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Effects of interleukin 2 receptor b chain (P75)-specific monoclonal antibody on the generation of cytotoxic T lymphocytes and suppressor T cells in mixid lymphocyte culture

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Abstract. The interaction of interleukin 2 (IL-2) with its receptor (IL-2R) plays an essential role in the proliferation and differentiation of T cells. The IL-2R β-chain is considered to function directly in the intracellular signal transduction. In this study, we investigated using a newly established IL-2R β-chain-specific monoclonal antibody (MAb) (TU-25) and an IL-2R α-chain-specific MAb (H-31). The IL-2-induced proliferation of concanavalin blasts and the mixed lymphocyte reaction (MLR) were suppressed by TU-25 in combination with H-31. This combination had a greater suppressive effect than each of them alone. The generation of cytotoxic T lymphocytes (CTL) using a cell-mediated lympholysis (CML) assay, was not inhibited by TU-25 alone. TU-25 in combination with H-31 suppressed the generation of CTL completely in this assay even if recombinant IL-2 (rIL-2) was added. Although the CTL generation was inhibited, cells that suppressed a fresh MLR were preserved. Our study suggests that the combination of TU-25 with H-31 completely blocks the functional high-affinity binding site of IL-2 but does not inhibit the generation of suppressor cells. This may lead to immunosuppressive therapy using an IL-2R β-chain-specific MAb in combination with an IL-2R α-chain-specific MAb in clinical organ transplantation.

Key words: Interleukin 2 (IL-2) – IL-2 receptor – Monoclonal antibody – Suppressor cell

Interleukin 2 (IL-2) is produced by allostimulated T cells and acts via its receptor (IL-2R) on the surface of T cells. The binding of IL-2 to the IL-2R leads to the proliferation and the differentiation of T cells. There are three forms of receptor, which have three different affinities to IL-2. The high-affinity one has been shown to be composed of at least two distinct subunits, IL-2R α-chain (p55) and IL-2R β-chain (p75), each of which exhibits low-affinity and intermediate-affinity to IL-2 [13, 21, 22]. The IL-2R β-chain has a larger intracellular domain than the IL-2R α-chain. The interaction between IL-2 and the intermediate- or high-affinity receptor can induce intracellular signal transduction, indicating that the IL-2R β-chain directly functions in the signal transduction pathway [3, 4, 14].

Recently, monoclonal antibodies (MAb) directed against the IL-2R β-chain have been produced [9, 17, 21]. In this study, we used a newly established IL-2R β-chain-specific MAb (TU-25) and an IL-2R α-chain-specific MAb (H-31) and observed their effects on the proliferation of T cells by allostimulation or IL-2 and the generation of cytotoxic T lymphocytes (CTL) or suppressor T cells.

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Materials and methods

Antibodies. The H-31 and TU-25 are IgG_1 MAb directed against the IL-2R α- and β-chains, respectively, and were kindly given by Dr. Sugamura (Tohoku University, Sendai, Japan). The 2-3D1, which was used as a control MAb, is also an IgG_1 MAb directed against *Escherichia coli.*

IL-2-induced proliferation of ConA blasts. Peripheral blood lymphocytes (PBL) were isolated by Ficoll-Hypaque gradient density centrifugation. Concanavalin A (ConA) blasts were obtained by stimulating PBL with 5 μg/ml ConA (Gibco Laboratories, Grand Island, N.Y.) for 3 days in RPMI 1640 medium containing 25 m*M* HEPES (Difco Laboratories, Detroit, Mich.) and 10% fetal calf serum (FCS; Difco Laboratories) at 37 °C, 5% CO_2. The ConA blasts were washed twice and plated out at a concentration of 1×10^4 cells/well in 200 μl of culture medium in 96-well, flat-bottomed plates (Falcon tissue culture plate, 3072). The H-31 and TU-25 were tested in the presence of different concentrations of recombinant IL-2 (rIL-2). After 48 h incubation (37 °C, 5% CO_2), the cultures were pulsed with 1.0 μCi/well of tritiated thymidine (TdR) for 18 h, harvested, and then counted for radioactivity.

Inhibition of the MLR. Equal numbers (5×10^4) of responder cells and MMC-treated stimulator cells were plated in 96-well, round-bottomed plates (FALCON tissue culture plate, 3077). In the presence or absence of MAb, the cells were cultured for 6 days, harvested, and pulsed with 1 μCi/well of ^{3}H-TdR 18 h before harvesting.

Induction of CTL and the CML assay. The induction of CTL was carried out in mixed lymphocyte culture (MLC). Equal numbers (1×10^7) of responder cells and MMC-treated stimulator cells were

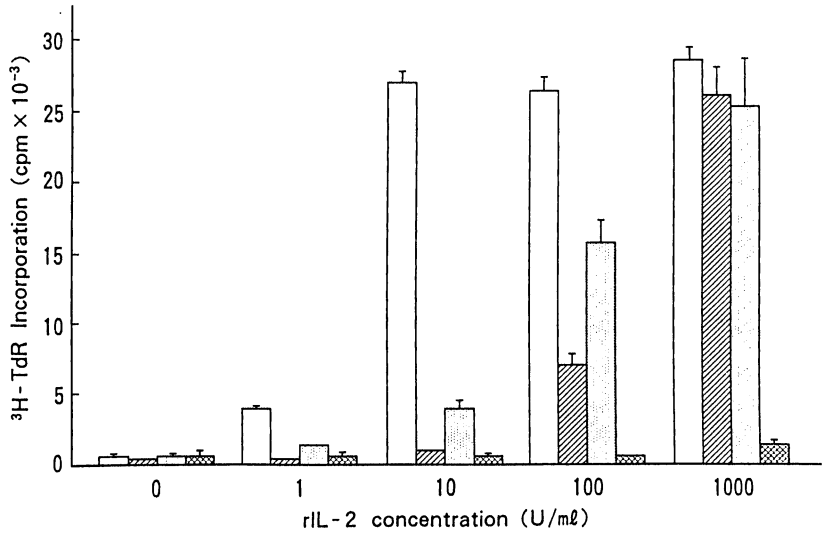

Fig. 1. Inhibitory effect of H-31 and TU-25 on interleukin 2 (IL-2)-induced proliferation. Concanavalin A (ConA) blasts were cultured without monoclonal antibody, MAb (□) or with H-31 (50 μg/ml; ▨), TU-25 (50 μg/ml; □), or H-31 (50 μg/ml) + TU-25 (50 μg/ml) (▩) in the presence of rIL-2

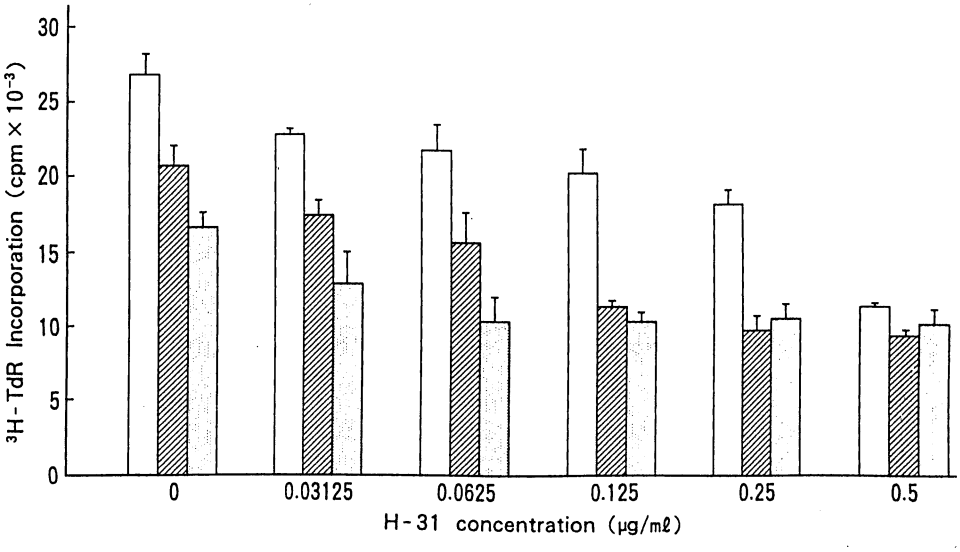

Fig. 2. Inhibitory effect of H-31 and TU-25 on the mixed lymphocyte reaction (MLR) which was performed in the presence of H-31 without TU-25 (□) or with TU-25 (1.25 μg/ml) (▨) or TU-25 (2.5 μg/ml) (□)

cocultured for 7 days in a total volume of 20 ml in the presence or absence of MAb. These induced cells were used as effector cells in a cell-mediated lympholysis (CML) assay. Target cells, fresh stimulator PBL, or third-party PBL were cultured for 3 days in culture medium containing 50 μg/ml phytohemagglutinin (PHA-P; Difco Laboratories) and labeled with ^{51}Cr. Target cells (1×10^4/well) were added to effector cells in 96-well, round-bottomed plates. After 6 h incubation, the supernatant from each well was harvested using a supernatant collection system (Skatron, Lier, Norway), and ^{51}Cr release was determined using an autowell gamma-system. Spontaneous release was determined by incubating the target cells in medium alone, while maximum release was determined by target cells exposed to 1 *N* NaOH. The percentage lysis of target cells was calculated according to the formula: % Cytotoxicity = (Experimental release − Spontaneous release) × 100 (Maximum release − Spontaneous release)

CTL generation in the presence of exogeneous rIL-2. Various concentrations of rIL-2 were added at the initiation of the MLC in the presence of H-31 (1.0 μg/ml) or H-31 (1.0 μg/ml) and TU-25 (2.0 μg/ml) or in the absence of MAb. The percentage lysis of target cells was determined and calculated as described above.

Generation of suppressor cells. Equal numbers (1×10^7) of responder cells and MMC-treated stimulator cells were cocultured for 10 days in a total volume of 20 ml in the presence of 1.0 μg/ml of H-31 and 2.0 μg/ml of TU-25 or in the absence of MAb. After 10 days, the cells were harvested, washed twice, and restimulated using the same allogeneic stimulator cells for 4 days in the absence of MAb. After 14 days from the initiation of culture, the induced cells were harvested, MMC-treated, and added as regulators in a primary MLR. Regulator cells were mixed with 5×10^4 fresh autologous PBL and 5×10^4 MMC-treated PBL (as specific stimulators or third-party stimulators). To measure the control response, autologous fresh PBL were MMC-treated and added as regulators. Cells were cultured for 6 days and pulsed with 1 μCi/well of ^{3}H-TdR 18 h before harvesting. The percentage suppression was calculated by the formula: % Suppression = 1 − cpm (responder + stimulator + induced regulator) × 100 cpm (responder + stimulator + fresh autologous regulator)

Results

As shown in Fig. 1, the rIL-2-induced proliferation of ConA blasts was suppressed by either H-31 or TU-25 alone at low concentrations of rIL-2. H-31 showed a stronger suppression action than TU-25. Proliferation was completely inhibited in the presence of both H-31 and TU-25 despite a high concentration of rIL-2 (1000 U/ml).

Figure 2 indicates that both H-31 and TU-25 showed suppressive effects on the MLR in a dose-dependent manner. Inhibition of the MLR by H-31 was stronger than that by TU-25 at the same concentration. H-31 brought about

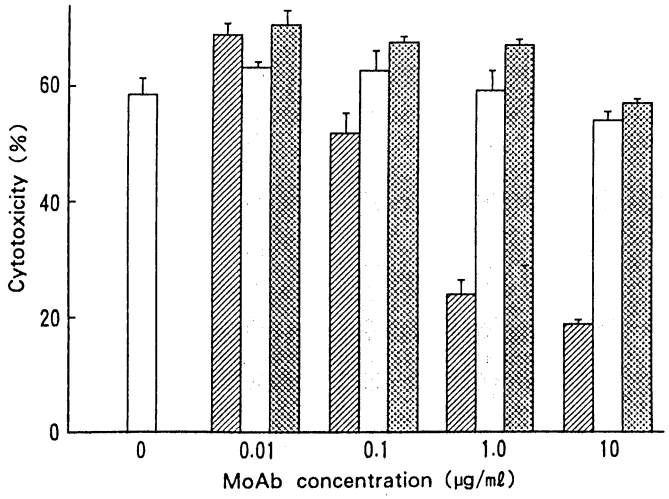

Fig. 3. Inhibitory effect of H-31 and TU-25 on cytotoxic T lymphocyte (CTL) generation. Induction of CTL was performed without MAb (□) or in the presence of H-31 (▨), TU-25 (□), or control MAb (2-3D1) (▩). Cell-mediated lympholysis (CML) assay was performed at 50:1 of effector-to-target ratio

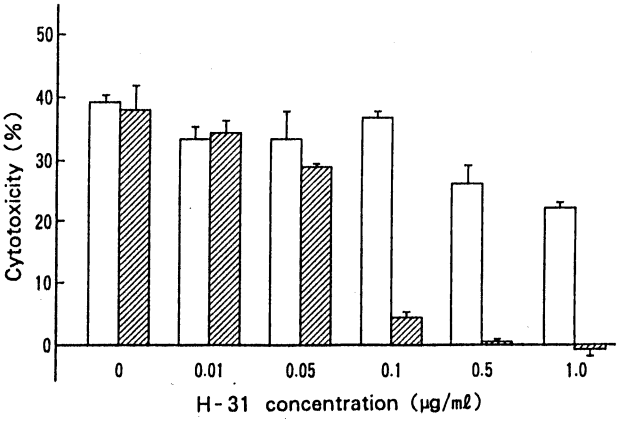

Fig. 4. Combined effect of TU-25 with H-31 on CTL generation. Induction of CTL was performed in the presence of H-31 without TU-25 (□) or with TU-25 (2.0 μg/ml; ▨). CML assay was performed at 50:1 of effector-to-target ratio

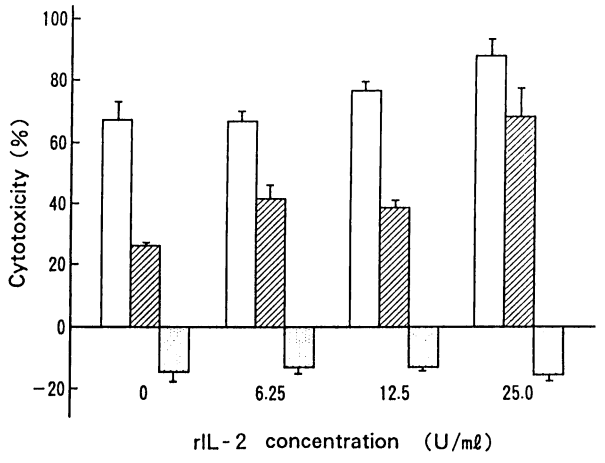

Fig. 5. Effect of H-31 and TU-25 on CTL generation in the presence of exogeneous recombinant IL-2 (rIL-2). Induction of CTL was carried out without MAb (□) or with H-31 (1.0 μg/ml; ▨) or H-31 (1.0 μg/ml) + TU-25 (2.0 μg/ml) (□) in the presence of rIL-2. CML assay was performed at 50:1 of effector-to-target ratio

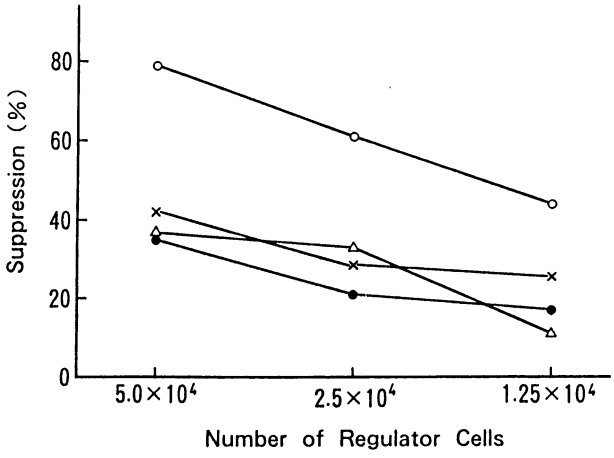

Fig. 6. Suppression of MLR by autologous cells primed in the presence of H-31 and TU-25. Control regulator cells, primed in the absence of MAb, were MMC-treated and added to fresh specific MLR (○—○) or third-party MLR (×—×). Modified primed cells, cultured in the presence of H-31 (1.0 μg/ml) + TU-25 (2.0 μg/ml), were MMC-treated and added to fresh specific MLR (●—●) or third-party MLR (△—△)

24% inhibition at a concentration of 0.125 μg/ml; in combination with TU-25 (1.25 μg/ml) it showed 57% inhibition, which was equal to the inhibition at a concentration of 0.5 μg/ml of H-31 alone.

To determine whether H-31 or TU-25 alone could inhibit the generation of CTL, they were added at the initiation of the MLC. As shown in Fig. 3, 2-3D1, a control MAb, did not affect the percentage cytotoxicity. H-31 alone inhibited CTL generation in a dose-dependent manner, and 59% inhibition was observed at a concentration of 1.0 μg/ml. In contrast, TU-25 alone had no inhibitory effect on CTL generation at any concentration.

To test the combined effect of TU-25 with H-31, they were added at various concentrations at the initiation of cultures. Figure 4 reveals that in the presence of various concentrations of H-31, the addition of TU-25 (2.0 μg/ml) reduced CTL generation much more effectively than the same concentration of H-31 alone. H-31 at a concentration of 0.5 μg/ml showed 43% inhibition of CTL generation, but 99% inhibition was observed in combination with TU-25 (2.0 μg/ml). Furthermore, TU-25 inhibited CTL generation in a dose-dependent manner in the presence of H-31 at a concentration of 0.1 μg/ml.

As shown in Fig. 5, when rIL-2 was added in the controls, the percentage cytotoxicity was augmented in a dose-dependent manner. The inhibitory effect of H-31 on CTL generation was reduced when rIL-2 was added. The percentage cytotoxicity was returned to the level of the control at a concentration of 25 U/ml. In the combination of H-31 (1.0 μg/ml) with TU-25 (2.0 μg/ml), the inhibitory effect on CTL generation was preserved despite the presence of rIL-2.

As shown in Fig. 6, the control regulator cells (R') showed 79% inhibition of fresh specific MLR (regulator cells 5×10^4). However, at the same time (as shown in

Table 1. Cytotoxicity 2 weeks after the initiation of culture

		% Cytotoxicity (Mean ± SD)			
		Effector to target ratio			
Effector	Target	50:1	25:1	12.5:1	6.25:1
R' (Control)	D (Specific)	78.4 ± 4.9	67.4 ± 1.3	53.9 ± 1.8	39.7 ± 1.6
R" (H-31 1.0 μg/ml + TU-25 2.0 μg/ml)	D	3.6 ± 0.5	4.0 ± 1.2	1.9 ± 0.9	0.5 ± 1.5
R'	C (Third party)	11.4 ± 0.8	3.3 ± 0.6	0.7 ± 0.8	−1.0 ± 0.7
R"	C	−5.4 ± 0.6	−2.2 ± 0.7	−0.5 ± 0.5	−1.7 ± 1.0

Table 1), 78% cytotoxicity was observed for the specific stimulator cells (effector-to-target ratio was 50:1). Modified cells primed in the presence of H-31 and TU-25 (R") also showed an inhibition of specific MLR and third-party MLR (35% and 37% inhibition, respectively). This inhibition was weaker than the control regulator cells (R") showed no cytotoxicity to both stimulator cells, although they were restimulated without MAb.

Discussion

MAbs directed against the IL-2R α-chain have been shown to inhibit the proliferation of allostimulated T cells and the generation of CTL by allogeneic stimulation in vitro [2]. In this study, we tested the effect of TU-25, a newly established IL-2R β-chain-specific MAb, on the proliferation of T cells and on the generation of CTL or suppressor cells.

H-31, an IL-2R α-chain-specific MAb, showed some inhibitory effects on the proliferation of T cells or the generation of CTL. Moreover, H-31 and TU-25 together showed synergistic effects in a dose-dependent manner. Interestingly, although the IL-2R β-chain is considered to be responsible for signal transduction, little inhibitory effect was observed with the addition of TU-25 alone. However, TU-25 showed inhibitory effects in the presence of H-31 in a dose-dependent manner which were synergistic.

These effects may be explained by several IL-2 binding studies [8, 24]. It was reported that when lymphocytes were stimulated in allogeneic cultures at 37°C, new β-chains were synthesized on the lymphocytes to form high-affinity receptors in cooperation with free α-chains. Once high-affinity receptors have formed, IL-2 can interact very rapidly with them, since the affinity of the receptors is much higher than that of the IL-2R β-chain-specific MAb.

Kamio et al. showed that an IL-2R β-chain-specific MAb completely inhibited the binding of the IL-2R β-chain with IL-2 at 4°C, but at 37°C the high-affinity IL-2R reappeared [5]. This was considered to be due to the replacement of the IL-2R β-chain-specific MAb by α-chain-mediated IL-2. They suggested that the IL-2R α-chain functions as a dimension converter of IL-2.

Treatment with antibodies against the IL-2R α-chain has been demonstrated to prolong graft survival or prevent allograft rejection in mice, rats, and monkeys [6, 10–12]. Recently, pilot studies and randomized trials have been performed in clinical human kidney transplantation [1, 7, 15]. The treatment was effective in preventing early rejection in combination with cyclosporin A or corticosteroids. An IL-2R α-chain-specific MAb was effective in acute rejection, but as shown in this study, the addition of exogenous rIL-2 to ConA blasts or CML assay reversed its inhibitory effect. Single therapy may not be sufficient to block the high-affinity IL-2R.

It has been shown that the IL-2R α-chain-specific MAb did not inhibit MLC-generated suppressor cells in vivo and in vitro [20]. Tan et al. reported that activation of antigen-specific T suppressor-inducer and T suppressor-effector cells appeared to be relatively IL-2 independent in their study using α-chain-specific MAb [19]. As shown above, primed cells in the presence of H-31 and TU-25 did not show such a strong and antigen-specific effect. Also, during stimulation without MAb they did not show any cytotoxicity. It was reported that in immunofluorescence analysis, the IL-2R α-chain and β-chain were preferentially expressed on $CD4^+$ and $CD8^+$ T cells with $CD45RO^+$ (memory) phenotypes, respectively [16]. These $CD45RO^+$ $CD4^+$ and $CD8^+$ T cells, unlike $CD45RO^-$ cells, proliferate in response to exogenous IL-2. This may explain why suppressor cells were not inhibited by IL-2R-specific MAbs.

When T cells are exposed to alloantigens, high-affinity receptors are synthesized, and the interaction of IL-2 with them stimulates mitosis and clonal expansion of the progenitors. Immunosuppressive therapy against the high-affinity IL-2R may be relatively specific and a target in the alloantigen-specific T-cell repertoire. Recently, the existence of the third component of IL-2R, the γ-chain (p64), was demonstrated [18]. This receptor is thought to be associated with the β-chain and to be concerned with intracellular signal transduction. The inhibition of this new receptor (γ-chain receptor) will be necessary for complete blockade of the high-affinity receptor.

Thus, our study suggests that immunosuppressive therapy using an IL-2R β-chain-specific MAb in combination with an IL-2R α-chain-specific MAb may be a new therapeutic strategy in clinical organ transplantation.

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