The Use of MHC Class I or Class II "Knock Out" Mice to Investigate the Role of These Antigens in Allosensitization

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ACCELERATED and hyperacute rejections in presensitized transplant patients constitute major causes of early allograft loss. Analysis of the distinct role of various transplantation antigens in the process of sensitization is therefore important in further understanding and subsequently preventing the occurrence of such events. The recent availability of "knock out" mice, which do not express major histocompatibility complex (MHC) class I or class II molecules,[1,2] has provided a tool to analyze the role of these antigens in the initiation of immune responses to allosensitization. In this study, we have presensitized mice with skin grafts from either class I or class II MHC-deficient donors, and then examined the sensitization status of the recipients by subsequent heart or liver grafting. The cellular and humoral immune responses of the sensitized recipients were also analyzed.

MATERIALS AND METHODS

Animals and Surgical Procedures

Male 10- to 12-week-old inbred C57BL/10 (B10, *H-2*^{b}), C57BL/6 (B6, *H-2*^{b}), B10.BR (*H-2*^{k}), C3H (*H-2*^{k}), b2m (*H-2*^{b}, class I deficient), and BALB/c (*H-2*^{d}) mice were purchased from the Jackson Laboratory (Bar Harbor, Me). C2D (*H-2*^{b}, class II deficient) mice were purchased from GenPharm International (Mountain View, Calif). Orthotopic liver transplantation was performed as described previously.[3] Abdominal heart transplantation was carried out as described elsewhere.[4] Total cessation of palpable contraction of the heart graft was defined as rejection. Allosensitization by skin grafting was done 14 to 21 days prior to heart or liver transplantation, by implanting a full-thickness tail skin graft on the dorsal side of the recipient trunk.

Mixed Lymphocyte Reaction (MLR)

Equal numbers (2×10^5) of responder and γ-irradiated stimulator spleen cells were cultured at 37°C in 5% CO_2 in air and pulsed with 1 μCi of [^{3}H]TdR for the last 18 hours of a 4-day culture period. The cells were harvested onto glass fiber filters and [^{3}H]TdR uptake was measured by liquid scintillation counting. Results were expressed as mean counts per minute (cpm) ± 1 SD.

Lymphocyte-Mediated Cytotoxicity Assay

Freshly isolated spleen cells were used as effectors. The EL4 (*H-2*^{b}) lymphoma cell line (ATCC, Rockville, Md) or Con A–activated B10 spleen cells were used as targets. The P815 (*H-2*^{d}) tumor cell line or Con A–activated BALB/c spleen cells were used as third-party target controls. The target cells were labeled with $Na_2{}^{51}CrO_4$ (NEN, Boston, Mass), washed, and plated at 4×10^3 cells per 200 μL/well at different E/T ratios. Killing by cytotoxic T cells was quantitated by determining the percent specific ^{51}Cr release over a 4-hour period.

Antibody-Dependent Cellular Cytotoxicity (ADCC)

Antibody titers were measured by modification of method described previously.[5] Briefly, serially diluted and decomplemented sera were incubated with target cells (5×10^5) in microtiter plates for 1 hour. The cells were then washed and incubated for 30 minutes with 100 μL of baby rabbit complement. At the end of this incubation the cells were washed and incubated for an additional 3 hours in HBSS supplemented with 20 μL of MTT (Sigma Chemical Co, St Louis, Mo). Dimethylsulfoxide (150 μL) was then added to each well, and the optical density was measured using a kinetic microplate reader (Molecular Devices, Menlo Park, Calif).

RESULTS

Skin graft–induced sensitization was tested in a fully allogeneic B10 → C3H liver transplantation model. As previously reported, B10 liver allografts were spontaneously accepted and survived >100 days in C3H recipients without any immunosuppressive therapy.[6] However, after skin presensitization, the liver grafts were uniformly rejected in 4 to 5 days. Kinetic studies demonstrated that donor skin grafts took at least 2 weeks to fully sensitize the recipients, and sensitization status was stable for up to 3 months.

As shown in Table 1, B10 heart or liver grafts, when transplanted into C3H recipients that had received a skin graft from B10, B6, or C2D mice 14 to 21 days earlier, were rejected in an accelerated fashion; however, this phenomenon was not observed in recipients that had been presensitized with either b2m, B10.BR, or BALB/c skin grafts. These results suggest that a skin graft expressing donor-specific MHC class II antigens alone and/or donor specific non–MHC-histocompatibility antigens (non–MHC-HA) is incapable of sensitizing the recipient sufficiently to induce accelerated rejection of a subsequently transplanted whole organ. On the other hand, skin grafts expressing only donor MHC class I antigens were able to induce significant sensitization in vivo, as evidenced by the accelerated rejection of the subsequently transplanted heart or liver allografts.

The immune status of the skin-sensitized C3H mice was

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Table 1. Survival of B10 Heart or Liver Allografts in Skin-Presensitized C3H Recipients

Skin donor	Sensitization loci	Survival (MST) (days)	
		Heart	Liver
—	—	8, 8, 8, 8, 9 (8)	35, >100, >100, >100, >100 (>100)
B10	MHC, non-MHC-HA	4, 4, 5, 5, 5 (5)	4, 4, 4, 4, 5 (4)
B6	MHC	3, 3, 4, 4, 5 (4)	4, 5, 5, 5, 8 (5)
b2m	II	7, 7, 7, 8, 8, 9 (7, 8)	20, 35, >100, >100, >100, >100 (>100)
C2D	I	3, 3, 4, 4, 4 (4)	3, 4, 4, 4, 4 (4)
B10.BR	Non-MHC-HA	6, 7, 7, 7, 7, 8 (7)	21, 28, >100, >100, >100, >100 (>100)
BALB/c	Third-party	8, 8, 9, 9, 9, 9 (9)	15, 51, >100, >100, >100, >100 (>100)

tested in vitro MLR, CTL, and ADCC assays. An elevated level of $H\text{-}2^b$-specific cytotoxicity was detected in fresh splenocytes isolated from C3H recipients that had received skin grafts from either B10, B6, or C2D mice, but not from b2m, B10.BR, or BALB/c donors. Furthermore, this elevated donor-specific cytotoxicity correlated well with the in vivo observations in which accelerated rejection of a subsequently transplanted B10 heart or liver allograft was observed. MLR and ADCC assays did not correlate with *these* in vivo findings, although b2m skin grafts induced a similar level of donor-specific ADCC in response to skin allografts from C2D mice (Table 1).

DISCUSSION

It has been demonstrated that presensitization with fully disparate donor skin grafts can lead to accelerated rejection of a subsequently transplanted heart or liver allograft. The sensitizing antigens appear to be restricted to MHC, since B10.BR skin (which shares the same non–MHC-HA with B10) does not lead to accelerated rejection of either B10 heart or liver grafts. Within the MHC loci, mismatched class I antigens appear to be crucial in sensitizing the recipients leading to accelerated rejection of heart and liver grafts, whereas class II antigens alone are inefficient in generating sufficient sensitization to cause subsequent accelerated rejection.

It has long been debated whether MHC class I and class II antigens play different roles in the immune response to allografts.[7] In the present study, presensitization by C2D skin grafts (MHC class II deficient) led to generation of CTL, while exposure to b2m skin (class I deficient) did not. This correlated with the pattern of rejection seen in a subsequently transplanted vascularized whole organ. These results confirm previous findings that the accelerated ("second set") rejection is caused primarily by presensitized cytotoxic T cells[8] in contrast to the primary ("first set") rejection which is initiated mainly by the donor MHC class II antigens which are known to stimulate helper T cells.[9]

Furthermore, presensitization with b2m and C2D skin grafts induced a similar level of donor-specific cytotoxic antibody response; however, sensitization with class I–deficient skin grafts failed to induce accelerated rejection of the subsequently transplanted whole organ. This suggests that the presence of donor-specific cytotoxic antibodies does not correlate with accelerated rejection. In fact, the presence of circulating donor-specific MHC class II antibodies have been shown to be unlikely mediators of accelerated or hyperacute rejection and, in some circumstances, they have been documented to prolong allograft survival.[10] It is interesting to note that, in clinical histocompatibility testing, the presence of donor-specific class II antibody is not considered a contraindication for transplantation.

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