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Monoclonal antibodies which differentiate high- and low-affinity binding sites of interleukin-2

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Five monoclonal antibodies (L15, L20, L23, L34, and L61) against human recombinant interleukin-2 were tested for their effects on the interleukin-2 bioactivity and binding. Four of these monoclonal antibodies, L15, L20, L34, and L61, which had neutralizing activity, completely blocked interleukin-2 binding to the high-affinity receptor. On the other hand, L23, which had a very weak neutralizing activity, blocked interleukin-2 binding to the low-affinity receptor. These results suggest that there are at least two distinct binding sites on the interleukin-2 molecule; those for the high-affinity receptor and those for the low-affinity receptor. These monoclonal antibodies should be useful tools in the study of the interaction between interleukin-2 and interleukin-2 receptor.

Monoclonal antibody; Interleukin-2; Interleukin-2 receptor

1. INTRODUCTION

Interleukin-2 (IL-2) plays pivotal roles in the immune system [1–3]. IL-2 induces proliferation and activation of T cells. It also induces growth and differentiation of B cells [4]. Cumulative evidence supports the idea that IL-2 exerts these effects through interaction with a specific receptor present on the surface membrane of responsive cells. IL-2 receptor (IL-2R) has been detected in high- and low-affinity forms and only the high-affinity receptor could transduce the signals of IL-2 [5]. Tac antigen (p55) was initially identified as the IL-2R using anti Tac monoclonal antibody [6]. The gene for Tac protein has been cloned [7,8] but on transfection induced only the expression of low-affinity IL-2R [9]. Recently, a novel IL-2R, p70/75 was detected by affinity cross-linking study [10–13]. The p70/75 IL-2R identified on LGL cells by itself has an intermediate affinity for IL-2 but is capable of transducing the signal of IL-2. It is assumed that the high-affinity state is induced by the complex formation between p55 and p70/75, but nothing yet is known about the nature of p70/75, except for the behavior of the cross-linked protein on sodium dodecyl sulfate polyacrylamide gel. Neither is anything known about the mechanism of complex formation or interaction of IL-2 with these receptor subunits.

Monoclonal antibodies (mAbs) to IL-2 should be useful tools for studying the relationship between the structure and function of IL-2. We previously established mouse hybridoma cell lines producing mAbs against recombinant human IL-2 (rIL-2) [14]. In this report, we show that IL-2 bind these receptor subunits through different binding sites on the IL-2 molecule and that the high- and low-affinity binding sites on the IL-2 molecule can be differentially recognized by these mAbs.

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2. MATERIALS AND METHODS

2.1. *Cell culture condition*

The IL-2 dependent murine CTLL-2 was kindly donated by Professor Kumagai of Tohoku University. CTLL-2 was maintained in RPMI 1640 medium (Nissui & Co.) supplemented with 10% fetal calf serum (Boehringer Mannheim), 4 mM L-glutamine, 1.4 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 10 mM Hepes, pH 6.8, and 100 U/ml rIL-2 (Biogen).

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Cultures were maintained in a humidified incubator at 37°C with an atmosphere of 5% CO_2 in air.

2.2. *mAbs*

Five mouse mAbs, L15, L20, L23, L34, and L61 are directed against rIL-2 [14]. They were purified from ascitic fluid by protein A agarose affinity chromatography (BioRad, MAPS II kit). Affinity constants of purified mAbs against rIL-2 were calculated from Scatchard plots using [^{125}I]IL-2 as described [14].

2.3. *Bioassay of mAbs*

mAbs were assayed for their activity to neutralize the proliferative effect of rIL-2 on IL-2 dependent murine T cell line, CTLL-2 as described elsewhere [15]. The cells were washed with RPMI 1640 medium containing 10% fetal calf serum, 50 μM 2-mercaptoethanol, and 1.4 mM sodium pyruvate followed by incubation for 16 h and seeded into 96-well microtiter plates (10^4 cells/well). Serially diluted mAbs and a constant level of rIL-2 (4 U/ml, i.e. 25 pM) were added in a total volume of 200 μl. After 20 h incubation at 37°C, [^{3}H]thymidine (Amersham, 0.5 μCi/well) was added and pulse-labeled for 4 h. The cells were collected on glass fiber filters, washed with water, and counted for radioactivity in a liquid scintillation counter. The extent of [^{3}H]thymidine incorporation was expressed as the percentage of the count obtained in the absence of the mAbs.

2.4. *IL-2 binding assay*

The binding of radioiodinated IL-2 to CTLL-2 cells was measured according to the methods described by Robb et al. [5]. Before the binding experiment, prebound IL-2 was removed by incubating the cells in 10 mM sodium citrate, pH 4.0, containing 0.14 M NaCl for 20 s followed by centrifugation at 3000 × *g* for 1 min. After washing, the cells (2×10^6) were resuspended in RPMI 1640 medium containing 10% fetal calf serum, 4 mM L-glutamine, 1.4 mM sodium pyruvate, 50 μM 2-mercaptoethanol, and 10 mM Hepes, pH 6.8. Serial dilutions of ^{125}I-labeled IL-2 (Amersham) were added and incubated for 1 h at 4°C in a total volume of 200 μl. Next, cells were centrifuged for 5 min at 1000 × *g* and resuspended in 100 μl of the same medium. Residual unbound radioactivity was removed by centrifugation for 5 min at 1000 × *g* through a 300-μl layer of a mixture of 20% olive and 80% di-*n*-butyl phthalate. The tips of the tubes containing the cell pellets were cut off, placed in 4-ml polystyrene tubes, and counted for radioactivity in a gamma counter. Non-specific binding was determined in the presence of a 200-fold excess of cold IL-2.

3. RESULTS AND DISCUSSION

A series of hybridoma cell lines which produce mAbs against rIL-2 were established previously [14]. mAbs were assayed for their inhibitory activity on IL-2-dependent cellular proliferation. Titration curves of chromatographically purified mAbs are shown in fig.1. The ability of mAb to neutralize the IL-2 activity is in the order of L61 > L15, L20, L34 > L23. Next, the effect of mAbs on

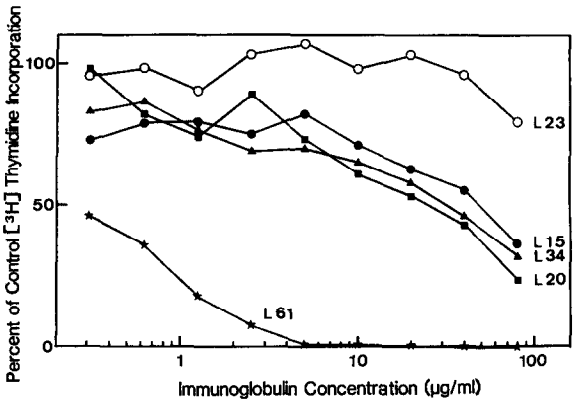

Fig.1. Neutralizing activity of mAbs against rIL-2. Serial dilutions of mAbs (●, L15; ■, L20; ○, L23; ▲, L34; ★, L61) were added to the bioassay medium containing CTLL-2 (10^4 cells/well) and rIL-2 (4 U/ml).

the binding of IL-2 to IL-2R was studied. Experiments were carried out under conditions permitting the binding of IL-2 to only high-affinity sites [5,10] (fig.2). The ability of mAb to block the IL-2 binding to the IL-2R is in the order of L61 > L15, L20, L34 > L23. These results suggest that the neutralizing activity of the mAbs correlated directly with their inhibitory activity on high-affinity binding. The difference of neutralizing activity of these mAbs could then be ascribed to the difference of their affinity to IL-2 or to the difference of recognition sites on the IL-2 molecule. Table 1 shows the affinity constants of these mAbs

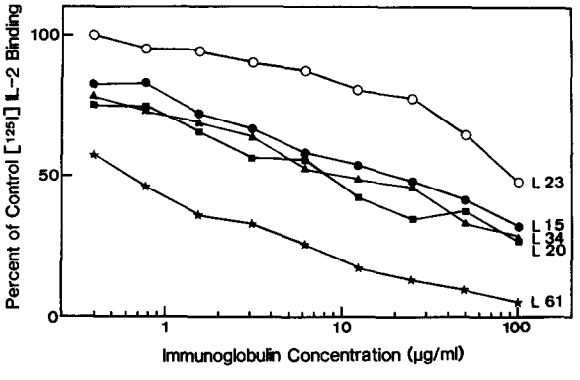

Fig.2. Inhibition activity of mAbs on IL-2 binding to CTLL-2. Serial dilutions of mAbs (●, L15; ■, L20; ○, L23; ▲, L34; ★, L61) were preincubated with 50 pM [^{125}I]IL-2 for 1 h at 4°C and then the reaction mixture was added to the binding assay medium containing CTLL-2 (2×10^6 cells/tube).

Table 1

Affinity constants of mAbs for rIL-2

mAbs	Affinity constant (K_a) (M^{-1})
L15	4.99×10^8
L20	1.61×10^8
L23	1.60×10^8
L34	3.19×10^7
L61	2.74×10^8

for rIL-2. Most of these mAbs exhibit the same order of affinity, which suggests that the difference in the neutralizing activity is not due to the difference in their binding ability, but rather to the difference in the recognition sites of these mAbs on the IL-2 molecule.

We followed up these observations by Scatchard analyses of IL-2 binding in the presence or absence of each mAb (fig.3). L15, L20, and L34 inhibited high-affinity binding at a concentration of 100 μg/ml. Few effects were observed on the low-affinity binding. L61 inhibited high-affinity binding even at a concentration of 1 μg/ml. At 10 and 100 μg/ml, L61 completely blocked the high-affinity binding and slightly reduced the affinity of the low-affinity binding. On the other hand, L23 did not affect both affinity bindings at 1 and 10 μg/ml, but completely blocked the low-affinity binding and reduced the affinity of the high-affinity binding at 100 μg/ml. Control mouse immunoglobulin was without effect up to 100 μg/ml.

The high-affinity IL-2R is thought to consist of the ternary complex of IL-2, p55, and p70/75 [9–13], and the p70/75 IL-2R subunit is generally thought to be necessary for the biological action of IL-2. The binding site(s) on the IL-2 molecule to these receptor subunits, however, is still speculative at present, and whether the same or different site is responsible for the binding has not been clarified. The present study clearly shows that high- and low-affinity binding sites exist in different regions on the IL-2 molecule. mAb L61 recognizes the high-affinity binding site of IL-2, thereby inhibiting binding of IL-2 to the high-affinity receptor complex (p55-p70/75) and to the intermediate-affinity receptor (p70/75), resulting in the inhibition of growth of CTLL-2 cells. On the

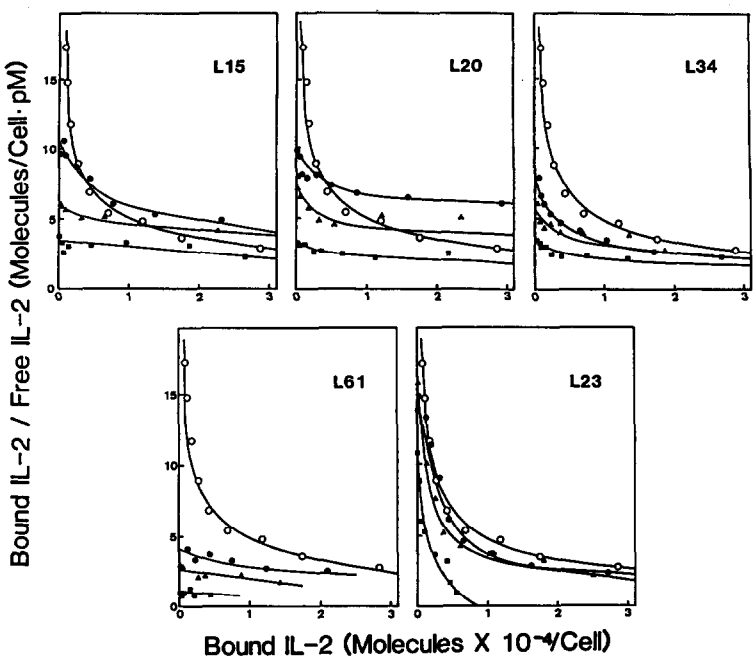

Fig.3. Scatchard plots of IL-2 binding to CTLL-2 (2×10^6 cells/tube). Serial dilutions of [^{125}I]IL-2 were preincubated for 1 h at 4°C without (○) or with mAb at concentrations of 1 μg/ml (●), 10 μg/ml (▲), and 100 μg/ml (■), followed by IL-2 binding assay as described in section 2.

other hand, L23 recognizes low-affinity binding site of IL-2 and inhibits the binding of IL-2 to the low-affinity receptor subunit (p55). The resulting IL-2-L23 immune complex still retains the ability to bind to p70/75, but at a reduced affinity since it cannot form the ternary complex. The weak inhibitory activity of L23 observed in figs 1 and 2 can be ascribed to the decrease in the affinity of IL-2 to the receptor. The effects of L15, L20, and L34 appeared to be qualitatively similar to that of L61, but a higher concentration was needed to achieve the same effect. A plausible explanation would be that these mAbs recognize a site close to but different from that recognized by L61; they bind to the high-affinity binding site of the IL-2 molecule in such a way as to decrease the affinity to the high-affinity receptor complex (p55-p70/75).

Our results demonstrate that anti IL-2 mAbs, L15, L20, L23, L34, and L61 recognize separate binding sites on the IL-2 molecule which participate in the binding to p55 and p70/75 subunits of the IL-2 receptor. These mAbs are expected to offer important clues to the elucidation of these binding sites and also to the investigation of the receptor subunits.

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