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The Role of Antibodies in Liver Graft–Induced Tolerance in Mice: Passive Transfer of Serum and Effect of Recipient B-Cell Depletion

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MOUSE liver allografts are spontaneously accepted without immunosuppression and induce donor-specific acceptance of subsequently transplanted skin or heart allografts[1] despite various degrees of MHC incompatibility. This in vivo tolerance is associated with donor-specific reactivity in vitro, a phenomenon called "split tolerance."[2] Therefore, clonal deletion is unlikely to be the explanation of graft acceptance in this model.

The same phenomenon[3–6] has been observed in some rat strain combinations. In the DA into PVG strain combination, the liver is spontaneously accepted,[3] whereas other organs, such as kidney, heart, or skin, are rapidly rejected. A liver allograft in the rat is able to overcome the effects of priming by donor antigen and to convert a state of immunological memory into one of specific transplantation tolerance as demonstrated by reversal of ongoing heart and/or skingraft rejection.[1,4] In the rat model, liver graft–induced tolerance (LGT) has been attributed to cellular factors, such as selective clonal deletion,[7–9] and development of suppressor cells,[10–12] as well as humoral factors. One popular explanation for these findings is the release of soluble donor class I antigen by the liver allograft, which in separate experiments has been shown to prolong modestly allograft survival upon passive transfer.[13–15] Antidonor class II antibodies were shown to exert an even more powerful effect.[16–18]

In this study, we tested the role of antibodies in LGT in the mouse model by analysing the biological activity of serum from liver-grafted mice (>100 days) and compared it to sera from heart and skin graft recipients using in vivo (serum transfer) and in vitro (MLR and CML) assays.

MATERIALS AND METHODS

Mice

Ten- to 12-week-old, male C57Bl/10 (B10) (*H-2*^{b}) and C3H/NeJ (*H-2*^{k}) mice, purchased from Jackson Laboratory (Bar Harbor, Me), were used as donors and recipients for all experiments. Animals were housed in the pathogen-free facility of the University of Pittsburgh Medical Center according to NIH guidelines.

Surgical Procedures

Liver transplantation was performed according to Qian et al[19] and liver graft function assessed by daily monitoring. Hearts were transplanted heterotopically into the abdomen, adapting the technique from Ono and Lindsay.[20] Skin grafts were performed by placing a full-thickness graft (8 × 8 mm) from the tail of the donor on the recipients dorsal side by the method of Billingham et al.[21] Serum was obtained and pooled from serial blood samples (>100 days after transplantation) and stored at −20°C until use.

In Vivo B-Cell Depletion

Neonate C3H/HeJ mice were intraperitoneally injected with 100 μg of purified polyclonal rabbit anti-mouse IgM μ-chain within 12 hours after birth and then of 100 μg once a week from the age of 14 days.[22] Anti-IgM treatment resulted in complete suppression of B-cell development, documented by the absence of surface Ig and B220-Ag$^+$ cells (flow cytometric analysis) and the lack of lipopolysaccharide-induced mitogenic responses of spleen and peripheral blood mononuclear cells.[22]

In Vitro Assays

To assess the inhibitory effect of serum from LGT, such serum was used in standard MLR assays. C3H (0.75%) or LGT (0.75%) serum or serum from the recipients of heart or skin graft, respectively, the IgG fraction was added to 2.5 × 10^5 cells in round-bottom plates in a final volume of 200 μL of supplemented DMEM medium and cultured for 3 to 5 days at 10% CO_2. The IgG fraction of serum was obtained by affinity purification over a Protein G column and low pH elution.

The inhibitory effect of serum on cytotoxicity was tested by preincubating 5 × 10^6 ^{51}Chromium-labeled Con A blasts with 5 μL of either serum for 1 hour. A total of 5 × 10^5 in vitro–sensitized C3H effector cells were then incubated with these target cells for 4 hours and 100 μL of supernatant measured in a gamma-counter.

Serum Transfer

The enhancing effect of LGT was studied by passive transfer of 200 μL of serum IV immediately after transplanting a B10 heart to a C3H recipient. Functioning of the heart was monitored by daily palpation through the abdominal wall. Rejection was defined by the cessation of the cardiac impulse and confirmed by exploration and histological examination.

RESULTS

In contrast to the rat model, transfer of serum from LGT mice resulted in consistent, but not donor-specific prolongation of heart graft survival from 9 to 15 days. Moreover, this effect was not liver-specific because serum from skin-grafted mice achieved the same results (Table 1). When serum from LGT mice was used in a mixed lymphocyte culture (MLR), it suppressed the proliferative activity to the same extent as serum from heart- or skin-grafted mice (about 50%), there-

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Table 1. Enhancing Activity of Serum From Liver Graft-Tolerant Mice on Heart Graft Survival

Serum	Graft Survival (days + SD)
None	7.4 + 0.40
C3H	9.0 ± 0.45
OLT B10 → C3H	15.2 ± 1.10
Skin B10 → C3H	14.8 ± 0.73
OLT BALB/c → C3H	13.2 ± 1.24
Skin BALB/c → C3H	15.2 ± 2.06

fore this inhibition was not donor-specific. Cytolytic activity in the CML was also nonspecifically reduced by half when the target cells were preincubated with serum from either LGT or skin grafted mice. This nonspecific immunosuppressive activity was largely contained within the immunoglobulin-rich fraction of the serum.

We further tested the role of antibodies and B cells in liver graft acceptance by performing liver allografting in B-cell–depleted recipients, which are defective in producing antibody upon immune stimulus. Despite the paucity of antibody-producing B cells in the depleted recipients (<5%), the rate of spontaneous long-term survival (>100 days) in B-cell–depleted animals (five of eight = 62.5%) was similar to that in naive recipients (eight of ten = 80%, $P > .05$), as demonstrated in Table 2.

DISCUSSION

In contrast to profound[23–26] effects in the rat, serum from LGT mice showed only modest and nonspecific enhancement of heart allograft survival of about 6 days. Moreover, this property was not specific to LGT serum but was also observed with serum from murine skin or heart allograft recipients. In both models the in vivo observations were strengthened by the results of the in vitro studies. Strong and donor-specific inhibitory effects on proliferation assays were demonstrated in the rat model,[27] whereas the inhibitory effect on proliferation and cytotoxicity in the mouse model was not donor-specific and not unique to the LGT serum. In both models, the inhibitory effect could be attributed to IgG fraction of the serum.

Although a liver graft can induce in vivo tolerance in select strain combinations in the rat and is a more general phenomenon in the mouse, the role of B cells, their production of antibodies, and their integrative effect in the immune network seem to follow different mechanisms. Using B-cell–depleted recipients, which are defective in producing antibody upon immune stimulation, is the ultimate way to analyse the role of antibodies in LGT.[22] Such a model has already been used to study the importance of recipient antibody production on skin allograft rejection in mouse.[28] In our model, long-term survival was achieved in five of eight recipients compared to eight of ten controls; the allograft loss in both possibly related to large liver infarcts observed on autopsy.

Table 2. Liver Graft Survival in B-Cell–Depleted Recipients

Recipients	Survival Time (Days)	Long-Term Survival (%)
C3H (n = 10)	8, 19, >120 (×8)	80.0%
$C3H^{-B}$ (n = 8)	12, 16, 16, >120 (×5)	62.5%

Since tolerance to liver allograft could be induced in B-cell–depleted mice and the "immunosuppressive" activity of the serum was nonspecific, we conclude that mechanisms other than antidonor antibody production by recipient B cells plays the pivotal role in liver graft–induced tolerance. Other studies in the mouse model,[1] reporting the association between the tolerance phenomenon and donor hematolymphoid chimerism, relate the tolerance induction to the incorporation of a functional fragment of the donor immune components into the recipient immune system.

REFERENCES

1. Qian S, Demetris AJ, Murase N, et al: Hepatology 19:916, 1994
2. Dahmen U, Qian S, Rao A, et al: Transplantation (in press)
3. Kamada N, Davies HS, Wright DGD, et al: Transplantation 4:29, 1980
4. Kamada N, Davies HS, Roser B: Nature 292:840, 1991
5. Kamada N, Wright DGD: Transplantation 38:217, 1984
6. Kamada N, Yamaguchi A, Ohuchi Y, et al: Transplant Proc 21:433, 1989
7. Davies HS, Kamada N, Roser BJ: Transplant Proc 15:831, 1983
8. Kamada N, Shinomiya T: Immunology 55:85, 1985
9. Kamada N, Teramoto K, Bacuerito A, et al: Transplantation 46:165, 1988
10. Yoshimura S, Gotoh S, Kamada N: Clin Exp Immunol 85:121, 1991
11. Yamaguchi A, Yamadera H, Matsuda M, et al: Transplant Proc 13:866, 1991
12. Gotoh S, Kamada N, Kim Y, et al: Transplant Proc 23:2017, 1991
13. Sumimoto R, Kamada N: Transplant Proc 23:86, 1991
14. Sumimoto R, Kamada N: Transplantation 50:678, 1990
15. Sumimoto R, Kamada N: Immunol Lett 26:81, 1990
16. Kamada N, Shinomiya T: Transplantation 42:7, 1986
17. Kamada N, Shinomiya T, Tamak T, et al: Transplantation 42:581, 1986
18. Bacquerito A, Kamada N, Hart J, et al: Transplant Proc 22:2020, 1990
19. Qian S, Fung JJ, Demetris AJ, et al: Transplantation 52:562, 1991
20. Ono K, Lindsey ES: J Thorac Cardiovasc Surg 7:225, 1969
21. Billingham RE, Brent L, Medawar PB: Nature 172:603, 1953
22. Cerny A, Hugin AW, Sutters S, et al: Cell Biol 53:301, 1985
23. Kamada N, Yamaguchi A, Yoshima S, et al: Transplant Proc 22:1971, 1990
24. Kamada N, Sumimoto R, Bacquerito A, et al: Immunology 64:315, 1988
25. Yamaguchi A, Kamada N: Immunology 72:79, 1991
26. Sumimoto R, Kamada N: 21:431, 1989
27. Tschuchimoto S, Kakita A, Uchiro J, et al: Transplant Proc 14:3064, 1987
28. Cerny A, Ramseier H, Bazin H, et al: Transplantation 45:2222, 1988