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Molecular basis for two different affinity states of the interleukin 2 receptor: Affinity conversion model

(Scatchard plot/monoclonal antibodies/converter protein/ternary complex)

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ABSTRACT **Two affinity species of the interleukin 2 (IL-2) receptor are different states of a single receptor molecule. We assumed that a binary complex between the IL-2 receptor and another lymphocyte-specific protein would constitute the high-affinity receptor. To test this assumption, we counted the numbers of IL-2 receptors with high and low affinity in a murine T-cell line CT/hR-1 that expresses not only murine but also human receptors by cDNA transfection. We found that human high-affinity receptors disappeared when the murine high-affinity receptors were already occupied by the ligand. The results were incompatible with a fixed number of human and murine receptors with high affinity in CT/hR-1 cells. We suggest that the high-affinity state of the IL-2 receptor is a ternary complex of IL-2, the IL-2 receptor, and a postulated "converter" protein, which is fewer in number than the receptors. The converter would be unable to form a complex with the IL-2 receptor unless IL-2 was already bound to it. The ligand binding to the receptor would cause a conformational change in the receptor, increasing its affinity to the converter. Ternary complex formation would, in turn, change the apparent affinity of the receptor to the ligand from low to high by reduction of the dissociation constant.**

Antigen-specific clonal proliferation of lymphocytes is an important selection mechanism of lymphocytes bearing specific antigen receptors. The physiological proliferation of T lymphocytes (T cells) requires interaction between the humoral growth factor interleukin 2 (IL-2) and its cell-surface receptor (1, 2). Normal resting T cells, which do not express the IL-2 receptor, fail to receive growth signals even in the presence of IL-2. Antigenic stimulation of T cells induces transient expression of the IL-2 receptor, indicating that regulated expression of the IL-2 receptor is the molecular basis for positive selection of antigen-specific T-cell clones (3, 4). IL-2 receptors are of two distinct populations with high and low affinities (5). Physiological signals by IL-2 seem to be mediated through binding of the growth factor to the high-affinity receptor (6).

Studies of human and murine IL-2 receptors started with cloning of their cDNA (7–11). The IL-2 receptors expressed on non-lymphoid cells by cDNA transfection all have low affinity and are inactive in signal transmission (12, 13). The IL-2 receptor proteins synthesized in non-lymphoid cells when analyzed by immunoprecipitation are indistinguishable from those in T cells (12). We have established, using cDNA transfection, an IL-2-dependent mouse T-cell line, CT/hR-1, that expresses the human IL-2 receptor as well as the murine receptor (14). Human IL-2 functions with human and murine receptors, so the activity of human IL-2 receptors expressed on CT/hR-1 could be tested by blockage of IL-2 binding to the endogenous murine IL-2 receptor by the anti-mouse IL-2 receptor antibody. Murine and human IL-2 receptors are functionally active in CT/hR-1 cells (14). Hatakeyama *et al.* (15) have shown that human IL-2 receptors expressed by cDNA transfection of a murine T-cell line, EL-4, that grows without IL-2 and does not express the murine IL-2 receptor contain the high-affinity species. Robb (16) found that murine low-affinity receptors expressed on a non-lymphoid cell were converted to high-affinity receptors by fusion of the cell membranes with human T cells. These results taken together make it reasonable to assume that high- and low-affinity IL-2 receptors arise from different states of a single receptor protein and that a second lymphocyte-specific molecule is required for growth signal transmission and conversion of the receptor affinity from low to high (14–16).

Further studies of CT/hR-1 cells gave several observations that contradicted the assumption that the IL-2 receptor forms a stable complex with a putative effector protein termed a "converter," which is essential for signal transmission and affinity change. These studies led us to suggest that the high-affinity state of the IL-2 receptor results from the formation of a ternary complex consisting of IL-2, the IL-2 receptor, and the converter.

MATERIALS AND METHODS

Cells. CTLL-2 (17) and ATL-2 (18) were provided by M. Maeda (Kyoto University). CT/hR-1 was made from CTLL-2 by transfection of human IL-2 receptor cDNA by the DEAE-dextran method (14). Cell lines were cultured in RPMI 1640 medium containing 10% fetal calf serum, 0.05 mM 2-mercaptoethanol, and 25 ng of human recombinant IL-2 per ml.

Monoclonal Antibodies. Monoclonal antibodies against the murine IL-2 receptor are AMT13 (19), PC61 (20), and 7D4 (21). Monoclonal antibodies against the human IL-2 receptor are anti-Tac (22) and HIEI (23).

IL-2. Purified human recombinant IL-2 was a gift from Takeda (Osaka, Japan). Mouse IL-2 purified from the supernatant of COS cells transfected with mouse IL-2 cDNA was a gift of Ajinomoto (Tokyo). ^{125}I-labeling of human IL-2 was done by the Bolton–Hunter method (24). Reagents for labeling were purchased from the New England Nuclear. The specific activity and molecular weight of labeled IL-2 were 4000 cpm/ng and 15,000, respectively.

Scatchard Plot Analysis. The binding of ^{125}I-labeled human IL-2 was assayed as described (6). Incubation with antibody or mouse IL-2 was as follows. Cells were incubated in IL-2-free medium for 4 hr before assay to prevent contamination by IL-2. Then cells were collected and suspended in culture medium containing 740 units of mouse IL-2 or 2 mg of PC61 per ml.

Thymidine incorporation into lymphocytes was measured as described (14).

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Abbreviation: IL-2, interleukin 2.

RESULTS AND DISCUSSION

The Number of High-Affinity Sites Is not Fixed. Based on several reports (13–16), we assumed that a complex of the IL-2 receptor and the converter protein would constitute functionally active IL-2 receptors with high affinity. A free IL-2 receptor would be functionally inactive although it binds IL-2 with low affinity. The total number of high-affinity sites of CT/hR-1 is the same or less than that of the parental line CTLL-2 (14), so the number of converter molecules is limited and controlled independently from the number of IL-2 receptors. At first we thought that CT/hR-1 had a fixed number of high-affinity sites for human and murine receptors.

We estimated the number of high- and low-affinity species of the human IL-2 receptor in CT/hR-1 by blocking IL-2 binding to the murine receptor with the monoclonal antibody PC61, as shown in Fig. 1*A*. The number of human low-affinity sites (2.8×10^5 molecules per cell) was about half of the total (human plus murine) low-affinity receptors (5.2×10^5 molecules per cell) in CT/hR-1, in agreement with earlier results (14). However, the total number of high-affinity sites (3.1×10^3 molecules per cell) was almost the same regardless of the presence or absence of the monoclonal antibody, PC61, against the murine IL-2 receptor. The results show that the number of human high-affinity receptors measured by blockage of the murine receptors with antibody is the same as that of the total of both kinds of high-affinity receptor. The result might mean that the number of high-affinity species of the murine IL-2 receptor is negligible in CT/hR-1, but that does not agree with CT/hR-1 growing in the presence of murine or rat IL-2. Note that mouse or rat IL-2 does not interact with the human IL-2 receptor, although human IL-2 interacts with both human and mouse receptors.

In contrast, the presence of a small amount of mouse recombinant IL-2 drastically reduced the number of human high-affinity sites in CT/hR-1 (Fig. 1*A*). The number of low-affinity sites was not much affected because the amount of murine IL-2 was not sufficient to compete with the ^{125}I-labeled human IL-2. In a control experiment, the addition of murine IL-2 did not affect the number of high-affinity sites in ATL-2 (18), a human T-cell line derived from T cells of a patient with adult T-cell leukemia (Fig. 1*B*). The result indicates that the human high-affinity receptors in CT/hR-1 disappeared when the murine high-affinity receptors were already bound by the ligand.

Affinity Conversion Model. These results are incompatible with the suggestion that a stable complex of the IL-2 receptor and the converter acts as a high-affinity receptor, which assumes a fixed number of high-affinity sites for human and murine receptors in CT/hR-1. The findings are easily explained by an alternative suggestion, which we call the "affinity conversion model," that the converter is unable to form a complex with the IL-2 receptor unless IL-2 is bound to the receptor. This model also presumes that the apparent affinity of the receptor for the ligand would become high after formation of the ternary complex of the IL-2 receptor, IL-2, and the converter. As schematically shown in Fig. 2, the ligand binding to the receptor would cause a conformational change in the receptor that would increase its affinity to the converter. Ternary complex formation would, in turn, convert the apparent affinity of the receptor to the ligand from low to high. The increase in the apparent affinity to the ligand would involve an extremely low dissociation constant of the ligand from the ternary complex. In fact, the affinity of the receptor estimated by Scatchard analysis reflects the dissociation constant of the ligand from the receptor. Signal transduction may be mediated by aggregation or internalization of the ternary complex. Aggregation of the complex would be facilitated by complex formation, assuming that the converter has multiple binding sites for the receptor–ligand complex.

When some of the murine receptor molecules of CT/hR-1 are occupied by murine IL-2, all of the converter molecules form ternary complexes with the murine IL-2 receptor because the number of converter molecules is less. When no converters are free, the high-affinity species of human IL-2 receptors seem to be absent. When the binding of IL-2 to murine receptors is blocked completely by antibodies, all of the converters are available for ternary complex formation with human receptors. Thus, the number of high-affinity species of the human receptor is almost equal to the number of the converter, which decides the total number of high-affinity sites.

Further Evidence for the Affinity Conversion Model. Properties of two monoclonal antibodies against the IL-2 receptor are consistent with our model. The HIEI antibody binds to

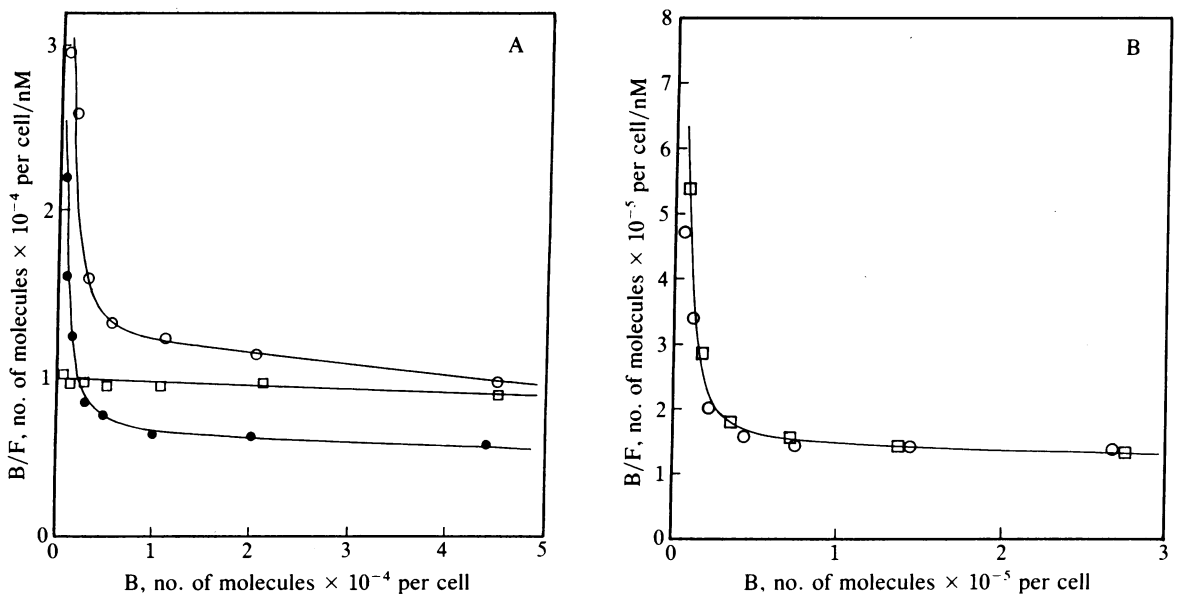

FIG. 1. Scatchard plot analysis of equilibrium binding of recombinant human ^{125}I-labeled IL-2 to CT/hR-1 (*A*) and ATL-2 (*B*). IL-2 binding was measured in the presence of 2 mg of PC61 per ml (●), 370 units of mouse IL-2 per ml (□), or neither (○). With 2 mg of PC61 per ml, IL-2 binding to CTLL-2 was not detected (data not shown). B and F, bound and free.

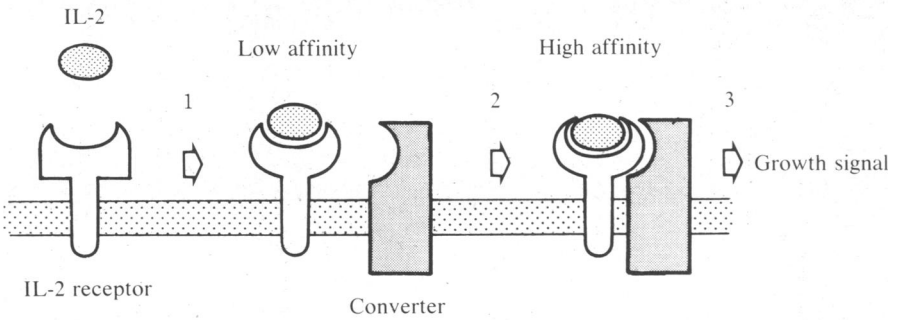

FIG. 2. Scheme of the affinity conversion model. The IL-2 receptor and converter have their top surfaces outside of the cell membrane (dotted horizontal bar). Blocking antibodies are AMT13, PC61, and anti-Tac (step 1), HIEI (step 2), and 7D4 (step 3).

the human IL-2 receptor but does not change the total number of IL-2 molecules binding to the receptor. However, high-affinity species are completely abolished in the presence of this antibody (25). HIEI antibody blocked the proliferation of CT/hR-1 in the presence of the anti-mouse IL-2 receptor antibody AMT13, as shown in Table 1. The observations are easily explained by the assumption that HIEI would block the formation of the ternary complex (step 2 in Fig. 2) after the ligand binds. It is reasonable that the HIEI antibody alone did not reduce the growth response of CT/hR-1 (Table 1), provided that this antibody blocks step 2 without trapping the converter. The slight reduction (by 19%) of thymidine uptake by HIEI alone was probably due to nonspecific contaminants in ascites fluid, as HIEI had a similar effect (8% reduction) on CTLL-2, which does not express the human receptor.

The 7D4 antibody bound to the murine IL-2 receptor but did not change the number of binding sites of either species (unpublished data and ref. 21). Naturally, 7D4 inhibited the IL-2-dependent proliferation of CTLL-2 (Table 1). Unlike the other antibodies, 7D4 reduced the thymidine incorporation of CT/hR-1 to 50% when added alone. This growth inhibition was not due to toxic materials contaminating the 7D4 culture supernatants, because 4-fold concentrations of 7D4 did not inhibit proliferation further. The effect of 7D4 can be explained by immobilizing the ternary complex of the murine IL-2 receptor, IL-2, and the converter, which would reduce the number of effective converters for human receptors. The 7D4 antibody seemed to block signal transduction (step 3 in Fig. 2) after formation of the ternary complex.

Table 1. Effect of antibodies on proliferation of CTLL-2 and CT/hR-1 cells in the presence of human IL-2

Cell line	Antibody: Against murine receptor	Antibody: Against human receptor	Thymidine incorporation, %
CTLL-2	None	None	100
	AMT13		6
	PC61		9
		HIEI	92
	7D4		26
	7D4 (×4)		24
CT/hR-1	None	None	100
	AMT13		111
	AMT13	HIEI	23
		HIEI	81
	7D4		52
	7D4 (×4)		51

IL-2-dependent thymidine uptake of CTLL-2 and CT/hR-1 in the presence of antibodies is shown. Values are means of two or three experiments. Background incorporation without IL-2 was subtracted from the data obtained, and relative responses are expressed as the percentage of [^{3}H]thymidine uptake without antibody. Final concentrations of antibodies are AMT13, 0.2 mg/ml; PC61, 0.1 mg/ml; HIEI, 1:1000 dilution of ascites fluid; 7D4 and 7D4 (×4), 1:10 and 1:2.5 dilutions, respectively, of culture supernatants of a 7D4 hybridoma.

The growth response of CT/hR-1 was constant even in the presence of antibodies (AMT13, PC61, or anti-Tac) that block IL-2 from binding to either the human or murine IL-2 receptor (Table 1 and ref. 14). This observation would appear to disagree with the finding that DNA synthesis is proportional to the extent of IL-2 binding to the high-affinity sites (6) if one assumes that the numbers of high-affinity sites for human and murine receptors are fixed. According to the affinity conversion model, however, the two observations are not contradictory, because the high-affinity sites would remain constant in number regardless of blockage of either the human or murine IL-2 receptors at step 1 (see Fig. 1*A*).

Interaction of the IL-2 Receptor and the Converter. The human IL-2 receptor expressed in the murine T-cell line is functionally active, so the murine converter may interact with the human IL-2 receptor at regions conserved between the two species. We thought that the converter would interact with the transmembrane and cytoplasmic regions as they are the best conserved portions of the IL-2 receptor between mouse and humans (10). However, mutant receptors that had large replacements and deletions in the transmembrane and cytoplasmic regions mediated strong thymidine-uptake responses (unpublished data). It is likely that the extra cytoplasmic region may be responsible, at least in part, for the interaction with the converter. The conserved sequence (residues 185–198) encoded by the 3′ end of exon 5 (10), which contains a cysteine residue, may be the region involved in this interaction. The idea that the converter interacts with the extracytoplasmic region of the IL-2 receptor is consistent with the finding that HIEI antibody abolishes high-affinity binding sites. The antibody should recognize determinants outside the membrane, which may be located in or near the region that interacts with the converter.

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