intrathymic maturation or selection of T cells and that XSCID can result from γ_c mutations that interfere with cytokine binding to γ_c or the ability of γ_c to associate with Jak3. If so, mutations in Jak3 may be found in some autosomal recessive cases of immunodeficiency that are phenotypically similar to XSCID or XCID, and reagents that disrupt Jak3-γ_c association may be immunosuppressive.

REFERENCES AND NOTES

1. W. J. Leonard, M. Noguchi, S. M. Russell, O. W. McBride, *Immunol. Rev.* **138**, 61 (1994); W. J. Leonard, *Curr. Opin. Immunol.* **6**, 631 (1994); T. Taniguchi and Y. Minami, *Cell* **73**, 5 (1993).
2. W. J. Leonard *et al.*, *Nature* **311**, 626 (1984); T. Nikaido *et al.*, *ibid.*, p. 631; D. Cosman *et al.*, *ibid.* **312**, 768 (1984).
3. M. Sharon, R. D. Klausner, B. R. Cullen, R. Chizzonite, W. J. Leonard, *Science* **234**, 859 (1986); M. Tsudo, R. W. Kozak, C. K. Goldman, T. A. Waldmann, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9694 (1986); K. Teshigawara, H. M. Wang, K. Kata, K. A. Smith, *J. Exp. Med.* **165**, 223 (1987); M. Hatakeyama *et al.*, *Science* **244**, 551 (1989).
4. T. Takeshita *et al.*, *Science* **257**, 379 (1992).
5. J. F. Bazan, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6934 (1990).
6. Y. Nakamura *et al.*, *Nature* **369**, 330 (1994); B. H. Nelson, J. D. Lord, P. D. Greenberg, *ibid.*, p. 333.
7. M. Noguchi *et al.*, *Cell* **73**, 147 (1993).
8. S. M. Russell *et al.*, *Science* **262**, 1880 (1993).
9. M. Kondo *et al.*, *ibid.*, p. 1874.
10. M. Noguchi *et al.*, *ibid.*, p. 1877; M. Kondo *et al.*, *ibid.* **263**, 1453 (1994).
11. J. G. Giri *et al.*, *EMBO J.* **13**, 2822 (1994).
12. W. L. Farrar and D. K. Ferris, *J. Biol. Chem.* **264**, 12562 (1989); M. E. Saltzman, K. White, J. Casnellie, *ibid.* **265**, 10138 (1990); G. B. Mills *et al.*, *ibid.*, p. 3561; H. Asao, S. Kumaki, T. Takeshita, M. Nakamura, K. Sugamura, *FEBS Lett* **304**, 141 (1992).
13. M. Kawamura *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6374 (1994); B. Witthuhn *et al.*, *Nature* **370**, 153 (1994).
14. J. A. Johnston *et al.*, *Nature* **370**, 151 (1994).
15. Unpublished observations.
16. R878 coprecipitates IL-2Rβ in the presence but not absence of IL-2 (Y. Nakamura and W. J. Leonard, unpublished observations).
17. Similar results were found with 3B5 mAb to γ_c.
18. T. Takeshita, H. Asao, J. Suzuki, K. Sugamura, *Int. Immunol.* **2**, 477 (1990).
19. M. Tsudo, F. Kitamura, M. Miyasaka, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1982 (1989).
20. 3B5 is an immunoglobulin G1 mAb to human γ_c, with properties similar to that of 1A11 mAb described in T. Nakarai *et al.* [*J. Exp. Med.* **180**, 241 (1994)].
21. E. G. Brooks *et al.*, *J. Clin. Invest.* **86**, 1623 (1990); F. C. Schmalstieg *et al.*, *Clin. Immunol. Immunopathol.* **64**, 71 (1992); A. S. Goldman, K. H. Palkowetz, H. E. Rudloff, E. G. Brooks, F. C. Schmalstieg, *Eur. J. Immunol.* **22**, 1103 (1992).
22. F. C. Schmalstieg *et al.*, in preparation.
23. N. Tanaka *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7271 (1994).
24. L.-M. Wang *et al.*, *ibid.* **90**, 4032 (1993); L.-M. Wang *et al.*, *Science* **261**, 1591 (1993); A. D. Keegan *et al.*, *Cell* **76**, 811 (1994); M. G. Myers Jr., X. J. Sun, M. F. White, *Trends Biochem. Sci.* **19**, 289 (1994).
25. Peripheral blood lymphocytes were activated for 72 hours with phytohemagglutinin (PHA), washed twice at pH 6.5, incubated for 3 hours in medium containing 0.5% human serum, and resuspended

in medium containing 10% fetal calf serum for 1 hour.
26. NK3.3 cells were used in Fig. 1, C and D, but IL-2 also failed to induce tyrosine phosphorylation of Tyk2 or Jak2 in PHA-activated T cells (S. M. Russell *et al.*, unpublished observations).
27. B. A. Wittuhn *et al.*, *Cell* **74**, 227 (1993).
28. We thank M. Tsang and R&D Systems for recombinant IL-9 and MO7E cells; J. Yodoi for YT cells; J. Kornbluth for NK3.3 cells; Cetus and G. Ju and J. Hakimi for recombinant IL-2; C. Reynolds for IL-2, IL-4, IL-7, and IL-9; M. Tsudo and J. Hakimi for mAb Mikβ1; K. Sugamura for mAb TU11 to IL-2Rβ and mAb TUGm2 to murine γ_c; J. Ritz and T. Nakarai for the mAb 3B5 to human γ_c; and T. A. Waldmann for anti-Tac mAb to IL-2Rα. Supported in part by National Cancer Institute Center Support (CORE) grant P30 CA21765 (J.N.I.), grant RO1 DK42932 from the National Institute of Diabetes and Digestive and Kidney Diseases (J.N.I.), the American Lebanese-Syrian Associated Charities (J.N.I.), a Postgraduate Research Grant, University of Sheffield (C.M.B.), and Fulbright Cancer Studentship (C.M.B.). We thank J.-L. Boulay and W. E. Paul for suggesting that the IL-9 receptor might contain γ_c (*8*). We thank W. E. Paul, M. Tsang, and J. Ritz for valuable discussions.

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Functional Activation of Jak1 and Jak3 by Selective Association with IL-2 Receptor Subunits

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The interleukin-2 receptor (IL-2R) consists of three subunits: the IL-2Rα, IL-2Rβ, and IL-2Rγ chains, the last of which is also used in the receptors for IL-4, IL-7, and IL-9. Stimulation with IL-2 induces the tyrosine phosphorylation and activation of the Janus kinases Jak1 and Jak3. Jak1 and Jak3 were found to be selectively associated with the "serine-rich" region of IL-2Rβ and the carboxyl-terminal region of IL-2Rγ, respectively. Both regions were necessary for IL-2 signaling. Furthermore, Jak3-negative fibroblasts expressing reconstituted IL-2R became responsive to IL-2 after the additional expression of Jak3 complementary DNA. Thus, activation of Jak1 and Jak3 may be a key event in IL-2 signaling.

IL-2 plays a pivotal role in immune responses by inducing the proliferation and differentiation of lymphocytes (*1*). The IL-2Rβ and IL-2Rγ subunits, but not the IL-2Rα subunit, are members of the cytokine receptor superfamily (*2*). A membrane-proximal cytoplasmic region of IL-2Rβ, termed the "serine-rich" region (S region) (*3*), and the cytoplasmic domain of IL-2Rγ are critical for IL-2 signaling (*4*, *5*). Mutations of IL-2Rγ are also associated with X-linked severe combined immunodeficiency (XSCID) (*6*). Although lacking intrinsic kinase activity, IL-2R couples ligand binding to induction of tyrosine phosphorylation of cellular substrates, including IL-2Rβ and IL-2Rγ (*7*). IL-2R is also linked to the serine-threonine kinase Raf-1 and phosphatidylinositol-3 kinase and to the hydrolysis of glycosyl-phosphatidylinositol (*1*). IL-2Rβ is physically associated with Src-family protein tyrosine kinases (PTKs) and Syk PTK (*8*, *9*). Both IL-2 and IL-4 induce the tyrosine phosphorylation and activation of the Janus kinases Jak1 and Jak3 (*10*, *11*), which suggests that these kinases might also associate with IL-2R and participate in IL-2 signaling.

To determine if Jaks associate with the IL-2R subunits, we used CD4 chimeric receptors that could be detected with the same monoclonal antibody to human CD4 (anti-CD4) (Fig. 1A). Extracts of COS cells cotransfected with each chimeric receptor complementary DNA (cDNA) and one of the Jak cDNAs were immunoprecipitated with anti-CD4 and subjected either to immunoblot analysis with antisera to the respective Jaks (Fig. 1B) or to in vitro kinase assay (*12*). Expression levels of the respective Jaks and chimeric receptors did not vary among cotransfected COS cells (*13*). Both assays showed that Jak1 could be coimmunoprecipitated with CD4β, but not with CD4γ or CD4γM1, whereas Jak3 could be coprecipitated with CD4γ, but not with CD4β or CD4γM1 (Fig. 1B) (*12*). In contrast, the association of Jak2 with any of the CD4–IL-2R chimeras was marginal (Fig. 1B) (*12*). Thus, the cytoplasmic domains of IL-2Rβ and IL-2Rγ possess regions capable of selectively associating with Jak1 and Jak3, and additional lymphoid-specific proteins are not required. Similar results were obtained in the IL-3–dependent cell line BAF-B03 that stably expresses each of the chimeric receptor cDNAs (*14*).

We examined the association sites within the respective cytoplasmic domains of the IL-2R subunits with the respective Jaks, using cDNAs encoding CD4 chimeras that contained deletions in their cytoplasmic β or γ domains (CD4βS, CD4βA, and CD4γM2; Fig. 1A). CD4γM1 and CD4γM2 bear the cytoplasmic domains of IL-2Rγ with COOH-terminal truncations of 79 and 48 amino acids, respectively. Jak1 can be detected, both by immunoblotting (Fig. 1C) and in vitro kinase assays (*12*), in anti-CD4 immunoprecipitates from COS cells cotransfected with the Jak1 cDNA and the cDNA for CD4β or CD4βA, but not CD4βS. A similar analysis was done for Jak3, which could associate with CD4γ but not with CD4γM1 or CD4γM2 (Fig. 1C) (*12*). Protein expression levels of the two Jaks, as well as of the different chimeric receptors, did not vary among the cotransfected COS cells (*13*). Thus, the cytoplasmic S region of IL-2Rβ is required for the association of IL-2Rβ with Jak1, whereas the COOH-terminal 48 amino acids of IL-2Rγ are necessary for the association of IL-2Rγ with Jak3. These two regions are known to participate in IL-2–induced cell proliferation (*3*, *5*, *15*). It is also worth pointing out that the COOH-terminal region of IL-2Rγ is deleted by nonsense mutations in many XSCID patients (*6*). The utilization of IL-2Rγ by a number of receptors (*16*) explains the activation of Jak3 by IL-4, IL-7, and IL-9 and predicts the activation of Jak3 in response to IL-13 or IL-15 (*17*). Jak1 associated with a region of IL-2Rβ that has structural similarity with the corresponding regions of other cytokine receptors (*3*, *18*). A comparable region in the erythropoietin receptor binds Jak2 (*19*); hence, the membrane proximal region can discriminate among Jaks.

We investigated the importance of these associations in the activation of Jaks by IL-2. We examined whether Jaks were activated after IL-2 stimulation in a BAF-B03–derived cell line, FWT-2, which expresses both human IL-2Rβ and human IL-2Rγ (*5*). Stimulation of FWT-2 cells by IL-2 induced tyrosine phosphorylation of endogenous Jak1 and Jak3, but not of Jak2 (*15*). The respective anti-Jak immunoprecipitates were tested for kinase activity in an in vitro kinase assay (Fig. 2A). Jak1

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