Pseudo–High Affinity Interleukin 2 (IL-2) Receptor Lacks the Third Component That Is Essential for Functional IL-2 Binding and Signaling

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Summary

Functional studies of the interleukin 2 receptor (IL-2R) of two (ED515-D and Kit225) IL-2-dependent and three (ED515-I, 3T3-αβ11, and Hut102) IL-2-independent cell lines were done. All of these cell lines appeared to express high as well as low affinity IL-2R. However, ED515-I and 3T3-αβ11, which expressed the IL-2R β chain, did not bind IL-2 at all when IL-2 binding to their IL-2R α chain was blocked with anti-Tac monoclonal antibody, whereas the intermediate affinity binding in ED515-D, Kit225, and Hut102 cells remained. We tentatively called the high affinity IL-2R of the former cells pseudo–high affinity IL-2R. The dissociation constant of pseudo–high affinity IL-2R was higher than that of ordinary high affinity IL-2R. Internalization of cell-bound ^{125}I-IL-2 into ED515-I and 3T3-αβ11 cells was less efficient than that into ED515-D cells. The addition of IL-2 neither promoted cell growth nor upregulated IL-2R α chain expression in ED515-I and 3T3-αβ11 cells. Furthermore, tyrosine phosphorylation of the cellular proteins (p120, p98, p96, p54, and p38) was induced or enhanced in response to the addition of IL-2 in ED515-D and Kit225 cells, but not in the cell lines expressing pseudo–high affinity IL-2R. Finally, ^{125}I-IL-2 crosslinking followed by SDS-PAGE analysis showed an 80-kD band corresponding to p65 + IL-2, in addition to bands corresponding to IL-2R α and β chain + IL-2 in cells bearing ordinary high affinity IL-2R but not in cells with pseudo–high affinity IL-2R. Taken together, we consider that another protein whose molecular mass is approximately 65 kD is functionally important in IL-2 binding and subsequent signal transduction and may be the third component of IL-2R.

IL-2 is a lymphokine produced by T cells that induces proliferation and differentiation of T, B, and NK cells, as well as thymocytes and monocytes. Cells bind IL-2 with three different affinities, designated as low ($K_d = 10^{-8}$ M), intermediate ($K_d = 10^{-9}$ M), and high ($K_d = 10^{-11}$ M) (1–9). Studies using mAbs and affinity crosslinking with radiolabeled IL-2 have identified and characterized the heterodimeric structure of IL-2R (6–15). A low affinity IL-2R consists of an α chain (p55, Tac) (2, 12), and association and dissociation of IL-2 for the low affinity IL-2R are very rapid ($t_{1/2} = 5$ and 6 s, respectively) (16, 17). The IL-2R α chain with a short intracytoplasmic portion does not solely transduce a growth signal (3, 4, 18). In contrast, the IL-2R β chain binds IL-2 with an intermediate affinity when expressed solely and appears to be more important in IL-2 signal transduction. The association and dissociation of IL-2 for the intermediate affinity IL-2R are much slower ($t_{1/2} = 45$ and 290 min, respectively). Because IL-2 binding to the high affinity IL-2R takes

on the characteristics of the low affinity IL-2R for its association and of the intermediate affinity IL-2R for its dissociation ($t_{1/2} = 37$ s and 285 min, respectively), IL-2/high affinity IL-2R complexes are most stable (16, 17). In cell lines that bind IL-2 with only intermediate affinity, doublet bands corresponding to p68 + IL-2 and p75 + IL-2 were detected by crosslinking with radiolabeled IL-2 (19, 20). Hermann et al. (21) and we (22) reported that these two bands differ not only in relative mobility in gel but also in the reactivity with anti-IL-2R β chain Ab, although these two bands had been considered to be due to the degradation of receptor-IL-2 complex or to a different posttranslational modification.

The cDNA cloning of the IL-2R β chain using Mik-β1 mAb (13) revealed that the IL-2R β chain contains no kinase domains, although it mediates IL-2 internalization and growth signal transduction when expressed on lymphocytes (23). However, transfected fibroblasts expressing the IL-2R β chain did not bind IL-2 (23–25), suggesting that the β chain must

be modified or associated with another chain for IL-2 binding. This possibility is supported by the fact that human NK cells obtained after in vivo IL-2 therapy expressed less IL-2 binding sites than the number of cell surface β chain (26). Saito et al. (27) also showed that the β chain expressed on a nonlymphoid cell line transfected with its cDNA can bind IL-2 when mixed with detergent-solubilized cell membrane of lymphocytes that cannot bind IL-2. A number of investigations have been done in an attempt to identify the third molecule of the IL-2R by chemical crosslinking or immunoprecipitation, and several candidate proteins have been reported (28–33). Although it is presumed to be close to the β chain or to the high affinity IL-2R, the function of the third molecule of IL-2R remains almost unknown.

In the present study, we identified a nontransfected cell line that seemed to lack the third component of IL-2R, and studied the characteristics of its IL-2R to know the role of the third component in IL-2 binding and subsequent signal transduction.

Materials and Methods

Cells and Antibodies. ED515-D is an IL-2-dependent leukemic T cell line established from an adult T cell leukemia patient (34). Kit225 is a human IL-2-dependent cell line derived from a chronic T lymphocytic leukemia patient (35). They were cultured in growth medium containing RPMI 1640, 10% FCS (Gibco Laboratories, Grand Island, NY), 60 mM tobramycin, 2 mM L-glutamine, and 0.5 nM rIL-2 (a gift from Shionogi Research Laboratories, Osaka, Japan), and incubated in IL-2-free medium for 24–48 h before use. ED515-I is a subclone of ED515-D and proliferates independently of IL-2. The 3T3-$\alpha\beta$11 cell line was established by transfecting cDNA for both human IL-2R α and β chains into NIH-3T3 cells (24). Hut102, ED515-I, and 3T3-$\alpha\beta$11 cells were cultured in growth medium without IL-2. Single cell suspensions of 3T3-$\alpha\beta$11 cells were made by treatment with PBS containing 1 mg/ml BSA and 5 mM EDTA, and then cells were resuspended in medium for each examination.

Binding Assay. The binding of ^{125}I-IL-2 to various types of cell lines at 4°C was measured as described (36). Human rIL-2 (Takeda, Osaka, Japan) was radioiodinated with Enzymobeads (Bio-Rad Laboratories, Richmond, CA) and the specific activity was 35,000 cpm/ng.

Association and Dissociation Kinetics of IL-2 Binding. To study the association kinetics, 4×10^5 cells were incubated with 50 pM of ^{125}I-IL-2 in 200 μl of the binding medium containing 10 mg/ml BSA, 1 mg/ml sodium azide, and 25 mM Hepes in RPMI 1640 (pH 7.4) at 4°C. At selected times, cells were centrifuged through a layer of a mixture of 20% olive oil and 80% di-n-butylphthalate, and the radioactivity of the cell pellet was counted by a gamma counter. To study dissociation kinetics, 4×10^5 cells were incubated with 50 pM of ^{125}I-IL-2 for 60 min at 4°C in 200 μl of the binding medium. After centrifugation, cells were resuspended in 200 μl of the binding medium supplemented with 10 nM cold rIL-2. At various times, the cell-associated radioactivity was measured as indicated.

Internalization of ^{125}I-IL-2. Cells (10^7 cells/ml for ED515-D and ED515-I, 5×10^6 cells/ml for 3T3-$\alpha\beta$11) were first incubated at 4°C for 20 min with 200 pM ^{125}I-IL-2 in RPMI 1640, 25 mM Hepes, pH 7.4, containing 10 mg/ml BSA, and 100 μM chloroquine (37), then quickly warmed to 37°C in a water bath. At

selected times, 200-μl aliquots of the cell suspension were centrifuged, and the radioactivity of the supernatant was measured to determine the level of free IL-2. The cell pellets were resuspended in 200 μl of 0.14 M NaCl, 20 mM glycine-HCl, pH 3.0, containing 1 mg/ml BSA. After 30 s, the cells were centrifuged through a layer of a mixture of 20% olive oil and 80% di-n-butylphthalate, and the radioactivity of the supernatant and the cell pellet was counted to determine the level of internalized or cell surface-bound IL-2 that was resistant or sensitive to the treatment with acidic (pH 3.0) buffer. Nonspecific binding was determined by adding a 1,000-fold excess of unlabeled IL-2.

Western Blotting of Tyrosine-phosphorylated Protein. Tyrosine-phosphorylated proteins were detected by immunoblotting as previously described with minor modifications (38). In brief, aliquots of 10^6 cells per sample were stimulated with rIL-2 (5 nM) and lysed at the indicated times. Whole cell lysates were loaded on to 7.5% SDS-polyacrylamide gels and subjected to electrophoresis. The proteins were transferred to a 0.45-μm Immobilon–polyvinylidene fluoride membrane (Milipore Corp., Bedford, MA) using a semi-dry transfer apparatus. The PVDF blots were blocked with 5% BSA in blotting buffer (20 mM Tris-HCl buffer, pH 7.4, 150 mM NaCl, 0.05% Tween 20) for 24 h, then incubated with purified rabbit antiphosphotyrosine Ab (Zymed Laboratories, Inc., San Francisco, CA) for 60 min followed by incubation with horseradish peroxidase–conjugated anti-rabbit IgG (Tago, Inc., Burlingame, CA) for 60 min. The membrane was washed in blotting buffer containing 1% BSA, then immunoblots were developed using the ECL System (Amersham Corp., Arlington Heights, IL).

Proliferative Response Assay. After preincubation in IL-2-free medium for 48 h, if necessary, cells were cultured at a density of 10^5 cells/ml in 200 μl growth medium for 48 h with serially diluted IL-2. Proliferation was measured by the incorporation of 19 kBq of [^{3}H]TdR (DuPont/NEN Research Products, Boston, MA) per well for the last 4 h of the culture. ED515-I cells in growth medium containing 0.5% FCS were examined in the same manner.

Flow Cytometric Analysis of the Regulation of IL-2R α Chain Expression by IL-2. After preincubation in IL-2-free medium for 24 h, cells were incubated at 3×10^5 cells/ml with or without 1 nM rIL-2 for 18 h at 37°C. Surface-bound IL-2 was removed by washing with acidic buffer for 30 s, and then 10^6 cells were incubated with a saturating quantity of FITC mAbs at 4°C for 30 min. The mean fluorescence intensity (MFI)[1] of the samples was measured using a FACScan® (Becton Dickinson Immunocytometry Systems, Fullerton, CA), and the difference between the MFI of cells stained with anti-Tac mAb and the MFI of cells stained with control mAb was calculated as the quantity of IL-2R α chain expressed on the cells.

Crosslinking Study. Affinity crosslinking was performed as previously described (22).

Results

^{125}I-IL-2 Binding Studies in the Presence of Anti-Tac mAb. 3T3-$\alpha\beta$11 cells bear both high and low affinity IL-2R. However, as reported by Tsudo et al. (24), 3T3-$\alpha\beta$11 cells do not bind IL-2 in the presence of anti-Tac mAb. We examined ^{125}I-IL-2 binding to various cell lines in the presence of anti-Tac mAb. ED515-D, Kit225, and Hut102 cells bound IL-2 with an intermediate affinity (K_d = 1.2–1.6 nM) in the presence of anti-Tac mAb (Table 1). The number of the ^{125}I-IL-2

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[1] *Abbreviation used in this paper:* MFI, mean fluorescence intensity.

Table 1. *^{125}I-IL-2 Binding Assay in the Presence or Absence of Anti Tac mAb*

Cell line	Types of receptor	Without mAb		With anti-Tac mAb	
		K_d	Sites/cell	K_d	Sites/cell
		pM		*pM*	
Kit225	High	5	7,000	–*	–
	Intermediate	–	–	1,200	6,000
	Low	12,000	300,000	–	–
ED515-D	High	4	25,000	–	–
	Intermediate	–	–	1,600	27,000
	Low	13,000	530,000	–	–
ED515-I	High	25	16,000	–	–
	Intermediate	–	–	–	–
	Low	10,000	240,000	–	–
3T3-$\alpha\beta$11	High	30	15,000	–	–
	Intermediate	–	–	–	–
	Low	9,000	640,000	–	–
Hut102	High	12	4,600	–	–
	Intermediate	–	–	1,200	870
	Low	13,000	260,000	–	–

* –, not detected.

binding sites detectable in the presence of anti-Tac mAb on ED515-D and Kit225 cells was almost equal to that of high affinity IL-2R. Intermediate affinity IL-2 binding in the presence of anti-Tac mAb was also detected on Hut102 cells, although the number of binding sites was unexpectedly smaller. On the other hand, anti-Tac mAb completely abolished IL-2 binding to ED515-I cells. These data indicate that ED515-I and 3T3-$\alpha\beta$11 cells appear to have a similar defect in IL-2 binding of the IL-2R β chain. We tentatively called the high affinity IL-2R of these cells (ED515-I and 3T3-$\alpha\beta$11) pseudo–high affinity IL-2R to distinguish from the ordinary high affinity IL-2R of ED515-D and Kit 225 cells in this paper.

The Association and Dissociation Kinetics of ^{125}I-IL-2. The dissociation constant value of the IL-2 binding to the high affinity IL-2R (K_d = 4–5 pM) of ED515-D and Kit225 cells is apparently smaller than that of the pseudo–high affinity IL-2R (K_d = 20–30 pM) (Table 1). Since these results were reproducible, we studied the association and dissociation kinetics of IL-2 for these cell lines. When 50 pM ^{125}I-IL-2 was added to 4 × 10^5 cells, the amount of radioactivity bound to Kit225 cells was the lowest among the four cell lines, since the number of high affinity IL-2R of Kit225 cells was two- to fourfold smaller than those of other cell lines. The association time courses of the IL-2 binding to the high affinity IL-2R were equal among four cell lines ($t_{1/2}$ association = 1 min) (Fig. 1, A and C). However, IL-2 dissociated rapidly from the pseudo–high affinity IL-2R ($t_{1/2}$ dissociation = 1 min) (Fig. 1 B). Though ∼10% of IL-2 dissociated from the high

affinity IL-2R in the first 5 min, the remaining bound IL-2 hardly dissociated during a further 240 min (Fig. 1 D). These data indicate that the higher dissociation constant value of pseudo–high affinity IL-2R is due to the faster dissociation of bound IL-2.
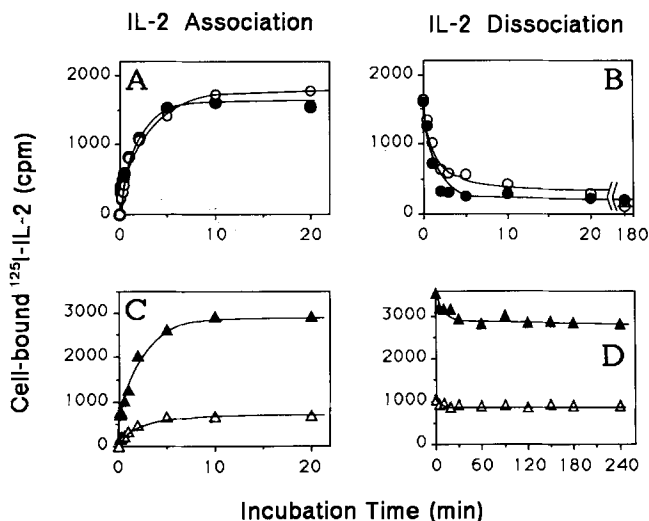

Figure 1. Time courses of association (*A* and *C*) and dissociation (*B* and *D*) of ^{125}I-IL-2 in each cell line. Kinetics were studied as described in Materials and Methods on ED515-I cells (○) and 3T3-$\alpha\beta$11 cells (●) (*A* and *B*), or ED515-D cells (▲) and Kit225 cells (△) (*C* and *D*).
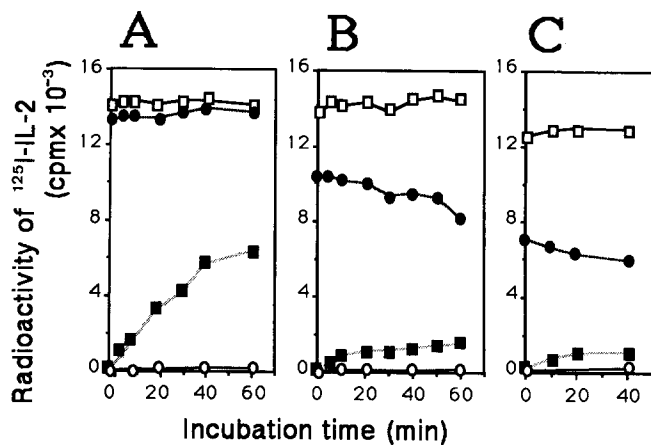
A   B   C

Radioactivity of ^{125}I-IL-2 (cpm x 10^{-3})

16   16   16

12   12   12

8   8   8

4   4   4

0   0   0

0 20 40 60   0 20 40 60   0 20 40

Incubation time (min)

Figure 2. Internalization of ^{125}I-IL-2 into ED515-D (*A*), ED515-I (*B*), and 3T3-$\alpha\beta$11 cells (*C*). At each time, the level of radioactivity in pH3-resistant and internalized ^{125}I-IL-2 (■), and pH 3-sensitive and cell surface-bound ^{125}I-IL-2 was measured. The total cell-bound ^{125}I-IL-2 (●) was measured as the sum of these two fractions. The level of radioactivity in the supernatant was also measured, and the sum of the counts of all three fractions (□) is plotted. After adding excess unlabeled IL-2, pH 3-resistant and internalized ^{125}I-IL-2 was measured (O) as nonspecific internalization. The specific internalization of IL-2 is, therefore, the difference between the total and nonspecific internalization.

IL-2 Internalization and IL-2-induced Tyrosine Phosphorylation of the Cellular Proteins. We examined whether cell-bound IL-2 was internalized into the cells after binding to pseudo-high affinity IL-2R. As shown in Fig. 2 *A*, IL-2 associated with the high affinity IL-2R of ED515-D cells was gradually internalized, and 41% of the total bound IL-2 was internalized at 40 min. Significant internalization of ^{125}I-IL-2 was also de-

tected in ED515-I and 3T3-$\alpha\beta$11 cells, but only 14–18% of total bound IL-2 was internalized at 40 min (Fig. 2, *B* and *C*). The total cell-bound IL-2 decreased in ED515-I and 3T3-$\alpha\beta$11 cells with increasing incubation time, because the dissociation constant value of pseudo-high affinity IL-2R is higher at 37°C than at 4°C (data not shown).

Next, we performed Western blotting using antiphosphotyrosine Ab to investigate whether IL-2 would increase tyrosine phosphorylation in the cells bearing pseudo-high affinity IL-2R. We consistently observed that IL-2 induced or enhanced tyrosine phosphorylation of proteins expressed as five bands on gels with molecular masses of 120, 98, 96, 54, and 38 kD in ED515-D and Kit225 cells (Fig. 3, lanes *3–8*). Similar induction or enhancement of tyrosine phosphorylation of proteins with apparent molecular masses of 98, 96, and 38 kD by IL-2 was detected in Hut102 cells (Fig. 3, lanes *11–13*). Tyrosine phosphorylation was detected within 1 min after the addition of IL-2, and the maximal increase was observed 10 min later. IL-2 was reported to induce the tyrosine phosphorylation of the IL-2R β chain (39, 40). The broad and vague band at ~84 kD in ED515-D and Hut102 cells may include the IL-2R β chain. On the other hand, IL-2 induced no increase in tyrosine phosphorylation in ED515-I cells (Fig. 3, lanes *1* and *2*). We observed several bands that were constitutively tyrosine phosphorylated in 3T3-$\alpha\beta$11 cells, but IL-2 induced no increase (Fig. 3, lanes *9–10*).

The Proliferative Response to IL-2 and the Upregulation of the IL-2R α Chain Expression by IL-2. As shown in Fig. 4 *B*, ED515-D and Kit225 cells displayed a proliferative response to rIL-2 as measured by [^{3}H]TdR uptake, whereas IL-2 did not enhance [^{3}H]TdR uptake by 3T3-$\alpha\beta$11 and ED515-I cells (Fig. 4 *A*). Since we supposed that ED515-I cells proliferated at their maximum in medium with 10% FCS and that
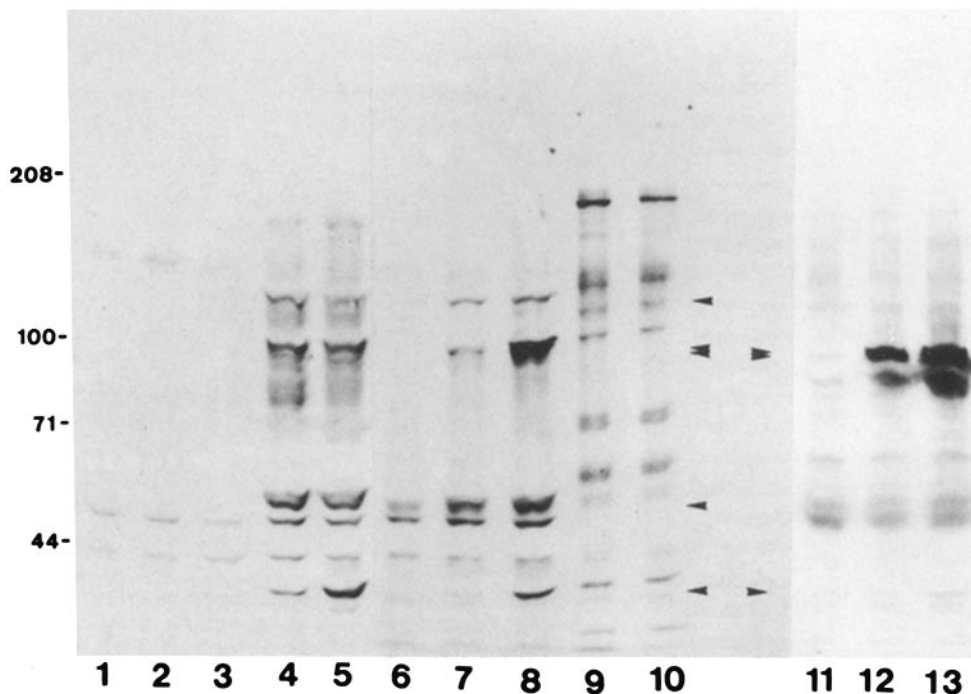

208-

100-

71-

44-

1 2 3 4 5 6 7 8 9 10   11 12 13

Figure 3. Effect of IL-2 on the phosphorylation of proteins at tyrosine residues in ED515-I (lanes *1* and *2*), ED515-D (lanes *3–5*), Kit225 (lanes *6–8*), 3T3-$\alpha\beta$11 (lanes *9* and *10*), and Hut102 cells (lanes *11–13*) incubated for 0 (lanes *1, 3, 6, 9,* and *11*), 1 (lanes *4, 7,* and *12*), or 10 min (lanes *2, 5, 8, 10,* and *13*) with 2 nM IL-2. The samples were then immunoblotted with Ab to phosphotyrosine as described in Materials and Methods. The arrowheads indicate the position of the 120-, 98-, 96-, 54-, and 38-kD proteins of which tyrosine phosphorylation was increased by IL-2.
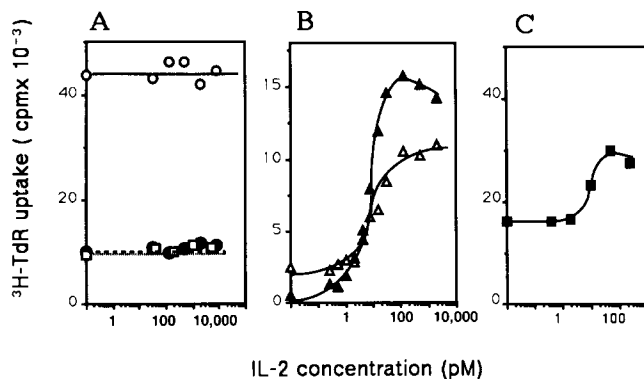
Figure 4. Proliferative response of various cell lines to IL-2. The IL-2 response was monitored by the [³H]TdR uptake in (A) ED515-I cells cultured with 10% FCS (○) or 0.5% FCS (□), and 3T3-αβ11 (●), (B) ED515-D (△), Kit225 cells (▲), and (C) Hut102 cells (■).

Figure 5. Affinity crosslinking of the IL-2R in various cell lines. Lane 1, ED515-D cells; lane 2, Kit225 cells; lane 3, ED515-I cells; lane 4, 3T3-αβ11 cells. The arrowheads indicate the presence of the middle band seen in ED515-D cells and Kit225 cells.

the addition of IL-2 no longer induced further thymidine incorporation into ED515-I, cell growth–promoting activity of IL-2 in these cells was examined in medium containing a low concentration of FCS (0.5%). However, ED515-I cells also did not respond to IL-2 in this culture condition. In contrast, Hut102 cells, which can be maintained without IL-2, showed IL-2-inducible [³H]TdR uptake in addition to the relatively high basic [³H]TdR uptake (Fig. 4 C).

It was reported that IL-2 upregulates the expression of the IL-2R α chain on T cells (41, 42), and we examined the upregulation of the IL-2R α chain expression by IL-2 stimulation in the cells bearing pseudo–high affinity IL-2R. In ED515-D and Kit225 cells, the addition of IL-2 resulted in a ~1.4–1.6-fold greater expression of the IL-2R α chain as measured by flowcytometric analysis (Table 2). In ED515-I cells, however, the upregulation of IL-2R α chain expression was not found.

¹²⁵I-IL-2 Crosslinking Studies. To explore the molecular properties of the two different types of high affinity IL-2R, a ¹²⁵I-IL-2 crosslinking study with chemical crosslinkers was

Table 2. *The Upregulation of the IL-2R α Chain by IL-2*

Cell line	MFI*	
	− IL-2	+ IL-2
Kit225	81.8	133.9 (164)‡
ED515-D	341.4	486.1 (142)
ED515-I	64.0	60.9 (95)

* Cells were stained with FITC-conjugated anti-Tac mAb or control mAb followed by FACScan® analysis, and the difference between MFI of cells stained with anti-Tac mAb and that with control mAb was calculated as the quantity of IL-2R α chain expression.
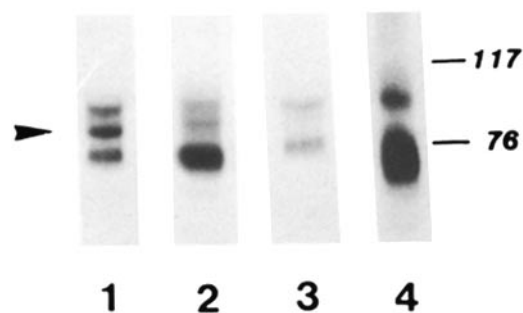‡ MFI of each cell line cultured with IL-2 is also shown as the percentage of that cultured without IL-2.

performed in these cells. Three bands, 72, 80, and 89 kD, were detected in ED515-D and Kit225 cells (Fig. 5, lanes 1 and 2). However, the middle p65 + IL-2 (15-kD) band was not detected in ED515-I and 3T3-αβ11 cells, although both the upper and lower bands corresponding to the IL-2R α and β chains were detected (Fig. 5, lanes 3 and 4).

Discussion

We demonstrated in the present study that ED515-I and 3T3-αβ11 cells have the following characteristics. (a) The IL-2R β chains of the cell lines were not fully functional as intermediate affinity IL-2R, because the anti-Tac mAb completely abolished IL-2 binding to these cell lines. (b) Their high affinity IL-2Rs had higher dissociation constants than those of ED515-D and Kit225. (c) IL-2 bound to them dissociated much faster than that bound to ED515-D and Kit225 cells, although the rate of IL-2 association was similar. (d) IL-2 bound to their high affinity IL-2R was internalized more slowly and to a less extent than in ED515-D and Kit225 cells. (e) IL-2 did not induce or enhance tyrosine phosphorylation of cellular proteins, which was demonstrated in ED515-D, Kit225, and Hut102 cells. (f) IL-2 neither promoted cell growth nor upregulated the IL-2R α chain expression. (g) The protein with a presumed molecular mass of 65 kD and crosslinked with IL-2 was not detected, unlike ED515-D and Kit225 cells.

In this paper we called the high affinity IL-2R of ED515-I and 3T3-αβ11 cells "pseudo–high affinity IL-2R" in comparison with ordinary high affinity IL-2Rs of ED515-D and Kit225 cells. However, we have no evidence that ED515-D and Kit225 cells have exclusively ordinary high affinity IL-2R. The IL-2 dissociation kinetics of high affinity IL-2Rs appeared to be biphasic in ED515-D and Kit225 cells (Fig. 1 D). About 10% of total cell-bound IL-2 that dissociated from the high affinity IL-2Rs of ED515-D and Kit225 cells in the first 5 min may have bound to the pseudo–high affinity IL-2Rs, if any, of these cell lines. Furthermore, Hut102 cells have a smaller number of the intermediate affinity IL-2-binding sites in the presence of anti-Tac mAb than that of high affinity IL-2-binding sites (Table 1). This result may indicate that Hut102 cells bear

a smaller number of the third components necessary for functional intermediate affinity IL-2R than that of the IL-2R β chain.

It has been controversial whether IL-2 can be internalized into fibroblasts transfected with cDNA of human IL-2R α and β chains (24, 25). Minamoto et al. (25) reported that L929$\alpha\beta$, a mouse fibroblast transfected with human IL-2R α and β chains, lacks IL-2 internalization. They, however, removed unbound ^{125}I-IL-2 after the initial incubation of cells and ligand. Considering our IL-2 dissociation kinetics data, ^{125}I-IL-2 may have dissociated from L929$\alpha\beta$ cells during cell washing or the incubation steps in their studies. Slower and less internalization of cell-bound IL-2 in ED515-I and 3T3-$\alpha\beta$11 cells demonstrated in the present study may suggest the different internalization mechanism or pathway in such cells bearing pseudo–high affinity IL-2R.

The signal transduction pathway of IL-2/IL-2R remains ill defined. The early activation of a tyrosine kinase occurring in response to IL-2 stumulation has been considered to be one of the key events in IL-2 signal transduction (39, 40, 43–45). Although the IL-2R β chain is phosphorylated on tyrosine and serine/threonine residues, the cytoplasmic portion of the IL-2R β chain lacks an obvious kinase domain. Fung et al. (45) reported that some tyrosine kinase physically associated with the IL-2R β chain. It was also reported that several proteins with molecular masses ranging from 38 to 180 kD were phosphorylated on tyrosine upon IL-2 stimulation. We showed that cellular proteins expressed as five bands on gels with molecular masses of 120, 98, 96, 54, and 38 kD were tyrosine phosphorylated upon IL-2 stimulation in ED515-D and Kit225 cells. Since such IL-2-induced tyrosine phosphorylation was undetectable in ED515-I and 3T3-$\alpha\beta$11 cells, the pseudo–high affinity IL-2R may be defective in some receptor-associated protein that is indispensable for not only the complete IL-2 binding but also IL-2 signaling. It is noteworthy that Hut102 cells, IL-2 independent in their cell growth and retaining intermediate affinity IL-2 binding in the presence of anti-Tac mAb, showed IL-2-inducible [^{3}H]TdR uptake and tyrosine phosphorylation in contrast to no response of ED515-I and 3T3-$\alpha\beta$11 cells lacking for p65. It is, therefore, unlikely that the loss or absence of p65 is closely associated with the acquisition of IL-2 independency in cell growth and that no response of ED515-I and 3T3-$\alpha\beta$11 cells is simply due to the loss of IL-2 dependency.

Hatakeyama et al. (46) reported that an IL-1R β chain mutant with a restricted cytoplasmic serine-rich region (amino acids 267–322) bound IL-2 with coexpression of the IL-2R α chain, followed by internalization, but its growth was not affected by IL-2. The similarity of the biological response between this IL-2R β chain mutant cell and pseudo–high affinity IL-2R-bearing cells as we reported here may show the importance of the serine-rich region (amino acids 267–322) of the IL-2R β chain for the interaction between the IL-2R β chain and the third component of IL-2R.

Studies using radiolabeled IL-2 and crosslinking reagents have indicated that a series of additional proteins of 22, 35–40, 90, 100, and 115 kD are associated with the IL-2R α and β chains in the murine cells (28–30). However, it was difficult to detect them reproducibly. The 80–90-kD non-Tac band easily detectable in the affinity crosslinking was not a single band, but a doublet (19, 20). As we recently reported, the lower band of the doublet appears not to be the IL-2R β chain, because polyclonal anti-IL-2R β chain Ab cannot react with it (22). By using limited proteolysis, Hermann and Diamant-stein also reported that this lower doublet band differs from the upper one, although they both displayed a high homology. As shown in Fig. 5, the lower band of the doublet was not detected in either ED515-I or 3T3-$\alpha\beta$11 cells, suggesting that the key molecule for functional IL-2R is the 65-kD molecule (p65). This is supported by the recent report by Takeshita et al. (33). They showed that the amount of p64 coprecipitated with the IL-2R β chain in the presence of IL-2 was proportional to the number of the IL-2 binding sites, but not to that of the IL-2R β chain. Our observation that p65 could be crosslinked with IL-2 in cells bearing high affinity IL-2R but not in cells bearing pseudo–high affinity IL-2R leads to two possible explanations with respect to the role of p65 in IL-2 binding. One is that p65 directly binds IL-2 as the supporter of the IL-2R β chain, and the other is that p65, located close to the IL-2R β chain, exerts a steric effect on the β chain without directly binding IL-2. The latter seems likely because the MT-β7 cell, which is MT-1 cell transfected with the human IL-2R β chain (47), bound IL-2 with an intermediate affinity when examined in the presence of anti-Tac mAb (data not shown), suggesting that MT-β7 cells have the third component of IL-2R. Parental MT-1 cells, however, bind IL-2 with only low affinity and IL-2 cannot be crosslinked with p65 in MT-1 cells (22). These data suggest that the third component cannot bind IL-2 without the help of the IL-2R β chain.

Finally, it would be of interest to determine whether there exist such cells having pseudo–high affinity IL-2R in vivo and which biological responses they, if present, are involved in.

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