Brief Definitive Report

INTERNALIZATION OF INTERLEUKIN 2 IS MEDIATED BY THE β CHAIN OF THE HIGH-AFFINITY INTERLEUKIN 2 RECEPTOR

BY RICHARD J. ROBB* AND WARNER C. GREENE‡

*From the *Medical Products Department, E.I. duPont de Nemours & Company, Glenolden Laboratory, Glenolden, Pennsylvania 19036; and the ‡Metabolism Branch, National Cancer Institute, Bethesda, Maryland 20892*

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High-affinity and low-affinity forms of the human interleukin 2 receptor (IL-2-R) have been identified on the surface of activated lymphocytes (1, 2). The high-affinity receptors appear to be essential for IL-2-dependent cellular proliferation (1, 2), while the biological function of the low-affinity binding sites remains undefined. The postbinding fate of IL-2 associated with high- and low-affinity receptors also differs. IL-2 binding to the high-affinity sites results in rapid receptor-mediated endocytosis of the growth factor–receptor complex (1, 3–5), while ligand binding to the low-affinity sites does not lead to internalization (4, 5).

The marked difference in ligand endocytosis mediated by the high- and low-affinity forms of the IL-2-R points to a basic structural variation. Recently, several laboratories have assembled chemical crosslinking and receptor reconstitution data which suggest that the high-affinity IL-2-R corresponds to a membrane receptor complex (6–9). This complex is composed of at least two polypeptide chains (termed α and β), each of which is independently capable of binding IL-2. The α chain corresponds to the well-characterized Tac antigen (10), while the β chain is a recently recognized 70–75 kD glycoprotein(s) that is not precipitated by the anti-Tac antibody (7–9). Cell lines have been identified that express either the α or β chain of the receptor in the absence of the other chain (4, 8, 9). Because only one of the chains is present, these cells do not express high-affinity IL-2-R, but rather bind IL-2 with a low or intermediate affinity. For example, HTLV-I-infected MT-1 cells express only the α chain and bind IL-2 with a low affinity (apparent K_d of 10–30 nM) (4). The gibbon ape MLA-144 T cell line and the human natural killer–like YT cell line (11), on the other hand, express predominantly or exclusively the β chain and bind IL-2 with an intermediate affinity (apparent K_d of 0.6–1.0 nM) (8, 9). We now report studies with these cell lines which demonstrate that the β chain alone is able to mediate endocytosis of surface-bound IL-2 as rapidly as the α/β heterodimeric high-affinity IL-2-R.

Materials and Methods

Cells. All human and primate cell lines were maintained in RPMI 1640 containing 10% heat-inactivated FCS. For certain experiments, the YT cells (3×10^5 cells/ml) were

treated with 10 μM forskolin (Sigma Chemical Co., St. Louis, MO) for 20 h at 37°C to induce increased expression of the Tac protein (YT^+).

Receptor Binding Assays. Receptor numbers and dissociation constants (K_d) were measured as previously described (1, 2) with minor modifications. The binding assays were performed in 100 μl RPMI 1640, 25 mM Hepes, pH 7.2, 5 mg/ml BSA (1640-BSA) containing serial dilutions of the labelled probes. IL-2 was radioiodinated as described (12), while anti-Tac antibody was labelled by reductive methylation (10). Cells and labelled probes were incubated at 37°C for 8–15 min, followed by separation of bound and free ligand by immediate centrifugation of the cells through a 400 μl layer of 81% silicone oil (phenylmethylpolysiloxane; Dexter Hysol, New York) and 19% paraffin oil (0–121; Fisher Scientific Co., Philadelphia, PA).

Internalization Time Course. Cells (10^7 cells/ml for YT, YT^+, HUT, and MT-1, and 5×10^7 cells/ml for MLA-144) were first incubated for 5 min at 37°C in 1640-BSA containing 100 μM chloroquine, a lysomotropic agent that prevents degradation of internalized IL-2 (1, 3). In the case of unstimulated YT cells, unlabelled anti-Tac antibody was added at a concentration (80 μg/ml) that eliminated all high- and low-affinity Tac-dependent binding, leaving behind only intermediate-affinity (K_d ~800 pM) β chain binding sites (9). ^{125}I-IL-2 was then added at final concentrations of 75 pM (YT^+, HUT) or 1 nM (YT, MT-1, MLA) to select for high-affinity (α plus β) and low- to intermediate-affinity (α or β) binding, respectively. After 20 min at 0°C, most of the cells (YT, YT^+, HUT, MLA) were washed twice with ice-cold 1640-BSA to remove unbound IL-2 and resuspended at 10^7 cells/ml (5×10^7 cells/ml; MLA) in prewarmed (37°C) 1640-BSA containing 100 μM chloroquine. Anti-Tac (80 μg/ml) was added to the suspension of unstimulated YT cells. At selected times, 100-μl aliquots of the cell suspension were removed and diluted with 1.2 ml ice-cold 1640-BSA. The cells were pelleted and the radioactivity in the supernatant was measured to determine the level of ^{125}I-IL-2 that had dissociated from the receptor sites. The cells were then resuspended in 200 μl of 10 mM citrate, pH 4, containing 0.14 M NaCl and 50 μg/ml BSA. After 15 s at 23°C, the cells were centrifuged through a 400 μl layer of silicone/paraffin oil. The radioactivity in the cell pellet and in the supernatant above the oil layer was measured to determine the level of pH 4–resistant, internalized IL-2 and the level of pH 4–sensitive, cell surface–bound IL-2, respectively.

Pilot experiments with MT-1 cells indicated that a substantial fraction of the ^{125}I-IL-2 that specifically bound to low-affinity Tac receptor sites dissociated during the cell washing and incubation steps. Therefore, the protocol for the MT-1 cells was modified. Unbound ^{125}I-IL-2 was not removed after the initial incubation of cells and ligand. Instead, the suspension was quickly warmed to 37°C and, at selected times, two 100 μl aliquots (10^6 cells each) were removed. The cells from one aliquot were pelleted through silicone/paraffin oil and the radioactivity in the cell pellet was measured to determine the level of cell-associated (internalized and surface-bound) IL-2. The second aliquot was centrifuged and the cells were resuspended in pH 4 buffer and processed as before. Radioactivity in the cell pellet was used to determine the level of pH 4–resistant, internalized IL-2.

Results and Discussion

Previous studies indicated that high-affinity receptors containing the Tac protein internalized bound IL-2 while low-affinity Tac binding sites did not (1, 3–5). The recent discovery of a second IL-2-binding molecule, β, and the demonstration of its role in forming high-affinity, Tac-dependent receptor sites (7–9) raised the question of whether the β chain was primarily responsible for ligand internalization. To examine this issue, cells and assay conditions were selected that allowed analysis of the individual IL-2-binding components. Binding assays confirmed that the YT cells that we had maintained in culture for 10 mo had almost exclusively intermediate-affinity (K_d ~820 pM) β chain binding sites

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TABLE I
Receptor Numbers and Affinities

Cell type	^{125}I-IL-2 binding: High affinity sites/cell	High affinity K_d (pM)	Intermediate affinity sites/cell	Intermediate affinity K_d (pM)	Low affinity sites/cell	Low affinity K_d (pM)	[^{3}H]Anti-Tac binding (sites/cell)
YT	≤150	~18.0	12,100	810	≤200	~11,000	285
YT$^+$	7,650	14.3	4,000	825	≤500	~11,000	7,890
HUT 102B2	7,900	7.2	—	—	212,000	12,100	285,000
MT-1	<50	—	—	—	143,000	13,400	172,000
MLA-144	<50	—	1,450	900	<200	—	<200

Binding sites numbers and dissociation constants (K_d) were determined by Scatchard analysis of IL-2 and antibody binding data (1, 2). The large number of low-affinity sites on HUT and MT-1 cells prevented accurate determination of their intermediate-affinity binding sites.

(Table I). The average level of Tac antigen on these cells had gradually declined until barely detectable levels of high-affinity, Tac-dependent IL-2 binding remained. This high-affinity component of binding was easily eliminated by inclusion of anti-Tac antibody in the assays (9). Stimulation of the YT cells with forskolin (YT$^+$), however, dramatically increased the level of Tac antigen and of high-affinity IL-2 binding (Table I), presumably by shifting the intermediate-affinity β component into high-affinity α/β receptor sites (9). The gibbon ape cell line MLA-144 appeared to exclusively express the intermediate-affinity β component of binding (Table I) (8). In contrast, the HUT 102B2 cell line displayed a mixture of high- and low-affinity IL-2-binding sites and the MT-1 cell line expressed only low-affinity Tac (α) protein. Based on the results of the binding assays, conditions were selected (see Materials and Methods) that favored IL-2 binding to either high-affinity receptors (YT$^+$, HUT) or to intermediate- and low-affinity β (YT and MLA) and Tac (MT-1)-binding sites.

Examination of the fate of bound IL-2 on unstimulated YT and MLA-144 cells demonstrated that ligand associated with intermediate-affinity β chain receptor sites was rapidly internalized with a $t_{1/2}$ of 10–15 min (Fig. 1). IL-2 bound to high-affinity receptors on forskolin-stimulated YT cells (YT$^+$) and on HUT 102B2 cells was internalized with similar kinetics (Fig. 1). In each case, the vast majority of the cell-associated IL-2 eventually entered the cells. In contrast to the rapid internalization by β protein, IL-2 bound to the abundant low-affinity Tac (α) sites on MT-1 cells was not internalized to a measurable extent (Table II). Thus, consistent with earlier observations (4, 5), Tac protein by itself was incapable of internalizing bound ligand. The IL-2 that was internalized by β receptors on unstimulated YT cells was converted into a TCA-soluble form ($t_{1/2}$ ~70–80 min) in a lysosomal-dependent fashion, sensitive to chloroquine (data not shown). This process mimicked degradation of IL-2 after binding to high-affinity receptors on murine and human cells (1, 3, 4). The similar fates of IL-2 bound to intermediate-affinity β chain binding sites and to high-affinity α/β heterodimers, coupled with the inability of Tac protein to mediate internalization, suggests that the β chain provides an essential element for ligand internalization by both types of receptor.

The cytoplasmic domains of cell surface receptors are believed to play an

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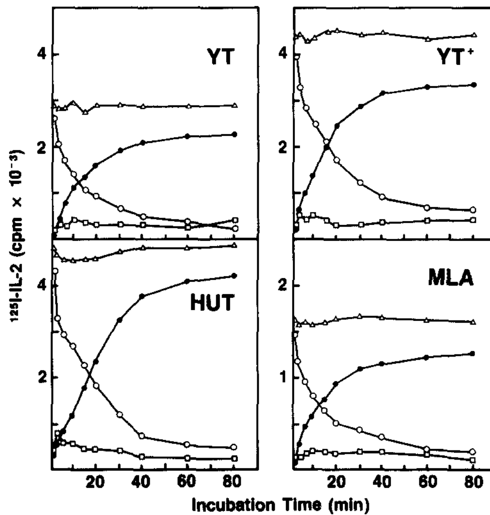

FIGURE 1. Receptor-mediated internalization of ^{125}I-IL-2 by human YT cells, YT cells treated for 20 h with 10 μM forskolin (*YT*$^{+}$), human HUT 102B2 cells, and gibbon ape MLA-144 cells. At each time, the level of radioactivity in the cell supernatant (□) was measured to determine the content of unbound ligand. The cells were then resuspended in pH 4 buffer and centrifuged through a layer of silicone/paraffin oil. The radioactivity in the cell pellet was measured to determine the level of pH 4–resistant, internalized ^{125}I-IL-2 (●), while the radioactivity in the supernatant above the oil layer was used to determine the amount of cell surface–bound ^{125}I-IL-2 that was dissociated by the pH 4 buffer (○). The sum of the counts of all three fractions (△) is also graphed. Anti-Tac antibody was included in the unstimulated YT culture to eliminate the small amount of high-affinity, Tac-dependent IL-2 binding associated with such cells.

TABLE II
Lack of Internalization of ^{125}I-IL-2 by Low-affinity Tac Protein on MT-1 Cells

Time	Total ^{125}I-IL-2 bound	Internalized ^{125}I-IL-2
min	*cpm*	*cpm*
0	19,045	312
10	19,337	497
20	19,727	389
30	19,436	595
60	18,555	473
80	19,145	381
CC*	441	299

* Unlabelled IL-2 (100 nM) was included at the outset of the incubation to determine the level of nonspecific binding (cold competition).

important role in the interactions that allow receptor-mediated endocytosis to occur (13). The failure of the Tac antigen to internalize IL-2 is not altogether surprising, as its cytoplasmic domain contains only 13 residues (10). The effective endocytosis of IL-2 by the β chain suggests that this protein either contains a larger cytoplasmic domain or that it is associated with another protein that permits efficient endocytosis of bound ligand.

In addition to IL-2 internalization, the isolated β chain also appears capable of transducing the ligand binding signal in certain lymphoid cells. For example, ^{125}I-IL-2 chemical crosslinking studies demonstrated that natural killer cells express small amounts of β chain.[1] Other studies have shown that cytolytic activity can be augmented in these cells by the addition of large quantities of IL-2 (14). This IL-2-induced response in NK cells was not blocked by anti-Tac antibody (14), but was inhibited by anti-IL-2 mAb 1H11-1A5 (R. J. Robb and M. E. Neville, unpublished observation), which interferes with IL-2 binding to

[1] Dukovick, M., Y. Wano, Le Thi Bich Thuy, P. Katz, B. R. Cullen, J. H. Kehrl, and W. C. Greene. Identification of a second human interleukin 2 binding protein and its role in the assembly of the high-affinity IL-2 receptor. Manuscript submitted for publication.

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the β chain receptor (9). Similarly, SKW6.4 B cells were shown to express the β chain receptor in the absence of Tac antigen,[1] and exposure of these cells to large quantities of IL-2 resulted in increased immunoglobulin secretion (15). Once again, this response was not blocked by anti-Tac, but was inhibited by the 1H11 antibody (15). Finally, IL-2 was found to induce the expression of Tac protein on YT cells in a manner sensitive to inhibition by 1H11 antibody, but insensitive to anti-Tac (our unpublished observation). Thus, it seems likely that the isolated β chain of the IL-2-R is capable of signal transduction. It remains unresolved, however, as to whether receptor endocytosis is required for these responses. Furthermore, it is unclear whether signalling through the isolated β chain is limited to the transduction of differentiation signals, or whether this IL-2 binding protein, like the high-affinity IL-2-R, is also capable of transducing signals resulting in cellular proliferation.

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Summary

High-affinity IL-2-R correspond to a membrane receptor complex composed of two different IL-2-binding proteins, the Tac antigen (α chain) and a 70–75 kD β chain. Using cell lines that express either the α or the β protein, we demonstrate that IL-2 internalization occurs when ligand is bound to the isolated β chain, but not when it is bound to the isolated α chain. The kinetics of IL-2 internalization mediated by the intermediate-affinity β chain were nearly identical to those of the high-affinity α/β heterodimer ($t_{1/2}$ of 10–15 min), and each type of receptor targeted the bound IL-2 for intracellular degradation in lysosomes. The β chain thus appeared to provide the essential element necessary for ligand internalization by both types of IL-2-R.

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References

1. Robb, R. J., A. Munck, and K. A. Smith. 1981. T cell growth factor receptors: quantitation, specificity, and biological relevance. *J. Exp. Med.* 154:1455.
2. Robb, R. J., W. C. Greene, and C. M. Rusk. 1984. Low and high affinity cellular receptors for interleukin 2: implications for the level of Tac antigen. *J. Exp. Med.* 160:1126.
3. Robb, R. J., and Y. Lin. 1984. T-cell growth factor: purification, interaction with a cellular receptor, and in vitro synthesis. *In* Thymic Hormones and Lymphokines, '83. A. L. Goldstein, editor. Plenum Press, New York. pg. 247.
4. Fujii, M., K. Sugamura, K. Sano, M. Nakai, K. Sugita, and Y. Hinuma. 1986. High-affinity receptor-mediated internalization and degradation of interleukin 2 in human T cells. *J. Exp. Med.* 163:550.
5. Weissman, A. M., J. B. Harford, P. B. Svetlik, W. L. Leonard, J. M. Depper, T. A. Waldmann, W. C. Greene, and R. D. Klausner. 1986. Only high-affinity receptors for interleukin 2 mediate internalization of ligand. *Proc. Natl. Acad. Sci. USA.* 83:1463.
6. Robb, R. J. 1986. Conversion of low-affinity interleukin 2 receptors to a high-affinity state following fusion of cell membranes. *Proc. Natl. Acad. Sci. USA.* 83:3992.

7. Sharon, M., R. D. Klausner, B. R. Cullen, R. Chizzonite, and W. J. Leonard. 1986. Novel interleukin 2 receptor subunit detected by crosslinking under high affinity conditions. *Science (Wash. DC)*. 234:859.
8. Tsudo, M., R. W. Kozak, C. K. Goldman, and T. A. Waldmann. 1986. Demonstration of a new non-Tac peptide that binds interleukin 2: a potential participant in a multichain interleukin-2 receptor complex. *Proc. Natl. Acad. Sci. USA*. 83:9694.
9. Robb, R. J., C. M. Rusk, J. Yodoi, and W. C. Greene. 1987. An interleukin 2 binding molecule distinct from the Tac protein: analysis of its role in formation of high-affinity receptors. *Proc. Natl. Acad. Sci. USA*. In press.
10. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Krönke, P. B. Svetlik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNA's for the human interleukin-2 receptor. *Nature (Lond.)*. 311:626.
11. Yodoi, J., K. Teshigawara, T. Nikaido, K. Fukui, T. Noma, T. Honjo, M. Takigawa, M. Sasaki, N. Minato, M. Tsudo, T. Uchiyama, and M. Maeda. 1985. TCGF (IL-2)-receptor inducing factor(s). I. Regulation of IL-2 receptor on a natural killer-like cell line (YT cells). *J. Immunol.* 134:1623.
12. Robb, R. J., P. C. Mayer, and R. Garlick. 1985. Retention of biological activity following radioiodination of human interleukin 2: comparison with biosynthetically labelled growth factor in receptor binding assays. *J. Immunol. Methods*. 81:15.
13. Schlessinger, J., A. B. Schreiber, A. Levi, I. Lax. T. Libermann, and Y. Yarden. 1983. Regulation of cell proliferation by epidermal growth factor. *CRC Crit. Rev. Biochem.* 14:93.
14. Ortaldo, J. R., A. T. Mason, J. P. Gerard, L. E. Henderson, W. Farrar, R. F. Hopkins, R. B. Herberman, and H. Rabin. 1984. Effects of natural and recombinant IL-2 on regulation of IFN γ production and natural killer activity: lack of involvement of the Tac antigen for these immunoregulatory effects. *J. Immunol.* 133:779.
15. Ralph, P., G. Jeong, K. Welte, R. Mertelsmann, H. Rabin, L. E. Henderson, L. M. Souza, T. C. Boone, and R. J. Robb. 1984. Stimulation of immunoglobulin secretion in human B lymphocytes as a direct effect of high concentration of IL-2. *J. Immunol.* 133:2442.

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