were fused with a mouse myeloma cell line SP2/0-Ag14 [T. Takeshita *et al.*, *J. Exp. Med.* **169**, 1323 (1989)]. We independently screened mAbs by two ways: inhibition on the intermediate-affinity binding of IL-2 to BWhβ-11 cells (*9*) and direct binding to cross-linked complexes of the IL-2 receptor with ^{125}I–IL-2 extracted from BWhβ-11 cells.

9. We established the BWhβ-11 cell line by cloning a cell highly expressing the human IL-2 receptor β chain in mouse T cell lymphoma BW5147 cells (obtained from Japanese Cancer Research Resources Bank) that were cotransfected with pSRB5, human β chain expression vector (*5*), and pSV2neo [P. J. Southern and P. Berg, *J. Mol. Appl. Genet.* **1**, 327 (1982)] by electroporation. The resulting cell line expressed the intermediate-affinity (2.4 nM) IL-2 receptor comprising the endogenous mouse γ chain and exogenous human β chain.
10. The mAb TUGm2 did not bind to a mouse fibroblast cell line NIH 3T3, a human T cell line MOLT4, or MOLT4 sublines exogenously expressing the human α and β chains.
11. ^{125}I–IL-2–labeled BWhβ-11 cells were cross-linked and lysed. The radiolabeled IL-2 receptor complex via IL-2 was used as a probe for mAbs to the γ chain. A positive one, TUGm3, gave 1041 cpm in the screening assay, whereas TUGm2 gave less than 30 cpm, indicating that the epitope for TUGm3 is on a region probably independent of IL-2 binding on the γ chain.
12. Y. Tanaka *et al.*, *Int. J. Cancer* **46**, 675 (1990).
13. T. Tanaka *et al.*, *J. Immunol.* **147**, 2222 (1991).
14. M. P. Beckmann *et al.*, *ibid.* **144**, 4212 (1990).
15. M. Kondo *et al.*, unpublished results.
16. B. Mosley *et al.*, *Cell* **59**, 335 (1989); N. Harada *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 857 (1990).
17. R. Fernandez-Botran, V. M. Sanders, E. S. Vitetta, *J. Exp. Med.* **169**, 379 (1989); O. M. Martinez, R. S. Gibbons, M. R. Garovoy, F. R. Aronson, *J. Immunol.* **144**, 2211 (1990); H. Lee, X. Xia, Y. S. Choi, *ibid.*, p. 3431.
18. K. A. Smith, *Science* **240**, 1169 (1988); T. Yokota *et al.*, *Immunol. Rev.* **102**, 137 (1988).
19. J. P. Disanto *et al.*, *J. Exp. Med.* **171**, 1697 (1990).
20. R. Kühn, K. Rajewsky, W. Müller, *Science* **254**, 707 (1991); M. Kopf *et al.*, *Nature* **362**, 245 (1993).
21. S. M. Zurawski, F. Vega Jr, B. Huyghe, G. Zurawski, *EMBO J.* **12**, 2663 (1993).
22. We thank H. Karasuyama for BCMGSNeo plasmid; T. Yoshiki for TART-1; M. Miyasaka for TM-β1; Y. Tanaka for REY-7; J. Hamuro for human recombinant IL-2; and B. Coffman for mouse recombinant IL-4. Supported by the Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture; grants from Special Coordination Funds of the Science and Technology Agency; a Grant-in-Aid from the Ministry of Health and Welfare of the Japanese Government for the Comprehensive Ten-Year Strategy for Cancer Control; and grants from Tokyo Biochemical Research Foundation and the Princess Takamatsu Cancer Research Fund.

13 September 1993; accepted 16 November 1993

Interleukin-2 Receptor γ Chain: A Functional Component of the Interleukin-7 Receptor

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The interleukin-2 receptor γ chain (IL-2Rγ) is a necessary component of functional IL-2 receptors. IL-2Rγ mutations result in X-linked severe combined immunodeficiency (XSCID) in humans, a disease characterized by the presence of few or no T cells. In contrast, SCID patients with IL-2 deficiency and IL-2–deficient mice have normal numbers of T cells, suggesting that IL-2Rγ is part of more than one cytokine receptor. By using chemical cross-linking, IL-2Rγ was shown to be physically associated with the IL-7 receptor. The presence of IL-2Rγ augmented both IL-7 binding affinity and the efficiency of internalization of IL-7. These findings may help explain the defects of XSCID. Given its role in more than one cytokine receptor system, the common γ chain (γ_c) is proposed as the designation for IL-2Rγ.

The interaction of IL-2 and IL-2 receptors regulates the magnitude and duration of the T cell immune response (*1*). Different combinations of three distinct chains (α, β, and γ) form three classes of IL-2 receptors (*1*). Low-affinity IL-2 receptors consist of IL-2Rα, intermediate-affinity receptors contain IL-2Rβ and IL-2Rγ, and high-affinity receptors contain all three chains. Thus, IL-2Rγ is a component of the functional intermediate- and high-affinity receptors (*2*). Mutations in IL-2Rγ result in XSCID in humans (*3*). In contrast to the low T cell numbers in this disease (*4*), IL-2–deficient SCID patients (*5*) and mice (*6*) have normal numbers of T cells. We therefore hypothesized that IL-2Rγ might also be a component of other cytokine receptors (*3*).

The IL-7 receptor was an attractive candidate. First, IL-7 is a growth factor for thymocytes (*7*) and T cells (*8*), activities which if defective might explain much of the XSCID phenotype. Second, since IL-7 is a growth factor for pre-B cells (*9*), a role for IL-2Rγ in the IL-7 receptor could help explain why B cells, although present in XSCID patients, are nonfunctional (*4*) and why the mature B cells of XSCID carrier females have nonrandom X chromosome inactivation (*4*) (that is, the X chromosome with the mutant IL-2Rγ chain is inactivated, indicating that IL-2Rγ participates in B cell maturation). Third, only a single chain of the IL-7 receptor has been identified (*10*), whereas most other cytokine receptor superfamily [also denoted hematopoietin or type I cytokine receptors (*11*)] members have two chains (one with a long cytoplasmic domain and the other with a much shorter one).

We labeled IL-7 with ^{125}I and cross-linked it to transfected COS-7 cells. No affinity-labeled band was seen when cells were transfected with pME18S (the vector control) or IL-2Rγ (Fig. 1A). Faint bands were seen in cells transfected with IL-7R alone, but stronger bands were seen in cells transfected with IL-7R and IL-2Rγ. Although the major affinity-labeled band seen when both subunits were transfected approximately comigrated with the major band seen when cells with only IL-7R were cross-linked, the labeling pattern in the former case was more complex. Nevertheless, it was impossible to determine whether IL-2Rγ, IL-7R, or both were affinity labeled (lane 4). A polyclonal antibody to IL-2Rγ (anti–IL-2Rγ) did not immunoprecipitate the affinity-labeled complexes from cells transfected only with IL-7R, but efficiently precipitated complexes from cells transfected with IL-7R + IL-2Rγ (Fig. 1B). These complexes may represent affinity-labeled IL-7R coimmunoprecipitated through association with IL-2Rγ, affinity-labeled IL-2Rγ itself, or both. Regardless of which possibility is correct, the ability of anti–IL-2Rγ to immunoprecipitate IL-7 affinity-labeled bands proved that IL-2Rγ was part of the complex. As expected, an antibody to IL-7R (anti–IL-7R) immunoprecipitated the bands from affinity-labeled IL-7R transfectants (Fig. 1C); however, the antibody was inefficient in immunoprecipitating bands from the double transfectant, suggesting either that much of the signal in Fig. 1A corresponded to affinity-labeled IL-2Rγ or that IL-2Rγ interfered with anti–IL-7R binding. No bands were immunoprecipitated by preimmune serum (Fig. 1D). Similar results were obtained in transfected L cells (*12*).

Because IL-2Rγ appeared to be part of both the IL-2 and IL-7 receptors, we used ^{125}I–IL-3 to find a cytokine that could not affinity label IL-2Rγ. Two major bands were detected when ^{125}I–IL-3 was cross-linked to COS-7 cells transfected with IL-3Rα and the common β chain [β_c, a chain that is also a component of the IL-5 and granulocyte-macrophage colony-stimulat-

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ing factor receptors] (Fig. 1E). The pattern was indistinguishable from that previously reported (*13*). No change in the affinity-labeled bands was detected when IL-2Rγ was cotransfected, and anti–IL-2Rγ did not immunoprecipitate any affinity-labeled band (Fig. 1E). Thus, IL-2Rγ is not a component of the IL-3 receptor.

We also analyzed ^{125}I–IL-7 cross-linked to phytohemagglutinin (PHA)-activated human peripheral blood lymphocytes (PBLs) and found a major affinity-labeled band which, like the analogous band from COS-7 cells transfected with IL-7R + IL-2Rγ, could be immunoprecipitated weakly by anti–IL-7R and strongly by anti–IL-2Rγ, but not by preimmune serum (Fig. 1F). Thus, IL-2Rγ is physically associated with IL-7R in normal PBLs as well as in transfected COS-7 cells.

To determine whether IL-7 was cross-linked to both IL-7R and IL-2Rγ, we analyzed two-dimensional gels of IL-7 affinity-labeled COS-7 cells transfected with pME18S, IL-7R, IL-2Rγ, IL-7R + IL-2Rγ, or IL-7R + IL-2Rγ-ΔCT (which retains only six residues of the IL-2Rγ cytoplasmic domain). No spots were seen with pME18S (Fig. 2A) or IL-2Rγ–transfected cells (*12*). Unexpectedly, two major affinity-labeled spots overlapping in molecular size (spots 1 and 2) were seen in IL-7R–transfected cells (Fig. 2B). Spots 1 and 2 could represent alternatively processed forms of IL-7R; it is also possible that one spot corresponds to a component of an endogenous IL-7 receptor present in COS-7 cells that was affinity labeled only in the presence of transfected IL-7R. As compared with spot 2, spot 1 has a molecular size closer to that expected for IL-7R; moreover, transfection of a truncated IL-7R construct resulted in a decrease in the size of spot 1 and a shift to a more basic pI, as expected on the basis of its amino acid composition (*12*). Although the molecular size of spot 2 appeared to decrease, there was no change in its pI. Thus, although spot 1 is derived from IL-7R, the identity of spot 2 remains unclear. Two minor spots (spots 3 and 4) were variably detected and migrated with isoelectric points and molecular sizes consistent with that for dimers of spots 1 and 2, respectively.

When IL-7R and IL-2Rγ were cotransfected, no new spots were observed, but the more acidic spot (spot 1) increased in intensity (Fig. '2C, arrow). The increased intensity presumably results from cross-linking of ^{125}I–IL-7 to IL-2Rγ (superimposed on the ^{125}I–IL-7 affinity-labeled IL-7R) because transfection of IL-2Rγ-ΔCT instead of full-length IL-2Rγ yielded a new spot, partially overlapping with spot 1, but of smaller molecular size (Fig. 2D, arrow). Thus, IL-7 could be cross-linked to both IL-7R (Fig. 2B) and IL-2Rγ (Fig. 2D).

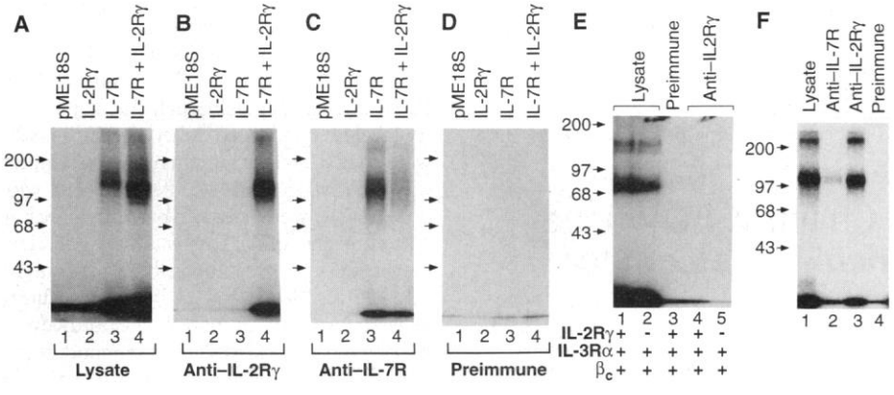

Fig. 1. Affinity labeling of IL-7R and IL-2Rγ with ^{125}I–IL-7. COS-7 cells were transfected with lipofectamine (BRL), with pME18S (lanes 1) or pME18S containing cDNAs encoding IL-2Rγ (lanes 2), IL-7R (lanes 3), or IL-7R + IL-2Rγ (lanes 4). Cells were washed three times in phosphate-buffered saline and affinity labeled with 5 nM ^{125}I–IL-7 and disuccinimidyl suberate (Pierce) as described (*24*). Cell lysates were either directly analyzed on gels (**A**) or immunoprecipitated first with anti–IL-2Rγ (**B**), anti–IL-7R (**C**), or preimmune serum (**D**). (**E**) ^{125}I–IL-3 was bound and cross-linked to COS-7 cells transfected with IL-3Rα + β_c (lanes 2 and 5) or IL-3Rα + β_c + IL-2Rγ (lanes 1, 3, and 4). Lysates were directly analyzed (lanes 1 and 2) or first immunoprecipitated with preimmune serum (lane 3) or anti–IL-2Rγ (lanes 4 and 5). (**F**) Peripheral blood lymphocytes activated with PHA were affinity-labeled with IL-7. The composition of the larger (>200 kD) band is unclear, but it was only variably detected and its immunoprecipitation by anti–IL-2Rγ suggests that it is a multimerized cross-linked form. Recombinant human IL-3 and IL-7 were iodinated with the Bolton-Hunter reagent (ICN) and Iodogen (Pierce), respectively. Polyclonal rabbit antibody to IL-2Rγ (R878) was generated to a peptide corresponding to the eight COOH-terminal amino acids of IL-2Rγ (NH_2-Cys-Tyr-Thr-Leu-Lys-Pro-Glu-Thr-COOH), which had been coupled to keyhole limpet hemocyanin. Molecular size markers are indicated at the left (in kilodaltons).

Table 1. Summary of binding data showing dissociation constants (K_d's) and receptor numbers for PBLs and for COS-7 cells transfected with pME18S or pME18S containing cDNAs encoding IL-2Rγ, IL-7R, or IL-7R + IL-2Rγ. The data for pME18S are from six experiments; all others are derived from four experiments. K_d's are shown as mean ±SD.

Transfectant	Low-affinity K_d (nM)	Receptors per cell	Intermediate-affinity K_d (pM)	Receptors per cell	High-affinity K_d (pM)	Receptors per cell
pME18S	148 ± 43	1.4×10^6				
IL-2Rγ	126 ± 11	1.2×10^6				
IL-7R	145 ± 31	2.6×10^6	253 ± 81	9.62×10^3		
IL-7R + IL-2Rγ	105 ± 41	2.3×10^6			36.9 ± 29.7	5.38×10^3
PBL	100 ± 46	3.7×10^4			64.0 ± 13.5	1.28×10^3

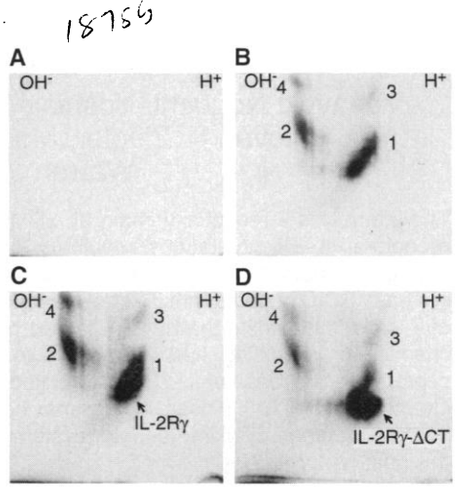

Fig. 2. Affinity labeling of IL-7R ± IL-2Rγ and analysis on two-dimensional IEF gels. Cells were transfected with pME18S (**A**) or pME18S containing cDNAs encoding IL-7R (**B**), IL-7R + IL-2Rγ (**C**), or IL-7R + IL-2Rγ-ΔCT (**D**), followed by affinity labeling with ^{125}I–IL-7. Cells were lysed in 50 mM tris (pH 7.4), 140 mM NaCl, 0.5% NP-40, nuclei were removed by centrifugation, and lysates were adjusted to 1% SDS and then boiled, followed by the addition of one-half volume of 100% NP-40. Lysates were then analyzed on 2D IEF gels. The positions of spots 1, 2, 3, and 4, IL-2Rγ, and IL-2Rγ-ΔCT are indicated. IL-2Rγ-ΔCT was prepared with the Bio-Rad Mutagene kit and the following oligonucleotide: 5′-CTGGCTGGAACGGACGATGCCCCGATGATCTAGACTGAAGAAACCTAGAG-3′ (stop codon and Xba I site are underlined). IL-2Rγ-ΔCT lacks all but six amino acids of the cytoplasmic domain.

Cross-linking to IL-2Rγ appeared as a more intense spot, suggesting that the efficiency of cross-linking ^{125}I–IL-7 to IL-2Rγ was greater than the efficiency of cross-linking ^{125}I–IL-7 to IL-7R (consistent with the results from Fig. 1).

Since IL-7 affinity labeling was more intense in COS-7 cells transfected with IL-7R + IL-2Rγ than with IL-7R (compare Fig. 1A, lane 4, with lane 3; see Fig. 2), we performed Scatchard analyses to evaluate whether IL-2Rγ augmented IL-7 binding affinity. Cells transfected with pME18S or IL-2Rγ expressed more than 1 million receptors with low affinity [dissociation constant (K_d) ≈ 125 to 150 nM] (Fig. 3, A and B, and Table 1). In contrast, IL-7R–transfected COS-7 cells (Fig. 3, C and D) expressed two classes of receptors: the low-affinity binding class seen on nontransfected cells and a higher affinity class (K_d ≈ 250 pM) (*14*). Cells transfected with IL-7R + IL-2Rγ had an even higher affinity (K_d ≈ 35 to 40 pM) receptor in addition to the low-affinity form (Fig. 3, E and F). PBLs from healthy donors expressed high- (K_d ≈ 65 pM) and low- (K_d ≈ 100 nM) affinity receptors (Fig. 3, G and H), similar to those on COS-7 cells transfected with IL-7R + IL-2Rγ. Thus, only transfection with both IL-7R + IL-2Rγ reconstituted the full binding affinity found on PBLs. These data and data from other experiments show that three classes of IL-7 receptors exist: low-, intermediate- (IL-7R), and high- (IL-7R + IL-2Rγ) affinity classes of IL-7 receptors (Table 1). Although spot 2 of Fig. 2B might correspond to the low-affinity endogenous COS-7 receptor, we have so far been unable to affinity label this spot from untransfected COS-7 cells, even using 72 nM ^{125}I–IL-7 (*15*).

Having established that IL-2Rγ increased in binding affinity, we investigated whether the association of IL-2Rγ with IL-7R was functionally significant by comparing the internalization rates of IL-7 in mouse fibroblasts (L cells) transfected with IL-7R or IL-7R + IL-2Rγ. Internalization efficiency was the assay first used to determine the functional significance of IL-2Rγ (*2*). ^{125}I–IL-7 at concentrations of 0.39 nM (Fig. 4A) or 2.5 nM (sufficient to saturate both high- and intermediate-affinity IL-7 receptors) (Fig. 4B) was efficiently internalized only when IL-2Rγ was present. An experiment done in COS-7 cells with 1 nM ^{125}I–IL-7 showed similar results (*12*). Little or no IL-7 was internalized in the absence of IL-2Rγ. Thus, the association of IL-2Rγ has functional significance for both the IL-2 and IL-7 receptors.

The sharing of IL-2Rγ may be fundamentally different from the sharing of a common β subunit among the IL-3, IL-5, and GM-CSF receptors (*13*) or of gp130 among the IL-6, leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor receptors (*16*). In those cases, the shared chain has a large cytoplasmic domain and is viewed as the major signaling component. In the case of the IL-2 and IL-7 receptors, IL-2Rβ and IL-7R are considered the major signaling molecules. Nevertheless, IL-2Rγ is critical for signal transduction (*3*, *17*). Because of its essential role in more than one cytokine system, we propose that IL-2Rγ be designated as the common γ chain (γ_c), analogous to the designation of the common β chain of the IL-3, IL-5, and GM-CSF receptors (*13*) as β_c.

Although the exact mechanisms by which a γ_c defect results in XSCID remain

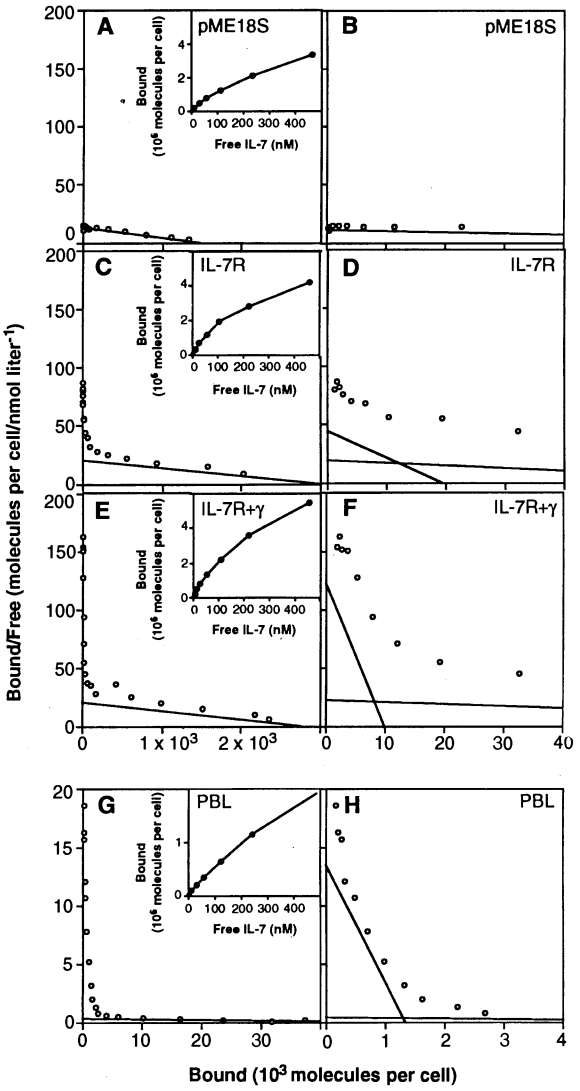

Fig. 3. IL-2Rγ + IL-7R form the high-affinity IL-7R. ^{125}I–IL-7 binding to COS-7 cells transfected with pME18S (**A** and **B**), IL-7R (**C** and **D**), or IL-7R + IL-2Rγ (**E** and **F**), or binding to PBLs (**G** and **H**). The insets in panels (A), (C), (E), and (G) show binding saturation curves; (A), (C), (E), and (G) show Scatchard transformations of binding data; and (B), (D), (F), and (H) show the early (high affinity) part of the data from (A), (C), (E), and (G) on an expanded scale to highlight the high-affinity binding of IL-7R + IL-2Rγ (*25*). Single representative experiments are shown; binding data from multiple experiments are summarized in Table 1.

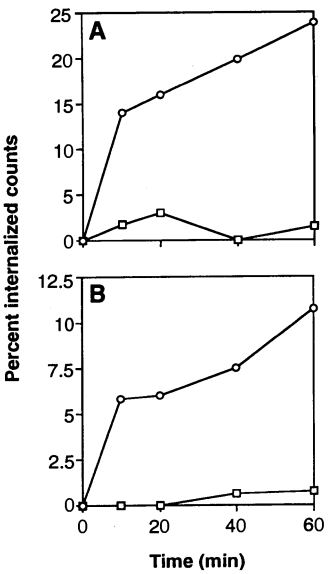

Fig. 4. Efficient internalization of IL-7 only when both IL-7R and IL-2Rγ are coexpressed. Transfected L cells (10^7 cells per milliliter) were incubated for 5 min at 37°C in RPMI 1640 medium containing 2% BSA, 20 mM Hepes (pH 7.4), and 100 μM chloroquine and then for 3 hours with 0.39 nM (**A**) or 2.5 nM (**B**) ^{125}I–IL-7 on ice (*26*). Circles, IL-7R + IL-2Rγ; squares, IL-7R.

unknown, the identification of γ_c as a component of the IL-7R may explain some features of XSCID, perhaps even more completely than the knowledge that γ_c is part of the IL-2 receptor. Although IL-2 stimulates proliferation of murine thymocytes (*18*), IL-2–deficient mice have normal thymic development (*6*). In contrast, mice treated with a monoclonal antibody to IL-7 have a significant decrease in thymic cellularity (*19*). The exact sites of γ_c function in T cell development remain unclear, but γ_c is expressed on all thymic populations studied (*20*). In addition to participation in T cell development, IL-7 promotes the growth of pre-B cells (*19*). Thus, a loss of IL-7 signaling might contribute to both the T cell and B cell defects found in XSCID. Nevertheless, the involvement of γ_c in the IL-2 and IL-7 receptors may not fully explain the defects of XSCID. Indeed, it is now clear that γ_c is also a part of the IL-4 receptor and perhaps part of other cytokine receptors as well (*21*).

REFERENCES AND NOTES

1. W. J. Leonard, in *Interleukin-2*, J. Waxman and F. Balkwill, Eds. (Blackwell, Oxford, 1992), pp. 29–46; T. Taniguchi and Y. Minami, *Cell* **73**, 5 (1993).
2. T. Takeshita, H. Asao, J. Suzuki, K. Sugamura, *Int. Immunol.* **2**, 477 (1990); T. Takeshita *et al.*, *Science* **257**, 379 (1992).
3. M. Noguchi *et al.*, *Cell* **73**, 147 (1993).
4. E. W. Gelfand and H. M. Dosch, *Birth Defects Orig. Artic. Ser.* **19** (no. 3), 65 (1983); M. D. Cooper and J. L. Butler, in *Fundamental Immunology*, W. E. Paul, Ed. (Raven, New York, 1989), pp. 1034–1039; M. E. Conley, *Annu. Rev. Immunol.* **10**, 215 (1992).
5. K. Weinberg and R. Parkman, *N. Eng. J. Med.* **322**, 24, 1718 (1990).
6. H. Schorle, T. Holtschke, T. Hünig, A. Schimpl, I. Horak, *Nature* **352**, 621 (1991).
7. J. D. Watson, P. J. Morrissey, A. E. Namen, P. J. Conlon, M. B. Widmer, *J. Immunol.* **143**, 1215 (1989); R. Murray, T. Suda, N. Wrighton, F. Lee, A. Zlotnik, *Int. Immunol.* **1**, 526 (1989); F. M. Uckun *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6323 (1991).
8. G. D. Chazen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5923 (1989); M. Londei *et al.*, *Eur. J. Immunol.* **20**, 425 (1990); P. J. Morrissey *et al.*, *J. Exp. Med.* **169**, 707 (1989); B. M. J. Foxwell *et al.*, *Eur. J. Immunol.* **23**, 85 (1993).
9. A. E. Namen *et al.*, *J. Exp. Med.* **167**, 988 (1988); A. E. Namen *et al.*, *Nature* **333**, 571 (1988).
10. R. G. Goodwin *et al.*, *Cell* **60**, 940 (1990).
11. J. F. Bazan, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6934 (1990); D. Cosman *et al.*, *Trends Biochem. Sci.* **15**, 265 (1990); A. Miyajima, T. Kitamura, N. Harada, T. Yokota, K. Arai, *Annu. Rev. Immunol.* **10**, 295 (1992).
12. M. Noguchi *et al.*, unpublished data.
13. T. Kitamura, N. Sato, K. Arai, A. Miyajima, *Cell* **66**, 1165 (1991); J. Tavernier *et al.*, *ibid.*, p. 1174.
14. Goodwin *et al.* (*10*) obtained similar binding affinities from COS-7 cells transfected with the IL-7R cDNA. They did not investigate binding in sham transfected COS-7 cells and therefore did not identify the endogenous IL-7 binding activity on COS-7 cells.
15. These experiments have limitations because the background on the gels increased significantly at high ^{125}I–IL-7 concentrations.
16. D. P. Gearing *et al.*, *Science* **255**, 1434 (1992); T. Taga *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10998 (1992).
17. H. Asao *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4127 (1993).
18. E. J. Jenkinson, R. Kingston, J. J. T. Owen, *Nature* **329**, 160 (1987); D. H. Raulet, *ibid.* **314**, 101 (1985); L. Tentori, D. L. Longo, J. C. Zuniga-Pflucker, C. Wing, A. M. Kruisbeek, *J. Exp. Med.* **168**, 1741 (1988).
19. K. H. Grabstein *et al.*, *J. Exp. Med.* **178**, 257 (1993).
20. X. Cao *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8464 (1993).
21. S. M. Russell *et al.*, *Science* **262**, 1880 (1993).
22. P. J. Munson and D. Rodbard, *Anal. Biochem.* **107**, 220 (1980).
23. N. Harada, G. Yang, A. Miyajima, M. Howard, *J. Biol. Chem.* **267**, 22752 (1992).
24. M. Sharon, R. D. Klausner, B. R. Cullen, R. Chizzonite, W. J. Leonard, *Science* **234**, 859 (1986).
25. Cells were harvested and resuspended at 10^6 cells in 100 μl of RPMI 1640, 25 mM Hepes (pH 7.4), bovine serum albumin (BSA) (5 mg/ml). ^{125}I–IL-7 (30 pM) was added to cells in the presence of 0 to 500 nM unlabeled IL-7 and incubated on ice for 2 hours. Cells were then overlayed onto 150 μl of 20% olive oil, 80% dibutylphthalate and centrifuged at 4500*g* for 4 min. Pellets and supernatants were counted. Receptor numbers and K_d's were determined with the Ligand computer program (*22*). Although it is reasonable to hypothesize that cells expressing both IL-7R and IL-2Rγ might express high- (IL-7R associated with IL-2Rγ), intermediate- (IL-7R not associated with IL-2Rγ), and low- (endogenous IL-7–binding activity) affinity receptors, the Ligand computer program could only fit the data to two binding affinities rather than three, even for the data in (H) where one might envision the existence of three different affinities. We present only the data "objectively" derived from the computer program.
26. Cells were washed twice with ice-cold medium and resuspended at 4×10^7 cells per milliliter in prewarmed (37°C) medium. At the indicated times, two 50-μl samples of the cell suspension were removed. One sample was treated with acid (*23*), the cells were centrifuged through olive oil–dibutylphthalate as in Fig. 3, and the cell pellet and supernatant were counted to determine the acid-resistant (that is, internalized) and acid-sensitive (that is, cell surface–bound + dissociated) IL-7. The other sample was immediately centrifuged through the oil layer, and the radioactivity in the supernatant was measured to determine the amount of dissociated IL-7. The acid-resistant counts at 10, 20, 40, and 60 min are expressed as a fraction of the ligand specifically bound at time 0 (percent internalized) to normalize for variations in transfection efficiency and for the higher affinity binding expressed by cells transfected with IL-7R + IL-2Rγ as compared with IL-7R alone.

27. We thank A. Miyajima and T. Kitamura for the IL-3Rα and β_c cDNAs; P. J. Munson, M. R. Bubb, M. Yanagishita, and K. Sakaguchi for assistance with analysis of binding data; A. Yamauchi for preparation of PBLs; and L. E. Samelson for valuable discussions.

12 October 1993; accepted 17 November 1993

Interleukin-2 Receptor γ Chain: A Functional Component of the Interleukin-4 Receptor

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The interleukin-2 (IL-2) receptor γ chain (IL-2Rγ) is an essential component of high- and intermediate-affinity IL-2 receptors. IL-2Rγ was demonstrated to be a component of the IL-4 receptor on the basis of chemical cross-linking data, the ability of IL-2Rγ to augment IL-4 binding affinity, and the requirement for IL-2Rγ in IL-4–mediated phosphorylation of insulin receptor substrate–1. The observation that IL-2Rγ is a functional component of the IL-4 receptor, together with the finding that IL-2Rγ associates with the IL-7 receptor, begins to elucidate why deficiency of this common γ chain (γ_c) has a profound effect on lymphoid function and development, as seen in X-linked severe combined immunodeficiency.

Interleukin-2 (*1*) and IL-4 (*2*) are multifunctional cytokines. Both can act as lymphocyte growth factors, although their ranges of actions are quite different. IL-2 is the principal T cell growth factor (*1*); IL-4 also exhibits T cell growth factor activity, but its principal actions are to regulate B cell growth and immunoglobulin class switching (*2*, *3*). The structure of each cytokine consists of a bundle of four α helices in which the first and second helices and the third and fourth helices are connected by long overhand loops, resulting in a characteristic "up, up, down, down" configuration (*4*). The prototypic molecule to exhibit this structure is growth hormone. Growth hormone transduces signals by homodimerization of the growth hormone receptor (*5*), whereas for IL-2 the formation of a heterodimer of the IL-2Rβ and IL-2Rγ

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