Brief reports

TRANSPLANT International

Transplant Int (1988) 1: 109-112

Induction of transplantation tolerance by intraportal injection of allogeneic bone marrow cells

Possible implications for intrauterine bone marrow transplantation across major histocompatibility barriers

Ahmed Eid[1], Shoshana Morecki[3], and Shimon Slavin[2]

[1] Department of Surgery, [2] Department of Bone Marrow Transplantation, and [3] Cancer/Immunobiology Research Laboratory, Hadassah University Hospital, IL-91120 Jerusalem, Israel

Abstract. Intraportal inoculation of C57BL/6 marrow cells into sublethally (400 rad) irradiated BALB/c recipients resulted in durable chimerism and the permanent acceptance of C57BL/6 skin allografts. Sublethally irradiated recipients of a similar number of marrow cells inoculated systemically did not develop chimerism or any significant prolongation of the survival of C57BL/6 skin allografts. Consequently, lethal graft-versus-host disease developed only in recipients of intraportal marrow allografts (80%). The intraportal injection of allogeneic C57BL/6 marrow cells into nonirradiated recipients resulted in significant, although not permanent, prolongation of skin allograft survival without durable chimerism, suggesting that the introduction of alloantigens intraportally may favor the induction of nonresponsiveness to alloantigens even across strong major histocompatibility barriers. The relevance of these findings is discussed regarding the intraportal inoculation of allogeneic bone marrow cells for the treatment of genetic disorders in utero through the induction of neonatal tolerance.

Key words: Transplantation tolerance - Intraportal injection - Allogeneic bone-marrow transplantation - Chimeras.

Several previously published experiments have suggested that the liver may be involved in nonspecific and alloantigen-specific immunological nonresponsiveness [2, 6, 10, 18]. Transplantation of the liver or even of isolated hepatocytes or extracts of liver cells can induce mild or complete nonresponsiveness to alloantigens, depending on the genetic barriers investigated [3]. Likewise, allografted rat livers not only survive in some strain combinations but have also induced nonresponsiveness to kidneys from the same donors [15]. Improved allograft survival has also previously been documented following organ grafting with drainage into the portal vein [2, 6, 7, 15, 18]. Sakai [18] and Boeck et al. [2] have demonstrated improved heart and renal allograft survival in the rat when these grafts were drained into the portal vein. This has also resulted in inhibition of the second set rejection of renal allografts, as has been shown by Fukada et al. [6].

Improved cardiac allograft survival in rats was recently documented by Kenick et al. [10] following the inoculation of allogeneic donor-type lymph-node and spleen cells 7-10 days prior to allografting: survival was extended from 15.0 ± 2.9 to >200 days in PVG-PVG-RT1^a and from 7.4 ± 0.5 to 33.5 ± 19.0 days in Wistar Furth-Lewis rats. Similarly, Qian et al. have documented the induction of nonresponsiveness to allogeneic tumor cells across major histocompatibility (MHC) barriers (C3H/He-BALB/c) by portal IV inoculation of allogeneic spleen cells followed by the injection of cyclophosphamide [17].

In the present study we examined the potential use of intraportal inoculation of bone marrow (BM) cells for the induction of durable chimerism and nonresponsiveness to donor-type skin allografts.

Materials and methods

Mice

Inbred 3- to 5-month-old BALB/c (H-2^d) (BALB) and 5- to 8-week-old C57BL/6(H-2^b) (C57) mice were purchased from the Hebrew University Animal Farm in Jerusalem, Israel. All mice

Offprint requests to: S. Slavin

were kept in standard, nonprotected animal facilities during all experiments.

Whole-body irradiation (WBI)

BALB mice were conditioned by 400 rad delivered from a Philips X-ray unit (250 kV, 20 mA) at a rate of 70 rad/min 1 day prior to the inoculation of BM cells. The source-to-skin distance was 40 cm and a 0.2 mm copper filter was used.

Bone marrow

BM cells were obtained from 6- to 8-week-old C57 mice by flushing femora and tibiae using a 25-gauge needle with 0.9% NaCl solution. Cells were washed and aliquots of 0.25 ml containing 30×10^6 live nucleated cells were injected intraportally under direct vision or systemically into the lateral tail vein of BALB recipient mice 1 day prior to skin allografting using a 32-gauge needle on a 1-ml plastic syringe.

Intraportal injection

BALB mice were anesthetized IP using 30 mg/kg pentobarbital. The abdomen was opened through an upper midline incision and the small bowel was placed on its left side. The portal vein was identified with the help of a magnifying glass. Each BM aliquot was slowly injected while a small piece of sterile gauze was applied with gentle pressure over the puncture site to prevent fatal bleeding. The incision was closed with a 3-0 continuous nylon suture.

Skin graft

A full-thickness skin graft (SG) from C57 donor mice was placed on the dorsum of BALB recipient mice as previously described [13]. The SG was considered rejected when most of the skin area became necrotic or turned into a black eschar.

Assay for chimerism

The percentage of donor-type cells in BALB recipients was assayed using BALB anti-C57 and C57 anti-BALB antisera in a complement-dependent microcytotoxicity test, as previously described [1].

Statistics

All values are expressed as the mean ± standard deviation. Differences between the various experimental groups were assessed by Student's *t*-test.

Results

Significant prolongation of SG survival (22 ± 0.8 days) was accomplished in BALB recipients following the intraportal injection of 30×10^6 C57 BM cells infused 1 day prior to skin transplantation (Table 1, group IV), as compared with 12.0 ± 1.5 days in controls (Table 1, group I), although none showed permanent graft acceptance. No significant prolongation of SG survival was documented in BALB mice conditioned with 400 rad WBI (Table 1, group II). Similarly, no significant prolongation of SG survival was documented in recipients of 400 rad WBI with subsequent IV inoculation of 30×10^6 C57 BM cells (Table 1, group III). In contrast, by combining both WBI and the intraportal injection of a similar number of histoincompatible C57 donor-type BM, a dramatic permanent SG survival was accomplished in all surviving recipients (Table 1, group V). Interestingly, skin allografts in this group were never rejected; al-

Table 1. Tolerogenic effects of intraportally inoculated donor-type BM cells on skin allograft survival. Group I, normal BALB control; group II, BALB recipients conditioned by WBI on day -2; group III, BALB recipients conditioned by WBI on day -2 and IV injection of 30×10^6 C57 BM cells on day -1; group IV, BALB recipients conditioned by intraportal injection of 30×10^6 C57 BM cells on day -1; group V, BALB recipients conditioned by WBI on day -2 and intraportal injection of 30×10^6 C57 BM cells on day -1. NS, not significant

Experimental group	No. of mice	Skin allograft survival (days)		
		Range	Mean ± SD	Significance
I	15	10-14	12 ± 1.5	-
II	10	13-17	15 ± 1.4	II vs I: NS
III	10	14-18	16 ± 1.6	III vs I, II; NS
IV	10	21-23	22 ± 0.8	IV vs I, II; $P<0.001$
V	8	≥30->360	124 ± 128	V vs I, II; $P<0.005$

Table 2. Survival of BALB mice conditioned by WBI followed by injection of 30×10^6 allogeneic BM cells of C57 origin, according to the route of administration. (For definitions, see Table 1)

Experimental group	Survival (days)	
	Range	Median
III	180->360	>360
IV	200->360	>360
V	30- 360	50

Table 3. Evidence for chimerism in BALB recipients of C57 bone marrow cells, according to route of administration

Experimental group	Percentage of net cytotoxicity (mean)		Chimerism (%)
	BALB anti-C57	C57 anti-BALB	
BALB	0	100	-
C57	100	0	-
III	0	100	0
IV	0	100	0
V	85	5	85

though 75% of the mice in this group died between days 30 and 60 posttransplantation, most likely due to graft-versus-host disease (GVHD), all had intact SG; all of the rest are still alive with perfectly intact SG (Table 2).

All long-term survivors belonging to group V were stable chimeras, with $\geq 85\%$ donor-type cells (Table 3). No evidence for chimerism could be obtained in nonirradiated BALB recipients inoculated intraportally with 30×10^6 C57 BM cells or in mice receiving an equal number of C57 BM cells IV following sublethal WBI (Table 3).

Discussion

In the present experiment, we showed that intraportal as opposed to intravenous administration of allogeneic BM cells leads to the development of nonresponsiveness to donor-type alloantigens, chimerism, and hence GVHD with permanent acceptance of donor-type SG across MHC barriers.

The factors responsible for intraportally mediated antigen-specific suppression are not known; functional clonal deletion secondary to hepatic entrapment of alloreactive cells, blockade by a suppressive serum factor or soluble MHC determinants, or even immunosuppression mediated by active GVHD may be relevant mechanisms in the induction of the nonresponsive state [4, 5, 9, 10, 16, 19]. Obviously, some of the above factors, as well as active suppression by suppressor lymphocytes, may play a role in concert in the establishment of the nonresponsive state, as previously suggested in other models of transplantation tolerance [4, 5, 8, 9]. Diminished delayed-type hypersensitivity (DTH) response to a number of antigens (heterologous erythrocytes and allogeneic cells) following the intraportal inoculation of cells has indeed been previously documented [20]. Our own data in another model of tolerance involving allogeneic chimeras induced by total lymphoid irradiation suggest that persistence of the donor's "milieu" is essential for maintaining a state of nonresponsiveness to the donor alloantigens [14], which implies that various components shed or induced by alloantigens (such as MHC products) may be essential for interaction with lymphocytes in the course of establishing clonal blockade against the specific inducing alloantigens. Similar mechanisms could in principle operate during the induction of nonresponsiveness following intraportal inoculation into a large and metabolically active organ such as the liver.

Data reported in this study are in agreement with the findings previously reported by Kenick et al. [10], demonstrating the immunosuppressive effects of the intraportal inoculation of allogeneic BM cells. Unfortunately, the tolerogenic effects of BM cells in the experimental conditions investigated are insufficient for the induction of absolute, long-lasting nonresponsiveness across MHC; the addition of sublethal WBI prior to BM inoculation was effective in establishing durable chimerism and tolerance to SG across MHC in animals not lost to GVHD.

The data reported on experiments involving the intraportal injection of immunocompetent allogeneic cells on GVH reactivity are far less conclusive, although most reports suggest less evidence of GVHD following the intraportal as opposed to systemic inoculation of allogeneic cells. Monchik and Russel [12] have observed consistently lethal GVHD following transplantation of the small bowel with caval venous drainage in the semiallogeneic combination of Lewis into the (Lewis × BN) F1 rat model.

In this study we investigated the tolerogenic effects of the intraportal injection of donor-type BM cells with and without prior exposure to sublethal WBI, using skin allografts across MHC as an indicator for the induction of the nonresponsive state. Skin allograft survival of C57BL/6 was significantly improved following the intraportal inoculation of BM cells, and permanent skin allograft acceptance was observed in all survivors when BM inoculation was preceded by WBI. The GVHD-induced mortality in C57BALB chimeras inoculated intraportally reflects the successful engraftment of the donor's immunohematopoietic system due to the intraportal inoculation of non-T-depleted BM allografts, since none of the BALB recipients of C57-BM-inoculated systemically showed sustained chimerism. Further studies are needed to help clarify the mechanisms involved in the induction and maintenance of nonresponsiveness to alloantigens; in this regard it should be remembered that the liver is also the major site of hematopoiesis during fetal life - a period involved in the generation of tolerance towards self antigens.

Regardless of the mechanism of nonresponsiveness to alloantigens induced by the adult liver tissue, the feasibility of the induction of tolerance to BM allografts across strong histocompatibility barriers by mild immunosuppression induced by sublethal WBI suggests that BM cells, or rather BM cells depleted of immunocompetent T lymphocytes, may be used for the induction of tolerance and chimerism in utero for the correction of a variety of genetic disorders through intraportal injection into the fetal cord veins during the first two trimesters of

pregnancy, during the period of relative immunoincompetence.

Acknowledgements. The authors wish to thank the Hebrew University of Jerusalem and the Hadassah Medical Organization for a joint research grant, the Israel Cancer Research Fund, the Israel Cancer Society for the Michael Koenigsberg Memorial Fellowship and a career development award (to S.S.), and the Israel Ministry of Health for a basic research grant.

References

1. Billingham RE, Medawar PB (1951) The technique of free skin grafting in mammals. J Exp Biol 28: 385-389
2. Boeck W, Sobis H, Lacquet A, Gruwez J, Vandeputte M (1975) Prolongation of allogeneic heart graft survival in the rat after implantation on portal vein. Transplantation 19: 145-149
3. Calne R, White HJO, Yoffa DE, Binns RM, Maginn RR, Herbertson RM, Millard PR, Molina VP, Davis DR (1967) Prolonged survival of liver transplants in the pig. Br Med J 4: 645-648
4. Cobbold SP, Waldmann H (1984) Therapeutic potential of monovalent monoclonal antibodies. Nature 308: 460-462
5. Fujiwara H, Qian JH, Sato S (1986) Studies of the induction of tolerance to alloantigens. II. The generation of serum factor(s) able to transfer alloantigen-specific tolerance for delayed-type hypersensitivity by portal venous inoculation with allogeneic cells. J Immunol 136: 2763-2768
6. Fukada A, Hanaoka T, Solowey AC, Rapaport FT (1969) Inhibition of second set renal allograft responses by portal vein drainage. Transplant Proc 1: 602-608
7. Gores PF, Rabe F, Sutherland DER (1987) Prolonged survival of intraportal versus subrenal capsular transplanted islet allografts. Transplantation 43: 747-748
8. Hall BM, Desaxe I, Dorsh SE (1983) The cellular basis of allograft rejection in vivo. III. Restoration of first-set rejection and heart grafts by T-helper cells in irradiated rat. Transplantation 36: 700-705
9. Kamada N (1985) Transplantation tolerance and immunosuppression following liver grafting in rats. Immunol Today 6: 336-342
10. 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
11. Monaco AP, Wood ML (1984) The use of antilymphocytic serum and donor antigen (bone marrow) for the induction of specific unresponsiveness to tissue allografts. In: Slavin S (ed) Tolerance in bone marrow and organ transplantation. Elsevier, Amsterdam, pp 17-74
12. Monchik GJ, Russel DS (1971) Transplantation of small bowel in the rat, technical and immunological considerations. Surgery 70: 693-698
13. Morecki S, Leshem B, Weigensberg M, Bar S, Slavin S (1985) Functional clonal deletion versus active suppression in transplantation tolerance induced by total-lymphoid irradiation. Transplantation 40: 201-210
14. Morecki S, Leshem B, Eid A, Slavin S (1987) Alloantigen persistence in induction and maintenance of transplantation tolerance. J Exp Med 165: 1468-1480
15. Nakano H, Nakajima Y, Segawa M, Kanehiro H, Murao Y, Nakagawa K, Shiratori T (1987) Immunologic significance of hepatocytes in tolerance induction after liver transplantation. Transplant Proc 19: 511-513
16. Qian JH, Hashimoto T, Fugiwara H, Hamaoka T (1985) Studies on the induction of tolerance to alloantigens. I. The abrogation of potentials for delayed-type-hypersensitivity responses to alloantigens by portal venous inoculation with allogeneic cells. J Immunol 134: 3656-3661
17. Qian JH, 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. Transplantation 43: 538-542
18. Sakai A (1970) Role of the liver in kidney allograft rejection in the rat. Transplantation 9: 331-334
19. Stephenson SP, Roser B (1985) The molecular basis of transplantation tolerance. Transplant Proc 17: 1145-1147
20. Triger DR, Cynamon MH, Write R (1973) Studies on hepatic uptake of antigen. I. Comparison of inferior vena cava and portal vein routes of immunization. Immunology 25: 941-950