These plasmids were each transformed into both JLY363 and its mutant counterpart JLY365 (*10*). Nine plasmids induced greater β-galactosidase activity in the wild-type reporter strain than the control. These plasmids were classified into five clones, *AAP1* through *AAP5*, on the basis of their Hind III restriction pattern. Each clone was retested in JLY360, JLY361, JLY387, JLY429, JLY431, JLY433, JLY435 (Fig. 4). The *AAP1* hybrid clone was called pJL720. The *AAP1* gene was later renamed *ORC6*.

12. The *ARS* function of the sequences in Fig. 4 was analyzed in the context of *ARS*1 domain B (Bgl II–Hinf I fragment, nt 853-734) in the following CEN-based URA3-containing plasmids: pJL347 (wt), pJL243 (multiple), pJL326 (A863T), pJL338 (T869A), pJL330 (T862C), and pJL316 (T867G). These plasmids were transformed into JLY106 (*MATα ura3 leu2 his3 trp1 lys2 ade2*) and its homozygous diploid counterpart JLY162. pJL243, pJL326, and pJL338 did not yield a high frequency of transformation and could not be assayed quantitatively for *ARS* function. pJL347, pJL330, and pJL316 transformed cells with high efficiency and were assayed for mitotic stability [D. T. Stinchcomb, K. Struhl, R. W. Davis, *Nature* **282**, 39 (1979)].
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14. The *ORC6* hybrid construct originally isolated from the YL3 library (pJL720) has two Bam HI sites. The 5′ site, which is created by the hybrid junction, corresponds to the Sau 3A site at nucleotide 843. Excision of the segment between the two sites generated pJL721, leaving amino acid residues 339 to 435 in frame with the $GAL4^{AD}$ (Fig. 5). pGAD3R (*11*), the parent vector for the YL3 library, contains no *ORC6* sequence. pRS425 [T. W. Christianson, R. S. Sikorski, M. Dante, J. H. Shero, P. Hieter, *Gene* **110**, 119 (1992)] contains no components of the fusion protein.
15. All sequencing was performed with Sequenase (USB) on collapsed double-stranded templates. The protein coding segments of the *AAP1* through *AAP5* hybrid clones were sequenced from their junction with the $GAL4^{AD}$ to their stop codon. Two of the *ORC6* sequencing primers were used as colony hybridization probes to screen a high copy number yeast genomic library [M. Carlson and D. Botstein, *Cell* **28**, 145 (1982)] for a clone of the full-length *ORC6* gene (pJL724). The full-length gene was sequenced on both strands with oligonucleotide primers positioned approximately 200 nucleotides apart. The accession number for the *ORC6* sequence reported in this paper is L23323.
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21. Marked *ORC6* deletions were constructed by replacing nucleotides 458-1721 (pJL731) or nucleotides 458-846 (pJL733) of the GenBank sequence with the URA3 Hind III fragment oriented in the opposite direction to that of the *ORC6* sequence. Each construct was used to generate heterozygous deletions of *ORC6* in diploid strains by one-step gene replacement. *ORC6* deletion analysis was performed in JLY461 (*MATa/MATα ura3/ura3 leu2/leu2 his3/his3 trp1/trp1 ade2/ade2 [cir°]*), JLY462 (*MATa/MATα ura3/ura3 leu2/leu2 trp1/trp1 his4/his4 can1/can1*), and JLY463 (*MATa/MATα ura3/ura3 leu2/leu2 trp1/trp1 his3/HIS3*); their respective genetic backgrounds are S288c, EG123, and A364a. Disruption of JLY461, JLY462, and JLY463 by pJL731 (full deletion) created JLY481, JLY475, and JLY469, respectively. Disruption of JLY461, JLY462, and JLY463 by pJL733 (NH_2-terminal deletion) created JLY485, JLY479, JLY473, respectively. These heterozygous marked deletion strains were sporulated, and 20 tetrads of each were dissected and grown on YEPD to assess viability.
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23. A point mutant (pJL766) was made by replacing the Bam HI-Sph I fragment of the full-length clone with a Bam HI-Sph I fragment generated by PCR from pJL720 with the primers

    5′-CAAGGATCCAAGAATTGA**T**CATTTTATAGT-CAG-3′

    5′-GTTATAGGGCTAAAGGCATGC-3′.

    The mutation, shown in bold, changes nucleotide 1471 of the GenBank sequence from C to T and was confirmed by sequence analysis.
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28. pJL749 contains the *GAL1* promoter (nucleotides 146 to 816) driving the expression of *ORC6* (nucleotides 443 to 2298) in the high-copy yeast shuttle vector RS425 [T. W. Christianson, R. S. Sikorski, M. Dante, J. H. Shero, P. Hieter, *Gene* **110**, 119 (1992)]. The sequence 5′-CCCG-GATCCC ATG GCC TAC CCA TAT GAT GTT CCA GAT TAC GCT TCT TTG GGT CCA GGG CTG CAG GAA TTC GGG CCC ATC-3′ lies between the *GAL1* promoter and *ORC6* and contains the influenza hemagglutinin (HA) epitope fused to the NH_2-terminus of ORC6. This construct complements a deletion of the *ORC6* gene. pJL772 is identical to pJL749 except that it lacks the *ORC6* sequence.
29. The *cdc* mutant strains listed in Table 1 have been backcrossed four to five times against two congenic strains derived from *A364a*, *RDY487* (*MATα leu2 ura3 trp1*) and RDY488 (*MATa leu2 ura3 trp1*). All are *ura3 leu2 trp1*. RDY510, RDY664, JLY310, and JLY179 are *MATα*; the rest are *MATa*. Additional markers can be found in JLY310 (*ade2*), RDY543 (*his3*), and RDY619 (*pep4Δ::TRP1 his3 ade2*). The RDY strains were a gift from R. Deshaies. pJL749, pJL772, and RS425 (*28*) were transformed into these strains and plated on SD-Leu at 22°C. Four colony-purified isolates from each transformation were patched onto SD-Leu plates and replica-plated to SGAL-Leu plates, all at 22°C. The patches on SGAL-Leu were replicaplated to a series of prewarmed SGAL-Leu plates at 22°, 25°, 27°, 30°, 32.5°, 35°, 37°, and 38°C. The viability of cdc mutants containing pJL749 was compared to those containing pJL772 and pRS425.
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32. We thank C. Peterson for preparation of library DNA, F. Banuett for oligonucleotide synthesis, and A. Lynn for superb help with the figures. We appreciate S. Fields, N. Hollingsworth, A. Sil, R. Deshaies, P. Sorger, P. Jackson, S. Sanders, A. Johnson, C. Detweiler, and A. Lynn for helpful discussions or useful suggestions on the manuscript. This work was supported by NIH grant AI18738 (to I.H.) J.J.L. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. J.J.L. also appreciates the early support from Bristol-Myers Squibb through the Life Sciences Research Foundation. The accession number for the *ORC6* sequence reported in this paper is L23323.

13 October 1993; accepted 17 November 1993

Sharing of the Interleukin-2 (IL-2) Receptor γ Chain Between Receptors for IL-2 and IL-4

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The γ chain of the interleukin-2 (IL-2) receptor is an indispensable subunit for IL-2 binding and intracellular signal transduction. A monoclonal antibody to the γ chain, TUGm2, inhibited IL-2 binding to the functional IL-2 receptors and also inhibited IL-4–induced cell growth and the high-affinity binding of IL-4 to the CTLL-2 mouse T cell line. Another monoclonal antibody, TUGm3, which reacted with the γ chain cross-linked with IL-2, also immunoprecipitated the γ chain when cross-linked with IL-4. These results suggest that the IL-2 receptor γ chain is functionally involved in the IL-4 receptor complex.

Functional high-affinity receptors for cytokines are generally complexes consisting of binding subunits (α chains) with low affinities to ligands and effector subunits (β chains) to transduce signals, both of which are members of the cytokine receptor superfamily (*1*). The same β chain is shared by receptors for IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (*1*, *2*). Another molecule, gp130, is shared as a signaling molecule by the receptors for IL-6, leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF) receptors (*3*). The IL-2 receptor is also a complex (*4*), but the low-affinity α chain is not a member of the cytokine receptor superfamily and the high-affinity receptor contains, in addition to the α and β chains, the γ

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chain, a member of the cytokine receptor superfamily (5). Dysfunction of the γ chain causes X-linked severe combined immunodeficiency (XSCID) characterized by profound defects of early T cell development (6). Mice lacking IL-2 production by disruption of the IL-2 gene can, however, develop mature T cell subsets (7). These observations prompted us to explore the possibility that the γ chain is a common subunit of receptor complexes for IL-2 and for other cytokines that may contribute to T cell development. In this study, we report that the IL-2 receptor γ chain is required for function of the IL-4 receptor complex.

To examine the biological significance of the γ chain, we produced monoclonal antibodies (mAbs) specific for the γ chain from Wistar rats immunized with TARTMG cells, a rat T cell line stably transfected with a mouse γ chain expression vector, BCMGSneo-mIL-2Rγ (8). The hybridoma TUGm2 produced a mAb (IgG2b) that completely inhibited IL-2 binding to BWhβ-11 cells (9). The specificity of TUGm2 was examined by flow cytometry (Fig. 1). The mAb TUGm2 bound TARTMG cells (8) that express the mouse γ chain gene, but not the parental TART-1 cells. The pair of cognate cell lines, Lmαmβ-7, a subline of the mouse fibroblast L929 cells expressing the mouse α and β chains from exogenously introduced genes, and Lmαmβmγ-1, derived from Lmαmβ-7 by transfection with the mouse γ chain gene (5), were also examined. The mAb TUGm2 associated with Lmαmβmγ-1 but not with Lmαmβ-7. A mouse IL-2–dependent T cell line, CTLL-2, also showed intense fluorescence when treated with TUGm2. Other mouse cell lines, which did not express mRNA for the γ chain, were negative for binding of TUGm2 (10), and TUGm2 did not bind to cells that expressed the high- and intermediate-affinity human IL-2 receptors (10), indicating that this antibody does not cross-react with a human component. The same results were obtained with the other hybridoma clone, TUGm3 (rat IgG2a), which directly bound the complex cross-linked with ^{125}I-labeled IL-2 and IL-2 receptor from BWhβ-11 cells (11). These results show that TUGm2 and TUGm3 are specific for the mouse γ chain.

During our preliminary examination of the effects of TUGm2 on cell growth response, we noticed that TUGm2 inhibited cell growth that was induced by IL-4 as well as by IL-2. Addition of TUGm2 alone did not affect the IL-2–dependent [^{3}H]thymidine incorporation of CTLL-2 cells, nor did a control rat mAb, REY-7 [specific for an HTLV-I *env* product (12)], or the mAb TM-β1 (specific for the mouse β chain) (Fig. 2A) (13). In contrast, simultaneous addition of TUGm2 and TM-β1 reduced [^{3}H]thymidine incorporation similar to the combination of TM-β1 and mAb for the IL-2 receptor α chain (13). The same combination of antibodies was used for IL-4–dependent growth of CTLL-2. CTLL-2 grows in response to IL-4 as well, although IL-4 supports growth for a shorter time and less effectively than IL-2 (14). TUGm2 alone inhibited [^{3}H]thymidine incorporation in response to IL-4 (Fig. 2B). TM-β1 did not synergize the TUGm2-induced inhibitory effect. Similarly, TUGm2 inhibited IL-4–induced growth of mast cell lines, MC9 and IC2 (15). Our results suggest that the γ chain is involved in the function of the IL-4–induced growth signal.

We determined whether TUGm2 affected the binding of IL-2 and IL-4 to their receptors. Scatchard plots showed that CTLL-2 cells expressed the high-affinity (130 pM) and the low-affinity (10 nM) IL-2 receptors (Fig. 3A). As expected from the screening method, addition of TUGm2, without affecting the numbers of receptors, reduced the high affinity to 370 pM. This was close to the value of the pseudo–high affinity generated with the α and β chains (5), implying that the effects of TUGm2 were focused on the γ chain. Binding of IL-4 was significantly suppressed by TUGm2 (Fig. 3B). CTLL-2 showed ~1200 IL-4 binding sites per cell with a dissociation constant of 130 pM. Similar to the high-affinity IL-2 receptor, TUGm2 lowered the dissociation constant to 850 pM; however, no significant change in the number of IL-4 binding sites was seen. MOLT4 cells expressing the mouse γ chain alone bound little or no IL-4, demonstrating that the γ chain itself does not have the ability to bind IL-4 directly (15).

There is a paucity of information about high and low affinities in IL-4 binding. A literature survey indicates that there is a difference in the IL-4 binding affinity between cells expressing the intrinsic IL-4 receptor, such as CTLL-2 and HT-2 cells, and COS-7 cells transfected with the cloned IL-4 receptor gene (16). CTLL-2 and HT-2 always showed IL-4 binding affinities higher than the transfected COS-7 cells, although actual values varied from report to report. The dissociation constant of 130 pM we observed may represent a high-affinity, and the dissociation constant of 850 pM might be a low-affinity binding to the conventional IL-4 receptor. If this is the case, the high-affinity IL-4 receptor is a complex of at least a heterodimer which contains the γ chain that has been identified as a component of the IL-2 receptor complex.

This hypothesis is supported by the results from the experiments in which competition of TUGm2 binding with IL-2 or IL-4 was examined (Fig. 3C). From Scat-

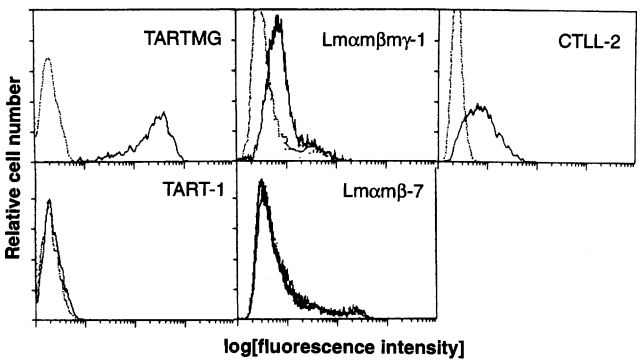

Fig. 1. Cell surface staining with TUGm2. TARTMG, TART-1,Lmαmβmγ-1,Lmαmβ-7, and CTLL-2 cells were incubated with (solid line) or without (dotted line) biotin-conjugated TUGm2 and then with R-phycoerythrin–conjugated avidine (Becton Dickinson) and analyzed with a FACScan (Becton Dickinson).

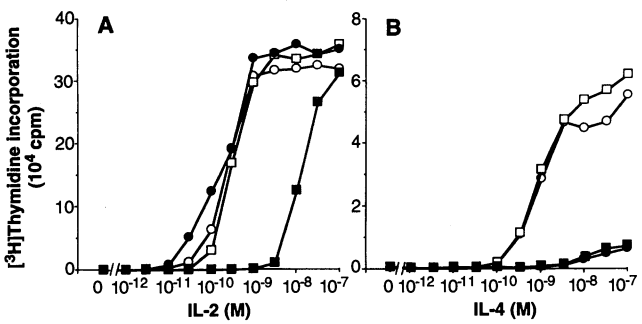

Fig. 2. Effects of TUGm2 on IL-2– and IL-4–dependent growth of CTLL-2 cells. Incorporation of [^{3}H]thymidine by CTLL-2 cells in response to either IL-2 or IL-4 was measured. Ascitic fluids containing the rat mAbs TUGm2 and TM-β1 specific for the mouse β chain and REY-7 specific for HTLV-I gp46 were added to CTLL-2 cultures to a final dilution of 1:40. Cells (1×10^4) were cultured for 48 hours with the indicated dosages of either IL-2 (**A**) or IL-4 (**B**) in the presence of REY-7 (○), REY-7 + TM-β1 (□), REY-7 + TUGm2 (●), or TUGm2 + TM-β1 (■), and 4 hours before harvesting, 1 μCi of [^{3}H]thymidine was added. Radioactivities incorporated into the cells were counted with a liquid scintillation counter. REY-7 did not affect the IL-2–dependent growth of CTLL-2.

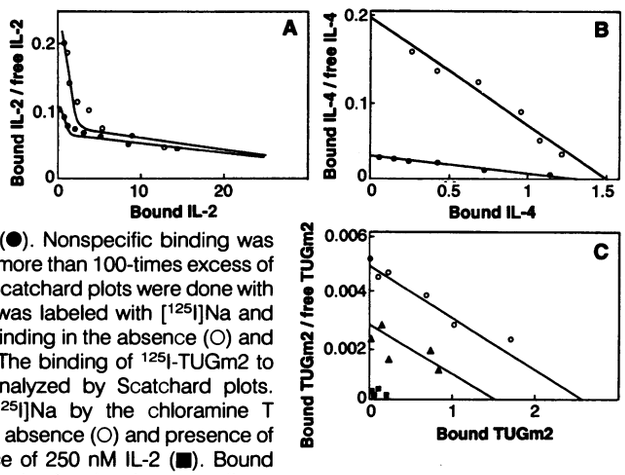

Fig. 3. Reciprocal interference in binding to CTLL-2 cells of TUGm2, IL-2, and IL-4. (**A**) Scatchard plots of the binding of ^{125}I–IL-2. Human recombinant IL-2 was labeled with [^{125}I]Na by the chloramine T method. IL-2 binding in the absence of (○) and presence of TUGm2 (●). Nonspecific binding was determined in the presence of more than 100-times excess of unlabeled IL-2. (**B**) The same Scatchard plots were done with mouse recombinant IL-4 that was labeled with [^{125}I]Na and Enzymobead (Bio-Rad). IL-4 binding in the absence (○) and presence of TUGm2 (●). (**C**) The binding of ^{125}I-TUGm2 to CTLL-2 cells was similarly analyzed by Scatchard plots. TUGm2 was labeled with [^{125}I]Na by the chloramine T method. TUGm2 binding in the absence (○) and presence of 250 nM IL-4 (▲) and presence of 250 nM IL-2 (■). Bound molecules recorded as thousands of molecules per cell.

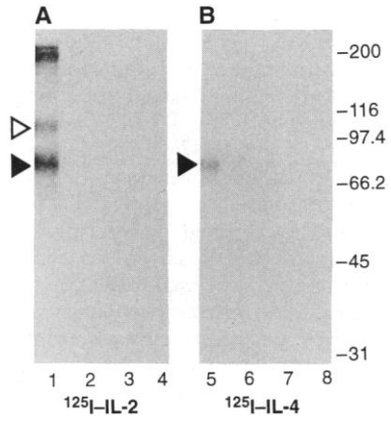

Fig. 4. Immunoprecipitation of the γ chain with TUGm3. CTLL-2 cells, after 2 hours of depletion of IL-2, were surface-labeled with either 1 nM ^{125}I–IL-2 (**A**) or .1 nM ^{125}I–IL-4 (**B**) and then chemically cross-linked with disuccinimidyl suberate (DSS, Pierce). Immunoprecipitation was done as described (*5*). In control experiments 100 nM of unlabeled IL-2 (lane 3) or IL-4 (lane 7) or a 1:10 dilution of TUGm2 ascites (lanes 2 and 6) was added to CTLL-2 culture before the addition of the radiolabeled ligands. REY-7 was used as a control mAb (lanes 4 and 8). Open and closed triangles indicate 105-kD and 85-kD ^{125}I-labeled ligand cross-linked complexes, respectively. For detection of ^{125}I–IL-4 and ^{125}I–IL-2 cross-linked complexes, 2 × 10^7 and 6 × 10^7 cells of CTLL-2 were used for each lane.

chard plot analysis with ^{125}I-labeled TUGm2, we estimated that CTLL-2 cells expressed 2800 molecules of the γ chain per cell. The binding of ^{125}I-TUGm2 was completely blocked by addition of IL-2, indicating that nearly all of the γ chain molecules participate in formation of the high-affinity IL-2 receptor with α and β chains. Thus, free γ chains capable of binding to TUGm2 are few in the presence of IL-2. On the other hand, addition of unlabeled IL-4 partially abolished ^{125}I-TUGm2 binding to CTLL-2 cells. Even with excess IL-4, 1600 sites remained free of the IL-4 receptor complex and, therefore, accessible to TUGm2 (Fig. 3B, C), presumably because the ~1200 conventional IL-4 receptors restricted the number of putative high-affinity IL-4 receptor complexes which included the same number out of the 2800 γ chain molecules.

Finally, to verify the involvement of the γ chain in the IL-4 receptor complex, we immunoprecipitated with another mAb, TUGm3, which did not block IL-2 binding to CTLL-2 but precipitated the ^{125}I–IL-2–cross-linked IL-2 receptor complex from CTLL-2. The TUGm3-directed precipitate included at least 105-kD and 85-kD bands, probably representing the IL-2–cross-linked β and γ chain complexes, respectively, on an SDS–reducing polyacrylamide gel (Fig. 4A). TUGm3-directed detection of the complexes including ^{125}I–IL-2 was abolished by addition of either TUGm2 or excess unlabeled IL-2 before cross-linking, because these impaired binding of ^{125}I–IL-2. Additionally, TUGm3 could precipitate the γ chain cross-linked with ^{125}I–IL-4 from cell lysate of CTLL-2 (Fig. 4B). Like the IL-2 receptor complex, addition of TUGm2 or unlabeled IL-4 failed to detect such a precipitation. These results suggest a physical association of the γ chain with the conventional IL-4 receptor.

The present study demonstrates the functional significance of the γ chain in the IL-4 receptor complex. The cytoplasmic domain of the γ chain is necessary for the IL-2–mediated signal transduction (*5*). In this study, IL-4 binding to the conventional IL-4 receptor, seen in the presence of TUGm2, does not permit enough cell growth. Collectively, the γ chain could participate not only in ligand binding but also in growth signal transduction of the IL-4 receptor complex.

The possible physical association between the IL-2 receptor γ chain and the IL-4 receptor may account for the previous observations that IL-4 can modulate IL-2–dependent cell growth and can down-regulate the expression of the IL-2 receptor (*17*). IL-2 and IL-4 may have synergistic effects on growth with cells expressing a large number of γ chains. However, inhibition may be seen with cells expressing few γ chains, which restricts the number of high-affinity IL-2 receptors. Cells in the latter case seem to reduce the number of IL-2 receptors in the presence of IL-4, because IL-4 consumes the γ chain.

With the implication of the γ chain in XSCID, we envisage its vital function as a receptor subunit for a cytokine that is pivotal in early T cell development (6). Both IL-2 and IL-4 are cytokines that could account for XSCID. Indeed, there are accumulating reports that implicate IL-4 as well as IL-2 in the proliferation of mature and immature T cells (*18*). However, patients whose T cells cannot produce IL-2 are known to have mature T cells (*19*), and mice having disrupted IL-2 or IL-4 genes develop mature T cell subsets (*7, 20*). IL-2 and IL-4 may compensate for each other in these cases to develop T cells. Alternatively, cytokines other than IL-2 and IL-4 may be responsible for the early T cell development through binding to a receptor complex that includes the γ chain. IL-13 is one such candidate, because receptors for IL-4 and IL-13 share a yet unidentified common receptor subunit (*21*), suggesting that the γ chain is also shared with the IL-13 receptor complex. The mAbs established in this study should provide powerful tools for elucidating the molecular basis not only of XSCID but also of multifunctions of cytokines capable of binding to the γ chain.

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8. BCMGSneo-mIL-2Rγ was constructed by insertion of the Eco RI–Bam HI fragment (1.2 kb) from pSRmG1 (*5*) into the Xho I and Not I sites of BCMGSNeo [H. Karasuyama *et al.*, *J. Exp. Med.* **172**, 969 (1990)]. TARTMG cells, a subline of a WKA/Hok rat T cell line TART-1 [M. Tateno *et al.*, *J. Exp. Med.* **159**, 1105 (1984)] that was transformed with human T cell leukemia virus type I (HTLV-I) and stably transfected with BCMGSneo-mIL-2Rγ, were intraperitoneally injected into Wistar rats. Spleen cells from the sensitized rats

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.
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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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