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Mouse Liver Transplantation Tolerance: The Role of Hepatocytes and Nonparenchymal Cells

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MOUSE orthotopic liver allografts are spontaneously accepted without immunosuppression in many strain combinations including those crossing major and minor histoincompatibility barriers. Liver allograft acceptance, moreover, leads to donor-specific acceptance of subsequent skin or heart allografts. Although the precise mechanisms of liver graft tolerance (LGT) are not completely understood, LGT has been attributed to: (1) donor nonparenchymal cell (NPC) migration leading to subsequent recipient/donor hematolymphoid chimerism[1,2]; and (2) hepatocyte (HC) release of soluble MHC molecules.[3]

We have used an experimental model, similar in principle to that of Rapaport et al,[4] and used previously by Sriwatanawongsa et al[5] to test whether the NPC or HC was the more important component in LGT by constructing *chimeric donor livers* whose NPC have been replaced by allogeneic bone marrow transplantation (BMT).

MATERIALS AND METHODS

Liver donor mice ($H\text{-}2^b$) were supralethally irradiated (9.5 Gy) and reconstituted via tail-vein injection with 1.5×10^6 syngeneic ($H\text{-}2^b$), or fully allogeneic liver recipient type ($H\text{-}2^k$), and third party ($H\text{-}2^d$) whole bone marrow cells harvested from mouse femur and tibia. Ninety days (90) days after BMT, splenocytes and peripheral blood mononuclear cells (PBMC) were phenotyped for recipient/donor cells by flow cytometry, and liver by immunohistochemistry. A mixed leukocyte reaction was also done to assess immune status in chimeric liver donor animals. Ninety-day-old chimeric donor liver grafts were transplanted according to techniques by Qian et al[2] into untreated naive $H\text{-}2^k$ recipients and challenged with $H\text{-}2^b$ and $H\text{-}2^d$ skin grafts 30 days after transplantation (Table 1) or on the same day as liver transplantation (Table 2). Two combinations of mouse strains were analyzed: (1) The C57BL/10 ($H\text{-}2^b$), BALB/c ($H\text{-}2^d$), and C3H ($H\text{-}2^k$) combination, which crosses all major and minor histoincompatibility barriers; and (2) the congenic C57BL/10, B10.D2 ($H\text{-}2^d$), and B10.BR ($H\text{-}2^k$), which cross all major loci.

RESULTS AND DISCUSSION

The donor preparation resulted in >99% replacement of PBMC and splenocytes with cells of bone marrow donor origin 90 days after BMT as determined by flow cytometry. Liver phenotyping by immunohistochemical staining with class I and II specific antibodies showed hepatocytes and bile duct cells to be of bone marrow recipient haplotype, while NPC to be >99% of bone marrow donor haplotype. However, rare residual NPC from the original liver persisted. At 90 days, chimeric animals also showed specific unresponsiveness to bone marrow donor and recipient haplotypes but not to third party as determined by the mixed leukocyte culture, suggesting immunological donor–recipient stability in these chimeras.

The transplantation of chimeric donor livers with either NPC of donor ($H\text{-}2^b$), third-party ($H\text{-}2^d$), or liver recipient ($H\text{-}2^k$) haplotype resulted in spontaneous acceptance of liver grafts in either of the combinations of mouse strains. However, the donor-specific tolerance normally induced for C57BL/10 skin by the unaltered C57BL/10 liver in this model (see Qian et al[2] and group 1 of Table 1) was no longer evident when the donor livers were taken from irradiated animals that had been reconstituted with syngeneic (C57BL/10) bone marrow. This suggested that the liver NPC (or some other factor in the organ) had been changed in the first stages of these control experiments. The reduced skin graft protection was now no different than when the donor NPC were converted to those of the recipient (group 4) or to a third party (group 3). Although the skin survival

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Table 1. Tolerance Induction of Donor Skin by Chimeric Livers in Mouse Liver Transplantation in C57BL/10, BALB/c, C3H Combinations

Group	Donor Liver		Recipient	Skin Graft Survival (Days)*	
	NPC	HC		C57BL/10†	BALB/c†
1	Normal C57BL/10	Normal C57BL/10	C3H	71, 80, >100, >100, >100	16, 17, 17, 23
2	C57BL/10	C57BL/10	C3H	29, 35, 44, 47, 86	15, 17, 19, 20
3	BALB/c	C57BL/10	C3H	44, 45, 46, 48	16, 17, 20
4	C3H	C57BL/10	C3H	34, 50, 53, 56	12, 12, 14, 14

*Isolated skin graft survival in the unaltered C3H recipient is 9 to 10 days using C57BL/10 donor, 9 to 10 days with BALB/c, and permanent with C3H.
†Skin grafts 30 days after liver transplantation.

Table 2. Tolerance Induction of Donor Skin by Chimeric Livers in Mouse Liver Transplantation in C57BL/10, B10.BR B10.BR, B10.D2 Combinations

Group	Donor Liver		Recipient	Skin Graft Survival (Days)*	
	NPC	HC		C57BL/10†	B10.D2†
5	Normal C57BL/10	Normal C57BL/10	B10.BR	>100, >100, >100	
6	C57BL/10	C57BL/10	B10.BR	>100, >100, >100	28, 30, 16
7	B10.D2	C57BL/10	B10.BR	30, 20, >100, >100, >100	28, 19, 21, 20, 21
8	B10.BR	C57BL/10	B10.BR	24, 25, >100, >100	22, 21, 17, 17, 20

*Isolated skin graft survival in the unaltered B10.BR recipient is 12 to 14 days using C57BL/10 donor, 12 to 14 days with BALB/c, and permanent with C3H.
†Skin grafts placed on the same day as liver transplantation.

was indistinguishable, it was modestly prolonged in all compared to that of an isolated skin graft in the same strain combinations (see footnote to Table 1). Similarly, the animals bearing transplanted "parked" livers rejected BALB/c skin without regard for the strain used to repopulate the hepatic graft donor, but at a pace of rejection that again was marginally slower than that expected in these strains after a conventional isolated skin graft (see footnote to Table 1).

The MHC nonspecificity of these results is self-evident. The possibility that a procedural timing factor was responsible prompted further experiments using fully allogeneic C57BL/10 → B10.BR mice. With this strain combination, transplantation of an unmodified liver confers specific tolerance to concomitantly transplanted donor skin (see Qian et al[2] and group 1 of Table 1). Unlike the controls in Table 1, donor-irradiated and syngeneic reconstitution did not cause loss of specific tolerance to the concomitantly engrafted skin grafts (group 2, Table 1). This reaction increased the importance of the experiments in group 3 in which the C57BL/10 liver donor was reconstituted with third-party NPC (B10.D2) and group 4 in which the reconstitution was with recipient leukocytes (B10.BR). Skin acceptance was expected in group 4 but not group 3 if hepatocytes were potently tolerogenic. However, 40% of the skin grafts in both groups were rejected in 20 to 30 days (Table 2). In addition, C57BL/10 livers reconstituted with B10.D2 NPC did not prevent rejection of B10.D2 skin suggesting that hepatic tolerogenicity for skin could not be attributed to NPC alone under these experimental circumstances.

These results are largely confirmatory of recent observations by Calne et al[5,8] in rats, differing only in that the hepatocyte effect appears to us MHC nonspecific. In common with the findings of the Cambridge team, there was a dissociation of liver graft acceptance from tolerance to skin, analogous perhaps to the survival of NPC-free islets injected into the mouse thymus but without the systemic donor-specific tolerance that is produced by unmodified islets. As discussed before, there is room for speculation about parenchymal–nonparenchymal cell collaboration in the events of tolerance or other immune events,[5,8,9] including the cross-regulation of immunologic and growth factor networks.[10]

As we have discussed earlier,[11] the "parking" experimental models may not be appropriate for such inquiries. The rationale for their use to investigate tolerance mechanisms has faded in the face of mounting evidence that transplantation tolerance is the product of a two-way immunologic transaction (graft versus host as well as host versus graft). When an organ has been parked and is stable in the intermediary host, both graft and recipients have exchanged NPC and have genetic composites.[1,2,6,7,9,11] The organ already is tolerant to the carrier animal and vice versa, with a mixed NPC population in both that presumably has manifold changes (particularly in gene expression). Only at this time is the organ extirpated from the immunologic network into which its mini-immune system has been assimilated. Because we believe that these changes define per se the molecular secrets of tolerance, it is not surprising that the use of such an organ to study tolerance de novo in a new recipient has yielded confusing and perhaps misleading information.

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