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Immunology

Contribution of a p75 interleukin 2 binding peptide to a high-affinity interleukin 2 receptor complex

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ABSTRACT **There are at least two forms of cellular receptors for interleukin 2 (IL-2); one with a very high affinity and the other with a lower affinity. We identified a non-Tac IL-2 binding peptide with a relative molecular weight of 75,000 (p75). Cell lines bearing either the p55 Tac or the p75 peptide alone manifested low-affinity IL-2 binding, whereas a cell line bearing both peptides manifested both high- and low-affinity receptors. After the internalization of labeled IL-2 through high-affinity receptors, the p75 peptide could not be detected by cross-linking studies. Furthermore, fusion of cell membranes from low-affinity IL-2 binding cell lines bearing the Tac peptide alone with membranes from a cell line bearing the p75 peptide alone generated hybrid membranes bearing high-affinity receptors. These results suggest a multichain model for the high-affinity IL-2 receptor in which high-affinity receptors would be expressed when both Tac and p75 IL-2 binding peptides are present and associated in a receptor complex.**

There are at least two classes of interleukin 2 (IL-2) receptors that differ in their affinities for IL-2 (1). One has a very high affinity [10 pM dissociation constant (K_d)], whereas the other has a much lower affinity (10 nM). Both classes of receptors share the same Tac peptide (M_r, 55,000) defined by the anti-Tac monoclonal antibody (1, 2). The Tac peptide has been characterized in detail (3), and cDNA encoding this peptide has been cloned (4). However, the molecular basis for the marked difference in affinity between high- and low-affinity receptors has not been elucidated. Tac cDNA has been shown to reconstitute high-affinity receptors only when transfected into lymphoid cells (5), but not when transfected into nonlymphoid cells (6). Furthermore, there was a conversion of low-affinity IL-2 receptors to a high-affinity form following the fusion of membranes from human T cells with cell membranes from L cells transfected with cDNA encoding the p55 murine counterpart of the Tac peptide (7). These two observations supported the view that cofactors are present on T cells that combine with the p55 peptide to create high-affinity receptors (5, 7). However, the nature of these putative cofactors and their role in the generation of high-affinity receptors was not defined in these studies.

We (8) and others (9, 10) have reported a non-Tac IL-2 binding peptide with a M_r of 75,000 (p75). Using cross-linking methodology we demonstrated the p75 peptide on MLA 144 (11), a gibbon T-cell line, which does not express the Tac antigen but manifests 6800 low-affinity (K_d, 14 nM) IL-2 binding sites per cell. The p75 peptide was also identified in addition to the Tac peptide (p55) on both HUT 102, a human T-cell lymphotropic virus type I-induced T-cell leukemia line and phytohemagglutinin (PHA)-activated lymphoblasts (PHA blasts). These cell populations express both high- and low-affinity receptors. We proposed a multichain model for the high-affinity IL-2 receptor, in which an independently existing Tac or p75 peptide would represent low-affinity receptors, whereas high-affinity receptors would be expressed when both peptides are expressed and associated in a receptor complex. To test this working hypothesis, a variety of T-cell lines were examined in the present study for IL-2 binding and were subjected to IL-2 cross-linking studies to determine if there was a correlation between the affinity of IL-2 binding and the IL-2 binding peptides expressed. We show here that MT-1 (12), a human T-cell lymphotropic virus type I-induced human T-cell leukemia line, manifests only low-affinity IL-2 receptors and expresses the Tac peptide, but does not express the p75 peptide. Furthermore, there was a conversion of low-affinity IL-2 receptors to a high-affinity form following the fusion of cell membranes from MT-1 bearing the p55 peptide alone with membranes from MLA 144 bearing the p75 peptide alone.

MATERIALS AND METHODS

Cells and IL-2 Binding Assay. Cell lines were maintained in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum (FCS). Human peripheral blood mononuclear cells were isolated by Ficoll-Paque (Pharmacia) centrifugation. Lymphoblasts were generated by culturing these cells at 2×10^6 cells per ml in RPMI 1640 with 10% (vol/vol) FCS and 0.1% PHA-P (Difco) for 3 days. The binding studies were performed as described (8), using ^{125}I-labeled human recombinant IL-2 (specific activity: 28.6–44.4 μCi/μg; 1 Ci = 37 GBq) purchased from New England Nuclear.

IL-2 Cross-Linking Study. Cross-linking studies were performed as described (8). Briefly, for cross-linking to membranes, various cells were homogenized with a Brinkmann Polytron in 0.25 M sucrose, 10 mM Tris·HCl (pH 7.4) containing 0.2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM phenylmethylsulfonyl fluoride, and antipain at 1 μg/ml (Sigma). After centrifugation at 600 × *g* for 10 min, the supernatant was centrifuged at 12,000 × *g* for 30 min. The 12,000 × *g* supernatant was adjusted to 0.1 M NaCl and centrifuged at 100,000 × *g* for 90 min. All operations were carried out at 4°C. The 100,000 × *g* membrane pellet was suspended in phosphate-buffered saline (PBS = 10 mM phosphate/0.14 M NaCl, pH 7.4) and stored at −70°C. For cross-linking, membranes (0.1–0.5 mg) were incubated for 1 hr at 4°C with 5 nM of ^{125}I-labeled IL-2 in a total volume of 100 μl of PBS containing 10% (vol/vol) FCS and 25 mM Hepes (pH 7.3). The incubation mixtures were centrifuged at 10,000 × *g* for 4 min. The pellets were resuspended in 100 μl of PBS and centrifuged at 10,000 × *g* for 6 min through a 1-ml layer of 2.5% (wt/vol) sucrose in PBS. The pellets were suspended in 250 μl of PBS, and disuccinimidyl suberate (Pierce) was added to a final concentration of 0.5 mM. In the case of MT-1, even a 4-min wash led to a great decrease of the labeling.

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Abbreviations: IL-2, interleukin 2; PHA, phytohemagglutinin; FCS, fetal calf serum.

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Therefore, the washing steps were omitted for MT-1, and cross-linking was initiated immediately following the incubation with ^{125}I-labeled IL-2. Following a 15-min incubation with disuccinimidyl suberate at room temperature, the reaction was quenched by adding 750 μl of 10 mM Tris·HCl, pH. 7.4/1 mM EDTA/0.14 M NaCl. Membranes were pelleted and solubilized with 50 μl of $NaDodSO_4$ sample buffer [6.25 mM Tris·HCl (pH 6.8), 10% (vol/vol) glycerol, 1% $NaDodSO_4$] containing 5% (vol/vol) 2-mercaptoethanol, and subjected to $NaDodSO_4$/PAGE using 7.5% acrylamide gel. For cross-linking to intact cells, 5–10 × 10^6 cells were incubated at indicated temperatures with 5 nM ^{125}I-labeled IL-2 in a total volume of 200 μl of RPMI 1640 containing 10% (vol/vol) FCS and 25 mM Hepes (pH 7.3). After centrifugation (10,000 × *g*, 1 min) through 10% (wt/vol) sucrose in PBS, cross-linking was carried out as described above. Cells were solubilized with 50 μl of PBS containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. After centrifugation (10,000 × *g*, 5 min), the supernatants were mixed with the same volume of 2×-concentrated $NaDodSO_4$ sample buffer containing 10% (vol/vol) 2-mercaptoethanol and electrophoresed as above.

Membrane Fusion. Membrane fusion and IL-2 binding assay for membranes were carried out according to the methods described by Robb (7) with a slight modification. Twenty-five microliters of MT-1 membranes (10 μg/μl of PBS) were mixed with 25 μl of MLA 144 membranes (15 μg/μl) and 25 μl of a sonicated phospholipid mixture [phosphatidylcholine at 1 mg/ml (type II-S, Sigma), stearylamine at 0.1 mg/ml (Sigma), 1 mM EDTA, 2 mM dithiothreitol, 10 mM Tris·HCl, pH 7.5]. Following a 5-min incubation at room temperature, 0.6 μl of 1 M $CaCl_2$ was added to the mixture. After another 5-min incubation, the tubes were transferred to a 37°C water bath, and 125 μl of 50% (vol/vol) polyethylene glycol [PEG 8000 (Sigma)] in RPMI 1640/25 mM Hepes, pH 7.3 was added dropwise. For a negative control, RPMI 1640 was substituted for PEG. Following the incubation with periodic stirring at 37°C for 5 min, the mixture was slowly diluted with 1 ml of 37°C RPMI 1640/25 mM Hepes, pH 7.3, and centrifuged at 10,000 × *g* for 5 min. The pellets were resuspended in 1.0 ml of RPMI 1640/25 mM Hepes, pH 7.3, containing 10% FCS (assay medium) and incubated further for 30 min at 37°C. In the IL-2 binding assay, membrane preparations (50 μl) were incubated with 50-μl serial dilutions of ^{125}I-labeled IL-2 for 1 hr at 4°C in microtest tubes. Then, membranes were transferred to a 96-well Millititer GV plate (Millipore) and washed four times with cold assay medium. The filters were removed, and the filter-bound radioactivity was measured. Nonspecific binding was determined in the presence of a 500-fold excess of unlabeled IL-2. In certain tubes, the IL-2 binding assay was performed in the presence of the anti-Tac antibody at 300 μg/ml.

RESULTS AND DISCUSSION

IL-2 binding assays for three human T-cell lines under relatively high concentrations of ^{125}I-labeled IL-2 are shown in Fig. 1. Both HUT 102 and MT-1 are human T-cell lymphotropic virus type I-induced T-cell leukemia cell lines that constitutively express the Tac peptide (1, 13). As shown in Fig. 1 *A* and *C*, both lines express numerous (300,000–600,000 molecules per cell) low-affinity (K_d, 40–60 nM) IL-2 binding sites. However, a major difference between these two cell lines is that HUT 102 manifested 17,900 high-affinity receptors with a K_d of 57 pM, whereas no high-affinity receptors were detected on MT-1. On the other hand, HUT 78 (14), a T-cell line established from a patient with the Sezary syndrome, expressed 3450 IL-2 receptors per cell with a K_d of intermediate value (0.76 nM). When examined by flow cytometry, HUT 78 expressed the Tac antigen very faintly on only 15% of cells (data not shown).

Cross-linking studies were performed to define the size of the IL-2 binding peptide(s) on these cell lines. Cell membranes were incubated with ^{125}I-labeled IL-2 (5 nM), then covalently cross-linked with 0.5 mM disuccinimidyl suberate, washed twice, and analyzed by $NaDodSO_4$/PAGE under reducing conditions. As shown in Fig. 2 (lane E), the labeling of an M_r 90,000 band was observed with MLA 144, representing a non-Tac IL-2 binding peptide with a calculated M_r of 75,000 cross-linked with IL-2 [M_r, 15,000 (15)]. There was no peptide of the size of the Tac peptide. MT-1 that also bears only low-affinity receptors showed a band at M_r 72,000 but not the upper band (lane G). The calculated molecular weight of the IL-2 binding peptide (Tac) on MT-1 was 57,000. The labeling of both the Tac and p75 band was observed with HUT 102, a line that expresses both high- and low-affinity forms of the IL-2 receptor (lane A). A more heavily labeled lower band migrated at M_r 69,000 representing the Tac peptide (M_r, 54,000) slightly smaller than that on MT-1. The upper band at M_r 90,000 represents the (non-Tac) IL-2 binding peptide with a calculated M_r of 75,000. HUT 78, in turn, bearing IL-2 receptors with an intermediate K_d value showed both the M_r 90,000 and M_r 70,000 bands (lane C). However, the labeling of the lower band was very faint in accord with the very low expression of the Tac antigen on HUT 78.

As summarized in Table 1, using select human and gibbon T-cell lines in this study, we demonstrated that two kinds of

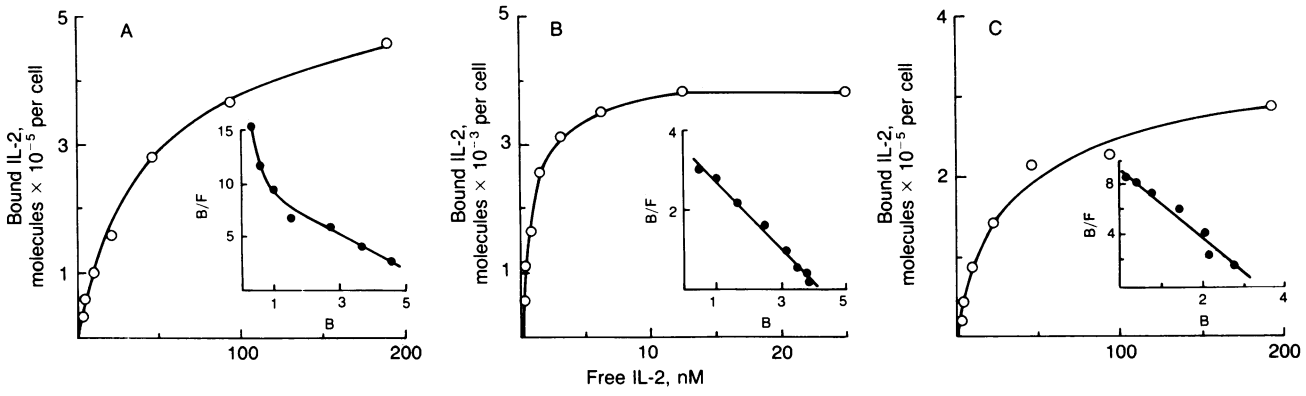

FIG. 1. The binding of ^{125}I-labeled IL-2 to various T-cell lines. (*A*) HUT 102. (*B*) HUT 78. (*C*) MT-1. (*Insets*) Scatchard transformations of data. Note the curvilinear feature of Scatchard plot of HUT 102. B/F, (Bound IL-2/free IL-2) × 10^{-3} for *A*–*C*. B, bound IL-2 expressed as 10^5 molecules per cell for *A* and *C* and as 10^3 molecules per cell for *B*.

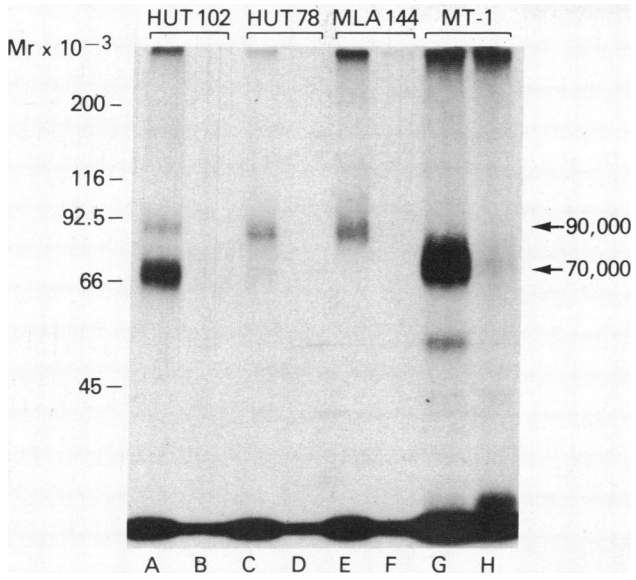

FIG. 2. Autoradiogram showing IL-2–receptor complexes produced by chemically cross-linking ^{125}I-labeled IL-2 to the cell membranes of HUT 102, HUT 78, MLA 144, and MT-1. Membranes were incubated with ^{125}I-labeled IL-2 alone (lanes A, C, E, and G) or in the presence of 300-fold excess of unlabeled IL-2 [recombinant human IL-2 from *Escherichia coli* (16), Cetus, Emeryville, CA] (lanes B, D, F, and H), then cross-linked.

IL-2 binding peptides exist and that these peptides are differentially expressed on cell lines that manifest different affinity forms of the IL-2 receptor. It appears that an independently existing p55 Tac peptide or a p75 peptide yields low-affinity receptors, whereas high-affinity receptors are generated when both peptides are fully expressed and associated in a receptor complex. For HUT 102 or activated lymphoblasts, the number of high-affinity receptors may be restricted by the number of the p75 peptides available to associate with the more numerous Tac peptides.

Since it has been reported that IL-2 undergoes internalization only through high-affinity receptors (17), we tested the effect of internalization on the band pattern of IL-2 binding peptides. Following the incubation of HUT 102 or human PHA blasts with ^{125}I-labeled IL-2 at 4°C for 30 min, the cells were incubated at 37°C for another 30 min and then cross-linked. As shown in Fig. 3, the upper bands of both HUT 102 and PHA blasts disappeared with a modest decrease of the radioactivity of the lower bands, suggesting that a high-affinity receptor complex including the p75 peptide that bound ^{125}I-labeled IL-2 was internalized and, therefore, was not cross-linked with disuccinimidyl suberate.

Furthermore, to determine whether the low-affinity Tac peptide combines with the low-affinity p75 peptide to generate high-affinity receptors, the membranes of MT-1 cells bearing the Tac peptide alone were fused with those of MLA 144 bearing the p75 peptide alone. The IL-2 affinity of hybrid membranes induced by PEG was assessed in an IL-2 binding assay. As shown in Fig. 4, a simple mixture of MT-1 and

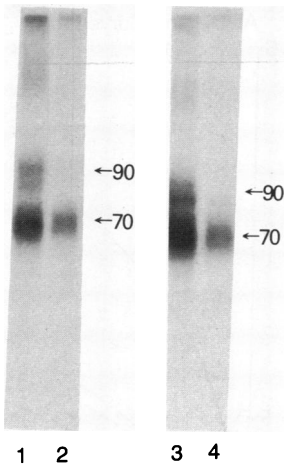

FIG. 3. Cross-linking studies after ligand internalization. Following the incubation with ^{125}I-labeled IL-2 at 4°C for 30 min, HUT 102 cells (lanes 1 and 2) and PHA blasts (lanes 3 and 4) were incubated at either 4°C (lanes 1 and 3) or 37°C (lanes 2 and 4) for another 30 min, then cross-linked. Molecular sizes in kDa are indicated.

MLA 144 membranes without PEG resulted in a linear Scatchard plot with an apparent K_d of 1.0 nM, which is lower than the K_ds of whole cells. However, a dramatic change appeared following the fusion of these membranes in the presence of PEG. The fused MT-1 and MLA 144 membranes resulted in a curvilinear Scatchard plot with high (K_d, 18 pM)—as well as low (K_d, 1.0 nM)—affinity binding sites. When anti-Tac antibody was present during the incubation of fused membranes with ^{125}I-labeled IL-2, no high-affinity binding was detected, indicating that the Tac peptide contributes to form the high-affinity IL-2 receptor. As predicted from the hypothetical model, high-affinity receptors were not generated following the membrane fusions of MLA 144 or MT-1 with MOLT-4, a human thymoma cell line that does not express either of the two IL-2 binding peptides (unpublished data). From these results, although the possibility of the involvement of other molecule(s) on MLA 144 membranes cannot be excluded, it is very likely that the p75 peptide participated in the formation of high-affinity IL-2 receptors on the fused membranes. Supporting this view, HUT 102 cells that express both high- and low-affinity receptors have the p75 peptide closely associated with the Tac peptide (8). Furthermore, high-affinity receptors were also generated following the membrane fusion of MLA 144 bearing the p75 peptide alone with a murine T-cell line expressing only low-affinity receptor and expressing p55 alone, suggesting that the p75 peptide can act across species lines (unpublished data).

In summary, the cross-linking studies and membrane fusion studies reported here lead us to the view that the p75 IL-2 binding peptide is a potential participant in a multichain high-affinity IL-2 receptor complex that plays a key role in lymphocyte proliferation and function.

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Table 1. IL-2 receptors on various T-cell lines

Species	Cell line	Cross-linking p55(Tac)	Cross-linking p75	High-affinity IL-2 binding Sites, no. per cell	High-affinity IL-2 binding K_d, pM	Low-affinity IL-2 binding Sites, no. per cell	Low-affinity IL-2 binding K_d, nM
Gibbon	MLA 144	−	+	−		6,800 ± 2,200	14 ± 3
Human	MT-1	++	−	−		499,000 ± 102,000	52 ± 10
	HUT 102	++	+	17,900 ± 2400	57 ± 21	519,000 ± 105,000	49 ± 6
	HUT 78	+/−	+	−		3,450 ± 1,900	0.76 ± 0.30

−, Not detectable. + or ++, Small or large quantities detectable, respectively. Results are mean ± SEM.

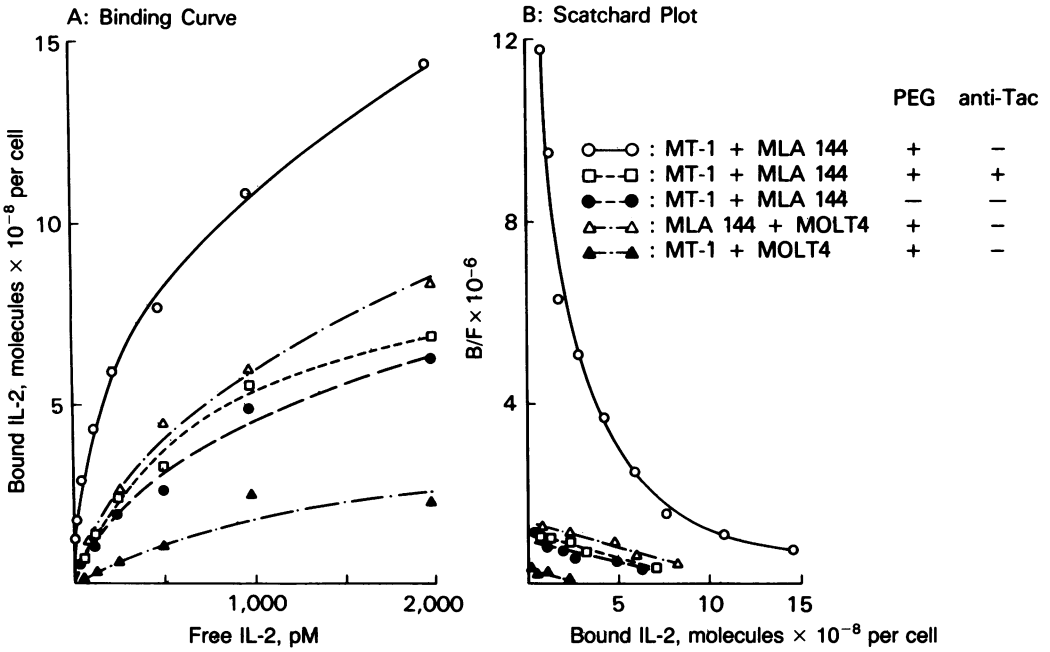

FIG. 4. (*A*) Binding of ^{125}I-labeled IL-2 to fused membranes. Membranes from MT-1, MLA 144, and MOLT-4 were mixed in various combinations as indicated in the presence or absence of PEG. The resulting membrane preparations were analyzed in the IL-2 binding assay either in the presence or absence of the anti-Tac antibody. (*B*) Scatchard transformation of the data in *A*. Symbols are same for *A* and *B*. B/F, bound IL-2/free IL-2.

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