*Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, Vol. 82, Suppl. II: 39-43, 1987.
*Intern. Symp. on Immunomodulators*

# STRUCTURE-FUNCTION RELATIONSHIPS FOR THE INTERLEUKIN 2 RECEPTOR SYSTEM

RICHARD J. ROBB

Medical Products Dept., Glenolden Laboratory, E. I. du Pont de Nemours & Co., Genolden, PA 19036, USA

*Receptors for interleukin 2 (IL-2) exist in at least three forms which differ in their subunit composition, their affinity for ligand and their ability to mediate a cellular response. Type I receptors occur following cellular activation and consist of the 55,000 m. w. glycoprotein Tac. These receptors bind IL-2 with a low affinity, do not internalize ligand and have not been definitively associated with any response. Type II receptors, on the other hand, consist of one or more glyproteins of 70,000 to 75,000 m. w. which have been termed "beta (β) chains." They bind IL-2 with an intermediate affinity and rapidly internalize the ligand. β proteins mediate many cellular IL-2-dependent responses, including the short-term activation of natural killer cells and the induction of Tac protein expression. Type III receptors consist of a ternary complex of the Tac protein, the β chain(s) and IL-2. They are characterized by a paricularly high affinity for ligand association. Type III receptors also internalize ligand and mediate IL-2-dependent responses at low factor concentrations. The identification of two independent IL-2-binding molecules, Tac and β, thus provides the elusive molecular explanation for the differences in IL-2 receptor affinity and suggests the potential for selective therapeutic manipulation of IL-2 responses.*

The original study of interleukin 2 (IL-2) receptors on activated lymphocytes by Robb et al. (1981) identified high-affinity binding sites which fit nicely with the very low levels of IL-2 which promoted cellular proliferation. Later studies, however, demostrated that the receptor existed in a number of forms with drastically different ligand affinities (Robb et al., 1984). Subsequent work has focused on explaining these differences in molecular terms and relating them to the function of this versatile growth and differentiation factor.

*Multiple Forms of the IL-2 Receptor* – IL-2 receptors exist in at least three forms which differ in their subunit composition, affinity for ligand and ability to mediate cellular responses (Fig. 1).

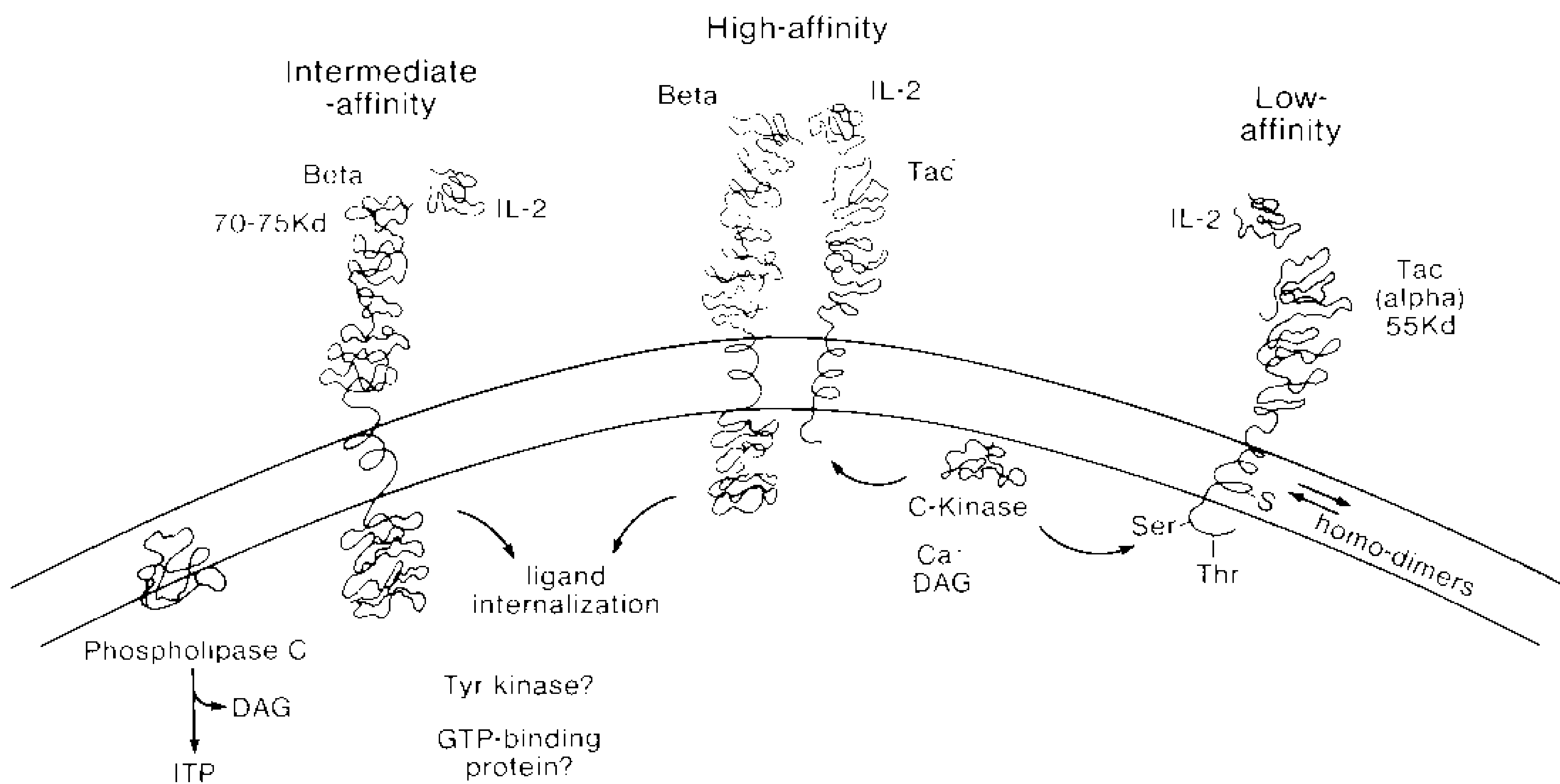


Fig. 1: Diagram of three forms of the IL-2 receptor consisting of various combinations of the Tac and β proteins. Also indicated are events (ligand internalization, activation of c-kinase [by diacylglycerol, DAG] and phospholipase C [yielding DAG and inositol triphosphate, ITP], and phosphorylation of the Tac protein) sometimes associated with binding of IL-2 to the different receptor forms. Reproduced from Robb (1987).

*A. Type I Receptors-The Tac Receptor Chain*

Activation of human lymphocytes and monocytes by antigens, lectins and certain viruses results in the surface expression of a 55,000 m. w. glycoprotein termed Tac (Leonard et al., 1982). The Tac protein is expressed in both high ($K_d \sim$ 5 to 20 pM) and low-affinity ($K_d$ 10 to 20 nM) configurations. A variety of experimental data indicates that this difference is due to the presence in high-affinity binding sites of a second receptor subunit (Hatakeyama et al., 1985; Kondo et al., 1986a, b; Robb, 1986). Thus, by itself, Tac binds IL-2 with a low affinity (Fig. 1). Such low-affinity receptors fail to internalize ligand and may be incapable of mediating IL-2-dependent responses (Fujii et al., 1986).

In crosslinking studies, low-affinity Tac receptors (55,000 m. w.) and IL-2 (15,000 m. w.) combined to form a 70,000 m. w. complex (Kuo et al., 1986). Proteolytic fragmentation of this complex demonstrated that IL-2 was covalently linked to a lysine amino group within the N-terminal 83 positions of the Tac molecule (Kuo et al., 1986). Thus, segments of the receptor protein encoded by exons 2 (residues 1-64) and 3 (residues 65-101) of the Tac gene appeared to be involved in contact with the ligand. Consistent with this notion, transfection of murine fibroblasts with a truncated form of Tac cDNA missing exons 5-8 led to the synthesis of a secreted form of the molecule (exon 7 encodes the transmembrane segment) which retained its ability to bind ligand (Neeper et al., 1987). Deletion of exon 4, however, gave rise to a form of the molecule which was incapable of IL-2 association. In fact, the shortest active form of Tac which we have thus far engineered consists of the N-terminal 163 amino acids (Robb, 1987). The requirement for segments encoded by exons 2 and 4 for the proper folding of the molecule is consistent with the presence of an intramolecular disulfide bond linking these two regions of the polypeptide chain (Fig. 2). Although it may thus be impossible to identify a short contiguous segment of amino acids sufficient for IL-2 binding, these and future studies will greatly facilitate the interpretation of crystallographic analyses of the Tac receptor protein.

*B. Type II Receptors – The Beta Receptor Chain*

Several reports describing the effect of IL-2 on natural killer (NK) cells and a particular B cell line (Ortaldo et al., 1984; Ralph et al., 1984) suggested the existence of an IL-2 receptor which did not contain the Tac protein. We obtained proof for such a novel IL-2-binding molecule during examination of an NK-like cell line termed YT (Robb et al., 1987). A Scatchard plot of binding to YT cells yielded a typical curvilinear plot indicative of high and intermediate-affinity binding (Fig. 3). Inclusion of anti-Tac antibody in the assay eliminated the high-affinity binding component, but left the predominant intermediate-affinity ($K_d \sim$ 800 pM) binding component intact. For convenience, we have termed this non-Tac IL-2 receptor "beta" ($\beta$).

That $\beta$ was distinct from Tac protein was confirmed by crosslinking studies with $^{125}$I-IL-2. Covalent attachment of IL-2 to low-affinity Tac receptors yielded a 70,000 m. w. complex on SDS gels while crosslinking to the $\beta$ receptors on unstimulated YT cells yielded a doublet at 83 and 90,000 m. w. (Fig. 4). By inference, the size of the $\beta$ protein(s) was thus 70-75,000 m. w. Several other laboratories have independently reached similar conclusions with regard to the $\beta$ protein(s) (Sharon et al., 1986; Tsudo et al., 1986; Teshigawara et al., 1987; Dukovich et al., 1987). The source of the size heterogeneity for $\beta$-IL-2 complexes on SDS gels is still under investigation. Treatment of the complexes with glycosidases reduced their size by 15-20%, but did not totally eliminate the heterogeneity (Robb et al., 1987). Thus, intermediate-affinity type II receptors may consist of two distinct proteins, although variable post-translational modification or partial proteolytic degradation also remain likely causes of the differences in electrophoretic mobility.

$\beta$ receptors occur on several different lymprocyte subpopulations in the apparent absence of activation signals (Dukovich et al., 1987). In contrast to the low-affinity type I receptors, the $\beta$ chain by itself mediates rapid ligand internalization and certain cellular responses (Robb & Greene, 1987; Robb, 1987). In particular, antibodies which selectively block $\beta$-IL-2 interactions also inhibit the IL-2-dependent increase in the cytotoxic activity of NK cells, the IL-2 dependent induction of immunoglobulin secretion on a Tac-negative B cell line, and the IL-2 dependent expression of Tac protein on YT cells (Robb, 1987). Thus, the $\beta$ chain is clearly a functional receptor for IL-2. It remains to be determined, however, whether type II receptors can mediate all IL-2 dependent responses or whether some responses, such as cellular proliferation, require high-affinity type III receptors.

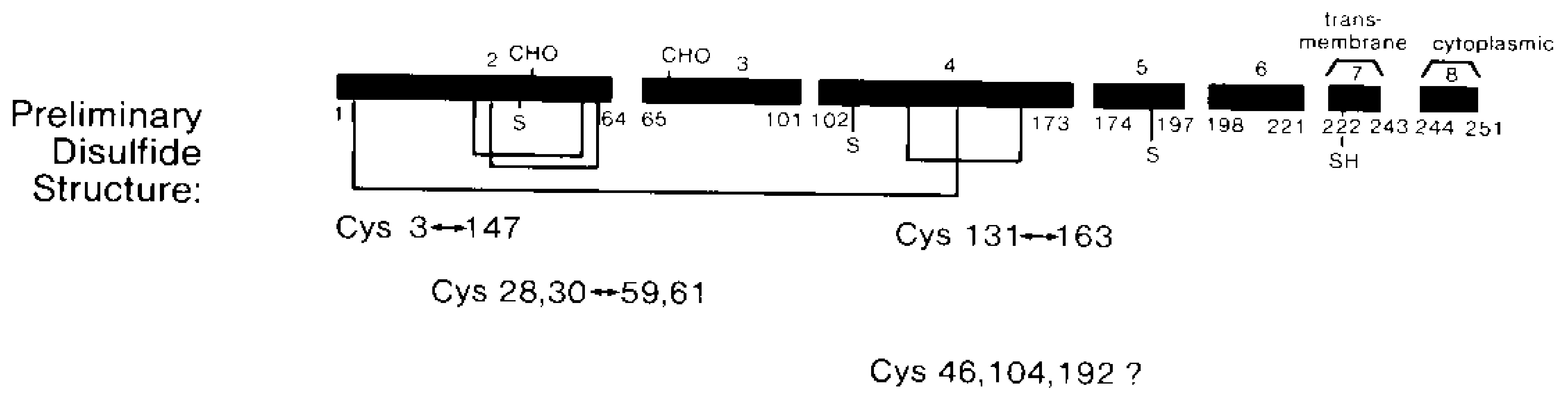


Fig. 2: Preliminary map of the disulfide structure of the Tac protein. The mature full-length Tac protein consists of 251 amino acids and is encoded by genomic exons 2-8. The disulfide structure shown here is based on the coelution of $^{35}$S-Cys-labelled peptides on reverse-phase chromatography (Robb, 1987). The proposed linkage of Cys 28 to Cys 59 and of Cys 30 to Cys 61 is based on the fact that Cys 30 and 61 occupy positions in exon 2 which are homologous to Cys 131 and 163 in exon 4. The recovery of peptides containing Cys 46, 104 and 192 was too low to determine their status. Reproduced from Robb (1987).

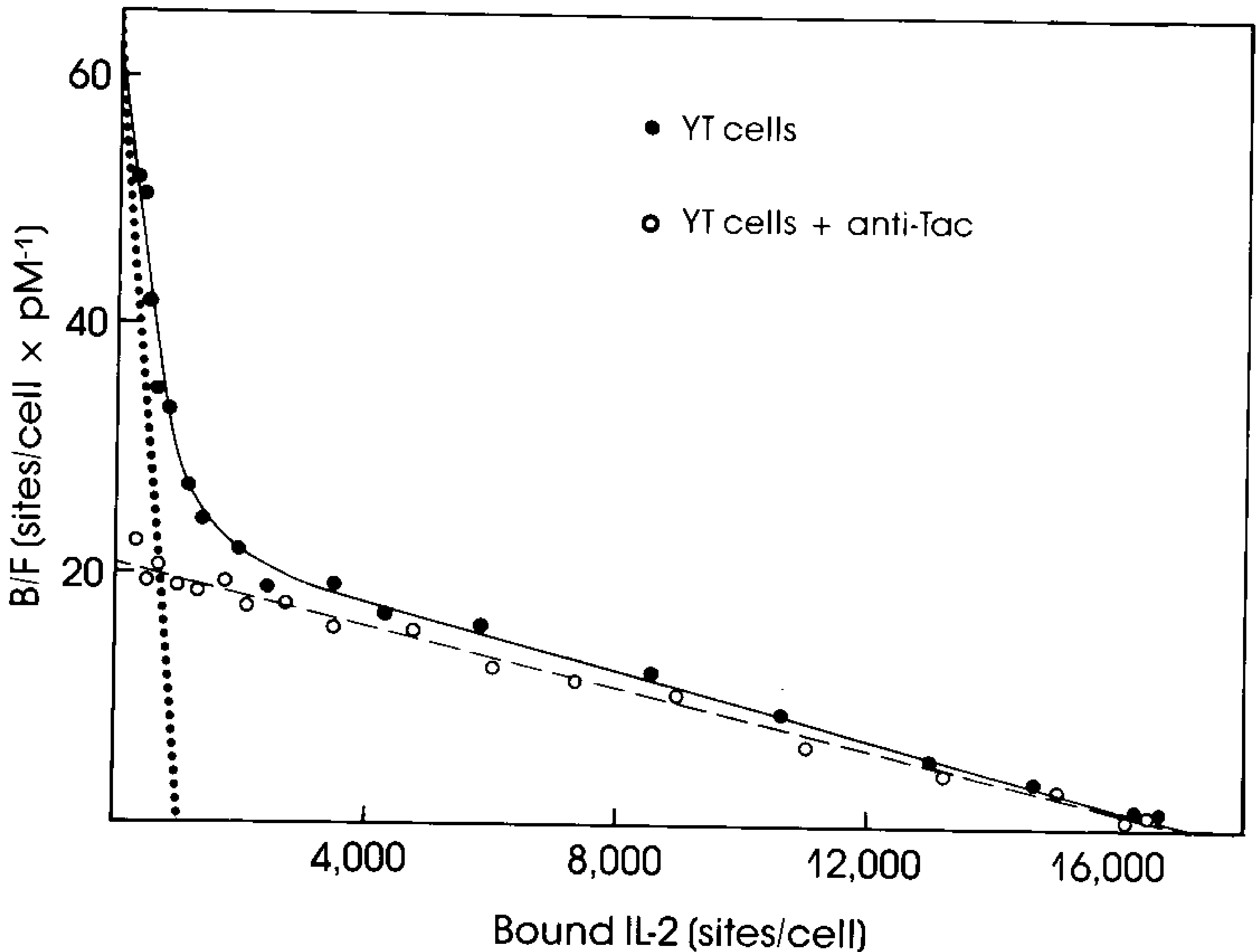


Fig. 3: Scatchard plot of the binding of $^{125}$I-IL-2 to unstimulated YT cells in the presence and absence of anti-Tac antibody. Note the loss of the high-affinity component of binding (dotted line) when anti-Tac was included in the assay. Reproduced from Robb et al. (1987).

*C. Type III Receptors – The Tac-IL-2-β Complex*

Due to their requirement for the Tac protein, high-affinity ($K_d$ ~5 to 20 pM) type III IL-2 receptors generally occur only after cellular activation. Their abundance on such cells, however, is limited by the low level of expression of β chains. Like the isolated β chains, type III receptors internalize ligand rapidly and mediate a variety of functional responses at low IL-2 concentrations (Robb et al., 1981; Robb, 1984; Fujii et al., 1986).

Several lines of evidence indicate that it is the simultaneous binding of IL-2 by both the

Tac and $\beta$ chains which provides the molecular basis for high-affinity binding sites. When YT cells were stimulated by forskolin, the number of Tac molecules on the cell suface and the number of high-affinity receptor sites increased dramatically (Table; Robb et al., 1987). At the same time, there was a parallel decrease in the original number of $\beta$ binding sites. Inclusion of anti-Tac in the assays for stimulated YT cells reversed these changes, eliminating the newly-formed high-affinity sites and restoring the original $\beta$ binding component. One explanation for these effects is that the induced Tac protein combined with $\beta$ to form high-affinity binding sites. Further supporting this concept, Dukovich et al. (1987) found that transfection of a cell line containing $\beta$ protein with cDNA for the Tac subunit resulted in the conversion of some of the $\beta$ binding sites to a high-affinity state. Crosslinking studies also suggested the involvement of Tac and $\beta$ in high-affinity binding sites. Following binding to high-affinity sites on either a T lymphocytic cell line (HUT) or stimulated YT cells, IL-2 was crosslinked to the receptor in complexes (70,83 and 90,000 m.w.) characteristic of association with *both* the Tac (70,000 m.w. complex) and $\beta$ proteins (83-90,000 m.w.) (Fig. 4). As a final piece of evidence, we found that antibodies capable of selectively blocking the binding of IL-2 to Tac or to $\beta$ could each block ligand binding to high-affinity receptor sites, implying that both Tac and $\beta$ proteins were involved (Robb et al., 1987; Robb, 1987).

*Concluding Remarks* - Molecular characterization of the three forms of the IL-2 receptor has provided a clear explanation for both the differences in ligand affinity originally noted by Robb et al., (1984) and the observation of cellular responses which were independent of the Tac receptor subunit (Ortaldo et al., 1984; Ralph et al. 1984; Robb, 1987). Looking to the future, cloning of the $\beta$ chain(s) will no doubt answer questions about the source of its heterogeneity and may provide clues as to the initial enzymatic steps in signal transducition mediated by functional receptors. In addition, mutagenesis and crystallographic analyses of both the Tac and $\beta$ subunits will aid the design of IL-2 mimicks and antagonists wich would facilitate therapeutic manipulation of the immune system.

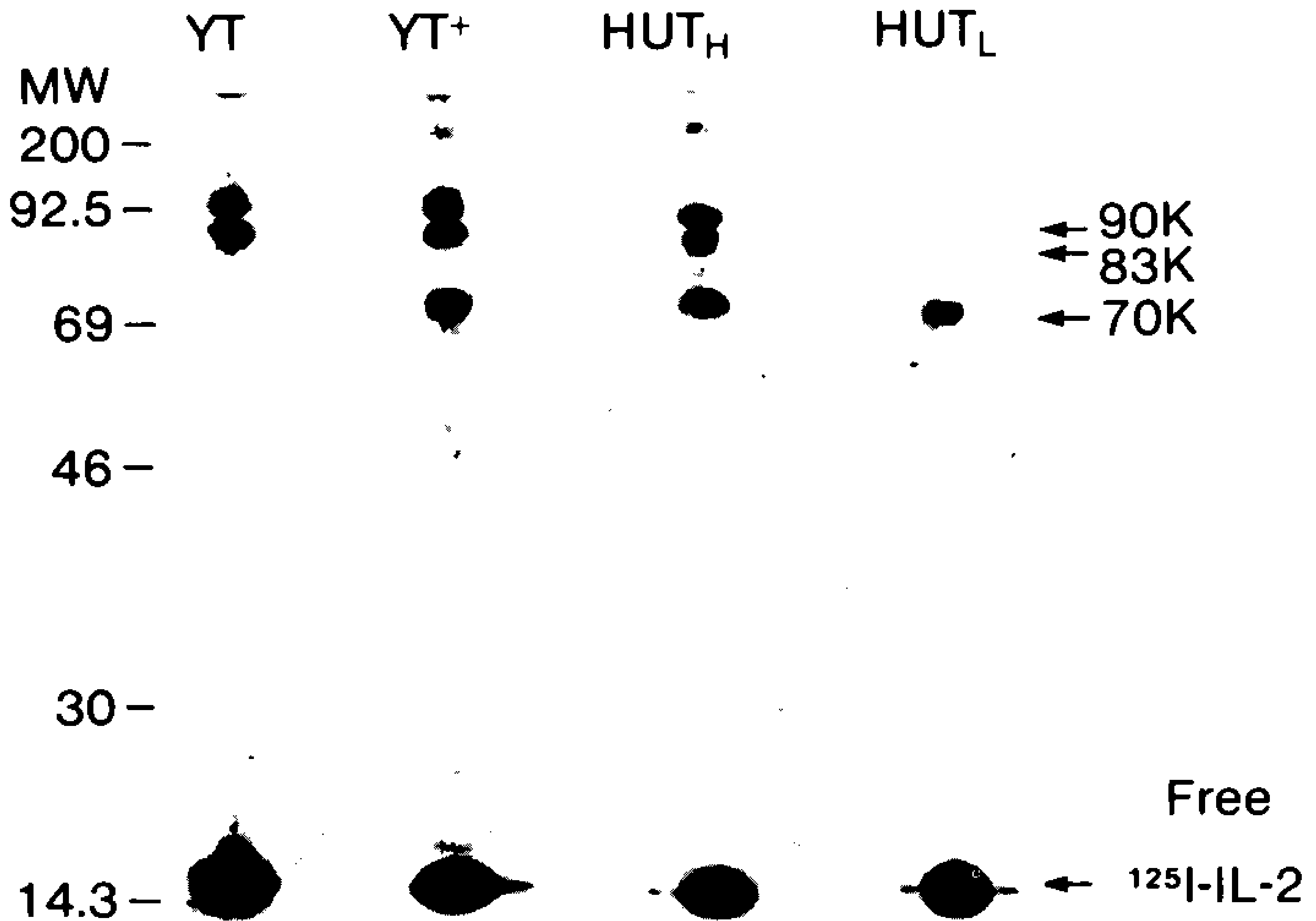


Fig. 4: SDS gel analysis of crosslinked complexes between $^{125}$I-IL-2 and receptor proteins on unstimulated YT cells (YT), forskolin-stimulated YT cells (YT$^+$) and HUT cells following high-affinity (HUT$_H$) and low-affinity (HUT$_L$) IL-2 binding. The unstimulated YT cells expressed predominantely intermediate-affinity $\beta$ chains while IL-2 binding to HUT cells under low-affinity conditions was predominantly dependent on low-affinity Tac proteins. In contrast, IL-2 association with forskolin-activated YT cells and HUT cells under high-affinity conditions required interaction with both the Tac and $\beta$ receptor subunits.

TABLE

Receptor Numbers and Affinities*

| Cell Type | Forskolin | Anti-Tac Antibody | IL-2 Binding | | | | | | Anti-Tac Binding |
|---|---|---|---|---|---|---|---|---|---|
| | | | *High-Affinity* | | *Intermediate-Affinity* | | *Low-Affinity* | | |
| | | | Sites/Cell | $K_d$ (pM) | Sites/Cell | $K_d$ (pM) | Sites/Cell | $K_d$ (pM) | Sites/Cell |
| YT | – | – | 1,160 | 17.4 | 13,100 | 810 | $<$200 | – | 1,100 |
| YT | | + | $\leqslant$20 | | 14,200 | 820 | $<$200 | – | $\leqslant$20 |
| YT | + | - | 8,950 | 18.5 | $\leqslant$2,000 | -800 | $\leqslant$1,000 | 11,000 | 10,200 |
| YT | + | + | $\leqslant$20 | – | 11,900 | 895 | $<$200 | – | $\leqslant$20 |

*Measurement of the binding $^{125}$I-IL-2 and $^3$H-anti-Tac antibody to unstimulated and forskolin-activated YT cells in the absence and presence of anti-Tac antibody.

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