

Acta Medica Okayama

Volume 42, Issue 1

1988

Article 5

FEBRUARY 1988

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Abstract

It is now well recognized that pre-transplant donor-specific blood transfusion (DST) has a beneficial effect on the survival of allografts. To determine the optimal interval between DST and transplantation, and to analyze the mechanisms of this effect, the survival of cardiac allografts to rats which received a single DST was examined. The cardiac allograft survival was found to be prolonged when the DST was performed 1 to 6 weeks before grafting. In addition, recipient rat sera collected 1 to 6 weeks after a single DST showed significant inhibition of a mixed lymphocyte reaction (MLR). This MLR inhibition correlated with prolongation of survival of histoincompatible rat cardiac allografts. It thus appears that a single DST given from 1 to 6 weeks before transplantation has a beneficial effect on allograft survival and that MLR inhibition may be essential for inducing the effect of transfusion on organ transplantation.

KEYWORDS: donor-specific blood transfusion, cardiac allografting, mixed lymphocyte reaction

*PMID: 2966542 [PubMed - indexed for MEDLINE]

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Effects of a Single Donor-Specific Blood Transfusion on the Survival of Rat Cardiac Allografts

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It is now well recognized that pre-transplant donor-specific blood transfusion (DST) has a beneficial effect on the survival of allografts. To determine the optimal interval between DST and transplantation, and to analyze the mechanisms of this effect, the survival of cardiac allografts to rats which received a single DST was examined. The cardiac allograft survival was found to be prolonged when the DST was performed 1 to 6 weeks before grafting. In addition, recipient rat sera collected 1 to 6 weeks after a single DST showed significant inhibition of a mixed lymphocyte reaction (MLR). This MLR inhibition correlated with prolongation of survival of histoincompatible rat cardiac allografts. It thus appears that a single DST given from 1 to 6 weeks before transplantation has a beneficial effect on allograft survival and that MLR inhibition may be essential for inducing the effect of transfusion on organ transplantation.

Key words : donor-specific blood transfusion, cardiac allografting, mixed lymphocyte reaction

The beneficial effect of pre-transplant donor-specific blood transfusions (DST) on the survival of human kidney allografts, as originally described by Salvatierra *et al.* (1), has been confirmed at many transplant institutions (2, 3). Clinically, three pre-transplant DSTs are administered approximately 2 weeks apart to potential recipients of kidneys from living related donors that are one haplotype mismatched. However, Persijn *et al.* (4) reported that even a single pre-transplant blood transfusion was effective in prolonging the survival of kidney allografts. Experimentally, single DSTs

have been shown to be effective in prolonging the survival of kidney, heart, and skin allografts in dogs (5), rats (6) and mice (7). The mechanism of the beneficial effect of pre-transplant blood transfusion on allograft survival is currently the subject of debate and intense investigation (8). However, the mechanism by which transfusions induce a state of immunological unresponsiveness and the significance of the time interval between the blood transfusion and transplantation are not fully understood. In the present study, examinations were made of the influence of a single DST and the time interval between a single DST and transplantation on the survival of rat cardiac

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allografts. The immune modulation at pre- and post-transplant periods in recipient rats was also examined.

Materials and Methods

Animals. Male ACI rats (RT1^{av1}) weighing 250–300 g (Hashimoto Exp. Animal Inc., Saitama, Japan) were used as recipients of single DSTs and cardiac allografts. Male (ACI×F344)_{F1} hybrid rats weighing 200–250 g (F344 rats (RT1^{lv1}) obtained from Charles River Japan Inc., Atsugi, Japan) were used as donors for cardiac allografts. WKA rats (RT1^k, Shizuoka Laboratory Animal Center, Shizuoka, Japan) were used as third-party donors.

Cardiac allografting. Heterotopic cardiac grafting was performed according to the method of Ono-Lindsey (9). Graft survival was monitored by direct palpation, and rejection was considered complete when no palpable ventricular contraction could be detected.

Single DST and transplantation. Donor-specific blood was collected from F344 rats. A single DST (1 ml of blood to each rat) was given i.v. to rats (5 animals per group) on day -1, or on week -1, -3, -6 and -12 with respect to transplantation (day 0 is the day of transplantation). As controls, rats were given a single transfusion of donor-nonspecific blood from WKA rats. In this experiment, the influence of the time interval between a single DST and transplantation on the survival of rat cardiac allografts was studied.

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

Experimental design for inhibition of mixed lymphocyte reaction (MLR). Cellular and humoral immune-modulation at pre- and post-transplant periods in rats receiving a single DST was studied by the inhibition assay of rat MLR as shown in Fig. 1. Briefly, a mixed lymphocyte culture (MLC) was maintained in flat-bottomed 96-well tissue culture plates (Costar, Cambridge, Massachusetts, U. S. A.), with 1×10^5 responder and 2×10^5 mitomycin C-treated stimulator cells in a total volume of 200 μ l containing 5×10^{-5} M 2-mercaptoethanol and gentamicin (10 μ g/ml). The

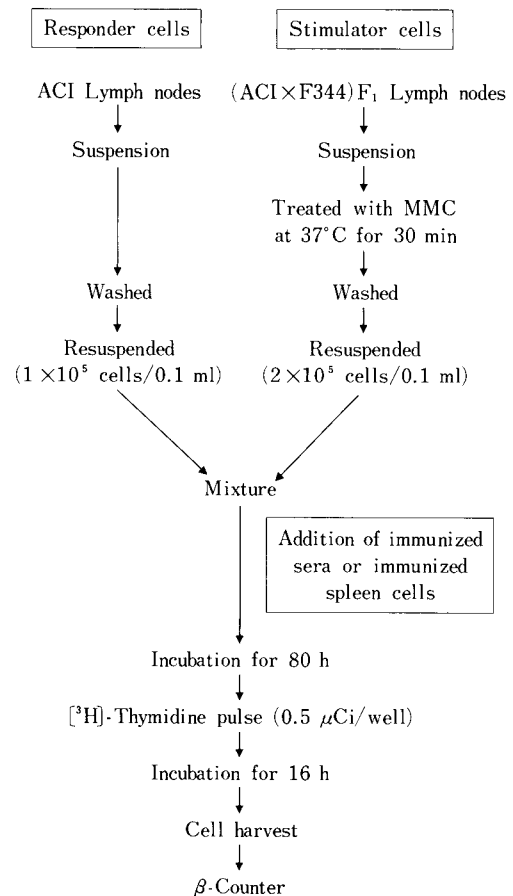


Fig. 1 Scheme of the inhibition assay of the rat mixed lymphocyte culture reaction. Lymphocytes from ACI rats were used as responder cells, and lymphocytes from F344 rats as stimulator cells. MMC; mitomycin C (Sankyo, Tokyo, Japan).

responder and stimulator cells were co-cultured with sera or spleen cells from transfused or transplanted rats. For the serum inhibition assay of MLR, 10 μ l of the serum to be tested or normal rat serum was added to the culture at the start of the mixed lymphocyte culture. For the suppressor cell assay of MLR, 1×10^5 spleen cells obtained at pre- and post-transplant periods from rats receiving a single DST or 1×10^5 normal rat spleen cells were added to the cultures at the start of the mixed lymphocyte culture. Cells were cultured for 4 days at 37°C in a humidified atmosphere of 5% CO₂ in air. [³H]-Thymidine (Amersham International, Amersham,

England, 1 $\mu\text{Ci}/\text{well}$) was added 18 h before the end of the culture. [^3H]-Thymidine incorporation was assessed by harvesting cells with a Mash II Automatic Harvester onto glass fiber filters and counting the radiation in a β -scintillation counter (LSC-703, Aloka, Japan). All cultures were performed in triplicate. The inhibition of MLR by serum and suppression of MLR by spleen cells were calculated as follows:

Inhibition of MLR by serum (%)

$$= \left(1 - \frac{\text{MLC with immunized rat serum}}{\text{MLC with normal rat serum}} \right) \times 100;$$

Suppression of MLR by spleen cells (%)

$$= \left(1 - \frac{\text{MLC with immunized rat spleen cells}}{\text{MLC with normal rat spleen cells}} \right) \times 100.$$

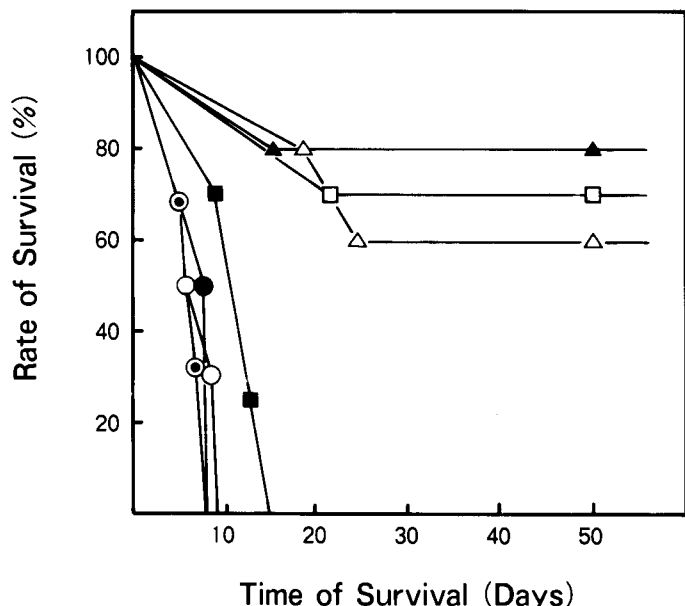
Results

Determination of the optimal time for a single DST to extend the survival of rat cardiac allografts. To determine the optimal interval between a single DST and cardiac grafting to extend the survival of

allografts, ACI rats were given a single DST from a F344 rat at week -1, -3, -6, -12, or on day -1 relative to cardiac grafting on day 0. A single DST was effective over a wide range of intervals, but not when given on day -1 and at week -12 (Fig. 2). The mean survival time of the untreated controls was 7.6 ± 0.8 days. A single DST from a F344 rat given at week -1, -3, and -6 resulted in the mean survival time of 68.2 ± 39.0 , 83.2 ± 33.6 , and 74.0 ± 36.8 days, respectively. A single DST on day -1 and at week -12 failed to prolong graft survival, the mean survival time being 8.3 ± 1.3 and 11.3 ± 2.6 days, respectively. A single donor-nonspecific transfusion of WKA rat blood failed to prolong cardiac allograft survival when given at week -1, the mean survival time being 8.7 ± 1.5 days.

Inhibition of MLR by the recipient serum after a single DST. To evaluate humoral immune modulation after a single DST, recipient rat sera obtained on day +1, and at week +1, +3, +6, +12 after a single

Fig. 2 Survival curves of (ACI $\times$ F344) F_1 hybrid cardiac allografts in ACI recipients treated with a single DST (5 animals per experimental group). Time between the single DST and cardiac allograft: 1 day ($\bullet$), 1 week ($\triangle$), 3 weeks ($\blacktriangle$), 6 weeks ($\square$), 12 weeks ($\blacksquare$). Untreated controls ($\circ$) and a single donor-nonspecific (WKA rat) blood transfused group ($\odot$).



DST given on day 0 were tested for their ability to inhibit MLR (Fig. 3). Recipient rat sera obtained at week +1, +3, and +6 after a single DST significantly inhibited MLR compared with normal ACI rat

serum. The most significant inhibition was noted in recipient rat serum obtained 1 week after a single DST. There was no significant inhibition of MLR in the recipient rat sera obtained 1 day or 12 weeks after a single DST.

Inhibition of MLR after cardiac allografting. To clarify the mechanism of cardiac allograft survival induced by a single DST, the effect of sera or spleen cells of recipient rats (harvested from enhanced and control ACI recipients) on the autologous MLR was tested (Fig. 4). Recipient rat sera harvested 1 week, 1 month, 3 months, and one year after the transplantation showed significant inhibition (32 to 68%) compared with normal ACI rat serum.

Spleen cells from ACI rats which received cardiac transplantation after a single DST at week -1 were co-cultured with normal ACI responder cells and F344 stimulator cells to examine the possibility of spleen cells inhibiting MLR. Spleen cells from the transplanted rats failed to suppress the normal MLC response to F344 lymph node cells at all stages.

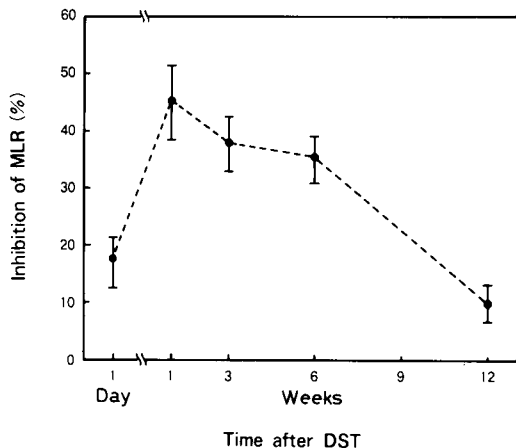


Fig. 3 Inhibition of mixed lymphocyte culture reaction (MLC) by the recipient rat serum following a single donor-specific transfusion (DST). Recipient rat sera obtained at various times after a single DST were tested for their capacity to inhibit MLR compared with normal rat (ACI) sera. Experimental details are described under Materials and Methods. Means $\pm$ SD of % inhibition obtained from 5 experiments.

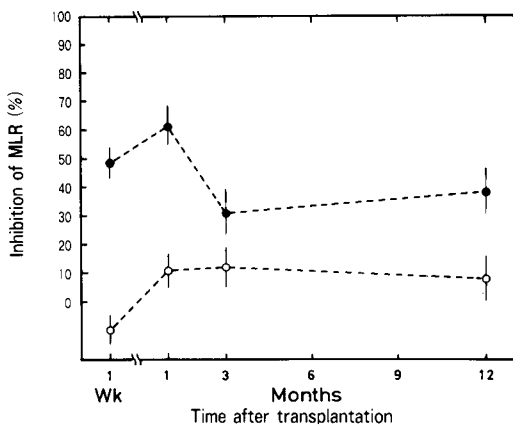


Fig. 4 Changes in the inhibition of MLR by recipient serum after a cardiac allograft performed 1 week following a single DST. Recipient rat sera (●) obtained at various times after the cardiac allograft were tested for the inhibition of MLR. Normal rat (ACI) sera were used as controls (○). For details, see Fig. 3.

Discussion

Three findings were obtained from the present study: (a) a single DST was effective in prolonging rat cardiac allograft survival over a wide range of time between DST and transplantation from 1 to 6 weeks, (b) recipient rat sera harvested from 1 to 6 weeks following a single DST significantly inhibited MLR (This inhibition of MLR correlated with the prolongation of histoincompatible rat cardiac allograft survival.), and (c) long-term survival of cardiac allografts achieved in rats which received a single DST was mediated by MLR inhibitory factors, not by suppressor cells. The data of the present study show that the

optimal interval between DST and transplantation is from 1 to 6 weeks, and that antibodies capable of inhibiting the MLC response can be induced by a single DST.

The importance of the time of blood transfusion was confirmed in the present study. Questions remain regarding the optimal time interval between blood transfusion and transplantation in clinical situations. In clinical kidney transplantation, Hourmant *et al.* (10) reported that patients who received the last blood transfusion within 3 months before grafting showed better graft outcome than those who received a transfusion more than 6 months before grafting. Marquet *et al.* (6) reported that a single blood transfusion of donor-specific blood given 1 week to 2 months before grafting gave better kidney allograft survival than that of a single transfusion 2 h to 2 days before grafting in a rat model. Also, Okazaki *et al.* (7) demonstrated that the effective interval for blood transfusion ranges from 30 days before grafting to 7 days after grafting in a mouse skin model. Persijn *et al.* (4) have shown, however, that the beneficial effect of a single blood transfusion lasts more than 120 months in clinical kidney transplantation. However, we and other investigators (6, 7) have confirmed that the enhancing effect of blood transfusion disappears after more than 3 months.

It is now well-established that pre-transplant blood transfusion has a beneficial effect on the subsequent survival of allografts. However, the mechanism of this effect remains obscure. Various suppressor mechanisms have been postulated, such as the action of suppressor T lymphocytes (6, 7) and antiidiotypic antibodies (11, 12). In the present study, sera from ACI rats which received a single DST were found capable of inhibiting the normal MLC response of ACI rat to F344 rat. This in-

hibition is possibly due to the formation of enhancing antibodies in the sera following a single DST. Interestingly, the induction of MLR inhibition correlated significantly with the prolongation of rat cardiac allograft survival. It thus appears that factors other than suppressor cells, such as anti-idiotypic antibodies or enhancing antibodies, may be essential to the suppression of the recipient immune response. Singal *et al.* (11) and Takeuchi *et al.* of our laboratory (12) have reported these antibodies to be present in patients following blood transfusion. Although the effect of transfusion may occur through a complex of different immunosuppressive mechanisms, the results of this study on a rat cardiac allograft model along with earlier data on renal transplant patients (12, 13, 14) demonstrate that a single DST induces MLR inhibition which may be important in bringing about the beneficial effect of transfusion.

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Received May 22, 1987; accepted October 28, 1987