Brief Definitive Report

CO-INTERNALIZATION OF THE p55 AND p70 SUBUNITS OF THE HIGH-AFFINITY HUMAN INTERLEUKIN 2 RECEPTOR

Evidence for a Stable Ternary Receptor Complex

By MARION R. FUNG, GRACE JU,* AND WARNER C. GREENE

*From the Howard Hughes Medical Institute, Departments of Medicine and Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710; and the *Department of Molecular Genetics, Roche Research Center, Hoffman-La Roche Inc., Nutley, New Jersey 07110*

Activated human T lymphocytes express both high-affinity (dissociation constant [K_d] ~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 complex comprised of at least two different IL-2-binding proteins, p55 (Tac, IL-2-Rα) and p70 (IL-2-Rβ). Expression of p55 or p70 alone results in low or intermediate (K_d ~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 indistinguishable 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 α and β 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 heterodimeric 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. 1 *A*. The p55 protein 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. 1 *B*). We now report studies designed 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 ($<3 \times 10^5$ 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 ^{125}I-Il-2 and ^{125}I-7G7/B6 was determined as previously described (1, 2). Purified human rIL-2 was obtained from Cetus Corp. (Emeryville, CA). Iodinated rIL-2 (^{125}I-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 (10^6) were preincubated at 4°C with 75 pM ^{125}I-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 ^{125}I-7G7/B6. ^{125}I-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^{125}I. 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 ^{125}I-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 NH_2-terminal p70-binding domain (15), did not have detectable

TABLE I

The Binding Characteristics of IL-2, Lys-20, and 7G7/B6

		Cell type					
		HUT 102B2		YT		YT^+	
Ligand*	Affinity	K_d	n	K_d	n	K_d	n
		pM		*pM*		*pM*	
IL-2	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

-, Undetectable

* 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.

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).

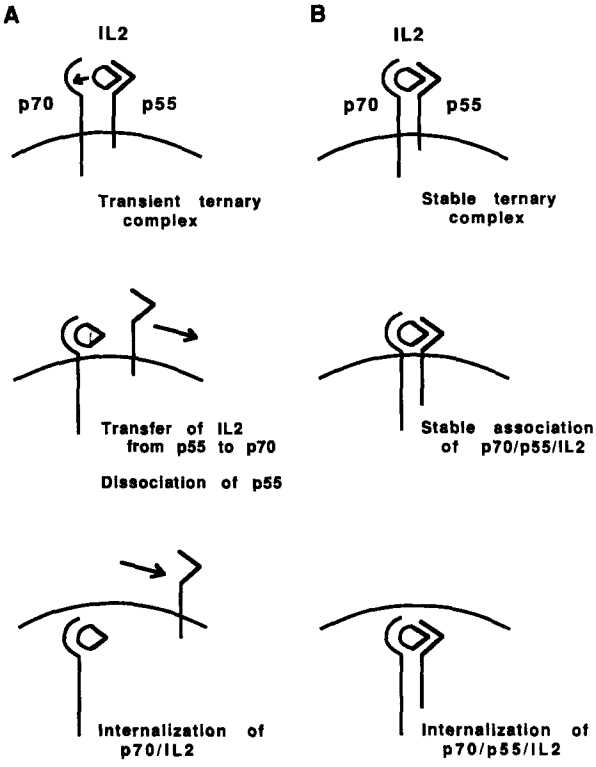

FIGURE 1. Two possible models for IL-2 internalization mediated by the high-affinity receptor. Fundamentally, these models differ in whether the p55 protein dissociates from a transient trimolecular complex before ligand internalization by p70, or alternatively, is cointernalized as part of a stable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

The internalization of radiolabeled IL-2 in the presence or absence of the unlabeled 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 internalization 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 demonstrate 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

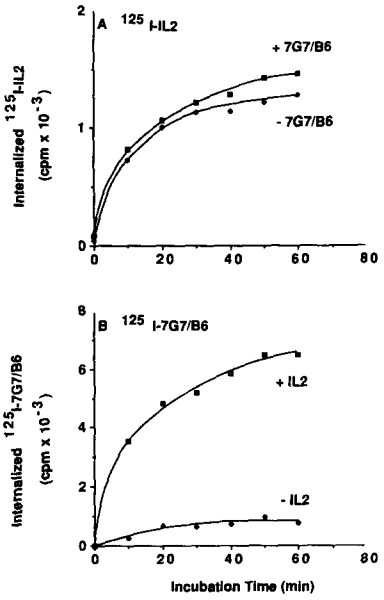

FIGURE 2. Receptor-mediated endocytosis of ^{125}I-IL-2 (*A*) and ^{125}I-7G7/B6 (*B*) in forskolin-stimulated YT cells (YT$^+$). Internalization was determined by binding of IL-2 or antibody to YT$^+$ cells at 4°C under high-affinity conditions for IL-2 (75 pM) and approximately half-saturating conditions 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 internalization of the antibody (*B*).

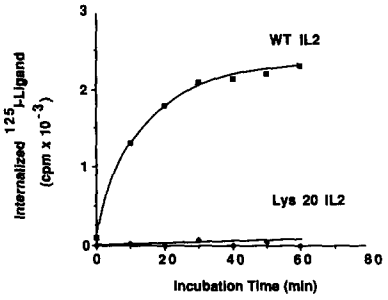

FIGURE 3. Receptor-mediated endocytosis of ^{125}I-IL-2 vs. ^{125}I-Lys-20 analogue of IL-2 in YT^{+} cells. Assays were performed at concentrations of these ligands approximating their K_ds.

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. 1 *B* 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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