CO-INTERNALIZATION OF THE p55 AND p70 SUBUNITS OF
THE HIGH-AFFINITY HUMAN INTERLEUKIN 2 RECEPTOR
Evidence for a Stable Ternary Receptor Complex

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Activated human T lymphocytes express both high-affinity (dissociation constant
\( [K_d] \approx 5-70 \) pM) and low-affinity (\( K_d = 5-30 \) nM) forms of membrane IL-2-Rs (1,
2). Cellular proliferative responses as well as receptor-mediated endocytosis of IL-2
appear to be mediated by the high-affinity IL-2-Rs but not by the low-affinity class
of receptors (1-3). Recent \(^{125}\)I-IL-2 crosslinking (4-8) and reconstitution studies
(6-8) have suggested that the high-affinity receptor corresponds to a membrane com-
plex comprised of at least two different IL-2-binding proteins, p55 (Tac, IL-2-Ra)
and p70 (IL-2-RB). Expression of p55 or p70 alone results in low or intermediate
(\( K_d \approx 0.5-1 \) nM) IL-2-Rs, respectively (6-8), while coexpression of the p55 and p70
proteins results in high-affinity heterodimeric-binding sites. The p70, but not the
p55, protein is capable of internalizing surface-bound IL-2 with kinetics indistin-
guishable from that of the high-affinity p55/p70 complex (9), suggesting that p70
is the primary component responsible for ligand endocytosis.

Although suggestive evidence has been presented for a ternary complex between
IL-2 and the noncovalently linked \( \alpha \) and \( \beta \) subunit equivalents of the murine IL-
2-R (10), chemical crosslinking studies have thus far failed to identify a similar
trimolecular complex composed of IL-2, p55, and p70 on the surface of human T
lymphocytes. Recent studies of the rates of IL-2 association and dissociation have
revealed striking differences in ligand binding to the p55, p70, and the heterodi-
meric high-affinity receptors (11, 12). The interaction of IL-2 with the p55 receptor
is characterized by very rapid rates of association and dissociation. In contrast, ligand
binding to the p70 protein occurs very slowly. The high-affinity receptor exhibits
a composite of these properties with the \( t_1/2 \) of association resembling p55 and the
\( t_1/2 \) of dissociation similar to p70 (11). These data raised a possible model for the
high-affinity receptor-mediated endocytosis of IL-2 shown in Fig. 1A. The p55 pro-
tein initially engages IL-2 at the cell surface and transfers IL-2 to p70 during the
formation of a transient complex, but then dissociates from the complex before ligand
internalization mediated by p70 alone. Alternatively, a stable IL-2/p55/p70 complex
may in fact exist such that on internalization, both the p70 and p55 receptor
subunits are removed from the cell surface (Fig. 1B). We now report studies de-
signed to distinguish between these two possible models. The human YT-1 T cell
line (13) was used with radiolabeled IL-2 and the non-neutralizing anti-p55 mAb,
7G7/B6 (14), to monitor internalization of the high-affinity IL-2-R and the p55 component. Furthermore, the IL-2 analogue, Lys-20, which binds p55 with wild-type affinity but lacks binding activity to p70 (15), was used to determine whether ligand association of the p70 subunit of the high-affinity p55/p70 receptor is essential for ligand internalization. We now demonstrate that: (a) the p55 protein is co-internalized with p70 after IL-2 binding; (b) IL-2 is either required for the assembly of the high-affinity complex or to initiate receptor-mediated endocytosis; and (c) an analogue of IL-2 that binds to only the p55 subunit (15) fails to trigger receptor-mediated endocytosis.

Materials and Methods

Cells. The YT-1, a subclone of YT (13), and HUT 102B2 cell lines were cultured in RPMI 1640, 10% FCS. YT cells (<3 × 10⁶ cells/ml) were stimulated with 10 μM forskolin (Sigma Chemical Co., St. Louis, MO) for 20–24 h to induce increased expression of the p55 (Tac) protein (YT⁺) and concommitantly, the expression of the high-affinity form of the IL-2 receptor (7).

Receptor Binding Assays. The binding of [125I]-IL-2 and [125I]-7G7/B6 was determined as previously described (1, 2). Purified human rIL-2 was obtained from Cetus Corp. (Emeryville, CA). Iodinated rIL-2 ([125I]-IL-2, 43.2 μCi/μg) was obtained from New England Nuclear, Boston, MA. The mAb 7G7/B6 specifically recognizes the p55 and not the p70 subunit of the high-affinity IL-2-R, but does not interfere with IL-2 or anti-Tac binding (14). The Lys-20 IL-2 analogue binds to p55 with a similar affinity as wild-type IL-2, but is markedly impaired with regard to p70-binding activity (15). The 7G7/B6 mAb and the Lys-20 IL-2 were iodinated to sp act of 8.2 and 50 μCi/μg, respectively.

Internalization Time Courses. Ligand internalization was measured as described elsewhere (9) with minor modifications. To determine the kinetics of IL-2 internalization under high-affinity conditions, YT⁺ cells (10⁶) were preincubated at 4°C with 75 pM [125I]-IL-2 and 10 nM unlabeled 7G7/B6. To monitor the fate of p55, cells were similarly incubated with 75 pM unlabeled IL-2 and 10 nM [125I]-7G7/B6. [125I]-7G7/B6 alone was incubated with cells to assess the level of p55 internalization in the absence of IL-2. For the experiments using Lys-20 IL-2, assays were performed under low-affinity binding conditions (10 nM).

Results and Discussion

To distinguish between the two possible models for the high-affinity IL-2-R presented in Fig. 1, IL-2, Lys-20 IL-2, and the anti-p55 mAb, 7G7/B6, were first radiolabeled with Na[125I]. The binding characteristics of each of these radiolabeled ligands on HUT 102, uninduced YT, or forskolin-induced YT cells (YT⁺) are summarized in Table I. Using [125I]-IL-2, both high- and low-affinity IL-2-R were detected on HUT 102 cells while uninduced YT cells primarily displayed intermediate-affinity p70 receptors. As previously reported (7), stimulation of YT cells with forskolin (YT⁺) induced the expression of the p55 protein and led to the almost complete conversion of the intermediate-affinity p70-binding sites to high-affinity p55/p70-binding sites. Thus, these YT⁺ cells provide a near homogeneous population of high-affinity receptors, permitting determination of whether the p55 component is internalized as a part of a stable receptor complex (Fig. 1 B) or alternatively, dissociates from a transiently formed complex and remains at the cell surface (Fig. 1 A). The binding of 7G7/B6 correlated with the level of p55 expression and no interaction of this mAb with p70 was observed. Lys-20 IL-2, an IL-2 derivative with a mutation in the NH₂-terminal p70-binding domain (15), did not have detectable
The number of binding sites (n) and the dissociation constants (Kd) were determined by Scatchard analysis of binding data performed at 4°C for each indicated ligand.

p70-binding capacity under these experimental conditions, but bound effectively to the p55 subunit. As engagement of both the p70 and p55 proteins is required for expression of high-affinity binding sites, only low-affinity binding was detectable with Lys-20 IL-2 (Table I).

**Figure 1.** Two possible models for IL-2 internalization mediated by the high-affinity receptor. Fundamentally, these models differ in whether the p55 protein dissociates from a transient trimolecular complex before ligand internalization by p70, or alternatively, is cointernalized as part of a stable complex with p70 and IL-2.

| Ligand*          | Affinity | HUT 102B2 | YT | YT' |
|------------------|----------|-----------|----|-----|
|                  | K_d      | n         | K_d| n   | K_d | n   |
| IL-2             | pM       | pM        | pM |     |     |     |
| High             | 70       | 7,000     | 10 | 350 | 74  | 9,600 |
| Intermediate     | -        | -         | 570| 10,000| -   | -   |
| Low              | 5,000    | 100,000   | -  | -   | -   | -   |
| LYS-20           |          |           |    |     |     |     |
| High             | ND       | ND        | -  | -   | -   | -   |
| Intermediate     | ND       | ND        | -  | -   | -   | -   |
| Low              | ND       | ND        | 9,000| 400 | 15,000| 9,500 |
| 7G7/B6           |          |           |    |     |     |     |
|                  | 12,000   | 200,000   | -  | -   | 12,000| 9,500 |

* The number of binding sites (n) and the dissociation constants (K_d) were determined by Scatchard analysis of binding data performed at 4°C for each indicated ligand.

- Undetectable

TABLE I

The Binding Characteristics of IL-2, Lys-20, and 7G7/B6
The internalization of radiolabeled IL-2 in the presence or absence of the unla-
beled 7G7/B6 antibody was determined first (Fig. 2 A). Surface-bound $^{125}$I-IL-2 was
rapidly internalized by YT' cells with a $t_{1/2}$ of 7-10 min, which is consistent with
the kinetics of ligand endocytosis mediated by both the intermediate- and high-affinity
class of receptors (3, 9, 12). Simultaneous binding of the p55 mAb, 7G7/B6, did
not alter the kinetics of IL-2 internalization. This finding permitted the use of $^{125}$I-
7G7/B6 and unlabeled IL-2 to clearly distinguish between models A and B of the
high-affinity IL-2-R presented in Fig. 1. As shown in Fig. 2 B, significant internali-
zation of $^{125}$I-7G7/B6 was detected in the presence of unlabeled IL-2. The antibody
was rapidly removed from the cell surface with kinetics similar to that for IL-2 bound
either to the high-affinity receptor complex or to the p70 protein alone (9). Approximately
40\% of surface-bound $^{125}$I-7G7/B6 was internalized upon IL-2 binding. This
is consistent with the concentration of IL-2 used (75 pM), which only titrates ~50\%
of the high-affinity binding sites present at the cell surface. These findings demon-
strate that the p55 subunit is endocytosed as a component of the heterodimeric high-
affinity human IL-2-R complex. In contrast, only low levels of internalized $^{125}$I-
7G7/B6 were detected in studies performed in the absence of IL-2. Occupation of
the IL-2-binding site on the p55 subunit by the antagonistic mAb, anti-Tac, instead
of IL-2 (data not shown), also failed to promote internalization of $^{125}$I-7G7/B6. The
requirement for IL-2 for 767/B6 internalization raises two possibilities. First, the
human high-affinity IL-2-R complex may be preassembled at the cell membrane,
but fails to cycle into the cell interior in the absence of ligand. Alternatively, the
p55 and p70 receptor subunits may not exist in a preassembled state, however, in
the presence of IL-2, the formation of the high-affinity receptor complex is induced.

Studies were also performed with the Lys-20 analogue of IL-2 to investigate whether
engagement of the p70 component of the high-affinity receptor was a prerequisite

Figure 2. Receptor-mediated endocytosis of $^{125}$I-IL-2 (A) and
$^{125}$I-7G7/B6 (B) in forskolin-stimulated YT cells
(YT'). Internalization was determined by binding of IL-
2 or antibody to YT' cells at 4°C under high-affinity con-
ditions for IL-2 (75 pM) and approximately half-saturating
conditions for 7G7/B6 (10 nM). Nonspecific binding was
determined by the addition of a 200-fold molar excess of
unlabeled ligand. Internalization was initiated by rapidly
increasing the temperature of the cells to 37°C. At selected
times, aliquots were removed, the cell pellets were washed,
and specific surface-bound ligand was completely dissociated
by resuspension in RPMI 1640, 10% FCS-HCl, pH 3. The
radioactivity associated with the cell pellet corresponded to
internalized ligand. $^{125}$I-IL-2 was incubated in the presence
and absence of cold 7G7/B6 to evaluate possible effects of
the anti-p55 antibody on IL-2 endocytosis (A). Similarly,
$^{125}$I-7G7/B6 was incubated with or without cold IL-2 to
assay levels of IL-2-inducible vs. noninducible internaliza-
tion of the antibody (B).
for ligand internalization. As noted, this analogue binds to p55 with low affinity either in the presence or absence of p70, but lacks p70-binding activity (15 and Table I). Under binding conditions permitting titration of nearly half of the available sites, no significant $^{125}$I-Lys-20 IL-2 internalization was detected (Fig. 3). In contrast, wild-type IL-2 was readily internalized. These findings argue that, despite the presence of or potential for high-affinity receptor display, effective ligand engagement of the p70 subunit is required for receptor-mediated endocytosis of IL-2.

Together, these findings clearly identify Fig. 1B as the correct model for the high-affinity IL-2-R. Not only are both the p70 and p55 subunits intrinsic to IL-2 binding of the high-affinity receptor, but both moieties are cointernalized together. These results support the existence of a stable p55/p70/IL-2 ternary membrane receptor complex. The nature of the interaction between the p55 and p70 chains, however, remains unknown. In particular, whether these subunits are associated in the absence of ligand is an unresolved issue in the human system. However, in the murine system, recent crosslinking studies suggest that the α and β receptor subunits do in fact interact in the absence of IL-2 (16). It is also possible that a more complex, multimeric receptor complex exists. Chemical crosslinking studies involving the murine IL-2-R have suggested a possible $M_r$ 100,000 γ chain (10), while resonance energy transfer studies in human T cells support the possible existence of a 95-kD receptor subunit (17). Further characterization of the full spectrum of receptor subunits, including p70, should provide additional insights into the structure of the high-affinity IL-2-R and its mechanism of signal transduction.

Summary

The high-affinity IL-2-R complex is composed of at least two distinct IL-2-binding subunits, including p55 (Tac, IL-2-Rα) and p70 (IL-2-Rβ). Using a radiolabeled mAb specific for the p55 receptor subunit and cells expressing a homogeneous population of high-affinity binding sites, we demonstrate that p55 is co-internalized with p70 after IL-2 binding to the receptor complex. Endocytosis of p55 depends upon the presence of IL-2 in a form capable of effectively interacting with the p70 subunit. Whether IL-2 is required for high-affinity receptor assembly or triggering of the internalization of preassembled receptors remains unresolved. Together, these findings support the existence of a stable, high-affinity human IL-2-R membrane complex composed of at least the p55 and p70 receptor subunits and IL-2.
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