

THE HUMAN INTERLEUKIN 2 RECEPTOR  $\beta$  CHAIN (p70)  
Direct Identification, Partial Purification, and Patterns of Expression on  
Peripheral Blood Mononuclear Cells

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The interaction of IL-2 with specific cellular receptors plays a key role in antigen-stimulated proliferation of T cells. IL-2 is secreted by activated T cells and can bind to low- and high-affinity receptors concomitantly expressed ( $K_d$  generally reported as 1–30 nM and 5–90 pM, respectively; for example see reference 1). High- but not low-affinity receptors mediate internalization of IL-2 (2).

Until recently (3–6) the biochemical features distinguishing these two classes of receptors were unknown, although on human cells, both classes were known to share the epitope of monoclonal anti-Tac antibody (7). Tac antigen is a 55-kD glycoprotein (p55) capable of binding IL-2 (7, 8). Crosslinking [<sup>125</sup>I]IL-2 to activated T cells provided indirect data for a 70–77-kD membrane protein (p70, IL-2-R- $\beta$ ), which upon associating with p55 (IL-2-R- $\alpha$ , Tac antigen) transforms it into a high-affinity site (3). A broad band at 68–72 kD represents IL-2 cross-linked to p55 (reference 3 and Fig. 1 A, lane a). An 85–92-kD doublet (reference 3 and Fig. 1 A, lane a) was immunoprecipitated with anti-IL-2 mAb 17A1 (Fig. 1 A, lane b) but not with anti-p55 antisera (3). This doublet was hypothesized to represent IL-2 complexed to a protein of 70–75 kD (3–6), which appeared capable of binding IL-2 directly (4–6).

We now report the direct identification of IL-2-R- $\beta$  (p70) as a 65–77-kD glycoprotein, distinct from p55. We detail a purification scheme for the IL-2/p70 complex and study expression of p70 on subsets of PBMC.

### Materials and Methods

*Cell Preparation and Cell Culture.* HUT-102B2, an HTLV-I-transformed human T cell line, was grown as described (3). YT, a human NK-like cell line (9), was grown in Iscove's modified Dulbecco's medium supplemented with 10% calf serum, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, and 3 g/liter sodium bicarbonate.

Human PBMC were isolated on Ficoll-Hypaque (LSM; Litton Bionetics, Kensington, MD) gradients. CD4<sup>+</sup> and CD8<sup>+</sup> T cells (98–99% pure) were prepared by depletion of plastic and nylon wool adherent PBMCs and subsequent flow cytometric sorting. B cells were purified by rosetting monocyte-depleted PBMC (<1% nonspecific esterase-positive

cells) with 2-amino-ethylisothiuronium bromide-treated SRBC, and removing the rosette-focusing cells. Such cells were 80% surface Ig<sup>+</sup>, latex noningesting cells. Cells were cross-linked before or after 48 h of culture in the presence of 1:10,000 *Staphylococcus aureus* Cowan I (SAC; Calbiochem-Behring Corp., San Diego, CA). Monocytes were prepared by elutriation of PBMC and were 95% pure by Giemsa stain and flow cytometry. Cells were crosslinked before or after 48 h of culture in the presence of 10 µg/ml LPS (Sigma Chemical Co., St. Louis, MO) and 50 U/ml of IFN-γ (Biogen, Cambridge, MA).

**Crosslinking <sup>125</sup>I-labeled IL-2 to Cells.** [<sup>125</sup>I]IL-2 (New England Nuclear, Boston, MA) was bound to cells, crosslinked with disuccinimidyl suberate (DSS; Pierce Chemical Co., Rockford, IL), and lysed in extraction buffer (3) before electrophoresis or immunoprecipitation.

**Direct Identification of p70.** HUT-102B2 cells were labeled overnight with 125 µCi/ml [<sup>3</sup>H]mannose (ICN K & K Laboratories Inc., Plainview, NY) in RPMI 1640 medium containing 10% dialyzed FCS, 200 mg/liter glucose, and 30 mg/liter L-glutamine. 2 × 10<sup>8</sup> cells were washed and resuspended in binding buffer (3), tumbled with 400 pM IL-2 for 1 h at 4°C, pelleted at 400 g, resuspended at 2.5 × 10<sup>6</sup> cells/ml of PBS, 1 mM MgCl<sub>2</sub>, pH 8.3, and crosslinked with dithiobissuccinimidyl propionate (DSP; Pierce Chemical Co.), as described for DSS (3). Cells were extracted, precipitated with 17A1 or control RPC5 (Litton Bionetics) mAbs as described (3), boiled in SDS, and mixed with molecular weight markers. A 7.5% two-dimensional nonreducing/reducing diagonal gel was performed as described (10).

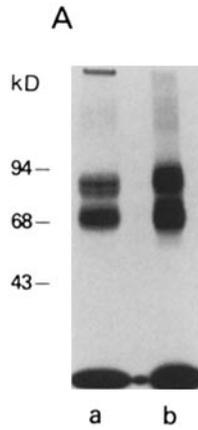
**Preparative Immunoaffinity Column.** 0.5–3.0 × 10<sup>10</sup> YT cells were incubated in binding buffer with 6 nM IL-2 for 1 h at 4°C, were resuspended in crosslinking buffer, and DSS (20 µg/ml) was added as described (3). Cells were extracted and extracts of YT cells were crosslinked with [<sup>125</sup>I]IL-2 added to the preparative lysate. Total lysate at 0.65% SDS was passed over a 17A1-Sepharose 4B column. The column was washed with 40 vol of 50 mM Tris, 300 mM NaCl, 0.5% NP-40, 0.65% SDS, pH 7.4; 20 vol of 10 mM Tris, 300 mM NaCl, 0.1% NP-40, 0.1% SDS, pH 7.4; and 10 vol of 10 mM Tris, 0.1% SDS, pH 7.4; and eluted with 2.5 vol of 0.1 M Tris, 500 mM NaCl, 0.1% SDS, pH 11.4. The eluate was neutralized with HCl, concentrated in Centricon-30 tubes (Amicon Corp., Danvers, MA) and 20% analyzed by silver staining of an SDS gel to assess yield and purity.

## Results

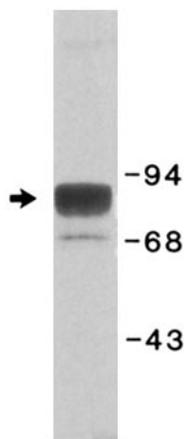
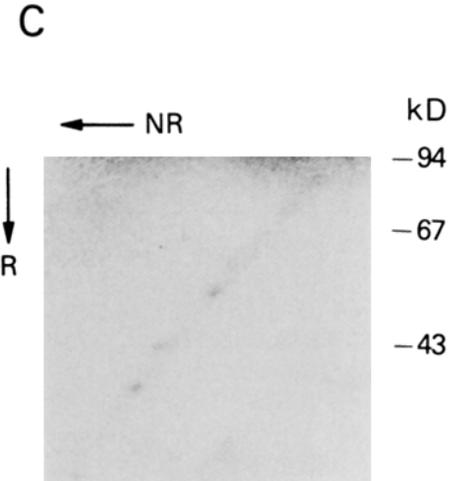
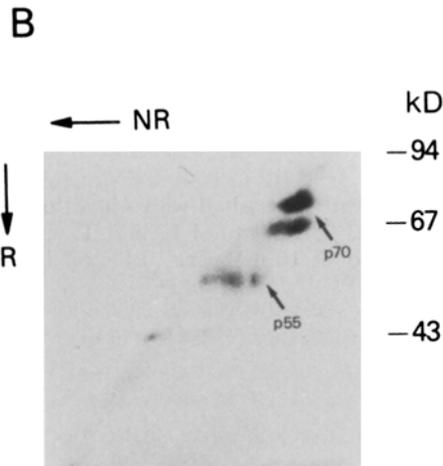
**Direct Identification of IL-2-R-β as a 65–77-kD Glycoprotein.** To directly identify the unknown species in the 85–92-kD complex, we bound 400 pM IL-2 to [<sup>3</sup>H]mannose-labeled HUT-102B2 cells, crosslinked using the cleavable crosslinker, DSP, and we analyzed anti-IL-2 (17A1) immunoprecipitates of cellular lysates on two-dimensional diagonal SDS gels (Fig. 1 B). IL-2-R-β was identified as a 65–77-kD doublet, most consistent with cleavage in the second dimension of a single, previously crosslinked IL-2 molecule (Fig. 1 B). This is the first demonstration of p70 as a distinct, uncrosslinked glycoprotein. p55 was also detected below the diagonal. Immunoprecipitation with control RPC5 yielded no spots below the diagonal (Fig. 1 C).

**Purification of IL-2/p70 Complexes.** With direct evidence that p70 was distinct from p55, we were interested in purifying p70. Because of diminished ability of solubilized p70 to bind IL-2 (unpublished observations) we isolated the IL-2/p70 complex using an anti-IL-2 immunoaffinity purification technique and YT cells, which express high levels of p70 (5). By comparison to standard protein markers, we estimate our yield to be on the order of 1 µg of the IL-2/p70 complex (~10 pmol) per preparation (Fig. 2). We are using this material as an immunogen and for microsequencing.

**Expression of p70 on PBMC.** Above, we studied p70 as a component of high-affinity IL-2-Rs. Yet, data suggest p70 can bind IL-2 independently of p55 (4–



**FIGURE 1.** (A) Crosslinking HUT-102B2 cells to 100 pM [ $^{125}$ I]IL-2 (high-affinity conditions) with DSS and analysis by SDS-PAGE. (Lane *a*) Extracts of treated cells. (Lane *b*) Anti-IL-2 (17A1) immunoprecipitates of these extracts. (B and C) Two-dimensional diagonal gel analysis of IL-2-R chains. HUT-102B2 cells were labeled with [ $^3$ H]mannose, bound to 400 pM IL-2, and cross-linked with the cleavable crosslinker, DSP. Extracts were immunoprecipitated with 17A1 (B) or a control antibody, RPC5 (C), and subjected to electrophoresis on a two-dimensional nonreducing (NR)/reducing (R) gel system.



**FIGURE 2.** Immunoaffinity-purified IL-2/p70 complex.  $2 \times 10^{10}$  YT cells were bound to 6 nM IL-2, crosslinked with DSS, extracted, and lysate was passed over a 17A1 column. An aliquot of the column eluate was analyzed by silver stain of an SDS gel. The arrow denotes a silver-impregnated band that comigrates with the [ $^{125}$ I]IL-2/p70 band that was copurified on the same 17A1 column.

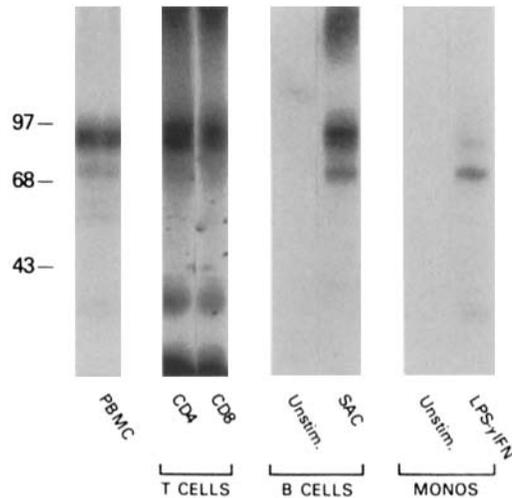


FIGURE 3. PBMCs were cross-linked using 1 nM [ $^{125}$ I]IL-2 and DSS. Lysates from  $10 \times 10^6$  cells were subjected to electrophoresis in each lane. PBMC and CD4 $^+$  and CD8 $^+$  T cell subsets were crosslinked immediately after preparation. B cells and monocytes were crosslinked before or after 48 h of culture with SAC or LPS and IFN- $\gamma$ . The additional bands in the T cell lanes represent high molecular mass material not entering the gel, free IL-2 at the dye front, and a band of  $\sim 35,000$ , which is seen with variable intensity.

6). Further, high-dose IL-2 can induce proliferation (11) and cytolytic activity (12, 13) on Tac-negative PBMC. We therefore evaluated subpopulations of human PBMC for the presence of p70 (Fig. 3). [ $^{125}$ I]IL-2 was crosslinked to PBMC, and cell extracts were analyzed by SDS-PAGE. p70 was indeed present on circulating PBMC. Densitometric analysis of several similar gels with samples derived from multiple donors indicated an upper/lower complex ratio of  $>10:1$ . Both CD4 $^+$  and CD8 $^+$  subpopulations of resting T cells expressed p70 and p55 in similar fashion to PBMCs. Thus, p70 can exist unassociated with p55 on these cells and can directly bind IL-2. Crosslinking to unstimulated B cells and monocytes showed little if any p70 or p55. Stimulation of B cells with SAC or monocytes with LPS induced expression of both p70 and p55. This effect of LPS was enhanced by IFN- $\gamma$ .

### Discussion

When IL-2 binds to activated T cells under high-affinity conditions it can be crosslinked into either 68–72-kD or 85–92-kD complexes. Although the 68–72-kD band was established as a dimer of IL-2 and p55 (3), only indirect evidence suggested the identity of the unknown component(s) in the 85–92-kD complex (3–6). We have now directly identified the IL-2-R  $\beta$  chain as a 65–77-kD glycoprotein (p70) by analysis on a two-dimensional diagonal gel, clearly establishing the existence of p70.

Immunoaffinity techniques have allowed us to partially purify IL-2/p70 cross-linked complexes, which have at least a doublet appearance on SDS-PAGE (Fig. 1 A). That isolated, uncrosslinked p70 (Fig. 1 B) also has a doublet character suggests heterogeneity, which we hypothesize is due at least in part to variable posttranslational modifications. Another (we believe less likely) possibility is that the doublet represents two distinct glycoproteins. When analyzed by a two-dimensional O'Farrell gel, the immunoaffinity-purified complex migrated within a limited pI range (5.3–5.9) in a way consistent with variable sialylation playing a part in the heterogeneity detected (unpublished observations).

Activated lymphocytes express ~10-fold more low-affinity Tac antigen (p55) sites than high-affinity sites (p55/p70-associated chains; reference 1). It is unclear, however, whether p70 can exist unassociated with p55 on the surface of such lymphoblasts. Crosslinking with  $^{125}\text{I}$ -IL-2, binding studies, flow cytometry with anti-Tac, and Northern blots with p55 cDNAs together suggest a good correlation between intensity of crosslinked bands and cell surface expression of p55 and p70 on both normal and leukemic T cells. On the basis of cross-linking, we now report that human PBMC express more p70 than p55 (see also reference 6). We also demonstrate that both the CD4<sup>+</sup> and CD8<sup>+</sup> subpopulations of T cells, but not B cells or monocytes, express p70 in the unstimulated state. However, activated B cells and monocytes express both p70 and p55, consistent with reports of high-affinity IL-2-Rs on such cells (14, 15).

rIL-2 alone can induce PBMC to proliferate (11) to evolve lymphokine-activated killer activity (12) and to augment the activity of NK cells (13). p70 plays a role in these responses and is the principal IL-2 binding protein expressed on large granular lymphocytes (16). In comparison with T cells, large granular lymphocytes express more p70 and are more responsive to IL-2 (16).

Although we have identified p55 and p70 as components of high-affinity IL-2-Rs, it is important to recognize the possibility that there may exist other subunits that are not cross-linked to IL-2. In this regard, bands of 113 and 180 kD have been observed in anti-Tac immunoprecipitations of lymphoblasts (7, 8) and studies of the murine IL-2-R provide data consistent with the possibility of a 100-kD protein as a component of the high-affinity receptor (17). We hope that the methodology described herein will facilitate development of antibodies to better define the biochemistry of p70 and its role in the immune response.

### Summary

IL-2 binds to high- and low-affinity receptors on activated T cells. The high-affinity receptor was hypothesized to consist of the noncovalent association between the  $\alpha$  chain (IL-2-R- $\alpha$ , p55) and a  $\beta$  chain (IL-2-R- $\beta$ , p70), whereas the low-affinity receptor consists of p55 without p70. We now directly identify p70 as a 65–77-kD glycoprotein doublet. Preparative quantities of the IL-2/p70 complex have been isolated. Further, we demonstrate that p70 is the principal IL-2 binding protein on both resting CD4<sup>+</sup> and CD8<sup>+</sup> T cells and that both p70 and p55 can be induced on normal B cells and monocytes.

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