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Donor-specific transfusion via the portal venous route induces prolongation of H-2-compatible but not H-2-incompatible cardiac graft survival

D. Górecki[1], M. Jakóbisiak[2], M. Łupińska[1], A. Kruszewski[1], and J. Czyżyk[1]

[1] Department of Histology and Embryology and [2] Department of Transplantology, Institute of Biostructure, Medical School, Chałubińskiego 5, PL-02-004 Warsaw, Poland

Abstract. In the H-2-compatible donor-recipient combination (BALB/c→DBA/2), pretransplant donor-specific blood transfusion (DST) via the portal venous (PV) route significantly prolonged cardiac graft survival. DST via the intravenous (IV) route (systemic circulation) also showed a marked prolongation of heart tissue transplant survival in this model. In the H-2-incompatible combination (BALB/c→CBA/H), DST via the IV - but not via the PV - route resulted in accelerated graft rejection.

Key words: Transfusion effect, intraportal route - Transfusion effect, experimental, heart - Heart transplantation, experimental - Portal venous route, transfusion effect.

It has been documented both in animal models and in clinical studies that pretransplant donor-specific blood transfusion (DST), with [15, 16, 33, 36] or without [8, 17, 24, 29] concurrent immunosuppression, exerts a beneficial effect on the survival of allografts. However, the immunologic mechanisms implicated in mediating this beneficial effect have not been fully elucidated, although several hypotheses have been presented [4, 18, 23].

A number of studies have suggested that orally presented antigen has been associated with suppression of systemic immune responses [3, 5, 35], a phenomenon referred to as oral tolerance. Observations showing that allogeneic cells injected into the portal circulation induced systemic hyporesponsiveness to the appropriate alloantigens [26, 27], as well as data suggesting increased responses to a variety of antigens after disruption of hepatic architecture [12, 34], have tended to support the notion that the liver plays an important role in the induction of oral tolerance. This phenomenon may be potentially useful in preventing the rejection of transplanted tissue.

In the present study, we examined the effect of pretransplant DST via the portal venous route on allogeneic graft survival in mice.

Offprint requests to: D. Górecki

Materials and methods

Animals

The following inbred mouse strains were used: BALB/c (H-2^d), DBA/2 (H-2^d), and CBA/H (H-2^k). Breeding pairs were originally obtained from Dr. A. Czarnomska (Institute of Oncology, Warsaw, Poland). All mice were kept in standard conditions during the experimental period. Recipients (DBA/2 and CBA/H) were 12-14 weeks old and heart donors (BALB/c) 2-4 days old at the time of grafting. Only males were used as graft recipients.

Blood transfusions

Recipient mice were given blood on the −9th day before grafting. Transfusions of 0.25 ml fresh, sex-matched, heparinized blood were given via the tail vein or via the portal venous route.

Injection via portal venous route

Animals were anesthetized with chloral hydrate given IP. A right side abdominal incision was made and the viscera exposed. Blood was slowly injected through a superior mesenteric vein with a 27-gauge needle. The hemostasis was performed using a cotton wool swab. Mice were occasionally excluded because of bleeding. The abdominal incision was secured with a chromic catgut 4/0 suture (Ethicon, Edinburgh, UK).

Sham operation

The sham operation was performed step-by-step like injection via the portal venous route except that mice were given 0.25 ml heparinized physiological saline.

Table 1. The effect of pretransplant donor-specific blood transfusion (DST) via the portal venous (PV) or the intravenous (IV) route on cardiac allograft survival in H-2 compatible donor-recipient combination (BALB/c→DBA/2)

Group	Recipient pretreatment[a]	Graft survival (days)	*P* value[b]
1	DST PV	7, 8, 8, 10, 10, 11, 11, 12, 13, 13, 14, 16, 16, 18, 18, 18, 20, 28, >100, >100, >100, >100, >100	1 vs 2: <0.005, 1 vs 3, 4: NS, 1 vs 5: <0.01
2	-	7, 7, 7, 9, 9, 10, 10, 11, 11, 11, 11, 11, 12, 12, 12, 12, 12, 13, 14, 14, 14, 16	2 vs 1: <0.005, 2 vs 3: <0.01, 2 vs 5: NS
3	DST IV	7, 8, 9, 9, 9, 9, 10, 11, 11, 11, 12, 13, 15, 15, 16, 20, 24, 26, 30, 30, 36, 75, >100	3 vs 1: NS, 3 vs 2: <0.01
4	DST IV + sham operation	7, 10, 10, 11, 11, 13, 20, 22, 22, 30, 32, 54	4 vs 1: NS
5	Sham operation only	8, 10, 11, 11, 11, 12, 12, 14, 14	5 vs 1: <0.01, 5 vs 2: NS

[a] 0.25 ml blood was transfused on the −9th day before transplantation
[b] Moses test for reciprocal values of survival times

Table 2. The effect of pretransplant donor-specific blood transfusion (DST) via the portal venous (PV) or intravenous (IV) route on cardiac allograft survival in H-2 incompatible donor-recipient combination (BALB/c→CBA/H)

Group	Recipient pretreatment[a]	Graft survival (days)	*P* value[b]
1	DST PV	7, 7, 9, 11, 11, 11, 11, 11	1 vs 2: <0.05, 1 vs 3: NS
2	DST IV	5, 6, 7, 7, 8, 8, 9	2 vs 1: <0.05, 2 vs 3: <0.01
3	-	7, 8, 8, 8, 8, 8, 9, 9, 10, 10 11, 12, 13, 17	3 vs 1: NS, 3 vs 2: <0.01

[a] 0.25 ml blood was transfused on the −9th day before transplantation
[b] As determined by Wilcoxon rank sum test

Technique of heart transplantation

The technique of heterotopic cardiac transplantation described by Fulmer et al. [9] was adopted. Briefly, hearts were removed from neonatal (2-4 day-old) mice and cut into two equal parts longitudinally. Each part of the heart tissue was placed into cold minimal essential medium (MEM). Meanwhile, recipient mice were anesthetized with chloral hydrate. A pocket on the dorsal side of the left ear lobe was made by an incision in the skin at the base of the ear lobe. The tips of small curved forceps were forced into the slit, bluntly dissecting the skin and the cartilage toward the distal edge of the ear. The heart fragment was placed into this pocket within 1 min of removal of the heart from the donor. Graft function was evaluated starting on the 5th or 7th day after grafting. For this, an electrocardiograph enhancer, recording electric heart impulses and transforming them into visual and sonic signals, was used. Animals were anesthetized with chloral hydrate. Electrodes made from thin pins were placed into the ear on both sides of the graft. The grafts were recorded daily during the first 2 weeks after grafting and thereafter on every second day; beginning on the 36th day, this occurred once a week. Grafts were regarded as rejected if no impulses were recorded on three consecutive examinations.

Statistical analysis

The reciprocal values of survival times for the H-2-compatible donor-recipient combination (BALB/c→DBA/2) were calculated and are presented in Table 1. For the graft surviving longer than 100 days, the reciprocal value of survival time was assumed to be zero [2]. Such transformed data were compared by means of the nonparametric Moses test of extreme reactions. In Table 2 these values for the H-2-incompatible donor-recipient combination (BALB/c→CBA/H) are shown. Here, the Wilcoxon rank sum test was used.

Results

The effects of DST via the PV or IV route on the survival of BALB/c heart tissue transplanted into H-2-compatible DBA/2 recipients are summarized in Table 1. In this model, DST via the PV route induced significant prolongation of graft survival resulting, in several cases, in permanent cardiac tissue survival (group 1 vs 2, $P<0.005$; group 1 vs 5, $P<0.01$). DST via the IV route also showed a marked prolongation of heart tissue transplant survival (group 3 vs 2, $P<0.01$). There was no statistically significant difference in the donor-specific transfusion effect induced via the PV or IV route (group 1 vs 3 and group 1 vs 4). In this model, surgical stress (sham operation) did not exert any influence on graft survival (group 2 vs 5 and group 4 vs 3).

DST via the IV route in a "strong" histoincompatibility barrier (BALB/c→CBA/H) resulted in accelerated graft rejection, as compared to control and DST via the PV route (Table 2: group 1 vs 2, $P<0.05$ and group 2 vs 3, $P<0.01$). On the other hand, there was no statistically significant difference in the effect of DST via the PV route in comparison to control (group 1 vs 3).

Discussion

Prolongation of graft survival can be achieved by interfering with the alloantigen recognition or graft rejection phases. Several different attempts have been made to shift the balance from positive response to a state of tolerance [31].

One of the major advances in transplantation has been the recognition of the beneficial effect of pretransplant blood transfusion [24]. Specific and nonspecific suppressor cells of a macrophage or lymphocyte line, blocking antibodies, anti-idiotypic antibodies, and antigen-antibody complexes have been proposed as participating in this effect [6, 10, 13, 30]. Potentiation of DST effect is one of the most important issues in clinical transplantation as it may decrease the post-transplant immunosuppressive requirements of graft recipients.

It is well-known that oral presentation of antigen has been associated with the suppression of systemic responses [3, 5, 35]. Both the intestine and the liver appear to play a role in the induction of oral tolerance. Several mechanisms have been described as being responsible for this type of immunologic unresponsiveness. First, the formation of antigen-specific suppressor cells in gut-associated lymphoid tissue (GALT) after oral administration of antigen has been reported [19]. Second, the predominant GALT response against immunogens is the production of IgA antibody. André et al. [1] suggested that circulating antigen-antibody IgA complexes are important for the induction of oral tolerance. Other investigators have reported that oral administration of antigen is followed by the generation of antigen-binding T-cell-derived suppressor factors that may be released into the serum and that can bind the relevant antigen [20, 21]. However, the effect of noncellular factors is difficult to interpret because other data suggest that oral immunological tolerance is not transferable by serum [11].

The interposition of the liver between the gastrointestinal tract and the general circulation may be immunologically relevant. If the portal circulation is diverted to the vena cava so that absorbed antigen bypasses the liver, systemic unresponsiveness is abolished [5]. This observation has emphasized the importance of the liver in the induction of this type of tolerance.

It has recently been shown that injection of allogeneic cells into the portal, but not systemic, circulation induces inhibition of delayed-type hypersensitivity [26]. Moreover, the combined treatment consisting of PV inoculation with spleen cells and cyclophosphamide specifically abrogated the alloreactivity against tumor cells. Intravenous presensitization plus cyclophosphamide, as well as PV inoculation alone, failed to induce such a tolerance [27]. On the other hand, Kenick et al. [14] and Rao et al. [28] have recently demonstrated prolongation of rat cardiac allograft survival when donor strain mononuclear cells were injected via the PV route. Moreover, Eid et al. [7] have obtained prolongation of skin graft survival by PV injection of allogeneic bone marrow cells in mice. Depending on the donor-recipient combination, the IV inoculation had only a marginal effect or was as effective as PV injection [14, 28]. The present study demonstrates the beneficial effect of PV DST without any nonspecific immunosuppression in a "weak" donor-recipient barrier: BALB/c→DBA/2 (Table 1). However, the beneficial effect of DST via the PV route was not significantly different than that via the IV route, although PV injection seems to be more effective in bringing about permanent graft survival (>100 days).

In the H-2-incompatible donor-recipient combination BALB/c→CBA/H, the DST via the PV route - unlike that via the IV route - did not result in accelerated graft rejection. This seems to suggest that the PV route could prevent sensitization. However, we have not observed the beneficial effect of PV DST as compared to control (Table 2).

Numerous reports have demonstrated that surgical stress can decrease: total peripheral blood leukocyte, B and T lymphocyte counts, mixed leukocyte reactivity on both responder and stimulator levels [32], NK activity [25], and, more importantly delayed hypersensitivity [22]. In our model, surgical stress-related suppression of the immune system did not seem to influence graft survival.

The potential advantages of oral tolerance induction has been largely unexploited in transplantation. Surprisingly, only a few reports documenting the positive influence of such an approach on graft survival have been published [7, 14, 28]. Before oral tolerance induction can be applied in clinical transplantation, further experiments aimed at strengthening the effectiveness of this method have to be undertaken.

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References

1. Andre C, Heremans JF, Vaerman JP, Cambiaso CL (1975) A mechanism for the induction of immunological tolerance by antigen feeding: antigen-antibody complexes. J Exp Med 142: 1509-1519
2. Armitage P (1971) Statistical methods in medical research. Blackwell, Oxford, pp 293-296
3. Battisto JR, Millen J (1962) Immunologic unresponsiveness produced in adult pigs by parenteral introduction of minute quantities of hapten or protein antigen. Proc Soc Exp Biol Med 111: 111
4. Brent L, Horsburgh T, Wood PJ (1980) Induction of suppressor cells and allograft tolerance. Transplant Proc 12: 464
5. Cantor HM, Dumont AE (1967) Hepatic suppression of sensitization to antigen absorbed into the portal system. Nature 215: 744-745
6. Caulfield MJ, Shaffer D (1987) Immunoregulation by antigen-antibody complexes. J Immunol 138: 3680-3683
7. Eid A, Morecki S, Slavin S (1988) Induction of transplantation tolerance by intraportal injection of allogeneic bone marrow cells. Transplant Int 1: 109-112
8. Fabre JW, Morris PJ (1972) The mechanism of specific immunosuppression of renal allograft rejection by donor strain blood. Transplantation 14: 634-640
9. Fulmer RI, Cramer AT, Liebelt RA, Liebelt AG (1963) Transplantation of cardiac tissue into the mouse ear. Am J Anat 113: 273-285
10. Hall M, Gurley K, Dorsch SE (1987) Specific unresponsiveness in rats with prolonged allograft survival is dependent upon the graft and suppressor T cell. Transplant Proc 19: 495-496
11. Hanson DG, Vaz NM, Maia LCS, Lynch JM (1979) Inhibition of specific immune responses by feeding protein antigens. III. Evidence against maintenance of tolerance to ovalbumin by orally induced antibodies. J Immunol 123: 2337-2343
12. Hauptman S, Tomasi TB (1974) Antibodies to human albumin in cirrhotic sera. J Clin Invest 54: 122-125
13. Hellstrom I, Hellstrom KE (1972) Cell-mediated immunity and blocking antibodies to renal allografts. Transplant Proc 4: 369-371
14. Kenick S, Lowry RP, Forbes RDC, Lisbona R (1987) Prolonged cardiac allograft survival following portal venous inoculation of allogeneic cells: what is "hepatic tolerance"? Transplant Proc 19: 478-480
15. Lasek W, Jakóbisiak M, Płodziszewska M, Szczytnicki W, Górecki D (1988) The role of drug timing and histoincompatibility barrier in prolongation of cardiac graft survival in mice treated with donor-specific blood and cyclophosphamide. Immunol Lett 17: 357-360
16. Lasek W, Jakóbisiak M, Grochowska M, Górecki D, Gniadecki R, Kaliński P (1989) The influence of pretransplant and posttransplant immunosuppression on cardiac graft survival in the donor-specific transfusion model in mice: comparison of effects of cyclophosphamide, procarbazine, cyclosporine and cortisone. Transplantation 47: 913-915
17. Maki T, Okazaki H, Wood ML, Monaco AP (1982) Mouse skin allograft model for study of pregraft blood transfusion. Transplant Proc 14: 394-396
18. Marquet RL, Haysteek G (1981) Induction of suppressor cells by donor-specific blood transfusions and heart transplantation in rats. Transplantation 31: 272
19. Mattingly JA, Waksman BH (1978) Immunologic suppression after oral administration of antigen. I. Specific suppressor cells formed in rat's Peyer's patches after oral administration of sheep erythrocytes and their systemic migration. J Immunol 121: 1878-1883
20. Mattingly JA, Waksman BH (1980) Immunologic suppression after oral administration of antigen. II. Antigen-specific helper and suppressor factors produced by spleen cells of rats fed sheep erythrocytes. J Immunol 125: 1044-1047
21. Mattingly JA, Kaplan JM, Janeway CA Jr (1980) Two distinct antigen-specific suppressor factors induced by the oral administration of antigen. J Exp Med 152: 545-554
22. McLoughlin GA, Wu AV, Saporoschetz I, Nimberg R, Mannick JA (1979) Correlation between anergy and a circulating immunosuppressive factor following major surgical trauma. Ann Surg 190: 297-303
23. Nagarkatti PS, Joseph S, Singal DP (1983) Induction of antibodies by blood transfusions capable of inhibiting responses in MLC. Transplantation 36: 695-698
24. Opelz G, Sengar DPS, Mickey MR, Terasaki P (1973) Effect of blood transfusion on subsequent kidney transplants. Transplant Proc 5: 253-259
25. Pollock RE, Lotzova E (1987) Surgical-stress-related suppression of natural killer cell activity: a possible role in tumor metastasis. Nat Immun Cell Growth Regul 6: 269-278
26. Qian J, Hashimoto T, Fujiwara H, Hamaoka T (1985) Studies on the induction of tolerance to alloantigens. I. The abrogation of potentials for DTH responses to alloantigens by portal venous inoculation with allogeneic cells. J Immunol 134: 3656-3661
27. Qian J, Kokudo S, Sato S, Hamaoka T, Fujiwara H (1987) Tolerance induction of alloreactivity by portal venous inoculation with allogeneic cells followed by the injection of cyclophosphamide. I. Specific suppression of alloreactive cytotoxic and DTH responses as well as allograft rejection. Transplantation 43: 538-543
28. Rao VK, Burris DE, Sollinger HW, Burlingham WJ (1988) Evidence that donor spleen cells administered through the portal vein prolong the survival of cardiac allografts in rats. Transplantation 45: 1145-1149
29. Rood JJ van (1983) Pretransplant blood transfusion: sure! but how and why. Transplant Proc 15: 915-916
30. Singal DP, Ludwin D, Joseph S (1986) Induction of antiidiotypic antibodies by blood transfusions. Transplantation 42: 632-635
31. Siskind GW (1984) Immunologic tolerance: In: Paul WE (ed) Fundamental immunology. Raven Press, New York, pp 537-573
32. Slade MS, Simmons RL, Yunis E, Greenberg LJ (1975) Immunodepression after major surgery in normal patients. Surgery 78: 363-371
33. Terasaki PI (1984) The beneficial transfusion effect on kidney graft survival attributed to clonal deletion. Transplantation 37: 119-125
34. Triger DR, Alp MH, Wright R (1972) Bacterial and dietary antibodies in liver disease. Lancet I: 60
35. Triger DR, Cynamon MH, Wright R (1973) Studies on hepatic uptake of antigen. I. Comparison of inferior vena cava and portal vein routes of immunization. Immunology 25: 941-950
36. Yoshimura N, Kahan BD (1986) The requirement for the renal transplant to induce unresponsiveness by the combination of extracted histocompatibility antigen and cyclosporine. Transplantation 42: 642-646