MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) RESTRICTION OF FOREIGN TRANSPLANTATION ANTIGENS IN RATS RENDERED TOLERANT AT BIRTH

By HIROMITSU KIMURA, LISE DESQUENNE-CLARK, MEGUMU MIYAMOTO*, AND WILLYS K. SILVERS

*From the Departments of Pathology and Laboratory Medicine and Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; and the *Department of Anatomy, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan*

When mice and rats are inoculated at birth with MHC-incompatible bone marrow cells (BMC),[1] they not only are rendered immunologically tolerant of the foreign transplantation antigens present on the donor cells, they also become unresponsive to donor strain skin grafts known to possess skin-specific (Skn) antigens (1). To account for this situation we have proposed that MHC restriction accompanies the induction of tolerance, i.e., because donor cells migrate to the thymus, their T cell repertoire is restricted by the host's MHC (1). We now present further evidence that this is the case by demonstrating that third party skin grafts survive significantly longer on tolerant rats if they are MHC compatible with the tolerance-inducing inoculum, than if they are MHC compatible with the host.

Materials and Methods

Rats. DA (MHC:$RT1^a$), PVG ($RT1^c$), ACI ($RT1^a$), F344 ($RT1^{lv1}$), Lewis ($RT1^l$), Lewis.1N ($RT1^n$), BN ($RT1^n$), BN.B2 ($RT1^u$), BN.B4 ($RT1^a$), and Wag ($RT1^u$, *rnu*/+) rats, as well as (DA × PVG)F_1, (DA × F344)F_1, (Lewis × DA)F_1, and (BN.B4 × Lewis)F_1 hybrid animals were used. The PVG, ACI, F344, (DA × PVG)F_1, and (DA × F344)F_1 animals, as well as some of the DA animals, were bred at Hamamatsu University. Other DA rats, as well as Lewis, Lewis.1N, BN, BN.B2, BN.B4, Wag, (Lewis × DA)F_1, and (BN.B4 × Lewis)F_1 animals stemmed from colonies maintained at the University of Pennsylvania.

Tolerance Induction. Tolerance was induced in the PVG and DA rats by inoculating them intravenously at birth with 80×10^6 (DA × PVG)F_1 BMC. Neonatal F344 and DA animals also were rendered tolerant with similar numbers of (DA × F344)F_1 BMC. The Lewis rats were rendered neonatally tolerant by an intravenous inoculation of 10^7 (Lewis × DA)F_1 BMC after receiving 200 rad (^{137}Cs irradiation at a dose rate of 81 rad/min). The Lewis.1N rats were irradiated at birth with 300 rad just before receiving 10^7 Wag (*rnu*/*rnu*) BMC. Neonatal Lewis and Lewis.1N rats were sublethally irradiated to facilitate tolerance induction. The different dosages we used were arbitrary. The procedures involved have been described elsewhere (2).

Skin Grafting. In the case of the PVG and DA animals inoculated at birth with (DA ×

This study was supported by grant CA-18460 from the National Institutes of Health (Bethesda, MD) and by a research grant from the Ministry of Education of Japan. L. Desquenne-Clark is supported by a training grant from the National Cancer Institute (CA-09140). Address correspondence to W.K. Silvers, Department of Human Genetics, University of Pennsylvania, Philadelphia, PA 19104

[1] *Abbreviations used in this paper:* BMC, bone marrow cells; Skn, skin-specific antigens.

TABLE I
MHC-restricted Skin Graft Rejection Responses in Rats

Recipients*	Survival times of ACI* skin grafts (d)	Number rejected per total
PVG tolerant of (DA × PVG)F_1	2 × >50, 2 × >85‡, 4 × >100	0/8
DA tolerant of (DA × PVG)F_1	10, 3 × 11, 2 × 12, 13, 19, 25, 3 × >100	9/12
(DA × PVG)F_1	9, 3 × 10, 13, 15	6/6
F344 tolerant of (DA × F344)F_1	71, 3 × >100	1/4
DA tolerant of (DA × F344)F_1	11, 14	2/2
(DA × F344)F_1	10, 3 × 11, 12	5/5

* MHC haplotypes: PVG, $RT1^c$; DA, $RT1^a$; F344, $RT1^{lvl}$; ACI, $RT1^a$.
‡ Previously sensitized against ACI.

PVG)F_1 BMC, tolerance was verified by the permanent acceptance of a (DA × PVG)F_1 hybrid skin graft and, in the case of the F344 and DA recipients of (DA × F344)F_1 BMC, by the permanent acceptance of a (DA × F344)F_1 hybrid graft, transplanted at least 50 d before the ACI test graft. Tolerance was verified in Lewis rats by the permanent acceptance of (Lewis × DA)F_1 hybrid skin grafts, also transplanted at least 50 d before the third party grafts. In the case of the Lewis.1N recipients, tolerance was verified by the permanent acceptance of a Wag skin graft, transplanted simultaneously with a BN.B2 or BN third party graft. Grafts varied from 2.25–4.0 cm^2 and were transplanted according to procedures described previously (3, 4). All recipients were at least 7 wk old when initially grafted.

Immunization. Recipients were immunized in each hind footpad with three inocula of 30×10^6 BMC at 8–12-d intervals. We performed skin grafting 7 d after the final injection.

Results

Survival of ACI Skin Grafts on PVG and DA Rats Rendered Tolerant with (DA × PVG)F_1 BMC and on F344 and DA Rats Rendered Tolerant with (DA × F344)F_1 BMC. If MHC restriction accompanies the induction of tolerance, rats rendered tolerant at birth with MHC-incompatible BMC should theoretically accept any graft that is homozygous for the bone marrow donor's foreign MHC. This follows from the fact that not only should such animals be immunologically tolerant of the donor's MHC but, because of MHC restriction, they should only be able to recognize the foreign antigens of the donor in terms of their own MHC. Indeed, for this reason, similar third party grafts should not fare as well on MHC-compatible hosts tolerant of the same antigens. To determine if this is the case, we challenged PVG and DA rats, rendered tolerant at birth with (DA × PVG)F_1 BMC and F344 and DA rats made tolerant with (DA × F344)F_1 BMC, with ACI skin grafts. We also challenged (DA × PVG)F_1 and (DA × F344)F_1 hybrids with these same grafts. The results (Table I) are clearly in accord with MHC restriction. Thus, we believe that all eight of the tolerant PVG rats, as well as three of four of the tolerant F344 animals, accepted their third party ACI skin grafts because their foreign transplantation antigens were recognized in association with the MHC of the host and not in association with the MHC of the graft. Indeed, it is especially noteworthy that even the two tolerant PVG rats that had been putatively sensitized against ACI failed to reject ACI skin.

We also presume that these same ACI skin grafts failed to do as well (only 3

TABLE II

Survival of BN.B4 and/or (BN.B4 × Lewis)F_1 Skin Grafts on Lewis Rats Rendered Tolerant at Birth with 10^7 (Lewis × DA)F_1 BMC (after 200 rad)‡ and on (Lewis × DA)F_1 Hybrids*

Origin of skin grafts	Number of hosts	Graft survival times (d)
(BN.B4 × Lewis)F_1/(BN.B4 × Lewis)F_1	8	16/16, 17/18, 18/22, 27/27, 30/30, 43/43, 2 × >50/>50
(BN.B4 × Lewis)F_1 /BN.B4	8	17/23,§, 19/22, 19/24, 23/26, 24/35, 25/>50, 29/>50, >50/>50
(BN.B4 × Lewis)F_1/(BN.B4 × Lewis)F_1	8‖	12/12, 13/13, 14/13, 14/14, 14/15, 16/16, 17/14, 18/16

* MHC haplotypes: BN.B4, RT1[a]; Lewis, RT1[l]; DA, RT1[a]

‡ All of these animals permanently accepted (Lewis × DA)F_1 hybrid skin grafts.

§ The survival of the F_1 hybrid graft is presented in the numerator.

‖ (Lewis × DA)F_1 hybrids.

of 14 were accepted) on similarly tolerant DA rats because, being MHC compatible with their hosts in this situation, their foreign transplantation antigens were recognized in the context of their proper MHC.

Nevertheless, it is important to note that ACI grafts survived significantly longer on DA rats tolerant of (DA × PVG)F_1 BMC than on (DA × PVG)F_1 hybrids. This observation is important because it indicates that the survival of third party grafts on genetically tolerant F_1 hybrid rats can not be equated with their survival on immunologically tolerant recipients. Indeed, because on the basis of assuming such equality we concluded in a previous study (1) that exposure of Lewis rats tolerant of DA to BN.B4 skin grafts attenuated their ability to reject (BN.B4 × Lewis)F_1 hybrid grafts, this situation was examined more closely.

Survival of BN.B4 and (BN.B4 × Lewis)F_1 Hybrid Skin Grafts on Lewis Rats Rendered Tolerant at Birth with (Lewis × DA)F_1 BMC. When adult mice are exposed to MHC-incompatible grafts devoid of APCs, not only are they accepted, but continuous exposure to these grafts may induce unresponsiveness to fresh grafts of the same genotype (5, 6). It thus appears that continuous exposure to the foreign transplantation antigens of a graft either directly or in association with the host's MHC (APCs), may induce unresponsiveness to the same antigens in association with the MHC of the graft. If this is the case then the same situation should apply to the transplantation antigens of third party grafts that are accepted by immunologically tolerant animals, only in this situation exposure to the grafts should render the hosts unresponsive to their foreign antigens in association with the MHC of the host. To determine if this was the case, a panel of 16 Lewis rats rendered tolerant at birth with (Lewis × DA)F_1 hybrid BMC and bearing (Lewis × DA)F_1 hybrid skin grafts for at least 50 d, was divided into two groups. One group received a (BN.B4 × Lewis)F_1 hybrid graft along with a BN.B4 graft. The other group received two (BN.B4 × Lewis)F_1 hybrid grafts. It was deemed important to keep graft dosage as constant as possible since it is known that the size of a test graft can influence its survival on a putatively tolerant animal (7). For comparison, (Lewis × DA)F_1 hybrids also were challenged with two (BN.B4 × Lewis)F_1 skin grafts.

The results of this analysis (Table II) gave no indication that the simultaneous

TABLE III

Survival of BN.B2 or BN Skin Grafts on Lewis.1N Rats Rendered Tolerant at Birth with 10^7 Wag (nude) BMC (after 300 rad)*‡

Donor	Number of rats	Graft survival times (d)
BN.B2	9	18, 19, 22, 28, 40, >61,§ 3 × >100
BN	6	2 × 11, 12, 2 × 13, 14

* MHC haplotypes: BN.B2 and Wag, $RT1^u$; BN and Lewis.1N, $RT1^n$.
‡ All of these recipients permanently accepted Wag skin grafts.
§ Animal died.

presence of a BN.B4 graft promoted the survival of a (BN.B4 × Lewis)F_1 hybrid graft on Lewis rats tolerant of DA. Indeed, two of eight recipients of two F_1 hybrid grafts, as opposed to one of eight that received an F_1 hybrid and a BN.B4 graft, accepted both for as long as they were followed. Moreover, it should be noted that in this situation as well, the (BN.B4 × Lewis)F_1 hybrid grafts did significantly poorer on the (Lewis × DA)F_1 hybrids than on the tolerant recipients.

Nevertheless, these results do provide further evidence that MHC restriction occurs when tolerance is induced. This evidence stems from the observation that in no case did a (BN.B4 × Lewis)F_1 hybrid graft survive longer than a BN.B4 graft on the same tolerant recipient, and this is exactly what one would expect if only the transplantation antigens of the hybrid graft were recognized in association with $RT1^l$. Thus, if one assumes that the BN.B4 grafts were rejected solely by already educated (Lewis × DA)F_1 hybrid T cells and their descendants in the tolerance-inducing inoculum, the more rapid rejection of the (BN.B4 × Lewis)F_1 hybrid grafts is best accounted for by the fact that they could serve as targets for host T cells as well.

Survival of BN.B2 and BN Skin Grafts on Lewis.1N Rats Rendered Tolerant at Birth with Athymic (rnu/rnu) Wag BMC. It stands to reason that if MHC restriction occurs when tolerance is induced, that Lewis.1N rats made tolerant with Wag BMC should be more likely to accept BN.B2 skin grafts, i. e., grafts that are MHC-compatible with Wag, than BN skin grafts, i. e., grafts with the same minor histocompatibility antigens of BN.B2 but with the MHC of Lewis.1N. It also follows that if the educated T cells in the tolerance-inducing Wag inocula are the only cells that can react against the BN.B2 grafts, that such grafts should survive indefinitely on animals rendered tolerant with inocula devoid of this population. In the hope of achieving this situation, Lewis.1N rats were rendered tolerant at birth with BMC prepared from athymic nude (*rnu/rnu*) Wag donors. 7 wk later these animals were challenged with either a BN.B2 or BN skin graft as well as with a Wag skin graft (all of which were permanently accepted, verifying the tolerant state of the hosts). Although the results (Table III) are clearly in accord with MHC restriction from the standpoint that the BN.B2 skin grafts survived significantly longer than the BN grafts (four of nine BN.B2 grafts failed to be rejected while all six of the BN grafts were rejected within 2 wk), the fact that five of the BN.B2 grafts were rejected indicates either that some crossreactivity occurred, i. e., some BN.B2 antigens recognized in association with an $RT1^n$ MHC may share specificities with these same antigens associated with an RT1[u] MHC and/or as discussed below, that the tolerance-inducing inocula were not entirely devoid of already educated T cells.

Discussion

It seems evident that not only when tolerance is induced to self antigens are these antigens recognized solely in terms of self-MHC (8–10), but when tolerance is induced to MHC-incompatible antigens they too are recognized only in association with the host's MHC. Evidence that such restriction operates in vivo with respect to transplantation antigens was first demonstrated by Miyamoto et al. (11). These investigators found that DA female rats rendered tolerant at birth with PVG female BMC, rejected H-Y-incompatible DA male skin grafts but not H-Y-incompatible PVG male grafts after sensitization with DA male BMC. Here we have shown that such restriction applies to other transplantation antigens as well. Thus, PVG rats inoculated at birth with (DA × PVG)F_1 BMC are not only rendered tolerant of the foreign transplantation antigens in the inoculum but, because donor cells migrate to the thymus, the entire T cell repertoire of the tolerant animal, including chimeric donor T cells, is restricted to the host's MHC (1). Accordingly, such rats accept third party ACI skin grafts because their foreign antigens are recognized in terms of RT1[c] and not RT1[a]. Indeed, what was surprising was that even tolerant PVG rats that had been putatively immunized against ACI, accepted ACI skin. The genetic similarity between DA and ACI undoubtedly contributed to this anergy.

Further evidence that MHC restriction accompanies tolerance induction is provided by the observation that BN.B2 skin grafts survive significantly longer than BN grafts on Lewis.1N animals tolerant of Wag. And this occurs despite the fact that the only difference between the third party skin donors involved is that one (BN.B2) is MHC-compatible with the tolerance-inducing inoculum, while the other (BN) is MHC-compatible with the host.

If MHC restriction occurs when tolerance is induced, some explanation must be provided for why some third party grafts that theoretically should be accepted are not. Thus, why did one F344 rat rendered tolerant with (DA × F344)F_1 hybrid BMC ultimately reject its ACI skin graft? Why were most of the BN.B4 grafts rejected by Lewis rats made tolerant with (Lewis × DA)F_1 BMC; and why did some Lewis.1N rats rendered tolerant with athymic Wag BMC reject their BN.B2 grafts? While it certainly is likely that transplantation antigens associated with different MHCs crossreact, another possibility is that they were rejected by already educated T cells in the tolerance-inducing inocula (12). Such cells are present in normal bone marrow and they appear to be present in the marrow of nude rats as well. Evidence for this is provided by the fact that some sublethally irradiated Lewis.1N rats that received 10^7 Wag nude BMC developed all the typical signs of a graft-vs.-host reaction, including skin exfoliation and runting, before dying between 2 and 3 wk of age. Moreover, similar observations have been reported (13) for lethally irradiated mice reconstituted with spleen cells from MHC-incompatible athymic nude donors.

The most enigmatic finding of this study was that, in at least some strain combinations, third party skin grafts survive significantly longer on tolerant recipients than on genetically comparable F_1 hybrids. Although the basis for this observation, which was first described by Zeiss (14, 15), remains to be elucidated, it probably is related to the fact that F_1 hybrid animals have two sets of immune response genes. Nevertheless, the facility with which some tolerant rats accept grafts known to express foreign antigens deserves special comment, especially since it indicates that there is another factor(s), independent of MHC restriction, that is involved in determining the fate of these grafts. Surely, if only MHC restriction were involved in determining the fate of third party grafts on tolerant animals, one would not have expected two of eight Lewis rats rendered tolerant at birth with (Lewis × DA)F_1 hybrid BMC (and bearing [Lewis × DA]F_1 hybrid skin grafts), to have accepted (BN.B4 × Lewis)F_1 hybrid skin grafts for >50 d. Accordingly, we believe that the heterozygous nature of these third party grafts, along with the amount of tissue transplanted (each recipient received two grafts), somehow induced unresponsiveness to their foreign antigens. This presumption is not without precedence, as it is in complete accord with a previous observation that, despite the fact that on the basis of MHC restriction Lewis rats rendered tolerant with (Lewis × BN)F_1 hybrid BMC should accept Skn-incompatible BN skin grafts more readily than (Lewis × BN)F_1 hybrid skin grafts, the opposite is the case, especially if the grafts are large (7).

Finally, we also believe it possible that because large F_1 hybrid skin grafts bearing Skn and other weak transplantation antigens can induce unresponsiveness to these antigens, that this may have obscured the tolerogenic influence of parental strain grafts transplanted along with them. Thus, the ability of (BN.B4 × Lewis)F_1 hybrid grafts to promote their own survival on Lewis rats rendered tolerant with (Lewis × DA)F_1 hybrid BMC, may have concealed any tolerogenic influence of the BN.B4 grafts that accompanied them. Indeed, there is evidence that H-Y-incompatible grafts that are accepted by tolerant animals because their transplantation antigens are recognized only in association with the MHC of the graft, can render their hosts unresponsive to H-Y in association with the MHC of the host. Thus, whereas, as noted above, DA females rendered tolerant with (DA × PVG)F_1 female BMC reject DA male skin grafts after exposure to DA male BMC, such grafts are accepted if the hosts are first exposed to a PVG male graft (16).

Summary

Evidence is presented that MHC restriction of foreign transplantation antigens occurs when tolerance is induced. Whereas PVG and F344 rats rendered tolerant at birth with (DA × PVG)F_1 and (DA × F344)F_1 hybrid bone marrow cells (BMC), respectively, accept ACI skin grafts, presumably because the foreign transplantation antigens of these third party grafts, which are MHC-compatible with DA, are recognized only in association with the MHC of the hosts, DA rats rendered tolerant with (DA × PVG)F_1 or (DA × F344)F_1 hybrid BMC usually reject ACI skin. Further support that MHC restriction accompanies the induction of tolerance is provided by the observation that Lewis.1N rats rendered tolerant at birth with athymic (nude) Wag BMC are much more likely to accept BN.B2 (MHC-compatible with Wag) skin grafts, than BN (MHC-compatible with Lewis.1N) grafts.

We express our appreciation to Susan Raab for technical assistance.

Received for publication 27 May 1986 and in revised form 21 August 1986.

References

1. Desquenne-Clark, L., H. Kimura, and W. K. Silvers. 1985. Evidence that major histocompatibility complex restriction of foreign transplantation antigens occurs when tolerance is induced in neonatal mice and rats. *Proc. Natl. Acad. Sci. USA.* 82:6265.
2. Billingham, R. E. 1961. The induction of tolerance of homologous tissue grafts. *In* Transplantation of Tissues and Cells. R. E. Billingham and W. K. Silvers, editors. Wistar Institute Press, Philadelphia, PA. 87–106.
3. Billingham, R. E. 1961. Free skin grafting in mammals. *In* Transplantation of Tissues and Cells. R. E. Billingham and W. K. Silvers, editors. Wistar Institute Press, Philadelphia, PA. 1–26.
4. Miyamoto, M., K. Furui, and H. Kimura. 1980. Male skin isografts can induce unresponsiveness in female rats. *Transplantation (Baltimore).* 30:180.
5. Donohoe, J. A., L. Andrus, K. M. Bowen, C. Simeonovic, S. J. Prowse, and K. J. Lafferty. 1983. Cultured thyroid allografts induce a state of partial tolerance in adult recipient mice. *Transplantation (Baltimore).* 35:62.
6. Bowen, K. M., S. J. Prowse, and K. J. Lafferty. 1981. Reversal of diabetes by islet transplantation: vulnerability of the established allograft. *Science (Wash. DC).* 213:1261.
7. Silvers, W. K., N. H. Collins, and M. Naji. 1983. Influence of size and gene dosage on the survival of skin allografts on rats rendered tolerant at birth. *J. Exp. Med.* 157:591.
8. Groves, E. S., and A. Singer. 1983. Role of the H-2 complex in the induction of T cell tolerance to self minor histocompatibility antigens. *J. Exp. Med.* 158:1483.
9. Matzinger, P., R. Zamoyska, and H. Waldman. 1984. Self tolerance is H-2-restricted. *Nature (Lond.).* 308:738.
10. Rammensee, H-G., and M. J. Bevan. 1984. Evidence from *in vitro* studies that tolerance to self antigens is MHC-restricted. *Nature (Lond.).* 308:741.
11. Miyamoto, M., T. Sano, K. Suzuki, and T. Fukumoto. 1984. Major-histocompatibility-complex-restricted male skin graft rejection responses in rats. *Transplantation (Baltimore).* 38:284.
12. Chen, H-D., C. Ma, J-T. Yuan, Y-K. Wang, and W. K. Silvers. 1986. Occurence of donor Langerhans Cells in mouse and rat chimeras and their replacement in skin grafts. *J. Invest. Dermatol.* 86:630.
13. Okunewick, J. P., W. E. Beschorner, M. J. Buffo, and D. L. Kociban. 1985. Histopathology of a possible graft-versus-host reaction induced by nude mouse spleen cells. *Transplantation (Baltimore).* 39:447.
14. Zeiss, I. M. 1966. An analysis of third-party unresponsiveness in immunologically tolerant rats. *Immunology.* 11:597.
15. Zeiss, I. M. 1969. Further evidence of third party unresponsiveness in immunologically tolerant animals. *Immunology.* 17:517.
16. Miyamoto, M., T. Sano, Y. Hattori, T. Fukumoto. 1984. MHC-unrestricted tolerance induction to male skin grafts in female rats. *Proc. Jpn. Soc. Immunol.* 14:143.