

T CELLS FROM FULLY H-2 ALLOGENEIC (A → B)
RADIATION BONE MARROW CHIMERAS ARE FUNCTIONALLY
COMPETENT AND HOST RESTRICTED BUT ARE
ALLOREACTIVE AGAINST HYBRID I_a DETERMINANTS
EXPRESSED ON (A × B)_{F₁} CELLS

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There exists a great deal of evidence (1-4) to support the concept that the H-2-restricted self-recognition repertoire expressed by T cells is not a function of the T cells' own genotype, but rather is determined by the H-2 phenotype of the thymic environment in which the T cells had differentiated. It is a necessary prediction of this "thymic hypothesis" (2-4) that T cells that had matured in a fully H-2-incompatible thymus would be functionally competent and would use as self-recognition elements the H-2 determinants of that thymus. However, some of the initial experiments examining the self-specificity of cytotoxic T lymphocytes (CTL) from fully allogeneic A → B radiation bone marrow chimeras failed to find any functionally competent CTL of donor origin, even when the chimeric T cells were sensitized to antigen in an irradiated short-term (A × B)_{F₁} host that should have provided a priming environment that expressed thymic type allogeneic B major histocompatibility complex (MHC) determinants (2, 3, 5). Consequently, the apparent failure of T cells to acquire functional competence in a fully allogeneic chimeric environment and to be sensitized to antigen in an appropriate priming environment represented a serious challenge to the thymic hypothesis.

However, in support of the thymic hypothesis and in contrast to these initial studies on CTL function, we recently reported (6) that T-helper (T_H) cells of donor bone marrow origin from fully allogeneic radiation bone marrow chimeras do acquire functional competence and do recognize and respond to antigen presented by accessory cells that express thymic type H-2 determinants. These experiments were performed in vitro using unprimed T_H cell populations and so avoided the necessity of exposing A → B chimeric T cells to antigen in a short-term irradiated F₁ host. Instead, A → B chimeric T cells were exposed in vitro to antigen presented by accessory cells of thymic H-2^B haplotype. Similar findings to ours (7) have subsequently also been reported for T cell proliferative responses from fully allogeneic chimeras. Thus, one critical difference between the experiments in which competent T cell function was observed (6, 7) and those performed originally in which competent T cell function was not observed (2, 3, 5) was the exposure of A → B allogeneic chimeric T cells to antigen presented by accessory cells that expressed thymus type homozygous B H-2

determinants rather than heterozygous (A × B)_{F1} determinants. This was intentionally done because of the possibility that T cells from A → B allogeneic chimeras, while tolerant to cells that expressed only parental H-2 determinants, might not be tolerant to cells that expressed (A × B)_{F1} determinants because A → B chimeric T cells had never been exposed during their development to the unique hybrid Ia determinants expressed by F₁ cells.

In this communication, it is demonstrated that (a) T cells from B10 → B10.A fully allogeneic chimeras are indeed alloreactive against unique hybrid Ia determinants that are expressed by (B10 × B10.A)_{F1} cells even though they are tolerant to the MHC determinants expressed by parental B10 and B10.A cells and (b) that CTL of donor bone marrow origin do differentiate into functional competence in a fully H-2-incompatible thymic environment. These findings suggest that the unsuccessful attempts to generate CTL from fully allogeneic chimeras by sensitizing them to antigen in an irradiated short-term F₁ host might have been confounded by an ongoing alloreaction against the hybrid Ia determinants expressed by F₁ cells.

Materials and Methods

Animals. C57BL10/Sn (B10), B10.A, B10.D2, B10.BR, B10.A(4R), and (B10 × B10.A)_{F1} mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.A(5R) and (B10.A(4R) × B10)_{F1} mice were provided by Dr. David H. Sachs, National Institutes of Health, Bethesda, MD.

Radiation Bone Marrow Chimeras. Radiation bone marrow chimeras are designated as donor bone marrow → irradiated recipient and were constructed as previously described (6). Briefly, recipient mice were irradiated with 950 rad cesium and were reconstituted with 12–15 × 10⁶ bone marrow cells that had been depleted of T cells by pretreatment with rabbit anti-mouse brain serum, a reagent specifically cytotoxic for T cells (6). All mice used in the construction of these chimeras were housed in a limited access facility in which the long-term survival of fully allogeneic chimeras was 60%. Chimeras were rested for at least 2 mo after irradiation and bone marrow reconstruction before use. Spleen cells as well as thymocytes from such chimeras have been extensively typed with strain-specific reagents by immunofluorescence on a fluorescence-activated cell sorter and have consistently been found to be essentially all (>98%) of donor bone marrow origin (8).

Mixed Lymphocyte Reaction (MLR). 4 × 10⁵ responder spleen cells were cultured with 4 × 10⁵ 2,000 rad irradiated spleen stimulator cells in a volume of 0.2 ml for 4 d, as previously described (6). Cultures were pulsed with 1 μCi [³H]thymidine 8–12 h before harvest. Each experimental point represents the mean response of three replicate cultures.

Generation of CTL. 4 × 10⁶ responder spleen cells were cultured with 4 × 10⁶ irradiated spleen stimulator cells in a volume of 2 ml, as previously described (9). Trinitrophenyl (TNP) modification of stimulator and target cells was performed with 10 mM trinitrobenzene sulfonate as described (9). After 5 d, the cultures were assayed for CTL generation by their ability to lyse ⁵¹Cr-labeled concanavalin A-induced splenic blasts as target cells in a 4-h ⁵¹Cr-release assay.

Anti-H-2K^k Plus Complement (C) Treatment of Spleen Cells. Monoclonal anti-H-2K^k reagent was a culture supernatant of the hybridoma 11-4.1, described by Oi et al. (10). 5 × 10⁶ cells/ml were treated with a 1/4 dilution of this reagent for 30 min at 37°C, followed by treatment with a 1/6 dilution of rabbit C for an additional 30 min at 37°C. This treatment lysed >97% of B10.BR spleen cells, while recovery of non-H-2K^k-treated spleen cells ranged from 60–80% and was not different from the C control.

Results and Discussion

The Ia molecules expressed on the cell surface are each composed of two polypeptide chains, termed β and α (11). It has been shown that the I-A subregion not only encodes the β and α chains of the I-A molecule (designated A_β and A_α), but it also

TABLE I
T Cells from Fully Allogeneic Chimeras Are Reactive against Determinants Expressed on Hybrid Ia Molecules

Irradiated stimulator spleen cells	Haplotype origin of I-A and I-E molecules*		Responder spleen cells			
	$A_{\beta}A_{\alpha}$	$E_{\beta}E_{\alpha}$	B10 → B10.A	(B10 × B10.A) F_1	B10	B10.A
			$\Delta \text{cpm} \times 10^{-3} \ddagger$			
B10	b b	None	0.0	0.8	0.0	23.0
B10.A	k k	k k	0.9	0.5	27.9	0.0
B10.D2	d d	d d	17.5	15.3	38.2	28.9
B10 × B10.A	b b	k k	37.8	0.2	31.8	21.3
	k k	<u>b k</u>				
	<u>b k</u>					
	<u>k b</u>					
B10.A(4R) × B10	k k	None	36.1	0.0	23.7	23.2
	b b					
	<u>b k</u>					
	<u>k b</u>					
B10.A(5R)	b b	<u>b k</u>	17.3	0.8	17.6	21.9

* Underlined Ia dimers represent Ia molecules that are encoded by I^k and I^b genes but that are not expressed on the surface of homozygous H-2^a or H-2^b cells.

‡ Experimental cpm minus media cpm. Background media responses were <2,000 cpm.

encodes the β chain of the I-E molecule (designated E_{β}). In contrast, the I-E subregion only encodes the α chain of the I-E molecule (designated E_{α}). Thus, cells from H-2^a mice express two sets of I region-encoded determinants on their surface, an I-A molecule composed of the dimer $A_{\beta}^k A_{\alpha}^k$ and an I-E molecule composed of the dimer $E_{\beta}^k E_{\alpha}^k$ (Table I). However, in some strains, such as H-2^b, the I-E subregion does not encode any gene product (11). In such strains, even though their cells do synthesize an E_{β}^b polypeptide, it is not expressed on the cell surface in the absence of an E_{α} polypeptide (11). Consequently, cells from H-2^b mice express on their surface only an I-A molecule that is composed of the dimer $A_{\beta}^b A_{\alpha}^b$ (Table I). Individual cells from an H-2^b × H-2^a not only express on their surface all three of the Ia molecules expressed by both parents (i.e., $A_{\beta}^k A_{\alpha}^k$, $A_{\beta}^b A_{\alpha}^b$, and $E_{\beta}^k E_{\alpha}^k$) but, in addition, also express Ia molecules that are dimers composed of one polypeptide chain encoded by each parental genome, i.e., $A_{\beta}^k A_{\alpha}^b$, $A_{\beta}^b A_{\alpha}^k$, and $E_{\beta}^b E_{\alpha}^k$. To examine the possibility that T cells from A → B fully allogeneic chimeras are alloreactive to determinants created by such hybrid Ia molecules, as are normal homozygous T cells (12), spleen cells from fully allogeneic B10 → B10.A chimeras were stimulated in vitro with stimulator cells expressing such determinants.

As can be seen in Table I, responding B10 → B10.A chimeric T cells, like (B10 × B10.A) F_1 T cells, were tolerant to B10 and B10.A stimulator cells. However, unlike F_1 responding cells, the chimeric T cells were strongly reactive against F_1 stimulator cells. To determine whether the chimeric T cells were alloreactive against hybrid $A_{\beta}A_{\alpha}$ I-A molecules as well as against hybrid $E_{\beta}E_{\alpha}$ I-E molecules, B10 → B10.A chimeric T cells were also stimulated with (B10.A(4R) × B10) F_1 cells that express only hybrid $A_{\beta}A_{\alpha}$

TABLE II
*TNP-specific CTL Responses Mediated by T Cells That Had Differentiated in a Fully
 H-2 Allogeneic Chimeric Host Can Be Elicited by TNP-modified Stimulators of Host H-2 Type
 but Not Donor H-2 Type**

Responder spleen cells	Treatment of responders	Effector to target cell ratio	Stimulator/target			
			B10-TNP	B10.BR-TNP	B10	B10.BR
			% specific ⁵¹ Cr release‡			
B10 × B10.BR	—	60	45	45	-2	0
		20	26	32	-3	-2
		7	10	24	-4	-2
B10.BR → B10	—	60	30	3	-4	-3
		20	21	2	-4	-5
		7	12	0	-4	-5
B10 → B10.BR	—	60	6	45	-2	-3
		20	0	29	-3	-5
		7	1	22	0	-1
B10 → B10.BR	C	60	4	51	-4	0
		20	-2	34	-3	-2
		7	0	26	-2	-2
B10 → B10.BR	anti-H-2K ^k + C	60	-2	47	-2	0
		20	-2	34	-2	-3
		7	-1	23	-3	-1

* This experiment is representative of over 30 individual experiments conducted over a 2-yr period.

‡ Results represent the means of triplicate determinations using 5×10^3 ⁵¹Cr-labeled target cells. Spontaneous release in presence of medium was always <20%; maximum release varied from 2,000 to 3,000 cpm for all targets.

I-A molecules and with B10.A(5R) cells that express only hybrid E_βE_α I-E molecules. As can be seen in Table I, B10 → B10.A responding cells reacted against both types of stimulator cells, whereas (B10 × B10.A)F₁ responder cells were tolerant to both types of stimulator cells. Thus, T cells from fully allogeneic A → B chimeras are alloreactive against both hybrid A_βA_α I-A molecules and hybrid E_βE_α I-E molecules that are expressed on F₁ cells.

Because these results suggested that previous failures to generate CTL from fully allogeneic chimeras by sensitizing them in an irradiated F₁ host would have been complicated by an ongoing in vivo alloreaction against the hybrid Ia determinants of the F₁ host, we examined the possibility that functionally competent CTL might be generated from the spleens of fully allogeneic chimeras if they were sensitized to antigen on homozygous stimulator cells. Consequently, spleen cells from B10 → B10.BR and B10.BR → B10 fully allogeneic chimeras were sensitized in vitro to TNP-modified stimulator cells of either donor or host haplotype. As can be seen in Table II, CTL responses were generated from these chimeric spleen cell populations, but only upon stimulation with TNP-modified stimulator cells of the host haplotype. These CTL responses were mediated by cells of donor bone marrow origin and not by residual host cells because pretreatment of responding B10 → B10.BR spleen cells with anti-H-2K^k + C had no effect (Table II), even though such treatment did lyse >97% of control B10.BR spleen cells treated simultaneously. Thus, anti-TNP-self CTL of donor bone marrow origin do differentiate into functional competence in a

fully allogeneic chimeric environment, and their activation is restricted to the recognition of TNP in association with the H-2 determinants of the chimeric host.

In and of themselves, these results do not establish with certainty that previous failures to generate anti-viral CTL from fully allogeneic chimeras by sensitizing them to virus in an irradiated F₁ host were due to alloreactions against the hybrid Ia determinants expressed in the F₁ host. Indeed, allogeneic effects can either positively or negatively influence different immune responses such that the influence of allogeneic effects on in vitro anti-TNP CTL responses would not necessarily be relevant for assessing their influence on in vivo anti-viral CTL responses. However, data in support of the concept that the allogeneic effects resulting from alloreactions against hybrid Ia determinants do interfere with the in vivo generation of anti-viral CTL were recently reported (13). Specifically, it was observed that even though anti-viral CTL from fully allogeneic (A → B) thymic chimeras were not generated by sensitizing them to virus in an irradiated F₁ host, anti-viral CTL from the same chimeras were successfully generated by sensitizing them to virus in a homozygous strain B host that did not express hybrid Ia determinants.

In conclusion, the present results demonstrate that the requirements for generating CTL responses from fully H-2-incompatible chimeras do conform to predictions of the thymic hypothesis and suggest that previous failures to sensitize CTL from fully allogeneic chimeras to antigen in a short-term irradiated F₁ host might have been complicated by an in vivo alloreaction against hybrid Ia determinants expressed in that F₁ host. Indeed, the present study demonstrates that allogeneic chimeras offer a potent tool for studying the T cell recognition of determinants created by such hybrid Ia molecules.

Summary

In this communication it is demonstrated that T cells from fully allogeneic A → B radiation bone marrow chimeras are alloreactive against the hybrid Ia molecules expressed on the surface of heterozygous A × B cells. These results suggested that previous failures to generate cytotoxic T lymphocyte (CTL) responses from fully allogeneic chimeras by sensitizing the chimeric T cells to antigen in an (A × B)F₁-priming environment might have been confounded by an ongoing alloreaction against determinants created by hybrid Ia molecules expressed on F₁ cells. Consequently, the ability to generate CTL responses from fully allogeneic chimeras was re-examined by sensitizing the chimeric T cells to antigen presented by homozygous rather than F₁ stimulator cells. It was found that T cells of donor bone marrow origin that mediate cytotoxic responses to trinitrophenyl-modified self determinants do differentiate into functional competence in an H-2-incompatible host environment and are restricted to the host H-2 haplotype.

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