A LARGER NUMBER OF L CHAINS (Tac) ENHANCE THE ASSOCIATION RATE OF INTERLEUKIN 2 TO THE HIGH AFFINITY SITE OF THE INTERLEUKIN 2 RECEPTOR

By YUJI SAITO, HISATAKA SABE, NOBORU SUZUKI, SHIGERU KONDO, TOSHIHIKO OGURA, AKIRA SHIMIZU, and TASUKU HONJO

From the Department of Medical Chemistry, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan

IL-2 is a major growth factor of mature T lymphocytes. Three distinct binding sites for IL-2, namely, low (K_d ~10 nM), intermediate (K_d ~1 nM), and high (K_d ~10 pM) affinity sites, have been found on T lymphocytes (1, 2). Chemical cross-linking experiments have shown that two polypeptide chains of the IL-2-R, the L (p55 or Tac) and H (p75 or converter) chains, bind IL-2 with low and intermediate affinities, respectively (1–4). The high affinity binding complex is composed of each mole of IL-2, the L and H chains (5). The association rate of IL-2 to the L chain was estimated to be as fast as that to the high affinity site and 100 times faster than that to the H chain, while the dissociation rate of IL-2 from the L chain was 1,000 times faster than that from the H chain (6, 7).

Experiments using mutants of the L chain cDNA have shown that the L chain is not directly involved in the growth signal transduction (8), whereas the H chain alone seems to be responsible for the growth signal transduction (6). The L chain is not expressed on mature T cells until they are stimulated by antigens or mitogens (9). Stimulated T cells express a large number (10^4 or more) of L chains per cell (9, 10). By contrast, a small number (10^3 or less) of H chains are constitutively expressed on unstimulated spleen T cells (11). These findings raised a series of interesting questions. For what is the L chain required in the IL-2-R system? Why is the number of L chains of stimulated T cells always over that of H chains? Why are the H and L chains regulated in different manners?

We have proposed an affinity conversion model that can provide a reasonable explanation for the above questions (5, 12, 13). This model assumes that IL-2 would first bind to the L chain and that the complex of the L chain and IL-2 would then associate with the H chain, forming the high affinity ternary complex. According to this model, a large number of L chains are useful and essential to accelerate the high affinity binding. Only a smaller number of H chains are required because the association of the H chain with the IL-2/L chain complex should be faster than the binding of IL-2 to the L chain. As the amplification of oncogenes related to the receptors responsible for the growth signal transduction often causes cell transformation (14, 15), expression of limited and stable numbers of H chains would be required for lymphocytes to escape from transformation. Induction of a large number of L chains

This work was supported by grants from the Ministry of Education, Science and Culture of Japan.

inert to the cellular activity yet efficient for binding IL-2 could be a strategy for lymphocytes to avoid transformation while transducing growth signal efficiently at physiological IL-2 concentrations that are usually very low.

On the other hand, others proposed that the high affinity site would be a preformed binary complex of the L and H chains (1, 2). These investigators have suggested that the binary complex of the L and H chains would use the faster association and the slower dissociation rates of the two chains to serve as the high affinity site (6, 7). However, the preformed binary complex model has not provided a reasonable explanation for the different regulatory systems and unbalanced numbers of the L and H chains.

In the present study we have measured association and dissociation rates of high affinity sites on T cells which express various numbers of L chains and a relatively constant number of H chains. We showed that expression of excess numbers of L chains contributed greatly to the increment of the association rate of IL-2 to the high affinity binding. These results, together with evaluation of kinetic constants, are favorable to the affinity conversion model and not compatible with the model that postulates the formation of the binary complex of the L and H chains before IL-2 binding.

Materials and Methods

Materials. ATL-2 and MT-1 are human T cell lines containing the HTLV-1 genome (16). YTC3 is a human thymoma cell line which has the NK-like activity (3, 4). These cells were cultured in RPMI 1640-10% FCS. PBMC isolated by Ficoll-Hypaque discontinuous gradient centrifugation were stimulated with 1 μg/ml PHA for 48 h, after which the cells were cultured in RPMI 1640-20% FCS and 0.05 mM 2-ME containing 12.5 ng/ml human rIL-2 (9). Purified rIL-2 was a gift from Takeda Chemical Industries (Osaka, Japan). ^{125}I-labeled human IL-2 was purchased from Amersham Corp. (Buckis, England). mAb against the human IL-2-R (anti-Tac) was provided by T. Uchiyama, Kyoto University (17).

Scatchard Plot Analysis. The binding of ^{125}I-IL-2 was assayed as described (8, 16). PBMC were centrifuged four times and incubated in IL-2-free medium for 4 h to remove bound IL-2, and were centrifuged twice. Cells were then suspended in RPMI-1640-10% FCS/25 mM Hepes, pH 7.4, containing ^{125}I-IL-2 at various concentrations. After 30 min at 37°C, free and cell-bound ^{125}I-IL-2 were separated by passing through an olive oil cushion and measured using a gamma counter.

Kinetics of IL-2 Binding. For the measurement of the kinetics of IL-2 binding, bound and free rIL-2 in medium were measured as in Scatchard plot analyses (8, 16). For association, cells were incubated with 50 pM ^{125}I-IL-2 at 37°C in 200-μl aliquots for sampling at various time intervals. Nonspecific binding was determined by the addition of 1,000-fold unlabeled rIL-2. To measure the kinetics of binding to the H chain, YTC3 was preincubated with the anti-Tac mAb (20% ascites or 50 μg/ml antibody). MT-1 cells were used to measure the kinetics of binding to the L chain. The concentrations of cells were 8.0×10^5/ml for YTC3, 3.5×10^5/ml for ATL-2, and 5×10^6/ml for PBMC. The experiments were designed to fulfill the following conditions: (*a*) the total number of ATL-2 H chains is about equal to that of YTC3 L chains; (*b*) the concentration of the ligand is in excess over the concentration of receptors so that the reaction follows the pseudo-first order; (*c*) the concentration of IL-2 is low enough (i.e., 50 pM) to occupy only the high affinity IL-2-R without virtual binding to the low and intermediate affinity sites. For dissociation, cells were preincubated with 50 pM ^{125}I-IL-2 at 4°C for 60 min and then 1,000-fold unlabeled rIL-2 was added. At various time intervals after shifting the temperature to 37°C, bound vs. free ^{125}I-IL-2 was determined by centrifugation of the cells through an oil cushion.

Results and Discussion

Kinetic Predictions of the Affinity Conversion Model and the Preformed Binary Complex Model. Both models agree with the overall reaction shown below but differ in their proposed partial reactions:

$$\text{IL-2} + \text{L} + \text{H} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{L·H·IL-2}$$

The affinity conversion model can be shown by two partial reactions as follows:

$$\text{IL-2} + \text{L} \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} \text{L·IL-2} \tag{1}$$

$$\text{L·IL-2} + \text{H} \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} \text{L·H·IL-2} \tag{2}$$

where L and H indicate the L and H chains of the IL-2-R, respectively.

We can determine above rate constants under the pseudo-first order conditions. We have confirmed that the k_1 and k_1' values are $\sim 1\text{–}2 \times 10^7$/M/s and $\sim 2\text{–}4 \times 10^{-1}$/s, respectively (6). To solve Eqs. 1 and 2 we have to introduce two assumptions as follows: (*a*) the k_2' value is negligible; and (*b*) the concentration of L·IL-2 is constant. Since the ternary complex (L·H·IL-2) is extremely stable (5), the assumption in *a* is reasonable. In fact, the dissociation rate (k_{off}) of the ternary complex was estimated to be 2.3×10^{-4}/s (6), which is far smaller than the k_1' value. The k_1 value is probably smaller than the k_2 value because the reaction (Eq. 2) takes place on the cell surface (two-dimensional reaction). If so, the concentration of L·IL-2 will not reach equilibrium, but will remain low at the steady state, and the assumption in *b* is also reasonable. Under the condition that the concentrations of IL-2 and L are excess over that of H, the concentration of the tenary complex (X) at a given time (t) is given by the following equation according to the Eqs. 1 and 2:

$$\ln \frac{H_0}{H_0 - X} = \frac{k_1 k_2}{k_1'} (I_0 L_0 - H_0 L_0 - H_0 I_0)\, t \tag{3}$$

where I_0, L_0, and H_0 represent the initial concentrations of IL-2, L, and H chains, respectively. Under the condition that the concentrations of IL-2 and H are over that of L, the following equation is drawn:

$$\ln \frac{L_0}{L_0 - X} = \frac{k_1 k_2 t}{\dfrac{k_2}{I_0 - L_0} + \dfrac{k_1'}{(H_0 I_0 - I_0 L_0 - H_0 L_0)}} \tag{4}$$

From the Eqs. 3 and 4 it is obvious that the velocity of the ternary complex formation, i.e., the high affinity binding, should be accelerated by increasing the L or H chain concentration.

On the other hand, the preformed binary complex model can be represented by the reaction as follows:

$$\text{IL-2} + \text{L·H} \underset{k_3'}{\overset{k_3}{\rightleftharpoons}} \text{L·H·IL-2} \tag{5}$$

Here, the initial concentration of L·H is equal to the concentration of a limiting component of the two chains. The following simpler equation can be drawn from Eq. 5 assuming that the concentration of IL-2 is over that of L·H:

$$\ln \frac{R_0}{R_0 - X} = k_3 I_0 t \tag{6}$$

where R_0 is the initial concentration of L·H. This equation indicates that the velocity of the high affinity binding would not be affected by increasing numbers of the more abundant component of the two chains according to the preformed binary complex model.

These kinetic predictions indicate that we can distinguish the above two models by testing whether the high affinity binding is accelerated by the increment of the L (or H) chain concentration. The k_3 values calculated by Eq. 6 should remain constant if the preformed binary complex model is correct. As the left sides of the Eqs. 3, 4, and 6 are identical, the apparent k_3 value would become larger by increasing the concentration of the abundant chain of the two if the affinity conversion model is correct.

Kinetic Comparison of ATL-2 and YTC3 Cells. We have compared binding kinetics with the high affinity IL-2-R of ATL-2 and YTC3 cells that express comparable numbers (3×10^4 and 6×10^4, respectively) of H chains per cell but different numbers (5×10^5 and 1×10^4, respectively) of L chains per cell. The binary complex model assumes that ATL-2 and YTC3 cells would have the same concentration of the L·H complex and so an equivalent association rate of IL-2 to the high affinity IL-2-R when the total numbers of the limiting chains, i.e., the H chain for ATL-2 and the L chain for YTC3, are matched by adjusting cell numbers in the assay system. On the other hand, the affinity conversion model predicts that ATL-2 cells would show more rapid association of IL-2 to high affinity sites than YTC3 cells because ATL-2 cells have a larger number of L chains under the same conditions.

An association profile of IL-2 binding to the high affinity IL-2-R on these cells are displayed in Fig. 1 *A*. The binding curves declined with prolonged incubation probably because IL-2 bound to the high affinity IL-2-R was rapidly internalized with a $t_{1/2}$ value of ~15 min and IL-2-R was downregulated (data not shown). The experiments were done practically under the pseudo-first order conditions because only 20% of added IL-2 was bound at 30 min when the maximal amount of IL-2 was bound to ATL-2. Under these conditions the k_3 value for ATL-2 (9.8×10^7/M/s) was about six times larger than that of YTC3 (1.7×10^7/M/s), which disagrees with

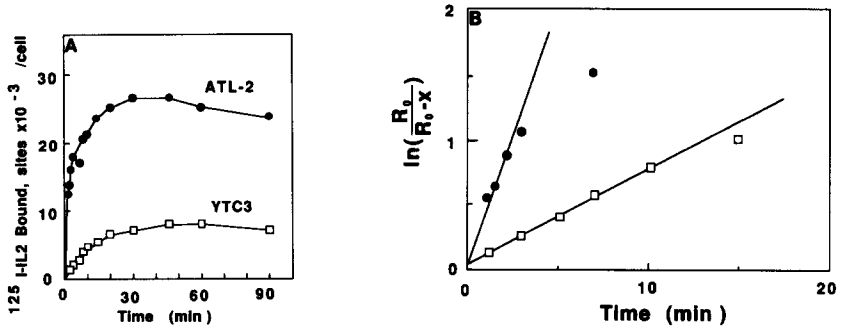

FIGURE 1. Kinetics of IL-2 association to the high affinity IL-2-R of ATL-2 (●) and YTC3 (□). (*A*) The time courses of IL-2 association were measured by incubating the cells with 50 pM ^{125}I-IL-2 at 37°C. At time intervals indicated, aliquots were taken and assayed for amounts of bound radioactivity. (*B*) Natural log plots of the association profiles shown in *A* according to Eq. 6. *X*, bound ^{125}I-IL-2 at time *t*. R_0, the total number of high affinity IL-2-R.

the binary complex model (Fig. 1 *B*). By contrast, the k_2 values of ATL-2 and YTC3 cells calculated by Eqs. 3 and 4, respectively, were essentially identical, i.e., 5.6 × 10^9/M/s for ATL-2 and 3.7 × 10^9/M/s for YTC3 (Table I). These results are consistent with the affinity conversion model and contradictory to the binary complex model.

Abundant L Chains Expressed on PBMC Accelerate the Rate of High Affinity Binding to IL-2. To confirm acceleration of the IL-2 binding to high affinity sites by abundant L chains, we investigated the binding kinetics of IL-2 to the high affinity IL-2-R on lectin-stimulated PBMC. The number of L chains on PBMC rapidly increased after lectin stimulation and progressively declined. Equilibrium and kinetic IL-2 binding studies were performed on days 5, 7, 9, and 11 after PHA stimulation. The numbers of L and H chains of each PBMC preparation were estimated by Scatchard plot analysis. The average numbers of L chains per cell were maximal on day 5 (3.3 ×

TABLE I
Summary of Kinetic Analysis

Cells	L chain	H chain	IL-2	k_2	k_3	k_{off} $=k_2'=k_3'=k_5'$	k_3'/k_3	k_4k_5/k_4'
	pM	*pM*	*pM*	*/M/s*	*/M/s*	s^{-1}	*M*	*/M²/s*
YTC3	13	80	50	3.7×10^9	1.7×10^7	1.2×10^{-4}	7.0×10^{-12}	3.2×10^{17}
ATL-2	290	17	50	5.6×10^9	9.8×10^7	1.4×10^{-4}	1.5×10^{-12}	5.6×10^{17}
PBMC								
Day 5	280	14	50	4.5×10^9	8.3×10^7	1.9×10^{-4}	2.3×10^{-12}	4.5×10^{17}
Day 7	81	12	50	2.7×10^9	1.3×10^7	1.5×10^{-4}	1.2×10^{-11}	2.7×10^{17}
Day 9	25	8	50	8.4×10^9	1.1×10^7	2.0×10^{-4}	1.8×10^{-11}	8.4×10^{17}
Day 11	34	10	50	7.8×10^9	1.3×10^7	1.9×10^{-4}	1.5×10^{-11}	7.8×10^{17}

The numbers of L and H chains were estimated by the numbers of low and high affinity IL-2-R by means of Scatchard plot analysis performed at 37°C. The concentrations of L and H chains were calculated from the products of the estimated numbers of L and H chains, and cell concentrations, which are described in the text and in Materials and Methods. The association and dissociation rate constants were calculated from log-linearized plots of the respective time course, according to equations described in the text. Data were from Figs. 1 and 2.

10^4) and gradually declined to 9.5 × 10^3 (day 7), 3.0 × 10^3 (day 9), and 4.0 × 10^3 (day 11). The average numbers of H chains per cell remained almost constant within the range between 900 and 1,600.

The association rate increased in parallel with the number of L chains per cell, as shown in Fig. 2 *A*. The association rate constants (k_3) on day 5, day 7, day 9, and day 11 were 8.3 × 10^7/M/s, 1.3 × 10^7/M/s, 1.1 × 10^7/M/s and 1.3 × 10^7/M/s, respectively (Fig. 2 *B*). This result also indicates that abundant L chains expressed on PBMC after lectin stimulation facilitate IL-2 binding to the high affinity IL-2-R. By contrast, the association rate constants (k_2) calculated by Eq. 3 were again almost constant among these PBMC, YTC3, and ATL-2 (Table I), except that the k_2 values for day 9 and day 11 PBMC deviated slightly from the others. This deviation may be due to the following two reasons: (*a*) the ratio of L/H is <3.5 and does not fulfill the pseudo-first order condition completely; and (*b*) the effect of internalization may not be negligible when the total number of receptors is less. These results taken together, our kinetic data are not compatible with the preformed binary complex model but are consistent with the affinity conversion model.

We have measured the dissociation rate (k_{off}) of the L·H·IL-2 complex by the release of IL-2 from IL-2-bound cells. The k_{off} values of 1.4 × 10^{-4}/s (ATL-2) and 1.2 × 10^{-4}/s (YTC3) are close to that (2.3 × 10^{-4}/s) reported previously (6). Dissociation profiles from the high affinity IL-2-R of PBMC also remained virtually constant, with k_{off} values of ~1.5–2.0 × 10^{-4}/s (Table I). According to the binary complex model the above k_{off} value must be equal to the k_3' value. As the independently determined k_1' value is much faster than the k_{off} value, it is reasonable to assume that the k_{off} value represents the k_2' value when we postulate that the Eqs. 1 and 2 are the major pathway. To confirm that IL-2 binding sites examined were mainly high affinity sites, the equilibrium dissociation constants calculated from the ratio of the two rate constants (k_3'/k_3) (Table I) were compared with the equilibrium dis-

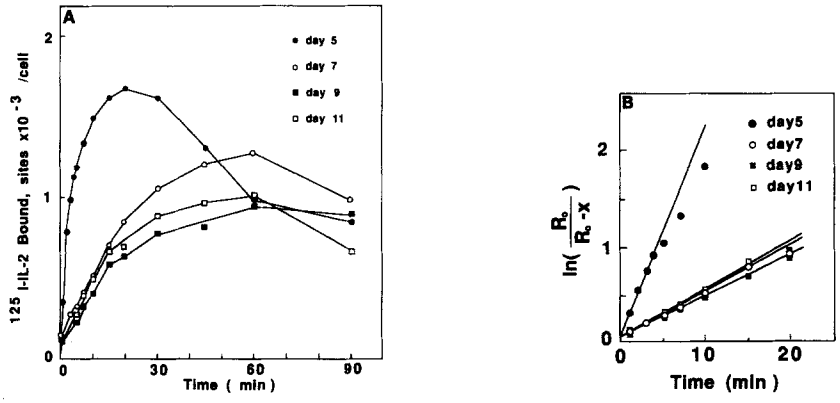

FIGURE 2. Kinetics of IL-2 association to the high affinity IL-2-R of lectin-stimulated PBMC. (*A*) The time courses of IL-2 association. Kinetic IL-2 binding study was performed on days 5, 7, 9, and 11 after PHA stimulation. Association kinetic experiments were initiated by incubating cells with 50 pM ^{125}I-IL-2 at 37°C. (*B*) Natural log plots of the association profiles shown in *A* according to Eq. 6. *X*, bound ^{125}I-IL-2 at time *t*. R_0, the total number of the high affinity IL-2-R.

sociation constant of the high affinity IL-2-R (K_{dh}), which was determined by Scatchard plot (8, 13, 18). The k_3'/k_3 values varied considerably, although they were closest to the K_{dh} value (10 pM) among the three K_d values of IL-2-R. The K_{dh} estimation by the previous Scatchard plot (8, 13, 18) has assumed the fixed number of high affinity sites. In this case, K_{dh} values would be calculated by k_3'/k_3, which should be constant. Therefore, the fact that the K_{dh} values per se are variable, with the range between 5 and 50 pM (8, 13, 18), is contradictory to the binary complex model.

According to the affinity conversion model, however, such K_{dh} values should vary depending on the number of L chains because the equilibrium of the overall reaction is determined by the combination of the Eqs. 1 and 2. The K_{dh} values determined by the previous Scatchard plot do not represent the real K_{dh} value, which should be approximately equal to the product of the K_d values of the Eqs. 1 and 2, i.e., $k_1'k_2'/k_1k_2$. When the k_1 value of 1.5×10^7/M/s is used (reference 6 and our data), the $k_1'k_2'/k_1k_2$ value is $\sim 6 \times 10^{-22}$M^2. The results confirm that IL-2 bound to the high affinity IL-2-R and give further support to the affinity conversion model.

Kinetic Evaluation of an Intermediate Model. We have also considered a variation of the preformed binary complex model, which postulates the following two partial reactions:

$$L + H \underset{k_4'}{\overset{k_4}{\rightleftharpoons}} L{\cdot}H \tag{7}$$

$$L{\cdot}H + IL\text{-}2 \underset{k_5'}{\overset{k_5}{\rightleftharpoons}} L{\cdot}H{\cdot}IL\text{-}2 \tag{8}$$

The equilibrium of the reaction (Eq. 7) is not entirely favorable to the binary complex formation and only a fraction of H chains are associated with L chains although the reaction (Eq. 8) is identical to the reaction (Eq. 5). To solve Eqs. 7 and 8 we have introduced two assumptions as follows; (*a*) the k_5' value is negligible and (*b*) the concentration of L·H is determined by the equilibrium of the reaction in Eq. 7. The assumption in *a* is reasonable because of the same observation as described above for the k_2' value. The reaction in Eq. 7, which takes place on the cell surface, is likely to proceed more rapidly than the reaction in Eq. 8 and unlikely to be the rate-limiting step of the overall reaction, which makes the assumption in *b* reasonable. Under the condition that the concentrations of IL-2 and L are excess over H, the reactions in Eqs. 7 and 8 will draw the following equation:

$$\ln \frac{H_0}{H_0 - X} = \frac{k_4k_5}{k_4'} (I_0L_0 - H_0I_0 - H_0L_0)\, t \tag{9}$$

Under the condition that the concentrations of IL-2 and H are excess over L, the reactions in Eqs. 7 and 8 will draw the following equation:

$$\ln \frac{L_0}{L_0 - X} = \frac{k_4 k_5}{k_4'} (I_0 H_0 - L_0 I_0 - L_0 H_0)\, t \qquad (10)$$

From Eqs. 9 and 10 we calculated k_4k_5/k_4' values, which were relatively constant and averaged at $5.4 \times 10^{17}/M^2/s$ (Table I). According to the intermediate model, the k_{off} value should represent the k_5' value and the K_{dh} value is equal to the k_5'/k_5 value. When the k_5' value of 2×10^{-4}/s and the K_{dh} value of 1×10^{-11}M were used, the k_5 value of 2×10^7/M/s was obtained. Since $k_4k_5/k_4k_5/k_4' = 5.4 \times 10^{17}/M^2/s$, the association constant of the reaction in Eq. 7, i.e., k_4/k_4', is equal to 2.7×10^{10}/M. With this association constant the majority of the H chains should be associated with the L chain under the experimental conditions used, which is contradictory to the original definition of the intermediate model.

On the other hand, the determined rate constants of the Eqs. 1 and 2, i.e., k_1 (1.5×10^7/M/s), k_2 (4.1×10^9/M/s), k_1' (3×10^{-1}/s) and k_2' (1.7×10^{-4}/s) satisfy the original assumptions introduced to calculate them. As expected, the reaction shown by Eq. 1 is far slower than the reaction of Eq. 2. Although the k_2 value seems to exceed the maximal k value that is limited between 10^8 and 10^9/M/s by diffusion in solution (19), this k_2 value is exaggerated by the increase of effective concentrations of L·IL-2 and H whose movement is limited within the two-dimensional cell surface.

Experimental conditions used for previous determination of the k_3 values were not clear (6) or not satisfactory for the pseudo-first order reaction (7). However, the k_1 and k_1' values were confirmed by our experiments. The association and dissociation rate constants for the H chain were also confirmed to be 1.5×10^5/M/s and 3×10^{-4}/s, respectively.

Physiological Significance of the Affinity Conversion Model. If the reaction of Eq. 2 takes place, it may be also likely to assume that the reaction of Eq. 7 occurs to some extent because the L and H chains are expected to have some affinities even without the ligand. Consequently, even the reaction of Eq. 8 may occur to some extent. Inversely, if we assume the reaction of Eq. 7, it is reasonable to assume that the reaction of Eq. 2 will also take place. In fact, the reactions of Eqs. 1, 2, 7, and 8 all may take place more or less. The most important question is what is the major pathway to form the L·H·IL-2 complex. The presence of the L·H complex alone does not prove the intermediate model. Our kinetic evaluation indicates that the reactions of Eqs. 1 and 2 are the major pathway to form the ternary complex of IL-2, the H and L chains.

According to the intermediate model, the kinetics of the L and H chains are identical except for their difference in the K_d value to IL-2. According to the affinity conversion model, however, the increase of the L chain has the stronger effect to the acceleration of the overall reaction than that of the H chain because the reaction of Eq. 1 is the rate-limiting step. Although the reaction of Eq. 1 is already proven to take place, the intermediate and binary complex models assume that the reaction of Eq. 1 per se would not contribute to the high affinity binding at all, whereas the affinity conversion model assumes that the reaction of Eq. 1 would be the initiation of the high affinity binding.

The two different ways of the regulatory expression of the L and H chains of IL-2-R (11) and the excess number of L chains on stimulated T cells suggest that the L and H chains may have different functional roles. Both kinetic and physiological

evaluations indicate that the affinity conversion model are more favorable than the other two.

Summary

The IL-2-R is composed of at least two proteins, that is, a 55-kD protein (p55, the L chain, or Tac) and a 75-kD protein (p75, the H chain, or converter). The high affinity binding of IL-2 results in the formation of the ternary complex consisting of IL-2, and the L and H chains. To distinguish the affinity conversion model and the binary complex model we have carried out kinetic studies on the IL-2 binding to the high affinity IL-2-R on T lymphocytes expressing various numbers of L chains and a relatively constant number of H chains. We found that expression of a larger number of L chains accelerated the association of IL-2 to the high affinity receptor. The results are not compatible with the binary complex model that assumes a fixed number of high affinity sites determined by the numbers of a limiting chain. Instead, the results are consistent with the prediction of the affinity conversion model that assumes association of IL-2 to the L chain as the first step of the ternary complex formation and they indicate that the possible role of excess L chains is to accelerate the formation of the ternary complex. The reaction rate constants calculated from the affinity conversion model were reasonably constant.

We are grateful to Dr. K. Izui for his kind advice about reaction kinetics, to Ms. K. Ikeno for her technical assistance, and to Ms. K. Hirano and M. Sugiura for their help in the preparation of this manuscript.

Received for publication 11 April 1988 and in revised form 8 August 1988.

References

1. Sharon, M., R. C. Klausner, B. R. Cullen, R. Chizzonite, and W. J. Leonard. 1986. Novel interleukin 2 receptor subunit detected by cross-linking under high-affinity conditions. *Science (Wash. DC).* 234:859.
2. Tsudo, M., R. W. Kozak, C. K. Coldman, and T. A. Waldman. 1986. Demonstration of a non-Tac peptide that binds interleukin 2: a potential participant in multichain interleukin 2 receptor complex. *Proc. Natl. Acad. Sci. USA.* 83:9694.
3. Teshigawara, K., H. Wang, K. Kato, and K. A. Smith. 1987. Interleukin 2 high-affinity receptor expression requires two distinct binding proteins. *J. Exp. Med.* 165:223.
4. Robb, R. J., C. M. Rusk, J. Yodoi, and W. C. Greene. 1987. Interleukin 2 binding molecule distinct from the Tac protein: analysis of its role in formation of high-affinity receptors. *Proc. Natl. Acad. Sci. USA.* 84:2002.
5. Ogura, T., M. Konishi, N. Suzuki, S. Kondo, H. Sabe, and T. Honjo. 1988. Molecular mechanism for the formation of the high-affinity complex of interleukin 2 and its receptors. *Mol. Biol. Med.* 5:123.
6. Wang, H., and K. A. Smith. 1987. The interleukin 2 receptor functional consequences of its bimolecular structure. *J. Exp. Med.* 166:1055.
7. Lowenthal, J. W., and W. C. Greene. 1987. Contrasting interleukin 2 binding properties of the α (p55) and β (p70) protein subunits of the human high-affinity interleukin 2 receptor. *J. Exp. Med.* 166:1156.
8. Kondo, S., M. Kinoshita, A. Shimizu, Y. Saito, M. Konishi, H. Sabe, and T. Honjo. 1987. Expression and functional characterization of artificial mutants of interleukin 2 receptor. *Nature (Lond.).* 327:64.

9. Cantrell, D. A., and K. A. Smith. 1983. Transient expression of interleukin 2 receptors. Consequences for T cell growth. *J. Exp. Med.* 158:1895.
10. Hemler, M. E., M. B. Brenner, J. M. Mclean, and J. L. Strominger. 1984. Antigenic stimulation regulates the level of expression of interleukin 2 receptor on human T cells. *Proc. Natl. Acad. Sci. USA.* 81:2172.
11. Nishi, M., Y. Ishida, and T. Honjo. 1988. Expression of functional interleukin 2 receptors in human light chain/Tac transgenic mice. *Nature (Lond.).* 331:267.
12. Kondo, S., A. Shimizu, T. Saito, M. Kinoshita, and T. Honjo. 1986. Molecular basis for two different affinity states of the interleukin 2 receptor: affinity conversion model. *Proc. Natl. Acad. Sci. USA.* 83:9026.
13. Kanamori, H., S. Kondo, M. Kinoshita, N. Suzuki, Y. Saito, H. Sabe, N. Matsunami, N. Ishida, A. Shimizu, and T. Honjo. 1986. Interleukin 2 Receptor: Structure, Function, and Expression. *Cold Spring Harbor Symp. Quant. Biol.* LI:739.
14. Ullrich, A., L. Coussens, J. S. Hayblick, T. J. Dull, A. Gray, A. W. Tom, J. Lee, Y. Yarden, T. A. Libermann, J. Schlessinger, J. Downward, E. L. V. Mayers, N. Whittle, M. D. Waterfield, and P. H. Seehing. 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature (Lond.).* 309:418.
15. Nilsen, T. W., P. A. Maroney, R. G. Goodwin, F. M. Rottman, L. B. Crittenden, M. A. Raines, and H. Kung. 1985. C-erbB activation in ALV-induced erythroblastosis: novel RNA processing and promoter insertion result in expression of an amino-truncated EGF receptor. *Cell.* 41:719.
16. Sabe, H., S. Kondo, A. Shimizu, Y. Tagaya, J. Yodoi, N. Kobayashi, M. Hatanaka, N. Matsunami, M. Maeda, T. Noma, and T. Honjo. 1984. Properties of human IL-2 receptors expressed on non-lymphoid cells by cDNA transfection. *Mol. Biol. Med.* 2:379.
17. Uchiyama, T., S. Broder, and T. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* 126:1393.
18. Robb, R. J., W. C. Greene, and C. M. Rusk. 1984. Low and high affinity cellular receptors for interleukin 2. *J. Exp. Med.* 160:1126.
19. Rosenberry, T. L. 1975. Acetylcholinesterase. *Adv. Enzymol.* 43:103.