T CELLS RECOGNIZE MINOR HISTOCOMPATIBILITY ANTIGENS ON H-2 ALLOGENEIC CELLS*

By JAMES FORMAN and J. WAYNE STREILEIN

From the Department of Microbiology and the Department of Cell Biology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Sensitized murine thymus-derived (T) lymphocytes possess the preferential ability to recognize extrinsic antigens on H-2 syngeneic cells (1). However, the T-cell repertoire also includes the capacity to recognize H-2 alloantigens (2). Because a relatively high proportion of cytotoxic T-cell precursors recognize H-2 alloantigens (3), the possibility exists that a portion of this alloreactivity includes clones of T cells that recognize extrinsic antigens in the context of H-2 allodeterminants. To determine whether there exist clones of T cells that can recognize antigens in the context of H-2 allodeterminants (antigen H-2 allospecific recognition) in an animal, we utilized animals rendered neonatally tolerant of H-2 alloantigens in which the degree of chimerism is exceedingly low. By eliminating the H-2 alloreactivity, we are able to demonstrate that cytotoxic T lymphocytes of host origin recognize minor histocompatibility (H)[1] alloantigens on the tolerated H-2 allogeneic cells.

Materials and Methods

Animals. All mice were produced and maintained in our colonies at the University of Texas Health Science Center at Dallas, Dallas, Texas. Neonatally tolerant animals were produced by a method previously described (4). Briefly, 15×10^6 semiallogeneic bone marrow and spleen cells were inoculated i.v. within 24 h of birth. Animals were assayed for tolerance at 8 wk of age by the application of an orthotopic skin graft identical to the donor of the tolerizing inoculum.

Immunization Protocols. Mice were sensitized against minor H-antigens by i.p. inoculation of 50×10^6 spleen cells. 3 wk to 3 mon later, the recipients were killed, their spleen cells were placed in culture with 3,000-rad-irradiated stimulator cells, and cytotoxic T-cell activity was assayed 5 d later.

Cytotoxicity Assay. This procedure has been described in detail in a previous communication (5). Data is expressed as the net isotope release, which equals the percentage of ^{51}Cr release from target cells in the presence of immune cells minus the percentage of ^{51}Cr release from target cells in the presence of control cells. Control cells represent cultures lacking stimulator cells.

Results

Minor H-Antigens Presented on Tolerated Haplotype Induce T-Effector Cells Restricted to Tolerated Haplotype. Strong alloreactivity to an unrelated *H-2* haplotype in normal animals precludes identifying reactivity to minor H-antigens in an *H-2*-allogeneic context. Therefore, adult B10.A ($H\text{-}2^a$) animals, rendered tolerant to $H\text{-}2^f$ antigens

* Supported by National Institutes of Health grants AI-13111, AI-10678, and CA-09082.

[1] *Abbreviation used in this paper:* H, histocompatibility.

TABLE I

B10.A Effector Cells Tolerant of B10.M Recognize Minor H-Antigens on H-2 Allogeneic Cells

Line	Responder	Immunization	Target cells	Minor-H antigen incompatibility between responder and target cells	*H-2*-region sharing between target cells and cells used for immunization	Net ^{51}Cr release* (effector-to-target cell ratio)		
						100:1	50:1	10:1
1	B10.M	A.CA	A.CA(K^fD^f)‡	yes	*K D*	31.1	18.9	4.7
2			A.SW(K^sD^s)	yes	— —	0.1	−0.2	−0.2
3			A(K^kD^d)	yes	— —	2.5	0.7	0.5
4			A.TFR1(K^sD^f)	yes	— *D*	28.3	19.4	4.9
5			B10.M(11R)(K^fD^d)	no	*K* —	1.5	1.2	2.5
6	B10.A tolerant to B10.M	A.CA	A.CA	yes	*K D*	18.8	10.4	2.5
7			A.SW	yes	— —	−0.2	0.9	−0.1
8			A	yes	— —	3.0	0.9	−0.2
9			A.TFR1	yes	— *D*	13.8	8.9	2.9
10			B10.M(11R)	no	*K* —	−0.4	0.1	1.0
11			B10.M	no	*K D*	−1.3	−1.7	−0.6
12	B10.A	B10.M	B10.M	no	*K D*	22.5	15.5	4.7

* ^{51}Cr release from target cells in the presence of nonimmune (control) effector cells ranged from 8.9 to 12%.

‡ *H-2K* and *H-2D* alleles.

by neonatal inoculation of (B10.A × B10.M)F_1 spleen cells, were inoculated i.p. with A.CA (*H-2^f*) spleen cells. Although B10.M and A.CA share the *H-2^f* haplotype, they have different alleles at multiple minor H-loci representing the difference between C57BL/10 and A strain background genes. 3 wk to 3 mon after in vivo inoculation of the *H-2^f*-tolerant B10.A animals with A.CA lymphoid cells, spleen cells from these mice were cocultured with irradiated A.CA spleen cells and subsequently tested for cytotoxic activity against a panel of target cells. As a positive control, normal B10.M animals were sensitized with A.CA spleen cells and the resultant cytotoxic effector cells were tested against the same panel of targets.

B10.M animals sensitized against minor H-antigen disparate spleen cells (A.CA) generate a cytotoxic effect against A.CA target cells (line 1, Table I). This response is *H-2* restricted in that H-2-incompatible target cells bearing the appropriate minor H-antigens are not lysed (lines 2 and 3); whereas a cytotoxic effect is observed if only the *H-2D^f* region is shared between the stimulator and target cell (line 4). B10.M(11R) target cells are not lysed because they lack the A strain background minor H-antigens (line 5).

B10.A animals tolerant to B10.M, sensitized in vivo and boosted in vitro with A.CA cells, display a strong cytotoxic effect against A.CA target cells (line 6). This response is *H-2* restricted to the same extent as that observed when testing the B10.M spleen cells. That is, H-2-incompatible target cells are not lysed (lines 7 and 8), whereas target cells sharing only the *H-2D^f* region with the stimulator cells are lysed (line 9).

TABLE II

Minor H-Antigens Presented on the Tolerated Haplotype Induce T-Effector Cells Restricted to Host Haplotype

Line	Responder	Cells used for in vivo priming	Cells used for in vitro challenge	Target cells	*H-2*-region sharing between cells used for in vitro challenge and target cells	Net ^{51}Cr Release* (effector-to-target cell ratio)		
						100:1	50:1	10:1
1	B10.A tolerant to B10.M	A.CA	A.CA	A.CA	*K D*	28.0	28.3	12.5
2				A	— —	−0.6	1.3	0.9
3				C3H(K^kD^k)‡	— —	2.2	2.9	3.6
4				C3H.SW(K^bD^b)	— —	0.5	0.4	0.4
5	B10.A tolerant to B10.M	A.CA	A	A	*K D*	35.3	27.2	11.7
6				A.CA	— —	1.0	0.6	3.4
7				C3H	*K* —	16.8	13.0	3.8
8				C3H.SW	— —	0.5	0.7	1.8

* ^{51}Cr release from target cells in the presence of nonimmune (control) effector cells ranged from 10.3 to 21.6%.

‡ *H-2K* and *H-2D* alleles.

B10.M(11R) target cells lacking the minor H-antigens of the A strain background are not lysed (line 10). These data also demonstrate that the $H\text{-}2^f$-tolerant B10.A animals do not generate anti-H-2 killer-cell activity against B10.M target cells (line 11); whereas nontolerant animals do generate such activity (line 12). Thus, a significant amount of specific cytotoxic T-cell activity can be generated in B10.A animals tolerant to $H\text{-}2^f$ antigens when these animals are sensitized against minor H-antigens in the context of the tolerated *H-2*-allogeneic haplotype.

Minor H-Antigens Presented on the Tolerated Haplotype Induce T-Effector Cells Restricted to Host Haplotype. Bevan (6) has demonstrated that minor H-antigens presented on H-2 semiallogeneic cells may be used to cross prime mice in vivo so that the antigen can be presented on the allogeneic *H-2* haplotype. In these experiments, we wished to determine whether animals tolerant to an *H-2* haplotype could be presented with minor H-antigens on the tolerated haplotype so that the antigens could cross-prime and restrict for the host haplotype. B10.A animals tolerant to B10.M were inoculated in vivo with A.CA lymphoid cells. When spleen cells from these tolerant animals were later cultured in vitro with A.CA cells, a cytotoxic response was generated against A.CA, but not H-2-incompatible target cells (Table II, lines 1–4), in agreement with the data shown in Table I. Furthermore, when freshly harvested spleen cells were challenged in vitro with A strain spleen cells, the cytotoxic effect generated was now restricted to K^kD^d target cells, including C3H, which indicates that the A and C3H strains share some minor H-alleles (lines 5 and 7). Importantly, A.CA cells and other H-2-incompatible target cells bearing the appropriate minor H-antigens were not lysed (lines 6 and 8). Thus, these results indicate that minor H-antigens presented on cells of an allogeneic *H-2* haplotype can prime the animal for minor H-antigen recognition on the host's *H-2* haplotype.

TABLE III

Minor H-Antigens Presented on Host Haplotype Fail to Induce T Effector Cells Restricted to Tolerated Haplotype

Line	Responder	Cells used for in vivo priming	Cells used for in vitro challenge	Target cells	*H-2*-region sharing between cells used for in vitro challenge and target cells	Net ^{51}Cr release* (effector-to-target cell ratio)		
						100:1	50:1	10:1
1	B10.A tolerant to B10.M	A	A	A	*K D*	15.8	8.5	2.9
2				A.CA	— —	−0.4	−3.5	−1.0
3	B10.A tolerant to B10.M	A	A.CA	A.CA	*K D*	−3.2	−1.1	−0.3
4				A	— —	−1.1	−1.7	0

* ^{51}Cr release from A and A.CA target cells in the presence of nonimmune (control) effector cells was 20.0 and 24.4%, respectively.

Minor H-Antigens Presented on Host Haplotype Fail to Induce T-Effector Cells Restricted to Tolerated Haplotype. We have previously determined that the extent of chimerism in animals rendered tolerant as neonates is extremely low (4, 7). Therefore, it would be expected that the number of (B10.A × B10.M)F_1 lymphoreticular cells able to present antigen in B10.A animals tolerant of the B10.M haplotype would be correspondingly low. In the following experiment, we determined whether the chimeric F_1 cells in tolerant animals could present minor H-antigens to the host's T-cell system. B10.A animals tolerant of B10.M were inoculated in vivo with A strain lymphoid cells followed by rechallenge in vitro with either A or A.CA cells. The specificity of effector cells generated following in vitro culture is shown in Table III. As expected, B10.A animals tolerant of B10.M and immunized with A strain cells in vivo and in vitro generated cytotoxic effector cells with specificity for A strain and not for A.CA target cells (lines 1 and 2). However, when the tolerant spleen cells were challenged in vitro with A.CA spleen cells, no cytotoxic effect was observed against A.CA target cells (line 3). Therefore, minor H-antigens presented on A strain spleen cells cannot be processed in vivo to allow for T-cell recognition of minor H-antigens on the tolerated $H\text{-}2^f$ haplotype. This finding suggests that host reprocessing of minor H-antigens is not a requisite for sensitization of cytotoxic T lymphocytes.

Minor H-Antigens Presented on Tolerated Haplotype Induce T-Effector Cells Restricted to Tolerated Haplotype. B10.M animals were made tolerant as neonates to B10.A cells, primed in vivo and challenged in vitro with A strain lymphoid cells. Their spleen cells were then tested against a panel of target cells (Table IV). The specificity of the cytotoxic cells generated was restricted to H-2-compatible target cells (lines 1–3). When the animals were challenged in vitro with A.CA rather than A strain lymphoid cells, a strong cytotoxic effect was observed against A.CA target cells (lines 4 and 5), demonstrating cross-priming similar to that shown in Table II. Therefore, B10.M animals tolerant to B10.A also possess T cells that can recognize minor H-antigens in the context of the tolerated *H-2* haplotype.

TABLE IV

B10.M Effector Cells Tolerant of B10.A Recognize Minor H-Antigens on H-2 Allogeneic Cells

Line	Responder	Cells used for in vivo priming	Cells used for in vitro challenge	Target cells	*H-2*-region sharing between cells used for in vitro challenge and target cells	Net ^{51}Cr Release* (effector-to-target cell ratio)		
						100:1	50:1	10:1
1	B10.M tolerant to B10.A	A	A	A	*K D*	33.4	24.6	9.8
2				A.CA	— —	2.3	0.4	0.9
3				A.TH(K^sD^d)‡	— *D*	32.2	24.0	10.2
4	B10.M tolerant to B10.A	A	A.CA	A.CA	*K D*	39.3	32.1	11.2
5				A	— —	6.0	4.7	3.4

* ^{51}Cr release from target cells in the presence of nonimmune (control) effector cells ranged from 13.8 to 15.8%.

‡ *H-2K* and *H-2D* alleles.

Discussion

The data in this report demonstrate that animals rendered tolerant as neonates possess a significant number of cytotoxic T-cell precursors that can recognize minor H-antigens in the context of the allogeneic *H-2* haplotype to which they were rendered tolerant. Secondly, because the specificity of the effector cells generated are restricted by the *H-2* haplotype of the cells used for the in vitro challenge, it is concluded that two different sets of cytotoxic effector cells co-exist in tolerant animals. Because we cannot find cytotoxic T cells (or their precursors) that can recognize the tolerated H-2 alloantigens themselves in these animals, it is surprising that T cells from these animals that recognize minor H-antigens can be restricted by the H-2 alloantigens to which they are tolerant.

This situation may be explained by postulating that alloreactive T cells bear receptors with a spectrum of avidities for H-2 antigens. In the tolerance protocol, high-avidity receptor bearing T cells are deleted so that only those with low-avidity receptors remain. These putatively low-avidity-receptor clones could bind alloantigen with high avidity, however, if the H-2 alloantigen is presented together with minor H-antigens. Alternatively, T cells recognizing minor H-antigens in the context of H-2 allodeterminants could represent a subset of anti-self clones that coincidentally cross-react with minor H-antigens along with the allo-H-2 determinants, and thus would not be expected to be affected by the tolerance procedure itself.

It is very unlikely that F_1 cells utilized for the tolerance induction are mediating the cytotoxic effects observed because we have previously shown that (*a*) the extent of lymphoid cell chimerism in animals rendered tolerant by this protocol is <5% (4, 7), and (*b*) treatment of the effector cells with antiserum against the F_1 cells does not affect the cytotoxic response (7).

It is possible that the activity of the effector cells from B10.A mice tolerant to

B10.M that were sensitized and tested against A.CA is *H-2* unrestricted and directed against Qa-Tla antigens (8) rather than A strain minor H-antigens restricted by *H-2K*^{f} or *H-2D*^{f}. This possibility is unlikely, however, because (*a*) both B10.M and A.CA have the same alleles at the *Qa-Tla* loci that control antigens that are recognized by cytotoxic T cells, and (*b*) attempts to demonstrate *H-2*-unrestricted anti-Qa-Tla effector cell activity by cross-sensitization of B10.M animals with A.CA cells and vice versa has yielded negative results (8; and James Forman, unpublished results).

Bevan (6) has demonstrated that cross-priming occurs in vivo when minor H-antigens are presented on an *H-2* haplotype that is different than that used for in vitro boosting. Therefore, it is possible that minor H-antigens have to be reprocessed on host cells to generate cytotoxic effector cells. However, our data demonstrate that this is not a requirement. Thus, B10.A animals tolerant of B10.M when primed in vivo and boosted in vitro with A.CA cells generate *H-2*^{f}-restricted anti-minor-H-antigen effector cells. It could be argued that A strain minor H-antigens are processed on the small number of (B10.A × B10.M)F_1 cells residing in the tolerant animals (<5%). If this occurs, then it would be expected that inoculation of A strain spleen cells into the same tolerant animals should allow for cross-priming, so that boosting in vitro with A.CA stimulator cells would result in *H-2*^{f}-restricted anti-minor-H-antigen cytotoxic effector cells. However, this does not occur, even though cross-priming does occur in the opposite direction. Therefore, we conclude that cytotoxic T-cell precursors can recognize minor H-antigens directly on the donor-cell inoculum without a requirement for reprocessing.

It is becoming more clear that the ability of T cells to recognize antigens on H-2-allogeneic cells is widespread and thus contrasts with the initial reports of Zinkernagel et al. (9), who did not find cytotoxic T cells in radiation chimeras that recognize antigen in the context of H-2 allodeterminants. Our data extend our previous findings which showed that trinitrophenyl could be recognized on H-2-allogeneic tolerated cells (7), and are in agreement with those of Matzinger and Mirkwood (10) who demonstrated the recognition of minor H-antigens in fully allogeneic radiation chimeras. Blanden and Andrew (11) also found that a certain portion of T cells in radiation chimeras could recognize ectromelia virus on H-2-allogeneic cells. Experiments are now in progress to determine whether T-cell recognition of minor H-antigens in the context of H-2 allodeterminants has in vivo relevance as demonstrated by the rejection of skin allografts bearing minor H-antigens in the context of the tolerated *H-2* haplotype.

Summary

B10.A animals were rendered tolerant to B10.M spleen cells by injection of (B10.A × B10.M)F_1 cells into neonates. Adult animals accepted B10.M skin grafts and failed to generate cytotoxic effector cells in vitro against B10.M H-2 antigens. In vivo inoculation of tolerant animals with A.CA spleen cells, followed by in vitro challenge with similar cells, resulted in the generation of cytotoxic effector cells that had specificity for the A strain minor histocompatibility (H)-antigens in the context of the *H-2*^{f} haplotype. If these animals were boosted in vitro with A strain spleen cells, cross-priming could be demonstrated, whereby the cytotoxic effect was restricted by the *H-2*^{a} haplotype. These data indicate that at least two sets of T cells co-exist in tolerant animals, one capable of recognizing antigens in the context of the host *H-2* haplotype,

and the other able to recognize antigens in the context of the tolerated *H-2*-allogeneic haplotype.

Because tolerant animals inoculated with A-strain spleen cells in vivo and boosted in vitro with A.CA spleen cells failed to generate a cytotoxic effect against A.CA, it is unlikely that minor H-antigens need to be processed by host lymphoreticular cells.

We wish to thank Julia Tsan for her excellent technical assistance.

Received for publication 4 May 1979.

References

1. Moller, G., editor. 1976. *Transplant. Rev.* **29:**1.
2. Dutton, R. W. 1966. Spleen cell proliferation in response to homologous antigens studied in congenic resistant strains of mice. *J. Exp. Med.* **123:**665.
3. Pimsler, M., and J. Forman. 1978. Estimates of the precursor frequency of cytotoxic T lymphocytes against antigens controlled by defined regions of the *H-2* gene complex: comparison of the effect of *H-2* differences due to intra-H-2 recombination *vs* mutation. *J. Immunol.* **121:**1302.
4. Streilein, J. W., and J. Klein. 1977. Neonatal tolerance induction across regions of H-2 complex. *J. Immunol.* **119:**2147.
5. Forman, J., and L. Flaherty. 1978. Identification of a new CML target antigen controlled by a gene associated with the *Qa-2* locus. *Immunogenetics.* **6:**227.
6. Bevan, M. J. 1977. Cytotoxic T-cell response to histocompatibility antigens: the role of H-2. *Cold Spring Harbor Symp. Quant. Biol.* **41:**519.
7. Forman, J., J. Klein, and J. W. Streilein. 1977. Spleen cells from animals neonatally tolerant to H-2K^k antigens recognize trinitrophenyl-modified H-2K^k spleen cells. *Immunogenetics.* **5:**561.
8. Flaherty, L. The *Tla* region antigens. *In* The Role of the Major Histocompatibility Complex in Immunobiology. M. Dorf, editor. In press.
9. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* **147:**882.
10. Matzinger, P., and G. Mirkwood. 1978. In a fully H-2 incompatible chimera, T cells of donor origin can respond to minor histocompatibility antigens in association with either donor or host H-2 type. *J. Exp. Med.* **148:**84.
11. Blanden, R. V., and M. E. Andrew. 1979. Primary anti-viral cytotoxic T-cell responses in semiallogeneic chimeras are not absolutely restricted to host H-2 type. *J. Exp. Med.* **149:** 535.