ROLE OF THE H-2 COMPLEX IN THE INDUCTION OF T CELL TOLERANCE TO SELF MINOR HISTOCOMPATIBILITY ANTIGENS

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The mechanisms by which T cells are tolerized to self antigens are unknown (1–3). It is known that most T lymphocytes only react to foreign antigens presented in the context of determinants encoded within the major histocompatibility complex (MHC)[1] (4). One possible explanation for the existence of MHC restrictions is that such T lymphocytes do not recognize nominal (i.e. non-MHC encoded) antigens per se, but rather recognize an antigenic complex composed of nominal antigen plus an MHC-encoded determinant. From this perspective, T cell tolerance to nominal self antigens could not result from the deletion of T cell clones that recognize and bind self antigens directly, but would have to result from the deletion of clones with specificity for complexes composed of nominal self antigens + self-MHC (5). Thus, T cell tolerance to nominal self antigens, like T cell reactivity to nominal foreign antigens, would be MHC restricted. In contrast, if individual T cells express two distinct recognition specificities, one for nominal antigens and one for MHC determinants, it is conceivable that T cells might be tolerized by recognition of nominal antigens alone. From this alternative perspective, T cell tolerance to nominal self antigens, as opposed to T cell reactivity to nominal foreign antigens, would not be MHC restricted. Thus, elucidating the possible role of MHC recognition in the induction of T cell tolerance to nominal self antigens has important consequences both for our concepts of self-tolerance by T cells and for our concepts of antigen recognition by T cells. Although most previous experimental attempts have failed to demonstrate a role for MHC recognition in the induction of T cell tolerance to self antigen (6, 7), data consistent with this possibility have recently been reported (8).

In the present study, the possible requirement for MHC recognition in the induction of T cell tolerance to self minor histocompatibility (minor H) antigens was investigated. The experimental approach involved the construction of Parent $\rightarrow$ F_1 radiation bone marrow chimeras of the type C3H.SW $\rightarrow$ B10 $\times$ B10.BR. The T lymphocytes from such experimental animals: (*a*) express C3H minor H antigens on their surface since they were derived from C3H.SW precursors

[1] *Abbreviations used in this paper:* APC, antigen-presenting cells; Con A, concanavalin A; Con A Sn, concanavalin A–induced supernatant; CTL, cytotoxic T lymphocyte; i.p., intraperitoneally; MHC, major histocompatibility complex; minor H antigens, minor histocompatibility antigens; pCTL, precursor cytotoxic T lymphocytes.

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present in the donor bone marrow inocula, (*b*) should be tolerant to both H-2^b and H-2^k determinants since they had differentiated in a B10 × B10.BR irradiated host, (*c*) should be able to utilize both H-2^b and H-2^k determinants as self-restriction elements since they had been educated in an H-$2^{b/k}$ thymus (9), (*d*) have been exposed to allogeneic Black minor H antigens in the context of both H-2^b and H-2^k determinants, but (*e*) have, in theory, only been exposed to self C3H minor H antigens in the context of donor H-2^b determinants. Thus, if tolerance to nominal self antigens were MHC restricted, it would be predicted that the T cell populations from these experimental animals would contain cytotoxic T lymphocyte precursors (pCTL) reactive to C3H self minor H antigens in the context of host H-2^k determinants (10), since they should not have encountered C3H self minor H antigens in the context of host H-2^k determinants during their differentiation into functional competence. Indeed, this is precisely what was observed: C3H.SW T cell populations from C3H.SW → B10 × B10.BR chimeras were found to be tolerant to allogeneic Black minor H antigens in the context of either H-2^b or H-2^k, were found to be tolerant to self C3H minor H antigens in the context of H-2^b, but were reactive to self C3H minor H antigens in the context of H-2^k. Consequently, the present study demonstrates that at least one component of T cell tolerance to self minor H antigens is genetically restricted in that these T cell populations are only tolerant to self minor H antigens in the context of self H-2. In addition, the present study demonstrates that MHC-restricted tolerance to nominal self antigens is expressed by both peripheral and intrathymic T cell populations.

Materials and Methods

Animals. C57BL/10Sn (B10), A/J, A.BY, C57BL/6 (B6), (B6 × A/J)F_1 (B6AF_1), B10.A, (B10 × B10.A)F_1, B10.BR (BR), (B10 × BR)F_1, and C3H.SW were obtained from The Jackson Laboratory, Bar Harbor, ME. C3H/HeN (C3H), (B6 × C3H)F_1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. BALB.B, BALB.K, and (BR × C3H.SW)F_1 mice were bred in our animal colony. The H-2 haplotypes and minor H antigens of the mice used in this study are shown in Table I.

TABLE I

Genetic Origins of the Mice Used in This Study

Strain	H-2	Minor H antigens
A/J	a	A
A.BY	b	A
B6AF_1	b × a	Black × A
B10.A	a	Black
C57BL/6 (B6)	b	Black
C57BL/10Sn (B10)	b	Black
B10.BR	k	Black
B10 × B10.BR	b × k	Black
C3H	k	C3H
C3H.SW	b	C3H
B6 × C3H	b × k	Black × C3H
B10.BR × C3H.SW	k × b	Black × C3H
BALB.B	b	BALB
BALB.K	k	BALB

Radiation Bone Marrow Chimeras. Radiation bone marrow chimeras are designated as bone marrow donor → irradiated recipient and were constructed as previously described (11). Recipient mice were irradiated with 950 rad from a ^{137}Cs source and reconstituted 2–6 h later with 1.5×10^7 bone marrow cells that had been pretreated with rabbit anti-mouse brain serum, a reagent selected to be specifically cytotoxic for all T cells (12), plus complement. Such chimeras were >95% of donor bone marrow origin as assessed by indirect immunofluorescence using strain-specific anti-H-2 reagents (11). Chimeras were first primed 12 or more weeks after irradiation and bone marrow reconstitution.

In Vivo Antigen Priming. Mice were routinely primed intraperitoneally (i.p.) with 2–4 $\times 10^7$ whole spleen cells 1 or more weeks before use. Where indicated, mice were boosted i.p. with 4×10^7 whole spleen cells 2 wk before use. Reactive thymocytes were obtained from animals using this same protocol.

In Vitro Generation of Cytotoxic T Lymphocytes (CTL). 4×10^6 responder and 4×10^6 2000 rad irradiated stimulating cells were mixed in 2-ml cultures containing RPMI 1640 supplemented with 10^{-5} M 2-mercaptoethanol, glutamine, and antibiotics in 7% CO_2 air atmosphere at 37°C (13). Thymocyte responder cultures were further supplemented with 1/8 vol/vol rat concanavalin A–induced supernatant (Con A Sn) (Collaborative Research, Waltham, MA) to which 0.2 M alpha-methyl-D-mannoside had been added to neutralize the remaining concanavalin A (14). After 5 d, the cultures were assayed for CTL generation by their ability to lyse ^{51}Cr labeled Con A–induced splenic blasts as targets in a 4-h ^{51}Cr release assay. Percent specific ^{51}Cr release = 100 × (experiment − spontaneous release)/(maximum − spontaneous release). Spontaneous release ranged from 17% to 22%. Each experimental point is the mean of triplicate cultures. Standard error for each point was <3% of the mean.

Results

Parent → F_1 Chimeric Splenocytes can be Primed to Allogeneic Minor H Antigens on Both Parental Haplotypes. In order to determine whether Parent → F_1 chimeras could be in vivo–primed to allogeneic minor H antigens in the context of either donor or host H-2 determinants, B10.A → B10 × B10.A and B10 → B10 × B10.A chimeras were primed in vivo with minor H allogeneic $B6AF_1$ spleen cells and restimulated in vitro with allogeneic strain A minor H antigens. Fig. 1 shows the splenocyte responses of such chimeras as well as the splenocyte responses of B10 × B10.A control mice primed in vivo with $B6AF_1$ cells. All three responder populations generated CTL specific for allogeneic strain A minor H antigens, which were restricted to either $H\text{-}2^a$ or $H\text{-}2^b$. The CTL generated by in vitro stimulation with A/J stimulators lysed A/J targets but did not significantly lyse either A.BY or B10 × B10.A targets (Fig. 1, *A*, *D*, and *G*); and the CTL generated by in vitro stimulation with A.BY stimulators lysed A.BY targets but did not significantly lyse either A/J or B10 × B10.A targets (Fig. 1, *B*, *E*, and *H*). Thus, these data demonstrate that it is possible to elicit from the spleens of Parent → F_1 chimeras CTL that are specific for allogeneic minor H antigens and that are restricted to either the donor or host H-2 haplotype.

Even though the priming inocula were $B6AF_1$ spleen cells and contained F_1 antigen-presenting cells (APC), it might be expected that in Parent → F_1 chimeras there would be more effective priming of T cells restricted to the donor H-2 haplotype than to the host H-2 haplotype since the APC resident in Parent → F_1 chimeras at the time of priming are virtually all of donor H-2 haplotype. In other words, allogeneic minor H antigens could be presented in the context of donor H-2 determinants by APC present in the F_1 priming inoculum as well as by APC resident in the chimera; in contrast, allogeneic minor H antigens could be

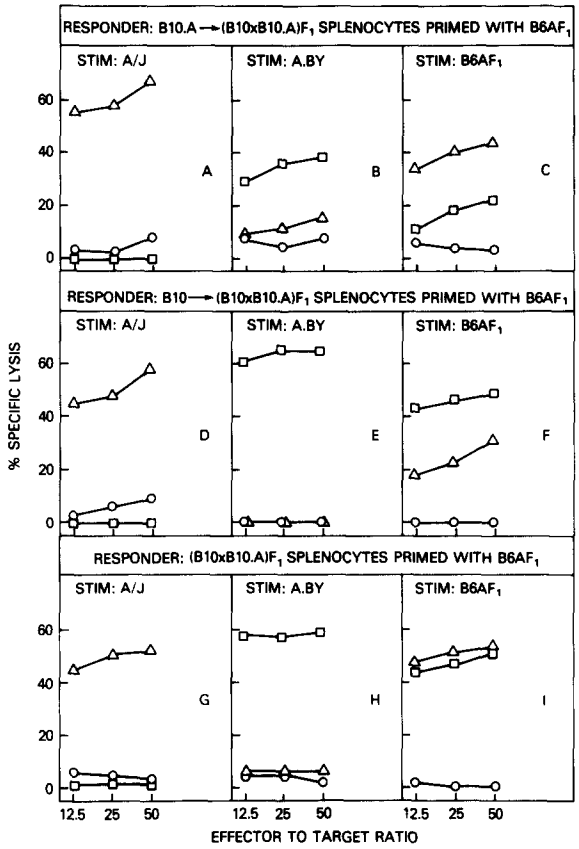

FIGURE 1. Parent → F_1 chimeric splenocytes can be primed to allogeneic minor H antigens on both parental haplotypes. CTL were generated from the indicated responder populations by in vivo priming with $B6AF_1$ spleen cells and by in vitro restimulation with 2000R irradiated stimulator spleen cells from A/J, A.BY, or $B6AF_1$ animals. Targets were Con A–induced spleen blasts from A/J (△), A.BY (□), and B10 × B10.A (○) animals.

presented in the context of host H-2 determinants only by APC present in the F_1 priming inoculum. In addition, exposure to environmental antigens might have selectively expanded the T cell subpopulation restricted to the donor H-2 haplotype. To assess the possibility that responses of in vivo–primed parent → F_1 chimeras is skewed toward the donor H-2 haplotype, the chimeric and normal F_1 responder cells in this experiment were also restimulated in vitro with $B6AF_1$ stimulators. Upon in vitro stimulation with $B6AF_1$ cells, B10 × B10.A responders generated equivalent strain A minor H–specific CTL responses restricted to H-2^b and H-2^a (Fig. 1*I*). However, upon stimulation with $B6AF_1$ cells the parent → F_1 chimeras generated asymmetric CTL responses, in that the minor H–specific responses restricted to the donor H-2 haplotype were greater than those restricted to the nondonor host H-2 haplotype (Fig. 1, *C* and *F*).

Thus, these results demonstrate that even though the CTL response magnitude is skewed toward responses restricted to the donor H-2 haplotype, it is possible to elicit from Parent → F_1 chimeras minor H–specific CTL restricted to either

the donor or host H-2 haplotype.

Tolerance to Self Minor H Antigens is H-2–Restricted: Direct Stimulation. Since minor H–specific CTL restricted to either donor or host H-2 determinants could be elicited from the spleens of Parent → F_1 chimeras, it was possible to assess the role of MHC recognition in the induction of tolerance to self minor H antigens. T cells from C3H.SW → B10 × B10.BR chimeras should in theory have never encountered and, hence not been tolerized to, C3H minor H antigens in the context of H-2^k. However, in practice, it is possible that residual host B10 × B10.BR APC could have taken up C3H minor H antigens shed from C3H.SW bone marrow cells and presented them in the context of either H-2^b or H-2^k, potentially tolerizing developing chimeric T cells to C3H minor H antigens in the context of either H-2^b or H-2^k (6). While there is no way to prevent such a possibility, most residual host APC in radiation bone marrow chimeras have disappeared before the chimeric T cell repertoire is fully established (15), so that the tolerization of T cells to C3H minors in the context of host H-2^k determinants should not be complete, if it occurred at all.

In an effort to maximize the possibility of eliciting C3H + H-2^k restricted CTL from the spleens of C3H.SW → B10 × B10.BR chimeras, the animals were simultaneously primed to the male H-Y alloantigen, a maneuver that allows the male H-Y antigen to serve as a potential carrier determinant for priming of minor H–specific CTL (16) (Fig. 2). Thus, C3H.SW♀ → B10 × B10.BR♀ chimeras were in vivo primed with B6 × C3H♂ spleen cells that expressed both Black and C3H minor H antigens, expressed both H-2^b and H-2^k determinants, and additionally expressed the male H-Y alloantigen as a potential carrier determinant. The primed spleen cells were restimulated in vitro with B6 × C3H cells and were assayed on female target cells that did not express the male H-Y alloantigen so that the CTL activity assayed could not be specific for the H-Y alloantigen. It can be seen in Fig. 2 that by this protocol CTL were generated from the spleens of C3H.SW → B10 × B10.BR chimeras which were specific for C3H minors + H-2^k. It might be noted that preliminary attempts to in vivo prime such chimeras with B6 × C3H♀ cells, which do not bear the H-Y carrier determinant, were unsuccessful (data not shown).

In Fig. 2, Expt. 1 the CTL lysed C3H targets but did not lyse C3H.SW, B10, or B10.BR targets. The failure to lyse C3H.SW targets was not due to the possibility that they were ineffective targets since they were lysed by CTL generated from control B10 × B10.BR responders. However, it was possible that the lysis of C3H targets by C3H.SW → B10 × B10.BR responders was not due to the generation of CTL specific for C3H minors + H-2^k, but instead was due to the generation of CTL specific for H-2–linked alloantigens only present on C3H cells (16–18). To rule out this possibility, in Fig. 2, Expt. 2 the CTL were assayed on B10.BR × C3H.SW target cells. If the CTL were specific for C3H minors + H-2^k they should lyse B10.BR × C3H.SW targets since these target cells express both C3H minor antigens and H-2^k determinants; however, if the CTL were entirely specific for an H-2–linked alloantigen present on C3H cells but absent on C3H.SW and B10.BR cells, they would not lyse B10.BR × C3H.SW target cells. It can be seen in Fig. 2, Expt. 2 that the CTL effectively lysed the B10.BR × C3H.SW target cells, demonstrating that they were specific

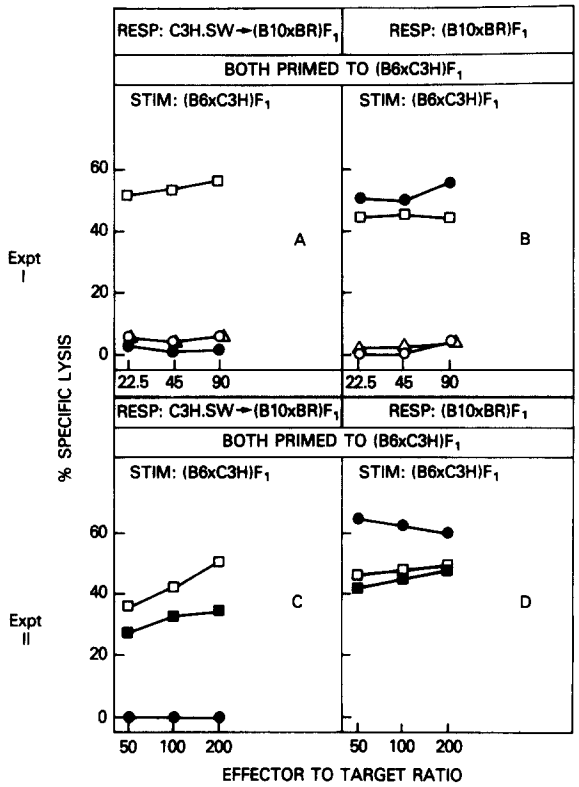

FIGURE 2. Tolerance to self minor H antigens is H-2 restricted as indicated by direct stimulation. CTL were generated from C3H.SW♀ → B10 × B10.BR♀ chimeras or B10 × B10.BR♀ normal controls that had been in vivo primed with B6 × C3H♂ spleen cells and restimulated in vitro with either B6 × C3H♀ (*A* and *B*) or B6 × C3H♂ (*C* and *D*) spleen stimulator cells. Targets were Con A–induced spleen blasts from female C3H (□), C3H.SW (●), B10 (△), B10.BR (○), or B10.BR × C3H.SW (■) animals.

for C3H minors + H-2^k.

Thus, the results of these experiments demonstrate that C3H.SW → B10 × B10.BR T cells are: (*a*) tolerant to both H-2^b and H-2^k determinants, (*b*) are tolerant to the allogeneic Black minor H antigens of the chimeric host in the context of either H-2^b or H-2^k, (*c*) are tolerant to self C3H minor H antigens in the context of H-2^b, but most importantly (*d*) are not tolerant to self C3H minor H antigens in the context of H-2^k. Since genotypically C3H.SW CTL were elicited with specificity for C3H minors + H-2^k but not for C3H minors + H-2^b, it can be concluded that T cell populations are not tolerant to self minor H antigens in an H-2 determinant context different from that in which they had encountered these antigens during their differentiation.

Tolerance to Self Minor H Antigens is H-2–Restricted: Cross-reactive Stimulation. Minor H antigens express only limited polymorphisms as indicated by the fact that H-2–restricted CTL specific for minor H alloantigens of one strain recognize the same or similar minor H alloantigens of other strains (10). We next exploited such cross-reactivity as an alternative approach to assessing the role of MHC recognition in tolerance induction to self minor H antigens and to

show that C3H.SW → B10 × B10.BR chimeras can react against allogeneic minor H antigens in the context of H-2^b. To demonstrate such cross-reactivity, B10 × B10.BR control mice were primed in vivo with BALB.K spleen cells and restimulated in vitro with either BALB.K or BALB.B spleen cells (Table II). Upon in vitro restimulation with BALB.K cells, B10 × B10.BR CTL were generated that lysed targets bearing BALB minors + H-2^k as well as targets bearing C3H minors + H-2^k (Table II *C*). Similarly, upon in vitro stimulation with BALB.B cells, B10 × B10.BR CTL were generated that lysed targets bearing BALB minors + H-2^b as well as targets bearing C3H minors + H-2^b, even though the responder cells had been in vivo–primed with BALB.K spleen cells (Table II *D*). This phenomenon has been referred to as "cross-priming" (19) and presumably reflects the in vivo presentation of shed BALB minor H antigens by responder B10 × B10.BR APC. Thus, these results indicate that many of the BALB minor H antigens recognized by Black responders are also present on C3H cells.

In the same experiment, the CTL responses, of C3H.SW → B10 × B10.BR chimeras that had been primed in vivo to BALB.K, were also determined. Upon in vitro restimulation with BALB.K cells, CTL were generated that were specific

TABLE II

Self Tolerance is MHC Restricted: Cross-reactive Stimulation

Expt.	Group	Responder splenocytes	Spleen cells for: In Vivo priming	Spleen cells for: In Vitro restimulation	E/T	H-2:	Targets* BALB.K k	C3H k	BALB.B b	C3H.SW b	B10 × B10.BR b × k
							% Specific lysis				
1	A	C3H.SW → B10 × B10.BR	BALB.K	BALB.K	120		63‡	40	−1	2	4
					60		55	31	−0	2	2
					30		42	28	0	2	0
	B	C3H.SW → B10 × B10.BR	BALB.K	BALB.B	120		1	−2	41	3	3
					60		2	−1	37	2	6
					30		0	−1	30	0	4
	C	B10 × B10.BR	BALB.K	BALB.K	120		50	48	15	9	5
					60		43	41	3	9	4
					30		41	40	4	10	4
	D	B10 × B10.BR	BALB.K	BALB.B	120		8	5	36	28	2
					60		3	4	37	23	1
					30		5	2	26	24	2
2§	E	C3H → B10 × B10.BR	BALB.B	BALB.B	110		0	5	72	20	7
					55		1	5	77	17	6
					28		1	2	71	17	5
	F	C3H → B10 × B10.BR	BALB.B	BALB.K	110		36	1	7	3	1
					55		34	0	5	2	0
					28		31	−1	2	4	1
	G	B10 × B10.BR	BALB.B	BALB.B	110		−5	0	71	73	0
					55		−1	0	66	78	0
					28		−2	1	65	67	1
	I	B10 × B10.BR	BALB.B	BALB.K	110		54	48	29	24	6
					55		51	48	23	24	8
					28		50	46	20	24	6

* All target cells were female.

‡ Boxed values represent comparative lysis of H-2 syngeneic BALB and C3H targets cells as referred to in the text and are not intended to imply statistical significance.

§ In this experiment animals were boosted in vivo with an additional 4 × 10^7 BALB.B spleen cells 3 wk before harvest.

for BALB minors + H-2^k (Table II *A*) and, upon in vitro restimulation with BALB.B cells, CTL were generated that were specific for BALB minors + H-2^b (Table II *B*), demonstrating that C3H.SW → B10 × B10.BR chimeras are reactive to allogeneic minor H antigens in the context of either H-2^k or H-2^b. More importantly, those CTL specific for BALB minors + H-2^k also lysed targets bearing C3H minors + H-2^k (Table II *A*), demonstrating again that there exist C3H.SW pCTL in these chimeras that are capable of recognizing self C3H minors + H-2^k. In contrast, the CTL specific for BALB minors + H-2^b did not lyse targets bearing C3H minors + H-2^b (Table II *B*). Thus, by cross-reactive lysis, these data demonstrate that it is possible to generate C3H.SW CTL that recognize self C3H minor H antigens + H-2^k but not self C3H minor H antigens + H-2^b, presumably because these T cell populations are tolerant to C3H minors + H-2^b. The reciprocal response pattern was observed with C3H → B10 × B10.BR chimeric spleen cells that had been in vivo primed to BALB.B (Table II *E–H*). That is, CTL generated from C3H → B10 × B10.BR chimeras that lysed BALB + H-2^b targets also lysed C3H + H-2^b targets (Table II *E*); however the C3H CTL from these chimeras which lysed BALB + H-2^k targets failed to lyse C3H + H-2^k targets (Table II *F*), presumably because these C3H T cell populations were tolerant to C3H minors + H-2^k. Thus, these data demonstrate in reciprocal chimera combinations that T cell populations contain pCTL that are reactive to self minor H antigens in other H-2 determinant contexts than those in which they encountered these antigens during their differentiation.

One final point raised by these experiments should be considered. It is clear that the lytic activity on C3H targets of BALB-specific B10 × B10.BR CTL was greater than the lytic activity on C3H targets of BALB-specific chimeric CTL (compare Table II Groups *A* and *C*; Groups *E* and *G*). This disparity most likely reflects the fact that the Parent → F_1 chimeric T cell populations have been partially tolerized to self C3H minor antigens in the context of host H-2 determinants since the chimeras may not have been completely devoid of (H-2^b × H-2^k)F_1 host APC during early T cell differentiation. However, the possibility cannot be ruled out that this disparity reflects the tolerization of a subpopulation of T cells to self C3H minor H antigens per se.

MHC-Restricted Tolerance to Self Minor H Antigens Is Also Expressed by Intrathymic T Cell Populations. In order to partially define the point in T cell differentiation that MHC-restricted tolerance to self minor H antigens is induced, we wished to next examine the reactivity of intrathymic T cells to minor H antigens. While thymocytes can be stimulated in vitro in the presence of added helper factors to generate allospecific and trinitrophenyl-modified self specific CTL (13, 14), it was not known whether thymocytes could be stimulated to generate anti-minor H–specific CTL responses even in the presence of added helper factors. Indeed, minor H–specific CTL responses cannot usually be elicited from unprimed T cell populations (10), and it is unclear whether intrathymic pCTL can be primed to exogenous antigens.

Nevertheless, we found that thymocytes from in vivo–primed mice did generate significant minor H–specific CTL responses in vitro in the presence of Con A SN and that thymocytes from unprimed mice did not (Table III). A detailed description of the ability of in vivo–primed thymocyte populations to generate

TABLE III

Generation of Minor H Specific CTL by Thymocytes from In Vivo Primed Animals

Responder cells	Spleen cells for:		Targets		
	In Vivo priming	In Vitro restimulation	E/T	B10.BR	C3H
				% Specific lysis	
C3H Thymocytes*	B10.BR	B10.BR	50	40	1
			25	31	2
			12	22	1
C3H Thymocytes*	None	B10.BR	50	−3	0
			25	−1	−2
			12	−2	−1
C3H Splenocytes	B10.BR	B10.BR	50	66	1
			25	68	1
			12	53	−1
C3H Splenocytes	None	B10.BR	50	4	4
			25	3	3
			12	2	0

*Cultures of thymocyte responders were supplemented with Con A SN 1/8 vol/vol.

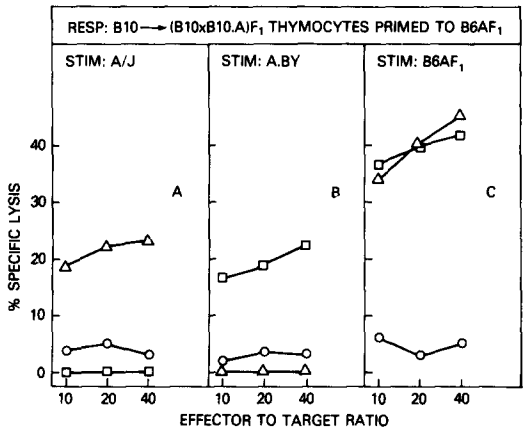

FIGURE 3. Parent → F_1 chimeric thymocytes can be primed to allogeneic minor H antigens on both parental haplotypes. CTL were generated from the indicated responder populations by in vivo priming with $B6AF_1$ spleen cells and by in vitro restimulation in the presence of Con A SN 1/8 vol/vol with 2000R irradiated stimulator spleen cells from A/J, A.BY or $B6AF_1$ animals. Targets were Con A–induced spleen blasts from A/J (△), A.BY (□), and B10 × B10.A (○) animals.

minor H–specific CTL will be given elsewhere.[2] Since it appeared that minor H–specific CTL could be generated from thymocyte populations, we wished to next determine whether thymocytes from Parent → F_1 chimeras could generate minor H–specific CTL restricted to the H-2 determinants of either the chimeric host or chimeric donor. To do so, B10 → B10 × B10.A chimeras were in vivo–

[2] Groves, E. S., S. O. Sharrow, and A. Singer. Manuscript in preparation.

TABLE IV

Tolerance of Thymocytes to Self Minor H Antigens is MHC Restricted

Expt.	Responder thymocytes‡	Spleen cells for: In Vivo priming	Spleen cells for: In Vitro restimulation	E/T	Targets*: C3H H-2:k	C3H.SW b	B10.BR k	B10 b	BR × C3H.SW k × b
							Specific lysis		
1	C3H.SW → B10 × B10.BR	B6 × C3H	B6 × C3H	80	28	−1	3	2	18
				40	20	0	0	2	10
				20	14	0	0	1	7
	B10 × B10.BR	B6 × C3H	B6 × C3H	80	53	47	−2	2	44
				40	51	33	−3	1	41
				20	40	29	−1	2	38
2	C3H.SW → B10 × B10.BR	B6 × C3H	B6 × C3H	100	33	−2	7	0	20
				50	29	−2	4	−1	20
				25	18	−2	3	1	17
	B10 × B10.BR	B6 × C3H	B6 × C3H	100	51	50	3	−2	49
				50	45	48	1	−3	38
				25	41	42	0	−3	37
3	C3H.SW → B10 × B10.BR	B6 × C3H	B6 × C3H	90	57	2	5	4	
				45	52	2	4	2	ND
				23	54	2	4	4	
	B10 × B10.BR	B6 × C3H	B6 × C3H	90	51	48	1	1	
				45	46	45	0	2	ND
				23	44	42	0	2	

* All target cells were female.

‡ Cultures of thymocyte responders were supplemented with Con A SN 1/8 vol/vol.

ND, not done.

primed with $B6AF_1$ spleen cells. Thymocytes from these chimeras were then restimulated in vitro in the presence of Con A SN with (*a*) A/J stimulators that expressed allogeneic A minor H antigens + host H-2^a determinants, (*b*) A.BY stimulators that expressed allogeneic A minor H antigens + donor H-2^b determinants, or (*c*) $B6AF_1$ stimulators that expressed allogeneic A minor H antigens + H-$2^{b/a}$ determinants (Fig. 3). It can be seen that Parent → F_1 chimeric thymocytes can generate minor H-specific CTL which are restricted to either donor or host H-2 determinants.

It was now possible to determine whether thymocytes from C3H.SW → B10 × B10.BR chimeras were reactive or tolerant to self C3H minors in the context of host H-2^k determinants, an antigen complex that most of these T cells had presumably not encountered during their differentiation. C3H.SW♀ → B10 × B10.BR♀ chimeras were in vivo primed with B6C3H♂ spleen cells so as to again utilize the male H-Y antigen as a potential carrier determinant for in vivo priming. The chimeric thymocyte populations were restimulated in vitro with B6C3H cells and were assayed on female target cells to preclude any possibility that the CTL generated were specific for H-Y. It can be seen in Table IV that such thymocyte populations did generate CTL that lysed C3H targets but not C3H.SW, B10.BR, or B10 targets demonstrating that the chimeric thymocytes were tolerant to all the complex specificities that they had encountered during their differentiation (i.e., Black + H-2^b, Black + H-2^k, C3H + H-2^b) but were

not tolerant to those complex specificities that they had not previously encountered (i.e., C3H + H-2^k). That the CTL which lysed C3H targets were specific for C3H minors + H-2^k was shown by the fact that they lysed B10.BR × C3H.SW target cells. The level of lysis of B10.BR × C3H.SW targets was somewhat lower than that on C3H target cells in all experimental groups, a finding consistent with the heterozygosity of these F_1 targets for the C3H minor H antigens as distinct from the homozygosity of C3H targets for the C3H minor H antigens. Thus, these data demonstrate that intrathymic T cell populations are also tolerant to self minor H antigens and that their tolerance to nominal self antigens is H-2 restricted.

Discussion

The induction of T cell tolerance to self antigens requires the functional inactivation of T cell precursors that are potentially reactive against self components. The present study has utilized CTL responses specific for minor H antigens as an experimental approach to determining whether recognition of self MHC determinants is involved in the induction of T cell tolerance to self antigens. It was demonstrated that T cell populations contain pCTL that are reactive against self minor H antigens in different H-2 determinant contexts than those in which the T cells encountered their self antigens during differentiation. Thus, it was observed that T cell populations are not solely tolerant to self antigens per se, but must be tolerant to self antigens in the context of self H-2. Such MHC-restricted T cell tolerance to self antigens was observed for both peripheral and intrathymic T cell populations.

C3H.SW → B10 × B10.BR radiation bone marrow chimeras were constructed for this study because T lymphocytes from such experimental animals, in principle, should have encountered self C3H minor H antigens only in the context of H-2^b determinants, and should be able to recognize and react against foreign nominal antigens presented in the context of either H-2^b or H-2^k determinants. Consequently, if the induction of T cell tolerance to self antigens were MHC restricted, T cell populations from C3H.SW → B10 × B10.BR chimeras should contain H-2^k–restricted pCTL specific for self C3H minor H antigens. However, in practice, it was possible that many of the H-2^k–restricted pCTL specific for self C3H minor H antigens could have been tolerized by encountering residual B10 × B10.BR APC, remaining either in the periphery or in the thymus of these chimeras, that had taken up and had presented shed C3H minor H antigens in the context of H-2^k determinants, thus reducing the number of pCTL specific for C3H minors + H-2^k. In an effort to maximize the likelihood of eliciting from C3H.SW → B10 × B10.BR T cell populations CTL specific for C3H minor H antigens + H-2^k that might have escaped such a potential tolerization mechanism, two maneuvers were performed. First, we utilized the male H-Y alloantigen as a potential carrier determinant for the in vivo priming of C3H + H-2^k specific T cells. It was observed that after such in vivo priming, CTL responses were elicited from the chimeric C3H.SW T cell populations that were specific for C3H minor H antigens + H-2^k. Second, we utilized the fact that minor H antigens are widely shared among different strains such that many BALB minor H antigens are also present on C3H cells. It was observed that CTL from C3H.SW → B10 × B10.BR

that were generated by stimulation with BALB minor H antigens + H-2^k lysed targets bearing C3H minor H antigens + H-2^k. Thus, both maneuvers resulted in the generation of C3H.SW CTL that were reactive against C3H minor H antigens + H-2^k, demonstrating that C3H.SW T cell populations from C3H.SW → B10 × B10.BR chimeras are, in fact, not tolerant to self C3H minor H antigens presented in the context of H-2^k determinants.

In contrast to the presence in C3H.SW → B10 × B10.BR of pCTL specific for C3H minors + H-2^k, no pCTL were detected in these chimeras which were specific for C3H minors + H-2^b, Black minors + H-2^b, or Black minors + H-2^k, even though these chimeras were primed in vivo and restimulated in vitro with B6 × C3H cells that expressed all of these antigenic combinations. These nonreactivities coincide with the antigenic complexes that were expressed in the chimeras and to which the developing T cells were exposed during their differentiation. While the nonreactivity of C3H.SW chimeric T cells against C3H minors + H-2^b could be argued to result from autologous cold target inhibition during effector phase of the CTL assay, such an explanation cannot account for the nonreactivity of these chimeric T cells against either Black minors + H-2^b or Black minors + H-2^k. Thus, it is most likely that these nonreactivities reflect the functional deletion of pCTL specific for these antigen H-2 complexes during their differentiation. Since the same T cell populations that were tolerant to C3H minor H antigens + H-2^b were not tolerant to C3H minor H antigens + H-2^k, it can be concluded that tolerance of these chimeric T cell populations to C3H minor H self antigens is MHC restricted. Furthermore, it should be noted that for C3H.SW → B10 × B10.BR chimeric T cells, C3H minor H antigens are self antigens probably expressed by the T cells themselves, since the target cells lysed by these chimeric CTL were T cells. Thus, it can be concluded from the present study that T cell tolerance to nominal self antigens is MHC restricted.

The results of the present study can be viewed from two perspectives with regard to the recognition requirements involved in the functional deletion, by whatever mechanism, of T cell precursors potentially reactive against self components. The first mechanism (referred to as the "H-2-signaling" model) requires that H-2 recognition be a necessary signal for triggering the clonal inactivation mechanism. In this model the H-2 molecule itself would act as a signal for triggering of the tolerization mechanism. Thus the recognition requirements for the functional deletion of immature T cells would be identical to the recognition requirements for the functional activation of mature T cells. In the second mechanism (referred to as the "simple exposure" model) simple binding of ligand by immature T cell precursors would be sufficient to lead to their functional deletion, whether or not the ligand included an H-2 determinant. For example, immature T cell precursors that only bound to self antigen alone would be deleted and those precursors that only bound to the complex of self antigen + self H-2 would be deleted, whereas those precursors that only bound the complex of self antigen + allogeneic H-2 would not be deleted. In the present experiments, this last population would be represented by those C3H.SW chimeric pCTL specific for C3H minors + H-2^k. Consequently, in this model some T cell precursors could be tolerized by having bound nominal antigen per se even though they could not be activated by nominal antigen per se. Thus, in the

simple exposure model the recognition requirements for the functional deletion of immature T cell precursors would not necessarily be identical with the recognition requirements for the functional activation of mature T cells. Regardless of which model is correct, the clear implication of the present data is that the functional inactivation of at least a subpopulation of self-reactive pCTL cannot be accomplished by recognition of nominal self antigen alone, but must involve simultaneous recognition of nominal self-antigen + self H-2 determinants.

The simplest way to envision the involvement of MHC recognition in the induction of T cell tolerance is from the perspective of a single receptor model of T cell recognition in which T cells recognize by one receptor an antigenic complex composed of nominal antigen + MHC. From this perspective, the complex of C3H minor H antigens + H-2^k would simply be another foreign antigenic complex recognized by reactive C3H.SW T cells, even though the nominal antigens in this case are self antigens probably expressed by the T cells themselves. Thus, the present study is consistent with the clonal deletion model of immune response gene regulation proposed by Schwartz (5), in which he suggested that MHC-linked nonresponsiveness to a nominal foreign antigen resulted from the deletion of T cell clones in the nonresponder animal as a consequence of being tolerant to self antigens + nonresponder MHC.

An esthetically less satisfying way to envision the involvement of MHC recognition in the induction of T cell tolerance is from the perspective of a dual receptor model of T cell recognition in which T cells recognize by distinct receptors nominal antigen and MHC. The present data preclude the possibility, as has been suggested (20), that all T cells are tolerized by simple binding via their antigen receptor and indicate that, for at least a subpopulation of pCTL, tolerization requires binding of both the antigen and H-2 receptors simultaneously. An additional constraint placed upon the dual receptor view is that it must now permit the existence of T cells that express antigen receptors specific for nominal antigens that the T cells themselves express. This theoretical problem has previously only existed for the anti-MHC receptor, in that antigen-specific CTL would express receptors specific for the recognition of H-2 determinants the CTL themselves express. Thus, while the present data do not exclude a dual receptor perspective, new limitations are placed upon the dual receptor model by these data, which may limit its conceptual usefulness.

The possibility that T cell tolerance to self antigens is MHC restricted has been addressed previously (6–8). Matzinger and Waterfield (6) addressed this issue by utilizing CTL responses specific for minor H antigens in radiation bone marrow chimeras in which the donor and host haplotypes were intentionally selected to differ in their minor H antigens. Unlike the present experiments, which utilized minor H–specific responses in Parent → F_1 chimeras and in which MHC-restricted self tolerance was observed, these investigators utilized F_1 → Parent chimeras and were unable to observe MHC-restricted self tolerance (6). The primary reason why the use of F_1 → Parent chimeras might have prevented these investigators from observing MHC-restricted self tolerance was noted by these investigators themselves, i.e. the minor H antigens of the chimeric host could have been shed and taken up by the long-lived F_1 APC derived from the donor inoculum, potentially tolerizing the developing F_1 T cells to host minor

H alloantigens in the context of both donor and host H-2 determinants. Thus, the difference in results between this earlier study and the present study most likely derives from differences in the H-2 haplotypes expressed by the long-lived donor-derived APC resident in $F_1 \rightarrow$ Parent vs. Parent $\rightarrow F_1$ chimeras. A more recent study, by Dos Reis and Shevach (8), utilized guinea pig T cell colonies specific for heterologous bovine insulin. It was found that a number of such T cell colonies were also reactive to a component of homologous guinea pig insulin when presented by allogeneic guinea pig APC. Thus this latter study is consistent with the concept that T cell tolerance to nominal antigens is MHC restricted.

In conclusion, the present study demonstrates that at least a component of T cell tolerance to self antigens is restricted by H-2–encoded determinants, and that H-2–restricted self tolerance is a feature of both postthymic and intrathymic T cell populations. Future studies will be directed at determining the role performed by intrathymic and prethymic elements in the induction of H-2-restricted T cell tolerance to self antigens.

Summary

The present study has utilized cytotoxic T lymphocyte (CTL) responses specific for minor histocompatibility (minor H) antigens as an experimental approach to determining whether recognition of self MHC determinants is involved in the induction of T cell tolerance to self antigens. It was observed that C3H.SW splenic T cells from C3H.SW $\rightarrow$ B10 × B10.BR radiation bone marrow chimeras contained CTL precursors (pCTL) reactive against self C3H minor H antigens + $H\text{-}2^k$ but were tolerant to self C3H minor H antigens + $H\text{-}2^b$. Precursor CTL with the reciprocal reactivity pattern were observed for C3H $\rightarrow$ B10 × B10.BR chimeras. In addition, it was observed that C3H.SW thymocytes from C3H.SW $\rightarrow$ B10 × B10.BR chimeras could generate minor H–specific CTL responses and were reactive against self C3H minor H antigens + $H\text{-}2^k$, but were tolerant to self C3H minor H antigens + $H\text{-}2^b$. Thus, the present study demonstrates that for peripheral and intrathymic T cell populations at least a component of T cell tolerance to self antigens is restricted by products of the MHC.

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