Interleukin 2 Receptor γ Chain Expression on Resting and Activated Lymphoid Cells

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Summary

The interleukin 2 receptor (IL-2R) is known to be comprised of at least three genetically distinct subunits termed α, β, and γ. These chains can be expressed individually or in various combinations resulting in distinct receptors with different affinities for IL-2. In contrast to α and β, the cell surface expression of the γ chain protein previously has not been well-characterized. To examine cell surface expresssion of IL-2Rγ on hematopoietic cells, we developed two new monoclonal antibodies (mAbs) specific for this protein. Both 1A11 (immunoglobulin [IgG1]) and 3G11 (IgM) specifically reacted with murine cells transfected with IL-2Rγ cDNA, and immunoprecipitation studies indicated that both antibodies precipitated a protein of approximately 62–65 kD. Scatchard analysis of IL-2 binding to murine cells transfected with cDNA-encoding combinations of IL-2R components demonstrated that neither β nor γ chain bind IL-2 with measurable affinity, but coexpression of both β and γ is sufficient to form an intermediate affinity receptor. In the absence of γ chain, β chain interacts with α chain to form a "pseudo-high" affinity receptor. In contrast, γ chain does not appear capable of interacting with α chain in the absence of β chain. Thus, γ chain appears to interact only with β, but β chain is capable of interacting with both α and γ. Using the newly developed mAbs to examine cell surface expression by immunofluorescence, resting T cells were found to express low levels of γ chain without detectable α or β. Early after mitogen stimulation, T cells expressed higher levels of α, β, and γ. However, at later time points, T cells expressed α and γ in marked excess over β. Thus, formation of high affinity IL-2R on activated T cells was primarily limited by β chain expression. In contrast, resting natural killer (NK) cells constitutively expressed IL-2Rβ without detectable α or γ. After activation with either IL-2 or IL-12, expression of both α and γ transiently increased and then returned to very low levels. Expression of functional IL-2R on resting and activated NK cells, therefore, appeared to be primarily limited by the expression of γ chain. IL-2 binding studies with resting NK cells confirmed the results of immunofluorescence studies indicating the presence of very low numbers of intermediate affinity (βγ) receptors for IL-2 on these cells. NK cells obtained from patients receiving IL-2 therapy were phenotypically similar to resting NK cells. These studies have identified marked differences in IL-2R composition in two different types of cytotoxic lymphocytes, further underscoring the complexity of this receptor/ligand system. With new reagents specific for IL-2Rγ, it will now be possible to examine further the functional significance of these differences.

IL-2 has pleiotropic effects on lymphocytes, including T, B, and NK cells (1–4), as well as other hematopoietic cells (5–8). The effects of IL-2 on these various cells are mediated through specific cell surface receptors. Over the past several years, our understanding of the IL-2R complex has increased substantially. It is now known that the IL-2R comprises at least three subunits encoded by distinct genes. These subunits can be expressed individually or in various combinations, resulting in receptors that bind IL-2 with markedly different affinity. The first IL-2R component to be identified, IL-2Rα

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(CD25, Tac antigen) (9) is a 55-kD protein that binds IL-2 with an equilibrium dissociation constant (K_d) of ~10 nM. IL-2Rα has a very small intracellular domain and does not exhibit homology to other known cytokine receptors. The second IL-2R component, IL-2Rβ, is a 75-kD protein with a large intracellular segment and areas of well-defined homology to other cytokine receptor superfamily members (10–12). IL-2Rβ is known to play an important role in receptor-mediated signaling, in part through association with cytoplasmic protein tyrosine kinases (13, 14). The third and most recently identified IL-2R component, IL-2Rγ, is a 64-kD protein that also has structural homology to other cytokine receptors (15, 16). Like IL-2Rβ, IL-2Rγ by itself has a very low affinity for IL-2 (17). However, when expressed together, these two chains form an intermediate affinity receptor with a K_d of ~1 nM. Expression of all three receptor components is required to form a high affinity IL-2 receptor (K_d, ~10 pM).

Although each component of the IL-2R complex is likely to play an important role in regulating the cellular response to IL-2, the central role of the γ chain is supported by the demonstration that X-linked SCID occurs as a direct result of γ chain mutations (18, 19). In addition, recent studies (20–22) have provided compelling evidence that the IL-2Rγ chain is also a component of the receptors for IL-4 and IL-7. Moreover, it is likely that the γ chain is a common component of additional cytokine receptors. However, in contrast to other IL-2R components, γ chain mRNA appears to be constitutively expressed in all lymphoid cells. This finding is consistent with the demonstration that the IL-2Rγ promoter sequence is GC-rich and lacks TATA motifs, and suggests that surface expression of γ chain is likely to be regulated primarily by posttranscriptional mechanisms (15, 23). However, lack of suitable reagents has previously prevented detailed examination of cell surface expression of IL-2Rγ.

In the present studies we developed two new murine mAbs specific for the IL-2Rγ chain. These mAbs have been used to examine IL-2Rγ expression by normal human lymphocytes as well as hematopoietic cell lines. To elucidate the functional consequences of expression of distinct IL-2R subunits, the surface expression of each IL-2R component on resting and activated T and NK cells was determined. T and NK cells are known to respond very differently to IL-2, and these studies provide further insight into the mechanisms whereby a single cytokine can mediate distinct functional activities in different cell types.

Materials and Methods

Construction of Plasmids. A full-length cDNA for human IL-2Rγ was isolated by RT-PCR using the following oligonucleotides: 5′-GAAGGCCTTCTGAACACGACAATTCTGACG (artificial StuI site) or GCTCTAGAGCGAAGAGCAAGCGCCATGTTG (XbaI) and 3′-GCTCTAGAGCCAAATGAAGGGGTGCTTACA (XbaI) from YT cell line mRNA. An expression vector containing IL-2Rγ cDNA (pMT2.UCHL1$^+$.IL-2Rγ.CD45) was derived from pMT2.LCA.1 (CD45 O-isoform) (24, 25), and synthetic oligonucleotides (5′-GCTTCCAAGGATCCGGAAG-3′ and 5′-CGCTTCCGGATCCTTGGAAGC-3′) by ligating appropriate restriction fragments in frame. The resulting hybrid protein, UCHL1$^+$.IL-2Rγ.CD45, contains portions of several proteins in the following order: (*a*) CD45 leader sequence; (*b*) CD45 amino acids 1-8 and 202-218; (*c*) IL-2Rγ amino acids (L1-N232); (*d*) seven amino acids (RFQGSGS) arising from the synthetic oligonucleotides; and (*e*) CD45 amino acids 214-1281 (numbering of CD45 and IL-2Rγ amino acid residues according to Streuli et al. [26] and Noguchi et al. [23], respectively).

The expression vector, pSRα2.IL-2Rγ, was generated by inserting full-length IL-2Rγ cDNA into the XbaI multilinker site of a slightly modified version of the pSRα2 expression vector (27). Structures of plasmid constructs were confirmed by restriction mapping and/or by nucleotide sequencing. Full-length cDNA encoding IL-2Rα and IL-2Rβ, were also introduced into the pSRα2 vector at the appropriate restriction enzyme sites, and designated as pSRα2.IL-2Rα and pSRα2.IL-2Rβ, respectively.

Transfection of cDNA. The Abelson virus–transformed murine 300–19 pre-B lymphocyte cell line was transfected with various constructs by electroporation with plasmid DNA using the Cell Porator Electroporation System (Bethesda Research Laboratories, Gaithersburg, MD). pMT2.UCHL1$^+$.IL-2Rγ.CD45, pSRα2.IL-2Rγ, and pSV2.neoSP (28) constructs were linearized before transfection with SspI, FspI, and PvuI, respectively. Transfected cells were monitored using available anti-CD45 mAbs, UCHL1 (CD45RO) and GAP8.3 (CD45). UCHL1 binds to the NH_2-terminal region of the 180-kD isoform, whereas GAP8.3 binds to a more COOH-terminal region of CD45. In subsequent experiments, cotransfection or sequential transfections were also performed as described above using various combinations of pSRα2.IL-2Rα, pSRα2.IL-2Rβ, and pSRα2.IL-2Rγ. α and β chain expression vectors were linearized with XmmI. pPGK.Hygro (Hygromycin B resistance gene) was used for sequential transfection.

Production of mAb to the IL-2Rγ Chain. BALB/c mice were immunized with 300-19(UCHL1$^+$.IL-2Rγ.CD45) transfectant cells expressing human IL-2Rγ and CD45 epitopes. Fusions were carried out as previously described (29), and hybridoma supernatants were screened by indirect immunofluorescence for binding to 300-19(UCHL1$^+$.IL-2Rγ.CD45) and lack of binding to 300-19(LCA.1) transfectants expressing only human CD45 (25, 30). Based on this screening, two hybridomas, designated 1A11 (IgG1) and 3G11 (IgM), were selected and subcloned by limiting dilution. These antibodies also react with 300-19(IL-2Rγ) cells expressing IL-2Rγ protein alone (data not shown), confirming that these antibodies react specifically with IL-2Rγ rather than with CD45RO or CD45 epitopes.

Immunoprecipitation. The 300-19(IL-2Rγ) transfectant cells were surface ^{125}I-labeled by the lactoperoxidase method or were metabolically labeled with [^{35}S]methionine (New England Nuclear, Cambridge, MA). Radiolabeled cells were washed and solubilized in 1 ml lysis buffer (1% NP-40, 50 mM Tris, pH 8.15, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 2 μg/ml PepstatinA, 10 μg/ml leupeptin, and 10 μg/ml aprotinin). Lysates were preincubated with rabbit anti–mouse Ig (RαM)–coupled protein A beads, followed by incubation overnight with specific antibodies and RαM-coupled protein A beads. Immunoprecipitates were washed extensively, followed by addition of sample buffer (60 mM Tris, pH 6.8, 5% β-MER, and 2% SDS) and subsequently subjected to SDS-PAGE (7.5% gel).

Cell Lines and Isolation of Normal Lymphocytes. T cell lines MT1, Jurkat, Molt4, H9, HPB-ALL, and Rex; B cell lines Daudi, SKW6.4, Nalm6, and CESS; NK cell lines NK3.3, YT-N17, and YT-2C2; myeloid cell lines K562, HL60, U937, KG1, TF1, and Mo7; colon cancer cell line Colo205; cervical cancer cell line HeLa;

small cell lung carcinoma cell line SW2; and melanoma cell line SKMEL were maintained in RPMI 1640 with 10% FBS. NK3.3 cells (31) were supplemented with 5–10% leukocyte-conditioned medium (LCM). TF1 and Mo7 were supplemented with 10 ng/ml GM-CSF or 20 ng/ml IL-3. The preparation of LCM was described previously (32).

PBMC were isolated on Ficoll-diatrizoate gradients from heparinized blood or from cytophoresis buffy coat cells obtained from normal volunteer donors. Adherent mononuclear cells were depleted by incubation on plastic petri dishes for 1 h at 37°C. Enriched NK cells were obtained by incubating PBL with a mixture of T1 (CD5), B1 (CD20), and My4 (CD14) mAb, and then separating antibody-bound cells using immunomagnetic beads (Advanced Magnetics, Inc., Cambridge, MA). In some experiments, enriched NK cells cultured in medium with 100 U/ml IL-2 or PBL cultured in medium with 1 μg/ml PHA were used for immunofluorescence experiments. PBMC obtained from patients receiving prolonged treatment with low dose rIL-2 (Amgen, Thousand Oaks, CA) were also examined (33, 34). Chronic administration of low dose IL-2 by either continuous i.v. infusion or daily subcutaneous injection has previously been shown to expand the total number of circulating NK cells and increase cytolytic activity against both NK-sensitive and -resistant target cells.

mAbs. FITC- or PE-conjugated murine mAbs were obtained from Coulter Immunology (Hialeah, FL), including T3 (CD3, IgG1), NKH1 (CD56, IgG1), IL-2R1 (CD25, IgG2a), B4 (CD19, IgG1), Mo2 (CD14, IgM), and isotype-matched control mAbs. T1 (CD5, IgG2a), B1 (CD20, IgG2a), My4 (CD14, IgG2b), N901 (CD56, IgG1), 3B8 (CD56, IgM), RW2 (CD3, IgG1), 2ad2 (CD3, IgM), UCHL1 (CD45RO), and GAP8.3 (CD45) were used as dilutions of ascites. Purified murine mAbs (FITC conjugated or unconjugated) directed against the p75 subunit (IL-2Rβ) (35) were kindly provided by Coulter Immunology. Purified 1A11 (IgG1) and 3G11 (IgM) antibodies were isolated from ascites by affinity chromatography on an Affi-Gel Protein A MAPS II Kit (Bio-Rad, Hercules, CA) or ImmunoPure IgM Purification Kit (Pierce, Rockford, IL), and were dialyzed against PBS.

Immunofluorescence Analysis. Samples of cells were stained directly or indirectly with FITC- or PE-conjugated mAb, washed, fixed in 1% formaldehyde, and analyzed by flow cytometry as described previously (36). Goat anti-mouse IgM-FITC (Southern Biotechnology Associates, Birmingham, AL) was used as secondary antibody for staining of 3G11 (IgM). NK3.3 and IL-2 activated NK cells were stained after the incubation in IL-2–free medium for 2 h at 37°C.

RT-PCR and Hybridization. RT-PCR and hybridization were performed as described elsewhere (37, 38). Primers specific for IL-2Rγ were the same as described above. Primers specific for β2-microglobulin were: (nucleotide 1543) 5′-ACCCCCACTGAAAAAGATGA and (nucleotide 3317) 3′-ATCTTCAAACCTCCATGATG. γ-[^{32}P]ATP-labeled oligomer probes, IL-2Rγ 5′-TCACATCCCTCTTATTCCTGC-3′, and β2-microglobulin, 5′-GCCCAAGATAGTTAAGTGGG-3′, were used for hybridization.

^{125}I-IL-2 Binding Assay. The ^{125}I-labeled IL-2 was obtained from New England Nuclear or made in one of our laboratories (T. L. Ciardelli) and had a sp act of 1.2–1.5 × 10^6 or 1.3–1.8 × 10^6 dpm/pmol, respectively. IL-2 binding was analyzed by scatchard plot (39). Briefly, cells (1–3 × 10^6 cells per aliquot) were incubated at 4°C for 120 min with ^{125}I-IL-2 at serial dilutions ranging from 1 pM to 64 nM in binding medium containing 1% BSA, 0.1% sodium azide, and 25 mM Hepes in RPMI 1640 medium, pH 7.4, in the presence or absence of a 500-fold excess of unlabeled rIL-2. The inhibitory effects of p75 (IL-2Rβ antibody), and 1A11 and 3G11 (IL-2Rγ antibodies) on IL-2 binding were also examined in the presence of excess amount of antibodies (50 μg/ml).

Results

Immunoprecipitation of Antigens Identified by Anti-IL-2Rγ mAb. To confirm the specificity of the newly generated mAb, immunoprecipitations were performed using 300-19(IL-2Rγ) transfectant cells. 1A11 and 3G11 precipitate a protein of ~62–65 kD from lysates of cells surface labeled with ^{125}I (Fig. 1 *A*) or metabolically labeled with [^{35}S]methionine (Fig. 1 *B*). Immunoprecipitation of surface-labeled transfectants also revealed an intense band at ~90 kD (see Fig. 3 *A*). This 90-kD band is also identified in immunoprecipitates of ^{125}I-labeled YT cells (data not shown). This protein

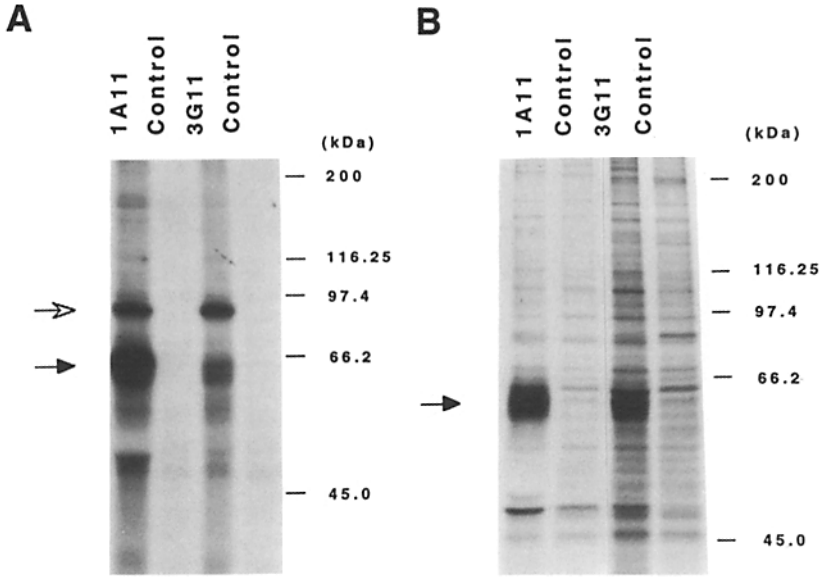


Figure 1. Immunoprecipitation of 300-19(IL-2Rγ) cell lysate with 1A11 and 3G11 mAb. (*A*) Cells were surface labeled with Na^{125}I or (*B*) metabolically labeled with [^{35}S]methionine. Cell lysates were precipitated with either specific mAb or negative control mAb and analyzed in 7.5% SDS-PAGE gel.

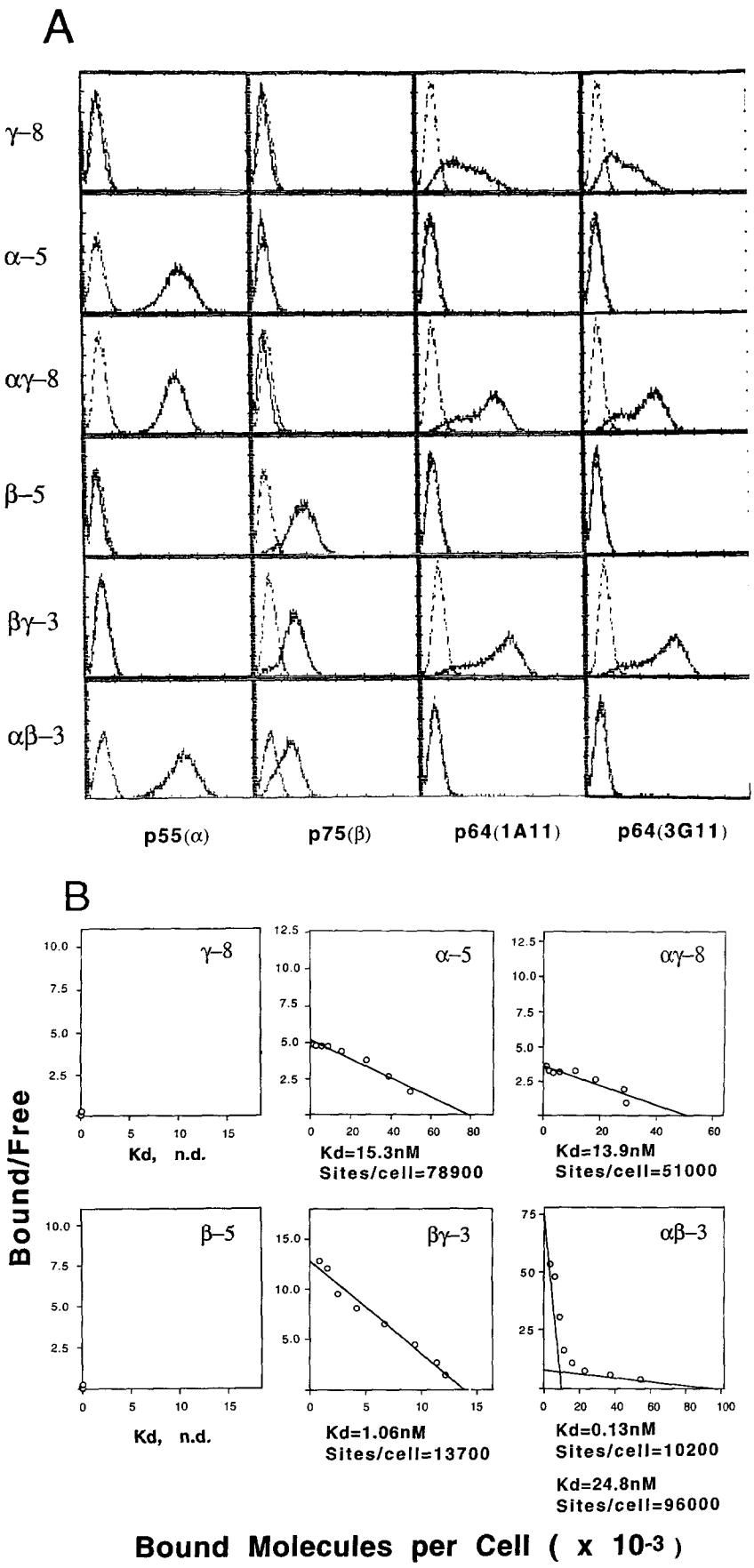


Figure 2. (*A*) Surface expression of IL-2R components in 300-19 transfectant cell lines. Five 300-19 sublines designated α, β, γ, αγ, and βγ were established by transfection with various combinations of expression vectors. (*Dotted lines*) Immunofluorescence reactivity with isotype-matched negative control mAb; (*solid lines*) cell surface binding of specific IL-2R antibodies indicated on the x-axis. (*B*) Scatchard plot analyses of ^{125}I–IL-2 binding on the same transfectant cell lines. Bound IL-2 (*molecules/cell*)/free IL-2 (pM) on the y-axis are plotted against bound molecules/cell on the x-axis.

does not appear to be either p75 or p55, but has not yet been further characterized.

Expression of IL-2R Components and IL-2 Binding to Transfectant Cell Lines. Fig. 2 *A* demonstrates the surface expression of the three different human IL-2R chains on a series of murine 300-19 cell lines transfected with cDNA encoding individual IL-2R components and a combination of IL-2R components. The reactivity of 1A11 and 3G11 antibodies are almost identical, but these antibodies appear to recognize distinct epitopes of IL-2Rγ (data not shown). Scatchard plots of IL-2 binding to the same transfectants are shown in Fig. 2 *B*. Although the IL-2Rγ and IL-2Rβ transfectants do not show detectable IL-2 binding, IL-2R$\beta\gamma$ transfectant cells demonstrate intermediate affinity binding (K_d, 1.06 nM). IL-2R$\alpha\beta$ transfectant cells demonstrate both pseudo-high affinity binding (K_d, 0.13 nM) mediated by the $\alpha\beta$ complex and low affinity binding (K_d, 24.8 nM) mediated by excess free α chains. Since the affinity of IL-2 binding to IL-2Rα and to IL-2R$\alpha\gamma$ transfectant cells is almost identical (K_d, 15.3 and 13.9 nM, respectively), expression of the IL-2Rγ chain does not appear to significantly affect IL-2 binding to IL-2Rα.

Expression of IL-2R Chains on YT Cells and Effects of Anti-IL-2Rγ mAb on IL-2 Binding. We next examined surface expression of IL-2Rγ by immunofluorescence on cell lines known to express high levels of intermediate affinity receptors for IL-2. As shown in Fig. 3 *A*, YT-2C2 cells express both IL-2Rβ and IL-2Rγ, but not IL-2Rα. YT-N17 cells express all 3 IL-2R components (data not shown). The fluorescence intensity of staining with 3G11 was slightly higher than with 1A11 (data not shown). As shown in Fig. 3 *B*, YT-2C2 cells express a relatively high number of intermediate affinity receptors for IL-2 (K_d, 1.47 nM, 13,900 sites/cell). The addition of excess mAb specific for IL-2Rβ completely inhibited binding of ^{125}I-labeled IL-2. In contrast, IL-2 binding was not significantly affected by addition of either mAb specific for IL-2Rγ (K_d after addition of 1A11 and 3G11 was 1.81 and 1.63 nM, respectively).

Expression of IL-2Rγ by Hematopoietic and Nonhematopoietic Cell Lines. Table 1 summarizes the surface expression of different IL-2R chains detected by flow cytometry and IL-2Rγ chain message detected by RT-PCR in a large number of hematopoietic and nonhematopoietic cell lines. Although most lymphoid cell lines revealed levels of IL-2Rγ chain expression detectable both by immunofluorescence and RT-PCR, surface expression of IL-2Rγ chain was generally at relatively low levels. Of all cell lines tested, surface expression of IL-2Rγ was highest in the YT cell lines. Four nonhematopoietic cell lines were not found to express IL-2Rγ by either immunofluorescence or RT-PCR.

Expression of IL-2Rγ by myeloid cell lines has not been previously reported. Although all six myeloid cell lines that we tested expressed IL-2Rγ mRNA by RT-PCR, relatively low surface expression of γ chain was detected by flow cytometry in two cell lines (Table 1).

Expression of IL-2R Components after Activation of T and NK Cells. We also examined changes in surface expression of the three IL-2R components on PHA-activated T cells ($CD3^+$ cells) and IL-2–activated NK cells ($CD56^+$ cells). As shown in Fig. 4, almost no α or β chain was detectable by flow cytometry on resting T cells, but γ chain was present at low levels. After PHA stimulation (48 h), surface expression of α chain increased markedly while β and γ chain expression increased to a smaller extent. By 5 d after activation, both α and β chain expression had declined. Although α chain expression remained increased compared with resting T cells, β chain expression had almost returned to very low baseline levels. In contrast, γ chain expression appeared to be increased compared with resting T cells and exceeded that of β chain.

Fig. 5 *A* shows the surface expression of the three IL-2R components on enriched NK cells after IL-2 activation. As previously shown, β chain was constitutively expressed by resting NK cells. Both $CD56^{dim}$ and $CD56^{bright}$ NK populations expressed IL-2Rβ, but neither population expressed

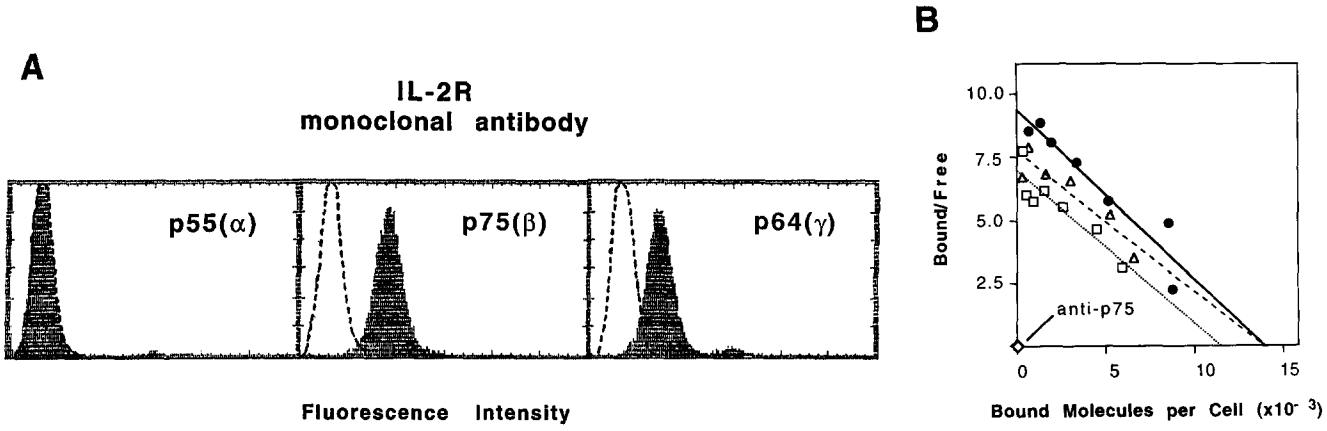


Figure 3. (*A*) Immunofluorescence reactivity of anti-p64 mAb with YT cell line. YT cells were stained directly with anti-p55-FITC (IgG1) and anti-p75-FITC (IgG2a) or indirectly with 1A11 and 3G11. Immunofluorescence reactivity was compared with negative controls. (*Dotted lines*) MsIgG1-FITC, MsIgG2a-FITC, MsIgG, and MsIgM. (*B*) Scatchard plot analyses of ^{125}I-IL-2 binding to YT cells. Binding studies were carried out in the presence of media (●) or excess mAb 1A11 (△), 3G11 (□), and anti-p75 (◇). Bound IL-2 (*molecules/cell*)/free IL-2 (pM) on the y-axis are plotted against bound molecules/cell on the x-axis.

Table 1. *Expression of IL-2 Receptor Components*

	Surface immunofluorescence			RT-PCR
	IL-2Rα p55	IL-2Rβ p75	IL-Rγ p64	IL-Rγ p64
T cell lines				
MT1	++	−	+	+
Jurkat	−	−	+	+
Molt4	−	−	+	+
H9	−	−	−	+
HPB-ALL	−	−	+	+
REX	−	−	+	+
B cell lines				
Daudi	−	−	+	+
SKW6.4	−	−	+	+
Nalm6	−	−	−	+
CESS	−	−	+	+
NK cell lines				
YT N17	++	++	++	+
YT 2C2	−	++	++	+
NK3.3	++	++	+	+
Myeloid cell lines				
K562	−	−	−	+
HL60	−	−	−	+
U937	−	−	+	+
KG1	−	−	+	+
TF1	−	−	−	+
Mo7	−	−	−	+
Nonhematopoietic cell lines				
Colo205			−	−
HeLa			−	−
SW2			−	−
SKMEL			−	−

Cells were stained directly with p55-FITC or p75-FITC, or indirectly with 3G11 ascites and goat anti-mouse IgM-FITC. Fluorescence intensity for each antibody was compared with isotype-matched negative control antibody. (−) <5% reactivity above negative control fluorescence; (+) 5–30% reactivity; (++) >30% reactivity.

detectable α or γ chain. 5 d after activation, NK cells demonstrated enhanced expression of both α and γ chain. However, expression of both α and γ chain remained at a lower level compared with IL-2Rβ. By 15 d after stimulation, expression of both α and γ chains had decreased to the baseline level of resting NK cells but β chain expression remained stable. Analysis of NK cells after activation with IL-12 gave similar results (data not shown). Thus, NK cells also demonstrated a discordance between expression of the β and γ chains but, in contrast to T cells, expression of β chain was always in excess and surface expression of γ chain appeared to represent the limiting component.

We also examined the expression of IL-2R components on PBMC obtained from patients receiving prolonged continuous infusions of low dose IL-2. Previous studies have demonstrated that such therapy results in the gradual expansion of circulating $CD56^+CD3^-$ NK cells, which become the predominant lymphocytes in peripheral blood after 4–6

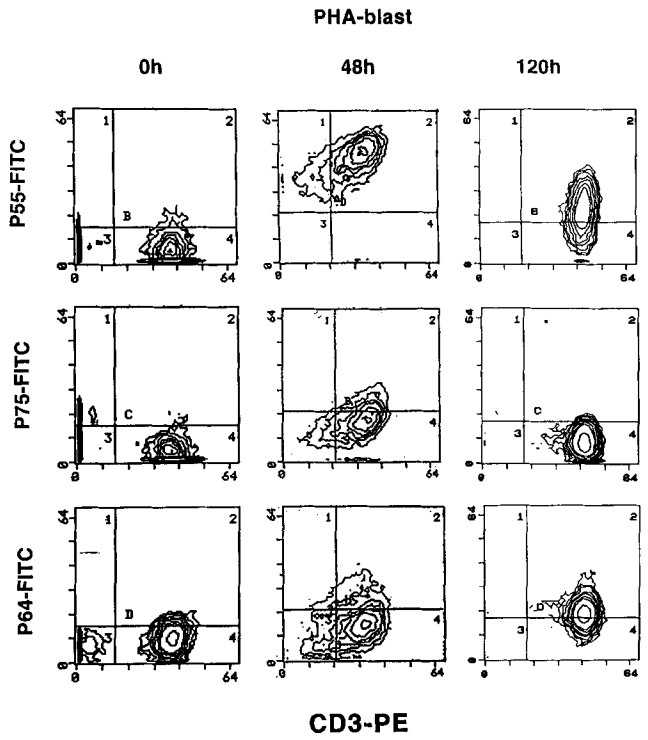
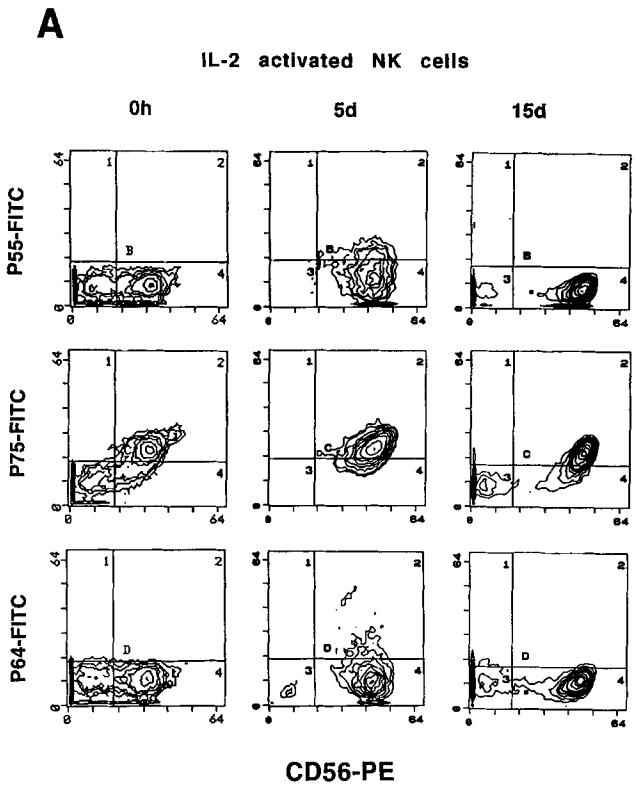


Figure 4. Surface expression of IL-2R subunits on resting and mitogen-stimulated T ($CD3^+$) cells. T cells were examined after 2 and 5 d of culture with 1 μg/ml PHA. IL-2Rγ expression was determined by staining cells indirectly with 1A11/anti-mouse IgG1-FITC or 3G11/anti-mouse IgM-FITC followed by CD3-PE. IL-2Rα and IL-2Rβ were examined directly with anti-p55-FITC and anti-P75-FITC in combination with CD3-PE. Quadrant settings distinguishing positive immunofluorescence from background fluorescence were determined by staining with isotype-matched control mAb (not shown).



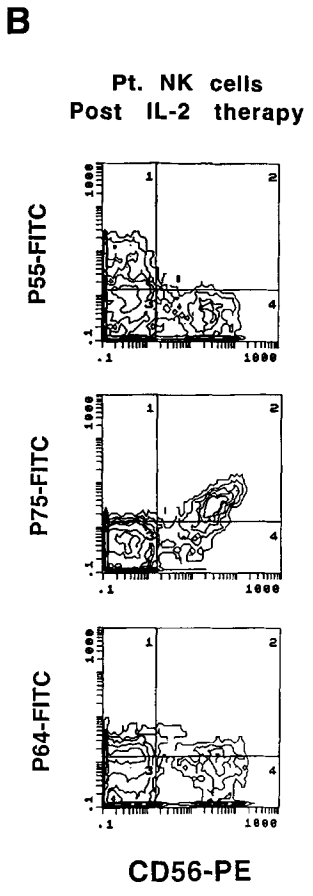


Figure 5. (*A*) Surface expression of IL-2R subunits on IL-2–activated enriched NK cells and (*B*) in vivo–expanded NK cells from patients receiving prolonged IL-2 infusion. Enriched NK cells were examined after 5 and 15 d of culture with 100 U/ml IL-2. NK cells from patients receiving IL-2 were examined without in vitro culture. IL-2Rγ expression was determined by staining cells indirectly with 1A11/anti-mouse IgG1-FITC or 3G11/anti-mouse IgM-FITC followed by CD56-PE. IL-2Rα and IL-2Rβ were examined directly with anti-p55-FITC and anti-P75-FITC in combination with CD56-PE. Quadrant settings distinguishing positive immunofluorescence from background fluorescence were determined by staining with isotype-matched control mAb (not shown).

wk of treatment (33, 34). As shown in Fig. 5 *B*, in vivo–expanded NK cells in such patients express IL-2Rβ but little IL-2Rα and IL-2Rγ. The pattern of expression of IL-2R components in in vivo expanded NK cells is thus similar to that seen in normal resting NK cells.

IL-2 Binding to Resting NK Cells. To confirm the low level of expression of γ chain on resting NK cells, we conducted three independent IL-2 binding experiments on enriched resting NK cells to determine the number of intermediate affinity IL-2 binding sites (Table 2). The percentage of NK cells ($CD56^+$) in these experiments was between 56 and 64%. Further analysis of these populations indicated that they contained 15–26% $CD3^+$ cells and ~20% other cells which were not identified. Scatchard analysis of IL-2 binding sites revealed the presence of both high and intermediate affinity IL-2 receptors. However, calculation of the number of IL-2 binding sites in these populations revealed only ~180–280 intermediate affinity binding sites and 10–35 high affinity binding sites per cell. These findings are consistent with very low levels of expression of both IL-2Rα and IL-2Rγ, despite relatively high expression of IL-2Rβ. Assuming that contaminating cells in this analysis have no IL-2 binding capa-

bility, the true number of IL-2 binding sites on resting NK cells may increase from one- to twofold. Nevertheless, these results remain consistent with our flow cytometric results indicating very low levels of expression of both α and γ chain in resting NK cells.

Discussion

The IL-2R is a complex receptor that includes at least three distinct subunits. Depending on the expression of these subunits, cells can possess receptors with markedly different affinities for IL-2 as well as different functional attributes. The β and γ chains are members of a large cytokine receptor family characterized by four conserved cysteine residues and a Trp-Ser-X-Trp-Ser (WSXWS) motif in two fibronectin type III modules. Both IL-2Rβ and IL-2Rγ possess large cytoplasmic domains that presumably contribute to signal transduction upon IL-2 binding (13, 14, 18, 40). In contrast, IL-2Rα has a very small cytoplasmic segment and has not been shown to mediate IL-2 internalization or signal transduction by itself. Nevertheless, IL-2Rα appears to facilitate IL-2 delivery to other IL-2R subunits and promotes the formation of high affinity receptors (41).

Previous studies have demonstrated that the gene encoding the IL-2Rγ chain appears to be constitutively transcribed by a variety of lymphoid and myeloid cells. This is compatible with data indicating that the γ chain gene does not include *cis*-acting elements typically associated with inducible expression. Thus, surface expression of the γ chain is probably regulated posttranscriptionally. However, studies of γ chain protein expression have heretofore been impeded by a lack of suitable reagents. In the present studies we describe two new murine mAbs, 1A11 and 3G11, specific for the human IL-2Rγ subunit, and have used these new reagents to examine expression of the γ chain in a variety of cell lines transfected with different IL-2R components as well as a large number of other hematopoietic cells.

Table 2. *Immunofluorescence Analysis and ^{125}I-IL-2 Binding to Enriched Resting NK Cells*

					^{125}I-IL2 binding	
	CD56	CD3	CD19	CD14	K_d	Sites/cell
		%			*pM*	
1.	62.7	15.9	ND	0.42	5.1	10
					236	277
2.	64.2	16.8	ND	0.97	6.9	27
					349	281
3.	56.4	26.1	0.1	4.2	3.2	34
					308	187

Enriched resting NK cells obtained after immunomagnetic bead depletion of T cells, B cells, and monocytes were stained directly with CD56-PE, CD3-FITC, CD19-FITC, CD14-PE, and isotype-matched control antibodies. Gates were set to include all mononuclear cells and percent positive cells represent percent total mononuclear cells. IL-2 binding assay was performed as described in Materials and Methods. ND, not detected.

Examination of surface expression of IL-2R components in a murine pre-B cell line transfected with different combinations of human cDNA encoding IL-2Rα, IL-2Rβ, and IL-2Rγ confirmed the specificity of the new mAbs. Immune precipitation studies also confirmed the reactivity of these mAbs with a cell surface protein with molecular mass of ~62–65 kD. It is interesting to note that further analysis of IL-2 binding to these transfectant cell lines demonstrated that neither β nor γ chain bind IL-2 with measurable affinity and that coexpression of these two chains is sufficient to form an intermediate affinity receptor. In the absence of γ chain, β chain clearly interacts with α chain to form a pseudo-high affinity receptor. In contrast, γ chain does not appear capable of interacting with α chain in the absence of β chain. In the formation of various IL-2R, these results indicate the γ chain only interacts with β, but that β chain interacts with both α and γ.

Despite the ubiquitous presence of γ chain message in the hematopoietic cells we examined, cell surface γ chain was detected primarily on lymphoid cells. We undertook detailed studies of IL-2R subunit expression on T and NK cells because these lymphocytes exhibit distinct functional responses to IL-2. Resting T lymphocytes were found to express very low levels of IL-2Rγ chain, whereas both α and β chains were essentially undetectable. After stimulation with PHA, virtually all T cells expressed IL-2Rα at high levels; IL-2Rβ and γ were more modestly upregulated. Thus, activated T cells appear to express a small number of heterotrimeric high affinity IL-2R that are limited by the number of β and γ chains on the cell surface. The expression of an excess of α chain appears to promote the formation of high affinity receptors capable of responding to very low concentrations of IL-2. Subsequently, β chain expression returns to undetectable levels and the number of α chains also declines, whereas γ chain expression remains elevated. Since α and γ do not appear to interact with each other, the downregulation of β expression appears to be an important mechanism for limiting the T cell response to IL-2 in the absence of further stimulation. In this setting, it seems likely that γ chains that persist after T cell activation are associated with other cytokine receptors, such as IL-4R and IL-7R. In fact, the selective downregulation of β chain may directly promote the interaction of the γ chain with other cytokine receptors, thus providing a mechanism for directing a sequential response to different cytokines. Similarly, the selective low level expression of γ chain without either α or β on resting T cells may indicate that γ chain is complexed with receptors for other cytokines before T cell activation.

Resting NK cells incubated with nanomolar concentrations of IL-2 exhibit augmented cytolytic activity and upregulation of cell surface adhesion molecules (4). These data imply that NK cells constitutively express intermediate affinity

IL-2R, and it has been established that virtually all resting NK cells express IL-2Rβ (39, 42). Although the β chain was clearly detected on resting NK cells, our immunofluorescence studies revealed almost no surface staining for the γ chain. Furthermore, our radiolabeled IL-2 binding studies detected only ~300 intermediate affinity IL-2R on resting NK cells, which is compatible with the immunofluorescence data. In this regard, normal NK cells resemble the expanded NK cell population in the peripheral blood of cancer patients receiving low dose IL-2 therapy. We found that such expanded NK cells also express IL-2Rβ in excess of IL-2α and IL-2Rγ by immunofluorescence analysis. Voss et al. (43) have previously shown that NK cells expanded in vivo after high dose IL-2 express abundant IL-2Rβ, but only a small fraction of the β chains appear to be associated with γ chains. Although Nagler et al. (42) have reported that NK cells express a relatively high number of intermediate affinity binding sites per cell, our results are more consistent with those of Voss et al. (43), indicating only a relatively low number of intermediate affinity IL-2R on resting NK cells.

After in vitro activation, expression of the β chain on NK cells remains relatively unchanged whereas both IL-2Rα and IL-2Rγ are upregulated. Thus, activated NK cells express a relatively low number of high affinity heterotrimers and intermediate affinity $\beta\gamma$ heterodimers together with an excess of presumably nonfunctional β chains. With more prolonged culture, α and γ chain expression declines to background levels, leaving only β chain detectable on the cell surface. In contrast to T cells, we did not find persistent γ chain on long-term activated NK cells. The very low expression of γ chain on either resting or activated NK cells also suggests that these cells would not be responsive to other cytokines that use the γ chain to facilitate cell surface interactions.

The differences in IL-2R subunit expression by T and NK cells underscore the distinct role of IL-2 in the biology of these lymphocytes. Naive, resting T cells do not express functional IL-2R. After stimulation with antigen or mitogen, T cells secrete IL-2 and express high affinity IL-2R heterotrimers. Furthermore, autocrine stimulation via the IL-2/IL-2R pathway supports the proliferation and functional differentiation of effector T cells. Upon withdrawal or clearance of antigen, T cells downregulate both IL-2Rα and β but appear to become unresponsive to exogenous IL-2 primarily because of the marked decrease in expression of β chain. In contrast to T cells, resting NK cells express intermediate affinity IL-2R and can respond to IL-2 in the absence of other stimuli. We have demonstrated that resting NK cells constitutively express a very small number of functional $\beta\gamma$ heterodimers and a large excess of isolated β chains. The isolated β chains would only bind IL-2 with extremely low affinity and may not be functional under physiologic conditions. Thus the IL-2 response of resting NK cells appears to be regulated primarily by the limited expression of both α and γ chain.

Despite constitutive expression of γ chain message by lymphoid cells, we have demonstrated that γ chain protein expression is tightly and differentially regulated on distinct lymphocyte subsets. It is likely that the precise composition of the IL-2R and of other multi-chain receptors that include the γ chain will be different on various other hematopoietic cells. In conjunction with mAbs specific for other IL-2R components, mAbs specific for IL-2Rγ should be useful reagents to elucidate the biology of complex hematopoietic cytokine receptors.

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