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Abstract

Interleukin-2 (IL2) is the obligatory signal for both T cell mitogenesis and in vitro generation of alloreactive cytotoxic T lymphocytes (CTL). An investigation was made to determine whether an antibody directed against IL2 would suppress the rejection reaction of rat cardiac allografts. Rabbit anti-interleukin 2 (anti-IL2) antiserum was obtained by immunizing at 2 week intervals over a period of 8 weeks with 10(6) U of recombinant human IL2 along with complete Freund's adjuvant. The bioassay for inhibition of IL2 activity by anti-IL2 antiserum was carried out in conjunction with the IL2-dependent cytotoxic T cell (CTLL cell) assay. Cardiac allografts of F344 rats were heterotopically transplanted into ACI rats. Seven daily doses of 1 ml of anti-IL2 antiserum were administered intravenously following transplantation. IL2-driven [3H]thymidine incorporation in CTLL cells was significantly inhibited by rabbit anti-IL2 antiserum. Graft survival in the anti-IL2 serum-treated group was significantly prolonged in a dose-dependent fashion compared to control groups. In conclusion, these results indicate that rabbit anti-IL2 antiserum may prove to be of significant value as an immunosuppressive agent in clinical organ transplantation.

KEYWORDS: anti-interleukin 2 antiserum, rat cardiac allograft, immunosuppressive agent

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Prolongation of Cardiac Allograft Survival in Rats by Treatment with Anti-Interleukin 2 Antiserum

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Interleukin-2 (IL2) is the obligatory signal for both T cell mitogenesis and *in vitro* generation of alloreactive cytotoxic T lymphocytes (CTL). An investigation was made to determine whether an antibody directed against IL2 would suppress the rejection reaction of rat cardiac allografts. Rabbit anti-interleukin 2 (anti-IL2) antiserum was obtained by immunizing at 2 week intervals over a period of 8 weeks with 10^6 U of recombinant human IL2 along with complete Freund's adjuvant. The bioassay for inhibition of IL2 activity by anti-IL2 antiserum was carried out in conjunction with the IL2-dependent cytotoxic T cell (CTLL cell) assay. Cardiac allografts of F344 rats were heterotopically transplanted into ACI rats. Seven daily doses of 1 ml of anti-IL2 antiserum were administered intravenously following transplantation. IL2-driven [^3H]thymidine incorporation in CTLL cells was significantly inhibited by rabbit anti-IL2 antiserum. Graft survival in the anti-IL2 serum-treated group was significantly prolonged in a dose-dependent fashion compared to control groups. In conclusion, these results indicate that rabbit anti-IL2 antiserum may prove to be of significant value as an immunosuppressive agent in clinical organ transplantation.

Key word: anti-interleukin 2 antiserum, rat cardiac allograft, immunosuppressive agent

It is well known that interleukin-2 (IL2), derived from activated T lymphocytes, is the obligatory signal for both T lymphocyte mitogenesis and *in vitro* generation of alloreactive cytotoxic T lymphocyte (CTL) (1). The role of IL2 in allograft rejection remains obscure. *In vivo* administration of IL2-containing supernatants is capable of greatly enhancing the cytolytic response to alloantigen challenge (2), and adoptive transfer of appropriately sensitized lympho-

cytes expanded in IL2 can mediate accelerated allograft rejection (3). Several authors reported that antibody directed against IL2 inhibited the differentiation of alloreactive CTL *in vitro* and *in vivo* (4-6). These investigators suggested the possibility of using anti-IL2 antibody to regulate the immune response *in vivo*. In the present study, we investigated the effect of antibody to highly purified human recombinant IL2 (rIL2) on the survival of rat cardiac allografts. This is the first report on anti-IL2 treatment for the prevention of

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allograft rejection.

Materials and Methods

Animals. ACI (RII^{av1}) and F344 (RT1^{lv1}) male rats between 10 and 20 weeks of age were used in the experiments. All inbred rats were obtained from commercial sources (ACI, Hashimoto Experimental Animal Inc., Japan; F344, Charles River, Japan). The ACI rats were used as recipients, and F344 rats, as cardiac allograft donors.

Cardiac allografting. Heterotopic cardiac grafting was performed according to the method of Ono-Lindsey (7). Graft survival was monitored by direct palpation, and rejection was considered complete when palpable ventricular contractions could no longer be detected.

Generation of anti-IL2 antiserum. Antibodies to IL2 were raised in rabbits. New Zealand white rabbits (Charles River, Japan) between 8 and 12 weeks of age were immunized at 2 week intervals over a period of 8 weeks with 10⁶ unit (U) (equivalent to 20 µg IL2 protein) of recombinant human IL2 (Ajinomoto Co. Inc., Yokohama, Japan), along with complete Freund's adjuvant (CFA). Each immunization consisted of 6 x 10⁵ U of rIL2 in CFA injected intraperitoneally and 4 x 10⁵ U of rIL2 in CFA injected subcutaneously into both hind legs. Three to 5 days after an intravenous booster with 10⁶ U of rIL-2, the immunized rabbits were bled. The serum was inactivated at 56°C for 30 min, and stored at -80°C until used. The sera were tested for anti-IL2 activity as described below.

IL2 neutralization assay. Anti-IL2 biologic activity was determined by the inhibition of the IL2-dependent proliferation of a cloned murine cytotoxic T lymphocyte line (CTLL-2). Following serial two-fold dilutions of rIL2 (50 U/ml) mixed with rabbit immune serum or normal serum, CTLL cells (4 x 10³/100 µl/well) were added to each well. After 20h at 37°C in a humidified atmosphere of 5% CO₂ in air, [³H]thymidine (0.5 µCi/50 µl/well) was pulsed for 4h. The cultures were harvested onto glass fiber filters, and [³H]-thymidine incorporation was determined by liquid scintillation counting. The results were expressed as [³H]thymidine incorporation in the presence of

test serum compared with a medium control or normal rabbit serum.

IL2 binding assay. Purified IL2 (10 µg/ml in phosphate-buffered saline) was distributed in 96-well microtiter plates (Tomy Seiko, Tokyo, Japan). After incubation for 18h at 4°C, the wells were rinsed with PBS (pH 7.4) and incubated with 300 µl of 0.5% bovine serum albumin (BSA) solution in PBS for 1h at 25°C. After washing with PBS, 20 µl of test serum or antibody were incubated for 2h at 37°C. The wells were washed three times with PBS followed by the addition of 30 µl of [¹²⁵I]-radiolabeled goat anti-mouse immunoglobulin ([¹²⁵I]-MIG). After 1h of incubation at 25°C, the wells were washed three times with PBS, cut with a hot wire and counted with a γ-counter.

Treatment groups. This experiment used rabbit anti-IL2 antiserum (No. 001) having strong neutralization and binding activity to IL2 molecules. Rats treated with anti-IL2 antiserum were divided into three groups of 5 animals each. Each experimental animal was intravenously administered with 1 ml of previously diluted (2⁰, 2³, and 2⁶-fold) anti-IL2 antiserum for 7 consecutive days. The controls consisted of untreated rats and those which had received normal rabbit serum.

Statistics. Allograft survival between groups within each experiment was analyzed using Student's *t* test.

Results

Inhibition of IL2-dependent CTLL cell proliferation. Anti-IL2 activity in rabbit sera was initially detected by IL2 neutralization assay. The activity of 5 pools of antisera is shown in Fig. 1, where it can be seen that 3 out of 5 immune sera completely suppressed the growth-promoting action of conditioned medium at a 2⁹-fold dilution. In contrast, the same amount of preimmune rabbit serum was inert.

Binding activity to human rIL2. Fig. 2 shows the results of the binding assay. Among 5 immune rabbit sera found positive

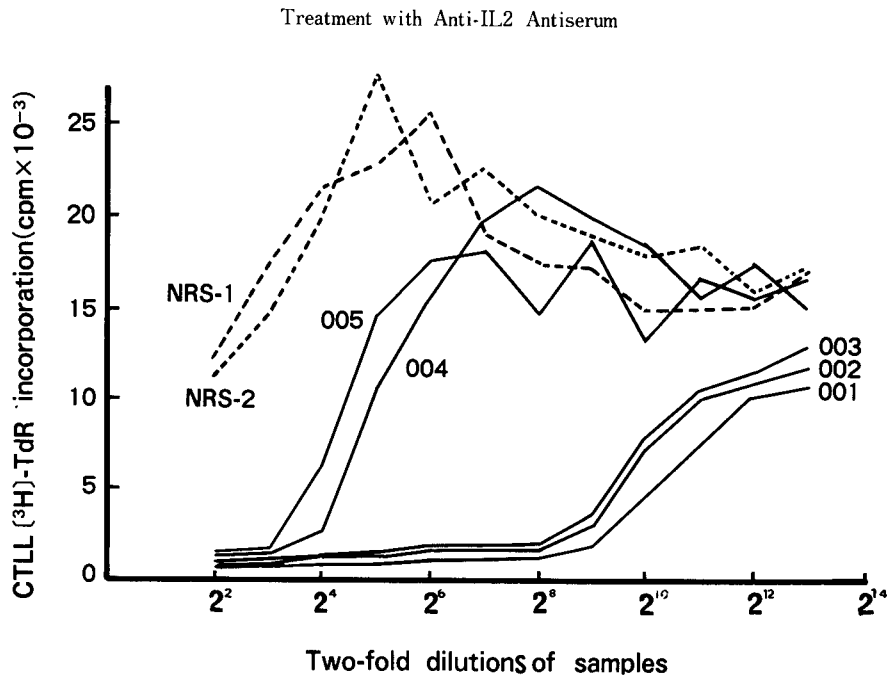


Fig. 1 Neutralization of IL2 biologic activity by immunized rabbit sera. Three (001, 002 and 003) out of 5 pooled immunized rabbit sera (—) show strong neutralization activity of rIL2. Normal rabbit sera (NRS-1 and NRS-2) (---).

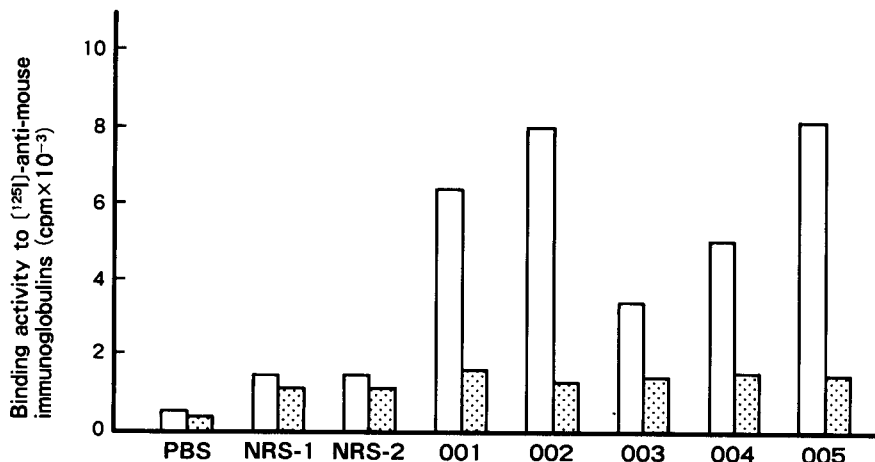


Fig. 2 Binding activity of immunized rabbit sera to rIL2. Three (001, 002 and 005) out of 5 pooled immunized rabbit sera show strong binding activity to rIL2 molecules ($\square$); Binding activity to bovine serum albumin ($\square$).

in the binding assay using recombinant human IL2 as ligand, antisera (001, 002 and 005) showed strong binding activity to human IL2. These antisera did not bind to the bovine serum albumin (BSA) used with recombinant human IL2 during the immunization. Normal rabbit serum showed no

binding activity to recombinant human IL2 or BSA.

Dose-dependent prolongation of cardiac allograft survival. The survival days of F344 cardiac grafts in the control and experimental groups are shown in Fig. 3. The mean survival time of the untreated

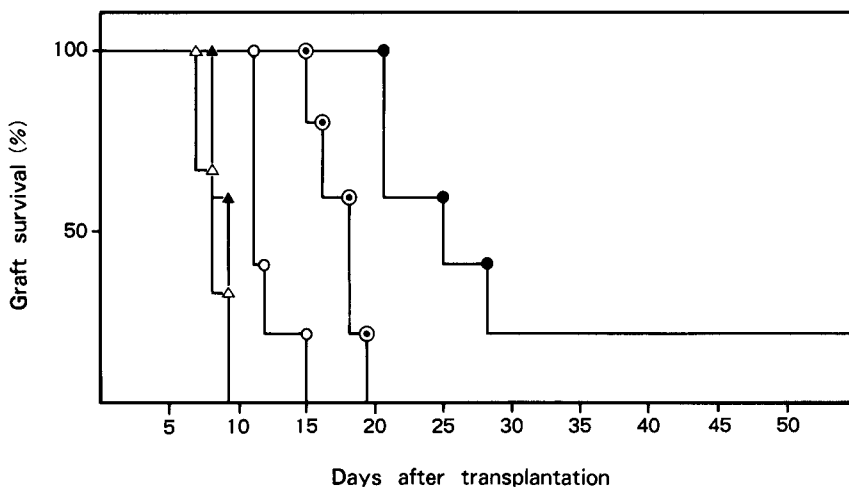


Fig. 3 Survival curves for F344 cardiac allografts in ACI recipients undergoing rabbit anti-IL2 antiserum treatment (5 animals per experimental group). Rabbit anti-IL2 antiserum treatment, started on the day of transplantation, consisted of 7 consecutive intravenous injections of 1 ml of 2⁶-fold diluted (○), 2³-fold diluted (◐), or 2⁰-fold diluted (●) anti-IL2 serum. △, untreated controls. ▲, normal rabbit serum-treated group.

rats was 8.0 ± 0.9 days, and of the rats treated with normal rabbit serum, 8.6 ± 0.7 days. A 7-day treatment with 2⁶-diluted anti-IL2 antiserum prolonged survival to 12.0 ± 2.2 days, 2³-diluted serum to 17.2 ± 2.0 days, and undiluted serum to 29.0 ± 15.0 days. One of the 5 grafts treated with undiluted anti-IL2 antisera survived indefinitely (>90 days). These results are statistically significant compared with those of the controls ($0.025 < p < 0.05$).

Discussion

Two striking findings emerged from the present study: (a) The *in vivo* administration of rabbit anti-IL2 serum significantly prolonged the survival of cardiac allografts in a fully allogeneic combination of rats in a dose-dependent fashion. (b) Rabbit anti-IL2 serum inhibited the proliferation of IL2-dependent CTLL cells and specifically bound to IL2 with high efficiency. These data indicate that the anti-IL2 antibody may be significant as an immunosuppressive agent

in clinical organ transplantation.

The mechanism of rabbit anti-IL2 serum-mediated graft prolongation is not entirely clear. IL2 from activated T lymphocytes is the chemical mediator for both T lymphocyte mitogenesis and the *in vitro* generation of alloreactive cytotoxic T lymphocyte (CTL) (1). We thus propose that the *in vivo* administration of antibody against the IL2 molecule inhibits the proliferation of CTL, and suppresses the rejection reaction. Recently, Lipkowitz *et al.* (8) suggested that there are at least three distinct phases of T lymphocyte activation. In the first phase, cells require mitogenic stimulation to induce both IL2 production and IL2 receptor expression. The second phase is an IL2-dependent phase requiring the interaction of IL2 with its receptor. The third phase is a commitment phase of the activated T lymphocytes that are proliferating independently of both mitogen and IL2. Based on the concept of Lipkowitz *et al.* (8), it might be speculated that rabbit anti-IL2 serum is responsible for the early phase (second phase of Lipkowitz's concept) of T

lymphocyte activation by interfering with IL2-IL2 receptor interactions.

However, little is known about the ability of exogenously administered anti-IL2 antibody to affect the *in vivo* immune response of transplanted recipients. Granelli-Piperno *et al.* (6) reported that lymph node cells from mice injected with rabbit anti-IL2 antibody show significant reduction of specific CTL responses to allogeneic cells compared with lymphoid cells from mice previously injected with saline or normal rabbit serum. Recently, we presented direct evidence for the specific accumulation of [¹²⁵I]-labeled monoclonal anti-IL2 antibody injected intravenously following transplantation in a grafted rat heart (9). With regard to the beneficial effects of rabbit anti-IL2 antiserum on rat cardiac allograft survival, we speculate that the rabbit anti-IL2 serum administered intravenously may bind to IL2 molecules released from IL2 producer cells infiltrating within the transplanted graft, thereby blocking the interleukin cascade, and delaying the rejection reaction. The anti-IL2 antibody may be useful as an immunosuppressive agent in clinical organ transplantation.

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