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Active Enhancement of Rat Cardiac Allografts by Donor-Specific B Lymphocytes

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ABSTRACT

In an attempt to induce the active enhancement of the cardiac allografts, recipient rats were injected intravenously with 1×10^7 donor-specific spleen cells or an equal number of their T or B cell subpopulations on each of 7 days before transplantation. Mean survival times (MST) in the group pretreated with donor-specific spleen cells (MST 27.5 ± 7.8 days) and the group pretreated with donor-specific B cells (MST 37.5 ± 15.5 days) were significantly prolonged ($p<0.01$) compared with the untreated control (MST 11.6 ± 2.0 days) and the group pretreated with donor-specific T cells. To investigate the mechanisms of the beneficial effect of donor-specific B cells on rat cardiac allografts, inhibition assay of mixed lymphocyte reaction (MLR) were carried out by the addition of recipient rat sera or spleen cells harvested 7 days following the intravenous administration of donor-specific spleen cells or their T and B cell subpopulations. Recipient rat sera harvested 7 days following the intravenous administration of donor-specific B cells showed significant inhibition of MLR; this inhibition was correlated to the prolonged survival of histoincompatible rat cardiac allografts. In contrast, MLR suppressor cells could not be detected in any experimental group. Thus, donor-specific B cell given 7 days before transplantation may possibly have beneficial effect on rat cardiac allografts and MLR inhibition induced by the intravenous administration of donor-specific B cells may be essential for prolonged allograft survival.

It is now well recognized that the active enhancement of a transplanted graft is due to specific suppression of cell-mediated immune responses by the induction of enhancing antibodies. Recently several investigators reported active enhancement could be achieved by preimmunization of the recipient with donor-specific B cells[3,6], or insoluble donor-specific RT1.B antigens[2]. In the present study, we investigated the effects of donor-specific spleen cells or their T and B cell subpopulations on the survival of rat cardiac allografts, and also, the changes in immune modulation in recipient rats pretreated with different cell populations.

MATERIALS AND METHODS

Animals and cardiac allografting.

F344 rats ($RT1^{lv1}$) were used as recipients, and (ACI × F344) F1 hybrid rats produced from ACI ($RT1^{av1}$) and F344 rats as cardiac allograft donors. Hetrotopic cardiac grafting was performed according to the method of Ono and Lindsey[5]. Graft survival was monitored by direct palpation, and rejection was considered complete when palpable ventricular contractions could no longer be detected.

Preparation and separation of a spleen cell suspension.

Spleen cells removed from ACI rats were minced with scissors, passed through a strain-

less steel mesh into a RPMI-1640 medium, and centrifuged at 1,000 $\times g$ for 5 min. The pellets were resuspended in 5 ml of Tris buffer and allowed to sediment by incubation for 3-4 min, and followed by removal of red cells The cells were washed with three changes of RPMI-1640 and adjusted to a concentration of 1×10^7 cells/ml. The spleen cell suspension thus obtained was placed over sterile nylon wool columns and separated into T cell- and B cell- enriched populations according to the method of Handwerger et al[2]. The cells that passed through the column were collected as the T cell- rich population (T cells). The nylon-wool adherent cells were collected as the B cell- rich population (B cells).

Pretreatment protocols.

F344 rats, recipients for cardiac allografts, were pretreated by an intravenous injection of a lymphoid cell suspension (1×10^7 cells/ml) from ACI rats, 7 days prior to cardiac allografting. Five experimental groups were set up including untreated groups or groups pretreated with donor-specific peripheral blood lymphocytes (PBL), spleen cells, T cells and B cells.

Statistics.

Allograft survival between groups within each experiment were analysed using the student's t test.

Rat MLC and its inhibition assay.

A one-way mixed lymphocyte culture (MLC) was made according to the method of Iwabuchi et al[6]. Briefly, the responder cells (1×10^5) of F344 rats in 0.1 ml were mixed with 2×10^5 of mitomycinC-treated stimulator cells of ACI and WKA rats ($RT1^k$) in flat-bottomed microtiter plates. The plates were cultured in a CO_2 incubator for 96 hr and pulsed with 0.5 μCi/well of [^{3}H]-thymidine 16 hr before harvest. For the MLC inhibition assay, 0.05 ml of recipient rat serum or 1×10^5 of mitomycinC-treated spleen cells obtained on the 7th day following immunization was added to the MLC. The results were expressed as [^{3}H]-thymidine incorporation in the presence of immunized serum or spleen cells compared with that of normal F344 rat serum or spleen cells.

RESULTS

Effect of pretransplant regimens on cardiac allograft survival.

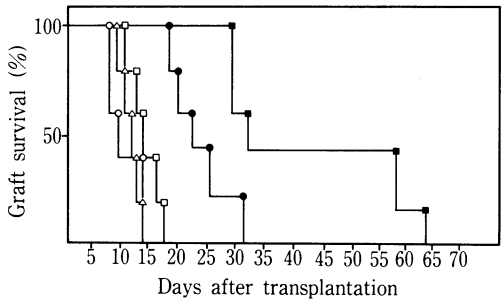

Fig. 1. Survival curves for (ACI × F344)F1 heart allografts in F344 recipients (5 animals per experimental group). Recipients were pretreated with F1 donor cells, either peripheral blood lymphocytes (○), spleen cell (●), T cells (□), or B cells (■), 7 days before grafting. Untreated controls (△).

The survival time of rat cardiac allografts in the control and experimental groups is shown in Fig. 1. The mean survival time (MST) of the untreated rats was 11.6 ± 2.0 days and of rats pretreated with donor-specific PBL, 11.4 ± 2.5 days. Rats pretreated with donor-specific spleen cells showed significant prolongation in a MST of 27.4 ± 7.4 days as compared with the untreated and PBL- pretreated rats ($p<0.01$). The effects of pretreatment with spleen cells in prolonging the graft survival was further assessed in groups of rats pretreated with either a donor-specific T or B cell populations of spleen cells. These groups displayed a MST of 15.1 ± 1.4 and 37.5 ± 15.5 days, respectively. Thus, MST in the donor-specific B cell-pretreated group was significantly longer ($p<0.01$) than that in the donor-specific T cell- pretreated and spleen cell-pretreated groups.

Inhibition of MLR by recipient rat spleen cells collected 7 days after immunization.

Spleen cells form F344 rat 7 days following immunization with various donor-specific lymphoid cells were co-cultured in MLC with normal F344 responder cells and normal ACI stimulator cells to assay the ability of spleen cells from immunized rats to inhibit MLR. Figure 2 shows the data from a MLR suppressor cell assay. spleen cells from rats immunized with donor-specific spleen cells, T cells or B cells did not suppress normal MLC response to ACI rat spleen cells.

Inhibition of MLR by recipient rat sera obtained 7 days after immunization.

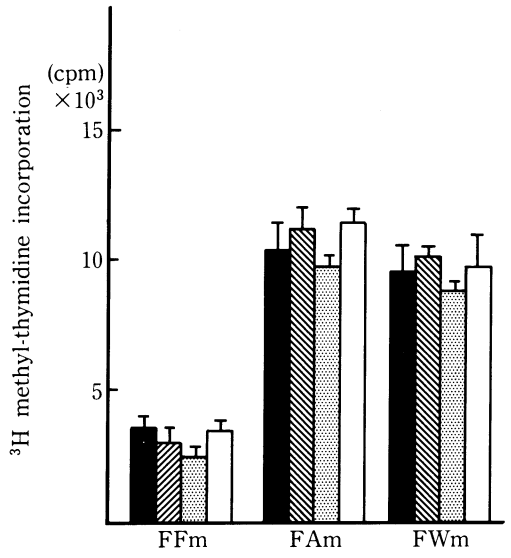

Fig. 2. Inhibition of MLR by normal F344 rat spleen cells (■) or immunized spleen cells 7 days following immunization with lymphoid cells of donor ACI rats as either spleen cells (▨), T cells (▩), or B cells (□). FFm (F344 × F344m)=autoMLC, FAm (F344 × ACIm)=specific MLC, FWm (F344 × WKAm)=nonspecific MLC.

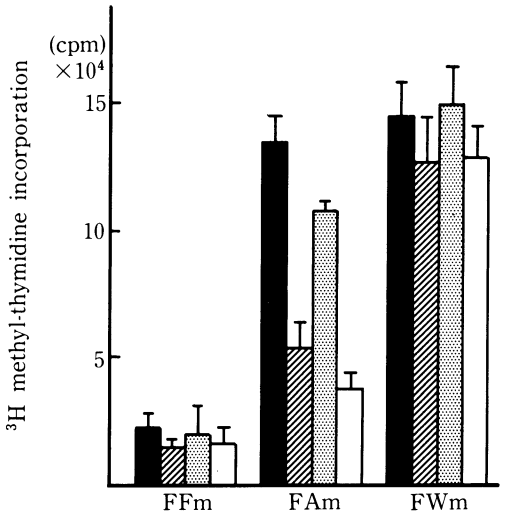

Fig. 3. Inhibition of MLR by normal F344 rat sera (■) or immunized sera 7 days following immunization with lymphoid cells of donor ACI rat as either spleen cells (▨), T cells (▩), or B cells (□ ⁚). FFm (F344 × F344m)=autoMLC, FAm (F344 × ACIm)=specific MLC, FWm (F344 × WKAm)=nonspecific MLC.

Sera from F344 rats 7 days following immunization were also tested for the ability to inhibit MLR. Figure 3 shows the results of inhibition of MLR by sera from F344 rats immunized with donor-specific spleen cells, T cells or B cells. The specific MLC (F344 × ACIm) was significantly ($p<0.01$) inhibited by sera from F344 rats immunized with donor-specific spleen cells (60.3%), and sera from those immunized with donor-specific B cells (71.5%). In contrast, immunized sera from F344 rats pretreated with donor-specific T cells caused only a 15.8% inhibition of MLR. The non-specific MLC (F344 × WKAm) was not suppressed by the addition of normal or immunized sera from F344 rats.

DISCUSSION

Two remarkable findings were obtained from the present study: (1) the intravenous administration at donor-specific B cells prior to transplantation was effective in prolonging rat cardiac allograft survival, and (2) recipient rat sera harvested 7 days after pretreatment with donor-specific B cells showed significant inhibition of MLR; this MLR inhibition was correlated to the prolonged survival of histo-incompatible rat cardiac allografts. Our data clearly show B cells to be essential for inducing active enhancement and T cells to be ineffective in our experiments. The present data accord with the earlier findings of Strom et al[6], Lauchart et al[3] and Kaldany et al[2].

It is well known that pretreatment of recipients with donor-specific antigen enhances the survival of subsequent allografts (active enhancement). Various methods for active allograft enhancement have been reported in organ transplantation in a rat models. Since Strom et al[6] reported that lymphoid cells, capable of inducing active enhancement in a rat renal allograft model, were the same cells which stimulate mixed lymphocytes cultures. Several investigator suggest the possibility that inducement of this active enhancement requires anti- class II antigen (Ia) immunity[2,6]. Lauchart et al[3] demonstrated that cardiac allograft rejection could be significantly suppressed only in rats pretreated with lymphocyte populations containing surface immunoglobulin-positive cells. Recently, Kaldany et al[2] presented evidence that membrane preparations from thymic lympho-

cytes exhibiting RT1.B (class II) antigenic properties, but lack detectable RT1.A (class I) alloantigen, can induce active enhancement of renal allografts in rats. Based on the present data and the findings of Lauchart et al[3] and Kaldany et al[2], active or passive immunity against donor class II antigens at the time of transplantation may be responsible for inducing active enhancement in an experimental rat model.

Little is known about the mechanisms involved in inducing active enhancement. Some data have been published on the relationship between transplant functions and MLR inhibition factors in the MLR[2,6], antiidiotypic antibodies[7] and suppressor T cells[4]. The conclusions, however, are contradictory. It is well-known that anti-Ia antibodies specifically inhibit mixed lymphocyte culture responses by interference at the level of the stimulatory cell expressed Ia antigen, possibly by interfering with B lymphocytes. Thus, the significant inhibition or MLR detected in sera at the time of transplantation following immunization of donor-specific B cells (present data) or subcellular RT1.B (Ia) antigens[2] supports further the presence of anti-Ia antibodies. Also, in the present study, suppressor cells could not be detected in rats on day 7 following donor-specific B cells. Although the beneficial effect of donor-specific B cells on the induction of active enhancement may be a complex of different immunosuppressive mechanisms, the present authors consider immunization with donor-specific B cells to induce anti-Ia antibodies which may perform some important function to bring about active enhancement.

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