

CYTOTOXIC T LYMPHOCYTE RESPONSES IN ALLOGENEIC RADIATION BONE MARROW CHIMERAS

The Chimeric Host Strictly Dictates the Self-Repertoire of Ia-restricted T Cells but Not H-2K/D-restricted T Cells

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The major histocompatibility complex (MHC)¹-encoded determinants that T cells recognize for responses to conventional (non-MHC) antigens are referred to as "self"-determinants and are not necessarily identical with the MHC determinants the T cells themselves express. Indeed, experiments with T cells from thymus-engrafted radiation bone marrow chimeras have demonstrated that the polymorphic MHC determinants that T cells recognize as self-determinants are those that they encountered on radiation-resistant thymic elements during their differentiation (1, 2). These experiments suggested the concept, referred to as the "thymic hypothesis," that the self-specificity of all MHC-restricted T cells is determined by the MHC phenotype of the thymus in which the T cells had differentiated.

MHC-restricted T cells can be divided on the basis of their specificity into two distinct subsets, those that are specific for the self-recognition of class I MHC determinants (e.g., H-2K, D, L, and Qa-encoded determinants) and those that are specific for the self-recognition of class II MHC determinants (e.g., Ia-encoded gene products). A large body of experimental evidence has been accumulated that demonstrates that the self-specificity of class II (Ia)-restricted T helper (T_H) cells for antibody responses conforms strictly to predictions of the thymic hypothesis (3-7). In contrast, there is a great deal of controversy regarding the influence of the thymus on the self-specificity of class I (H-2K/D)-restricted T cells that mediate cellular cytotoxicity (8-11). Indeed, H-2K/D-restricted cytotoxic T lymphocytes (CTL) have even been demonstrated in the spleens of congenitally athymic nude mice, which appear to lack a functioning thymus altogether (12, 13). Consequently, because of the discrepant results regarding the influence of the thymus on the self-specificity of H-2K/D-restricted CTL, the validity of the thymic hypothesis has been seriously questioned.

However, it seemed conceivable that the thymus might play a critical role in determining the self-specificity of Ia-restricted T cells, but might play a less central role in determining the self-specificity of H-2K/D-restricted T cells. To assess this possibility directly, it was necessary to examine the self-specificity of both Ia- and

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¹ *Abbreviations used in this paper:* C, complement; Con A SN, concanavalin A-induced spleen cell supernatants; CTL, cytotoxic T lymphocytes; MHC, major histocompatibility complex; pCTL, cytotoxic T lymphocyte precursors; RAMB, rabbit anti-mouse brain serum; T_H, T helper; TNP, trinitrophenyl.

H-2K/D-restricted T cells from the same animals in a single immune response, which required the participation of both T cell subpopulations. Consequently, the present study has used radiation bone marrow chimeras to assess the self-MHC recognition requirements for the *in vitro* generation of CTL to trinitrophenyl (TNP)-modified self determinants. The results of this study demonstrate that: (a) the *in vitro* generation of primary anti-TNP-specific and H-2K/D-restricted CTL requires the participation of self-Ia-specific T cells; and (b) the self-specificity of the Ia-restricted T cells is stringently restricted to the Ia determinants of the chimeric host, whereas the self-specificity of the H-2K/D-restricted T cells is not stringently restricted to the H-2K/D determinants of the chimeric host. Thus, these results demonstrate in a single immune response that the thymic hypothesis accurately predicts the specificity of T cells restricted to the self-recognition of class II Ia determinants but does not completely predict the specificity of T cells restricted to the self-recognition of class I H-2K/D determinants.

Materials and Methods

Animals. C57Bl/10sn (B10), B10.BR, (B10 × B10.BR)_{F1}, B10.A(2R), and C3H/HeJ mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.QBR, B10.MBR, B10.AQR, and B10.T(6R) mice were provided by Dr. David Sachs, National Institutes of Health, Bethesda, MD. 8–12-wk-old male mice were used in all experiments. The H-2 haplotypes of the mice used in this study are shown in Table I.

Radiation Bone Marrow Chimeras. Radiation bone marrow chimeras are designated as bone marrow donor → irradiated recipient and were constructed as previously described (5). Recipient mice were irradiated with 950 rad from a ¹³⁷Cs 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 (RAMB), a reagent selected to be specifically cytotoxic for all T cells (14), plus complement (C). RAMB plus C treatment of the bone marrow cell populations completely abrogated their ability to proliferate in response to the T cell mitogen phytohemagglutinin (15). Spleen cells were obtained from each chimera no earlier than 2 mo after irradiation and bone marrow reconstitution. Such chimeras were >95% of donor bone marrow origin as assessed by indirect immunofluorescence using strain-specific anti-H-2 reagents. All chimeric cell populations were specifically tolerant to both donor and host MHC determinants as assessed by both cell mediated lympholysis and mixed lymphocyte proliferation.

Anti-H-2K^k plus 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. (16). 5×10^6 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

TABLE I
H-2 Haplotypes of the Mice Used in This Study

Strain	Alleles		
	K	I	D
B10	b	b	b
B10.BR	k	k	k
B10.A(2R)	k	k/d	b
B10.QBR*	b	b	q
B10.MBR	b	k	q
B10.AQR	q	k/d	d
B10.T(6R)	q	q	d

* Sachs, D. H., manuscript in preparation.

cells, whereas recovery of treated B10 spleen cells was 80% and was not different from treatment with C alone.

Preparation of Concanavalin A-induced Supernatant (Con A SN). Con A SN was used as a source of soluble helper factors and was prepared from BALB/c spleen cells as previously described (17). The Con A SN was always supplemented with 0.2 M alpha-methyl-D-mannoside to neutralize the remaining Con A. Con A SN was used at a 25% vol/vol concentration in culture.

Generation of CTL. 5×10^6 responder spleen cells were cultured with 5×10^6 2,000-rad-irradiated spleen stimulator cells in a volume of 2 ml as previously described (18). TNP modification of stimulator and target cells was performed with 10 mM trinitrobenzene sulfonate (18). After 5 d, the cultures were assayed for CTL generation by their ability to lyse ^{51}Cr -labeled concanavalin A-induced splenic blasts as target cells in a 4-h ^{51}Cr release assay. Percent specific ^{51}Cr -release = (experimental - spontaneous release)/(maximum - spontaneous release) $\times 100$. Passage of responding spleen cells over G-10 Sephadex columns was performed as previously described (19).

In Vivo Priming of TNP-specific T_H Cells. Mice were skin painted with 7% trinitrochlorobenzene in 4:1 acetone-olive oil by a single application of 100 ml of this solution on shaved abdominal skin. Spleen cells from these mice were used as a source of TNP-specific T_H cells 2-3 wk after in vivo priming and were irradiated with 750 rad immediately before addition to culture.

Results

Host-restricted and TNP-specific CTL Responses from H-2 Fully Allogeneic Radiation Bone Marrow Chimeras Are Mediated by T Cells of Donor Bone Marrow Origin. It has recently been shown that T cells from H-2 fully allogeneic radiation bone marrow chimeras are competent but that their activation requires the recognition of conventional antigens in the context of host type MHC determinants (5, 20). To confirm these findings, spleen cells from B10.BR \rightarrow B10 and B10 \rightarrow B10.BR fully allogeneic chimeras were stimulated in vitro with TNP-modified B10 and B10.BR stimulator spleen cells and assayed for the generation of TNP-specific CTL. It can be seen in Fig. 1 that CTL were generated from the spleens of B10.BR \rightarrow B10 chimeric spleens upon stimulation with TNP-modified host type H-2^b spleen cells, but not upon

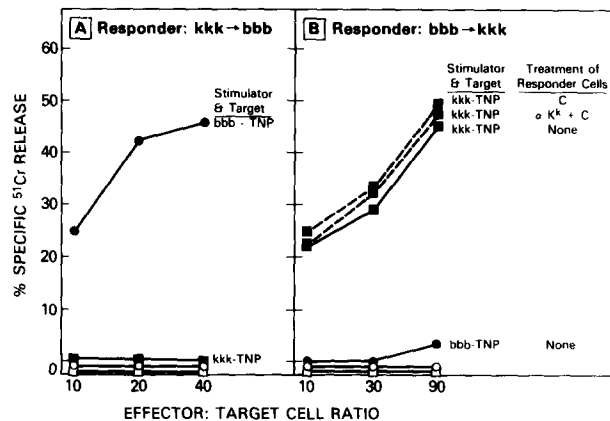


FIG. 1. T cells of donor origin from H-2 fully allogeneic radiation bone marrow chimeras are competent but restricted to host MHC determinants. Spleen cells from B10.BR \rightarrow B10 (A) or B10 \rightarrow B10.BR (B) fully allogeneic chimeras were stimulated in vitro with either B10-TNP (\circ) or B10.BR-TNP (\square) and assayed on either TNP-modified (closed symbols) or unmodified (open symbols) target cells. Some of the responder cells were treated with either C alone or anti-K^k + C as indicated.

stimulation with TNP-modified donor-type H-2^k spleen cells. Analogous results were obtained by stimulation of spleen cells from reciprocal B10 → B10.BR chimeras. These responses were TNP-specific in that the CTL only lysed target cells that had been modified with TNP. It should be noted that all the chimeras used in this study were judged to be specifically tolerant to both donor and host MHC determinants by their failure to either proliferate or generate CTL in response to unmodified stimulator cells expressing either donor or host-type MHC determinants (data not shown; 5, 20).

Even though significant numbers of residual cells of host origin were not detected in these chimeras by immunofluorescence, it was also demonstrated in the same experiment that the CTL responses generated from the spleens of fully allogeneic chimeras were mediated by cells of donor bone marrow origin. B10 → B10.BR chimeric spleen cells were pretreated with anti-H-2K^k plus C, a treatment that lysed >97% of normal B10.BR spleen cells that were treated in parallel with the chimeric spleen cells. It can be seen in Fig. 1 that even though such pretreatment should have eliminated any B10.BR host cells present, it had essentially no effect on the CTL responses generated from the spleens of B10 → B10.BR fully allogeneic chimeras.

These results confirm that donor-derived T cells from H-2 fully allogeneic chimeras are competent to generate TNP-specific CTL and that their generation strictly requires TNP-modified stimulators that express the MHC determinants of the chimeric host.

Existence of a Stringent Requirement for Recognition of Host Ia Determinants. To explore the possibility that the in vitro generation of anti-TNP CTL requires the participation of Ia-specific T cells, H-2 fully allogeneic chimeras were constructed using strain combinations for which intra-H-2 recombinant mice existed. Thus, B10.A(2R) → B10.T(6R) fully allogeneic chimeras were constructed and are designated by their H-2 K, I, D alleles as k,k/d,b → q,q,d (see Table I). Spleen cells from these chimeras were stimulated in vitro by TNP-modified stimulator cells of the host (q,q,d) or of B10.AQR (q,k/d,d), which expressed the K^qD^d determinants of the chimeric host but the Ia^{k/d} determinants of the chimeric donor. The chimeric spleen cells responded to host-type TNP-stimulators, but did not respond to B10.AQR TNP stimulators (Fig. 2A). The B10.AQR TNP-stimulator cells were immunogenic because they did stimulate the generation of TNP-specific CTL from the spleens of normal syngeneic B10.AQR responder mice (Fig. 2B). Since the only difference between the two stimulator cell populations was that the effective q,q,d TNP-stimulator cells expressed host I^q determinants, whereas the ineffective q,k/d,d TNP-stimulator cells expressed donor I^{k/d} determinants, it can be concluded that (a) the in vitro generation of TNP-specific CTL effectors requires the participation of Ia-restricted T cells, and (b) the self-specificity of these Ia-restricted T cells is stringently restricted to recognition of host Ia determinants.

In Vitro Generation of TNP-specific CTL Strictly Requires Recognition of Host Ia Determinants but Does Not Strictly Require Recognition of Host H-2K/D Determinants. The above experiment demonstrated that the in vitro generation of anti-TNP CTL effectors strictly required recognition of host Ia determinants. To explore whether a similarly stringent requirement also exists for recognition of host H-2K/D determinants, B10.T(6R) → B10.A(2R) fully allogeneic chimeras were constructed and are designated as q,q,d → k,k/d,b chimeras. Spleen cells from these chimeras were stimulated in vitro with TNP-modified host type (k,k/d,b), donor type (q,q,d), or B10.AQR

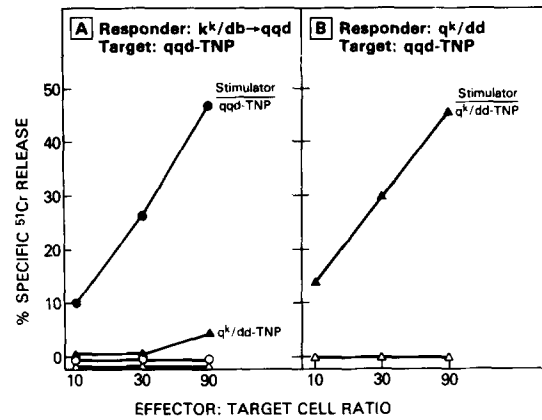


FIG. 2. Generation of anti-TNP CTL stringently requires recognition of host Ia determinants. (A) Spleen cells from B10.A(2R) \rightarrow B10.T(6R) chimeras were stimulated with either B10.T(6R)-TNP (\square) or B10.AQR-TNP (Δ) and assayed on either TNP-modified (closed symbols) or unmodified (open symbols) B10.T(6R) target cells. (B) B10.AQR spleen cells were stimulated with B10.AQR-TNP (Δ) or B10.T(6R)-TNP (\square) and assayed on either TNP-modified (closed symbols) or unmodified (open symbols) B10.T(6R) target cells.

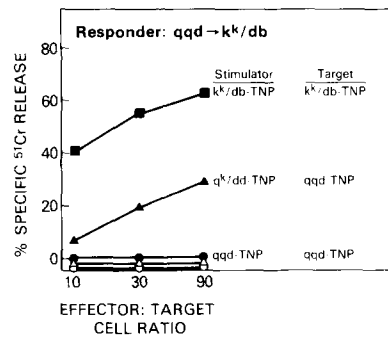


FIG. 3. Generation of anti-TNP CTL strictly requires recognition of host Ia determinants, but not host H-2K/D determinants. Spleen cells from B10.T(6R) \rightarrow B10.A(2R) chimeras were stimulated with B10.A(2R)-TNP (\square), B10.AQR-TNP (Δ), and B10.T(6R)-TNP (\square) and assayed on TNP-modified (closed symbols) or unmodified (open symbols) targets.

(q,k/d,d) stimulator spleen cells (Fig. 3). As expected the chimeric spleen cells did not respond to TNP stimulators of donor type, but did respond to TNP stimulators of host type (Fig. 3). In addition, the chimeric spleen cells also responded to B10.AQR TNP stimulators, albeit not nearly so well as they did to host type TNP stimulators (Fig. 3). Thus, CTL effectors were only generated by the chimeric spleen cells in response to TNP-modified stimulators that expressed host Ia^{k/d} determinants; however, if the TNP-modified stimulator cells did express host Ia^{k/d} determinants, CTL effectors were generated whether the stimulators expressed host K^kD^b determinants or donor K^qD^d determinants. It should be noted that the CTL effectors generated from the chimeric spleen cells in response to B10.AQR (q,k/d,d) TNP stimulators were not simply specific for host Ia^{k/d} determinants since the CTL in this experiment lysed q,q,d TNP target cells (Fig. 3).

This experiment demonstrates that, for the generation of TNP-specific CTL, there exists a stringent requirement for recognition of host Ia determinants, but there exists

only a relative preference for recognition of host H-2K/D determinants. The relative preference for host H-2K/D determinants observed in this experiment was, nevertheless, quite real since it cannot be simply accounted for by differences in the immunogenicity of K^kD^b and K^dD^d alleles. Indeed, in this experiment, k,k/d,b TNP stimulators and q,k/d,d TNP stimulators stimulated quantitatively similar TNP-specific CTL responses from the spleens of appropriate syngeneic responder mice (data not shown).

The TNP-specific CTL Effectors Generated in Response to TNP Stimulators Expressing Donor-Type H-2K/D Determinants Are Restricted to the Recognition of TNP in Association with Donor-Type H-2K/D Determinants. To determine the H-2 specificity of the CTL effectors generated in response to TNP-modified stimulator cells expressing nonthymic H-2K/D determinants, fully allogeneic B10.QBR \rightarrow B10.BR chimeras were constructed and are referred to by their K,I,D alleles as b,b,q \rightarrow k,k,k. Spleen cells from these chimeras were stimulated in vitro with TNP-modified donor-type (b,b,q TNP) or B10.MBR (b,k,q TNP) stimulator spleen cells. The specificity of the CTL effectors generated from each culture was then assayed on both k,k,k TNP and b,k,q TNP targets, which only differ in their H-2K/D alleles. It can be seen that no CTL of either specificity were generated by stimulation with donor-type TNP stimulators (Fig. 4A). In contrast, CTL effectors were generated in response to B10.MBR TNP stimulators that express host I^k determinants (Fig. 4B). It can also be seen in Fig. 4B that the anti-TNP CTL effectors generated in response to recombinant b,k,q TNP stimulators were specific for TNP-modified nonthymic K^bD^q determinants since they lysed only b,k,q TNP and not k,k,k TNP targets.

Thus, the CTL that are generated in response to TNP-modified stimulators expressing nonthymic type H-2K/D determinants are restricted to the recognition of TNP in association with those nonthymic type H-2K/D determinants. It can be concluded that once the strict requirement for recognition of host Ia determinants is fulfilled, H-2K/D-restricted CTL can be generated that recognize antigen in association with polymorphic H-2K/D determinants that are not expressed by the host thymus.

The Failure of Chimeric Spleens to Respond to Stimulators Expressing Donor Ia Determinants Is Not Due to Demonstrable Suppression. The observation that chimeric spleen cells could respond to stimulators expressing donor H-2K/D determinants but not to stimulators expressing donor Ia determinants suggested that the chimeric spleens might contain

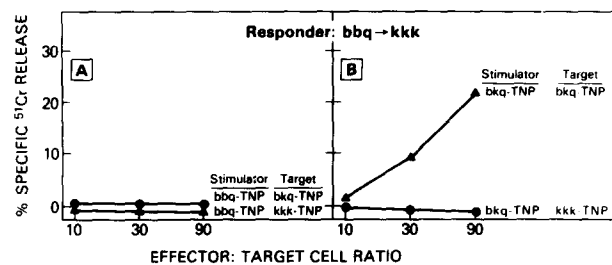


FIG. 4. Anti-TNP CTL generated in response to donor-type stimulators are restricted to donor-type H-2K/D determinants. Spleen cells from B10.QBR \rightarrow B10.BR chimeras were stimulated with either B10.QBR-TNP (A) or B10.MBR-TNP (B) and assayed on both B10.MBR-TNP and B10.BR-TNP targets. Specific lysis of unmodified targets was $<1\%$.

haplotype-specific suppressor cells which were specific for donor Ia determinants. To examine this possibility, the ability of B10.QBR \rightarrow B10.BR (b,b,q \rightarrow k,k,k) chimeric spleen cells to suppress the response of normal B10.QBR responders to B10.QBR TNP stimulators was examined. The B10.QBR TNP stimulators express donor I^b determinants and so should trigger haplotype-specific suppressors in the chimeric cell population if they are present. It can be seen in Table II that the chimeric spleen cells did not themselves respond to B10.QBR TNP stimulators and did not suppress the ability of normal spleen cells to respond. These results are consistent with previous failures to detect the presence of haplotype-specific suppressor cells in chimeras (21, 22). Thus, these results suggest that the failure of chimeric spleen cells to respond to stimulators expressing donor Ia determinants is not due to active suppression, but rather reflects the failure of T cells specific for the self-recognition of donor Ia determinants to differentiate into functional competence in an allogeneic differentiation environment.

Accessory Cells Can Fulfill the Stringent Requirement for Expression of Host Ia Determinants. Next, we examined whether the chimeric T cells specific for the self-recognition of host Ia determinants could be activated by recognition of host Ia determinants expressed by unmodified accessory cells as well as by TNP-modified stimulator cells. To do so, the responsiveness of spleen cells from fully allogeneic chimeras against TNP-modified stimulators of donor type was assessed in the presence and absence of unmodified accessory cells that expressed host Ia determinants. It can be seen in Fig. 5 that B10.T(6R) \rightarrow B10.A(2R) (q,q,d \rightarrow k,k/d,b) chimeric spleen cells did respond to donor type B10.T(6R) (q,q,d) TNP stimulators, but only in the presence of added accessory cells expressing host I^{k/d} determinants. In fact, stimulation with q,q,d TNP stimulators in the presence of accessory cells expressing host I^{k/d} determinants was as effective as stimulation with q,k/d,d TNP stimulators alone (Fig. 5). Thus, this

TABLE II
Failure to Detect the Presence of Haplotype-specific Suppressors in the Spleens of Fully Allogeneic Chimeras

Normal B10.QBR responders	B10.QBR \rightarrow B10.BR spleen cells	B10.QBR-TNP stimulators	Effector: target ratio	Percent specific ⁵¹ Cr release*
7.5×10^6 b,b,q	—	b,b,q TNP	90	40
			30	33
			10	20
5×10^6 b,b,q	—	b,b,q TNP	90	30
			30	26
			10	16
—	5×10^6 b,b,q \rightarrow k,k,k	b,b,q TNP	90	-5
			30	-4
			10	-5
5×10^6 b,b,q	2.5×10^6 b,b,q \rightarrow k,k,k	b,b,q TNP	90	36
			30	24
			10	15

* Targets were B10.MBR TNP (b,k,q TNP), which express K^bD^q determinants. Specific lysis of unmodified B10.MBR targets was <1%.

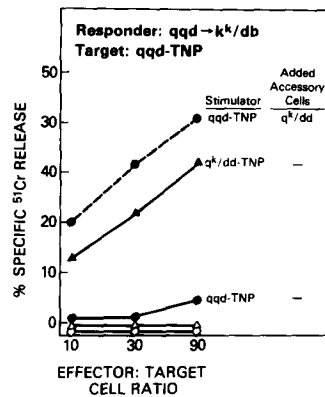


FIG. 5. Unmodified accessory cells fulfill the strict requirement for host Ia determinants. Spleen cells from B10.T(6R) → B10.A(2R) chimeras were stimulated with either B10.T(6R)-TNP (●) or B10.AQR-TNP (△) that had been pretreated with monoclonal anti-Thy-1.2 + C and assayed on either TNP-modified (closed symbols) or unmodified (open symbols) B10.T(6R) targets. Where indicated, B10.AQR spleen cells that had been pretreated with anti-Thy-1.2 + C and irradiated with 2,000 rad were added as a source of accessory cells.

experiment demonstrates that the presence in culture of accessory cells expressing host Ia determinants is sufficient to fulfill the strict requirement for host Ia determinants. In addition, although the simple transfer of TNP to the surface of the accessory cells from the surface of the stimulator cells is not excluded in this experiment, this result does suggest that accessory cells can reprocess TNP-modified stimulator cell surface determinants and efficiently present them in the context of the accessory cells' Ia determinants. This issue will be explored in greater detail elsewhere.

T_H Cells Can Fulfill the Strict Requirement for Ia Recognition. As an alternative to providing accessory cells expressing host Ia determinants, it might be possible to trigger donor-restricted anti-TNP CTL by the addition to culture of T_H cells capable of recognizing donor Ia determinants. To assess this possibility, in vivo TNP-primed C3H (H-2^k) spleen cells were irradiated and used in culture as a source of T_H cells. These irradiated T_H cell populations did not generate any CTL when cultured with the stimulator cells alone (data not shown). However, the addition of TNP-primed H-2^k T_H cells to cultures of responding B10.BR → B10 chimeric spleen cells (which are also genotypically H-2^k) resulted in the activation of anti-TNP CTL from the chimeras in response to donor type B10.BR-TNP stimulators (Fig. 6). It should be noted that responses were generated only upon the addition of irradiated T_H cells that had previously been primed to TNP. Thus, for the generation of anti-TNP CTL, the strict requirement for recognition of host Ia determinants can be circumvented by TNP-primed and radiation-resistant T_H cells specific for donor MHC determinants. Since this experiment demonstrates that their function can be replaced by TNP-primed T_H cells, it is likely that the chimeric T cells that are restricted to the recognition of host Ia determinants function as T_H cells for the generation of H-2K/D-restricted anti-TNP CTL responses.

Soluble Helper Factors Circumvent the Strict Requirement for Ia Recognition. If the Ia-restricted chimeric T cells are indeed T_H cells for the activation of H-2K/D-restricted anti-TNP CTL, it is possible that the addition to culture of soluble helper factors might bypass any requirement for T_H cell activation and, hence, might bypass any

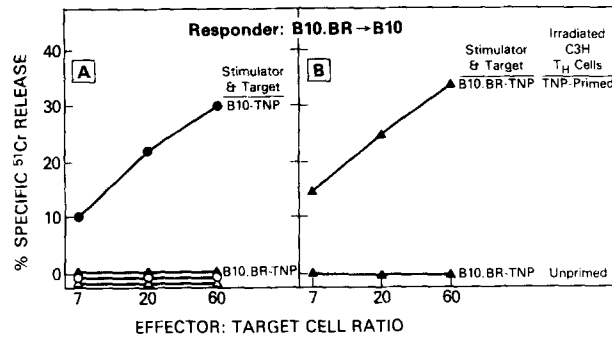


FIG. 6. Donor-specific TNP-primed T_H cells can activate donor-restricted anti-TNP CTL resident in the spleens of fully allogeneic chimeras. (A) Spleen cells from B10.BR \rightarrow B10 chimeras were stimulated with B10-TNP (\circ) or B10.BR-TNP (Δ) and assayed on TNP-modified (closed symbols) or unmodified (open symbols) targets. (B) 750-rad-irradiated spleen cells from either unprimed or TNP-primed C3H mice were added to the response cultures as a source of T_H cells. No CTL were generated when the irradiated C3H T_H cells were cultured with the TNP-modified stimulators alone.

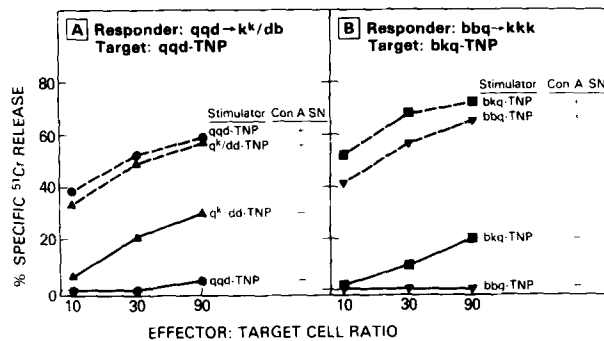


FIG. 7. Con A SN circumvents any requirement for Ia recognition. (A) Spleen cells from B10.T(6R) \rightarrow B10.A(2R) chimeras were stimulated with either B10.T(6R)-TNP (\circ) or B10.AQR-TNP (Δ) in presence or absence of Con A SN and assayed on B10.T(6R)-TNP targets. (B) Spleen cells from B10.QBR \rightarrow B10.BR chimeras were stimulated with either B10.QBR-TNP (∇) or B10.MBR-TNP (\square) in the presence or absence of Con A SN and assayed on B10.MBR-TNP targets. All the CTL in panel B, including those generated in the presence of Con A SN, were also assayed on B10.BR-TNP targets and were found to be specific for TNP-modified K^bD^b determinants since their lysis of B10.BR-TNP targets was one-fifth that of B10.MBR-TNP targets. Specific lysis of unmodified target cells was always $<1\%$.

requirement for Ia recognition. To examine this possibility, B10.T(6R) \rightarrow B10.A(2R) (q,q,d \rightarrow k,k/d,b) chimeras were stimulated with B10.T(6R) (q,q,d) and B10.AQR (q,k/d,d) TNP stimulators in the presence or absence of Con A SN (Fig. 7). As before, in the absence of Con A SN, anti-TNP CTL were only generated in response to B10.AQR TNP stimulators that express host $I^{k/d}$ determinants and were not generated in response to B10.T(6R) TNP stimulators that express donor I^q determinants. However, in the presence of Con A SN, virtually identical anti-TNP CTL responses were generated in response to both stimulators, regardless of which Ia determinants they expressed. Analogous results were obtained with B10.QBR \rightarrow B10.BR (b,b,q \rightarrow k,k,k) chimeras. It should be noted that the CTL effectors that were generated in the presence of Con A SN were still restricted to recognizing TNP in association with donor-type H-2K/D determinants. Thus, since exogenously added helper factors

circumvented any requirement for Ia recognition, this experiment supports the contention that the Ia-restricted chimeric T cells are T_H cells that, when activated, secrete nonspecific helper factors that act directly on antigen-stimulated H-2K/D-restricted precursor CTL (pCTL) (23).

Discussion

The present report documents in a single immune response system that radiation-resistant host restriction elements such as the thymus dictate the self-specificity of T cells restricted to Ia, but do not completely determine the self-specificity of T cells restricted to H-2K/D. Indeed, the only Ia-restricted T cells that could be detected in the spleens of experimental mice were those specific for the self-recognition of host Ia determinants. In contrast, there existed in the spleens of the same experimental mice pCTL that were specific for the self-recognition of donor H-2K/D determinants that were not expressed by the host animal. Thus, by distinguishing between the role that radiation-resistant host elements perform in determining the self-specificity of Ia-restricted T cells and H-2K/D-restricted T cells, the present report offers an explanation for much of the conflict regarding the role of the thymus in determining the self-specificity of MHC-restricted T cells. In addition, the present report also provides insights into the specificity of the cell-cell interactions that are involved in the generation of antigen-specific CTL responses.

Activation of Ia-specific T_H Cells Is Required for the Generation of H-2K/D-specific CTL. It was shown in this report that the generation of H-2K/D-restricted anti-TNP CTL effectors requires the activation of Ia-specific T_H cells. Since two distinct T cell subpopulations are involved in the generation of anti-TNP CTL responses, the possibility that the T-T cell interaction between T_H cells and pCTL is Ia restricted can be considered. Indeed, it has been previously suggested that even the differentiation of pCTL into functional competence requires an Ia-restricted interaction with T_H cells (24, 25).

The present results are not consistent with a requirement for an Ia-restricted T-T cell interaction between T_H cells and pCTL either during their differentiation into functional competence or, once functionally competent, for their activation. T_H cells from the spleens of fully H-2 allogeneic radiation bone marrow chimeras are restricted to the recognition of host Ia determinants, but the pCTL are all of donor origin and can encode only donor Ia determinants. Since the T_H cells are unable to recognize the Ia determinants encoded by the pCTL, the fact that spleen cells from fully allogeneic chimeras are competent to generate anti-TNP CTL effectors demonstrates that T_H cell recognition of pCTL-encoded Ia determinants is neither required for the differentiation of pCTL into functional competence nor for the activation of functionally competent pCTL. The possibility cannot be excluded that T_H cells do promote the differentiation and activation of pCTL by recognizing host Ia determinants that the pCTL might have acquired from the chimeric host thymus during their differentiation (26). However, since the soluble helper factors present in Con A SN are able to activate such pCTL, it is likely that the activation of H-2K/D-specific pCTL by Ia-specific T_H cells is mediated, at least in part, by nonspecific helper factors that are secreted by activated T_H cells.

Thus, the generation of anti-TNP CTL responses involves two parallel sets of MHC-restricted cell interactions: one between Ia-specific T_H cells and Ia-bearing

accessory cells which results in the elaboration of nonspecific helper factors such as IL-2; and one between H-2K/D-specific anti-TNP pCTL and H-2K/D bearing TNP-modified stimulator cells which, together with the soluble factors secreted by activated T_H cells, leads to the differentiation of pCTL into H-2K/D-restricted anti-TNP CTL effectors.

Role of the Thymus in Determining the Self-Specificity of MHC-restricted T Cells. Previous studies of Ia-restricted T_H cells involved in antibody responses have shown that the self-specificity of such T_H cells is dictated by the thymus in which they differentiated (3-7). The present experiments extend these previous results by demonstrating that the self-specificity expressed by Ia-restricted T_H cells functioning in CTL responses is also determined by radiation-resistant elements of the host in which these T cells differentiate. In addition, the present experiments demonstrate that there exist in the spleens of the same mice anti-TNP specific pCTL that are restricted to H-2K/D determinants that are not expressed by the chimeric host.

How can we understand this observation that the chimeric host completely dictated the self-specificity of those T cells restricted to Ia but did not completely dictate the self-specificity of those T cells restricted to H-2K/D? It is conceivable that the H-2K/D-restricted T cell repertoire is extensively degenerate and that the Ia-restricted T cell repertoire is not, so that the pCTL that recognize TNP in association with donor-type H-2K/D determinants are actually specific for some unknown antigen *X* in association with host thymic type H-2K/D determinants (27). However, this explanation is insufficient to explain why it has previously been observed that only splenic anti-TNP pCTL recognize nonthymic type-H-2K/D determinants but intra-thymic anti-TNP pCTL do not (15, 17, 28, 29). Another possibility is that T cells capable of recognizing donor MHC determinants expand to detectable numbers in the post-thymic periphery by encountering those determinants on donor-type accessory cells that repopulated the periphery of radiation bone marrow chimeras. However, this possibility does not explain why only donor-specific H-2K/D-restricted T cells, but not donor-specific Ia-restricted T cells, expand to detectable numbers in the post-thymic periphery. Thus, although it is true that the H-2K/D-restricted T cell repertoire is degenerate and that donor MHC determinants are expressed on extra-thymic chimeric elements, neither of these explanations is sufficient to account fully for all the available data.

One trivial explanation is that the donor-specific pCTL present in the spleens of radiation bone marrow chimeras had actually differentiated in the thymus of the bone marrow donor and had been inadvertently transferred into the irradiated chimeric recipient. Such a possibility is extremely unlikely because the donor bone marrow inoculum was always pretreated with a broadly specific anti-T cell reagent, RAMB + C, which is cytotoxic for all T cell subpopulations (14). Indeed, RAMB + C-treated bone marrow cell populations were essentially devoid of mature T cells, as indicated by their failure to respond to T cell mitogens. Nevertheless, if a few mature T cells had contaminated the donor bone marrow inoculum, only H-2K/D-restricted pCTL but not Ia-specific T_H cells would have had to survive the RAMB + C treatment. In any event, if mature pCTL had selectively survived the RAMB + C treatment, such mature pCTL would be alloreactive against the H-2K/D determinants of the allogeneic chimeric host; but no CTL capable of lysing unmodified host-type target cells were ever detected in these animals. Thus, it seems likely that all the

functionally competent T cells resident in the spleens of the experimental mice used in this study had in fact differentiated into functional competence in the chimeric host, even though some of the pCTL were restricted to the H-2K/D determinants of the chimeric donor.

The hypothesis we favor is that (a) radiation-resistant thymic elements determine the self-specificity of both Ia-restricted and H-2K/D-restricted T cells which differentiate intra-thymically, (b) that all Ia-restricted T cells differentiate intra-thymically, and (c) that H-2K/D-restricted T cells can differentiate either intra-thymically or extra-thymically. The H-2K/D-restricted T cell repertoire expressed by those T cells that differentiate extra-thymically can either represent the "education" of those T cells on donor bone marrow-derived peripheral elements or can represent the expression of germ-line-encoded (i.e., not environmentally influenced) self-specificities. This hypothesis is consistent with the observations that (a) Ia-specific T cells are restricted to host thymic-type Ia determinants, and there are resident in the periphery, but not the thymus, H-2K/D-specific T cells that are not restricted to host thymic-type H-2K/D determinants; and (b) there exists an H-2K/D-specific, but apparently not an Ia-specific, T cell repertoire in congenitally athymic nude mice (12, 13). From the perspective of this model, the marked preference for host-type over donor-type H-2K/D determinants expressed by anti-TNP CTL generated from the chimeric spleen cell populations reflects the fact that, even though H-2K/D-restricted T cells can differentiate extra-thymically, the predominant differentiation pathway for these T cells is intra-thymic. It might be noted that this hypothesis also helps explain why anti-minor H and anti-viral CTL responses generated from the spleens of $F_1 \rightarrow$ parent chimeras are, in general, only preferentially specific for thymic MHC determinants (2, 29), i.e., the spleens of $F_1 \rightarrow$ parent chimeras contain F_1 accessory cells expressing thymic Ia determinants, which promote the activation of thymically restricted Ia-specific T_H cells, which then provide helper factors for the activation of pCTL that had differentiated extra-thymically.

In conclusion, the present results demonstrate that the thymic hypothesis does accurately predict the self-specificity of Ia-restricted T_H cells, but less accurately predicts the self-specificity of H-2K/D-restricted pCTL. These results support the concept that Ia-restricted T cells can only differentiate into functional competence intra-thymically, whereas H-2K/D-restricted T cells can differentiate into functional competence either intra-thymically or extra-thymically. It remains to be determined whether Ia-restricted pCTL, as opposed to H-2K/D-restricted pCTL, are stringently restricted to the self-recognition of thymic MHC determinants, as would be predicted by this concept.

Summary

The present report has used fully H-2 allogeneic radiation bone marrow chimeras to assess the role of host restriction elements in determining the self-specificity of Ia- and H-2K/D-restricted T cells that participate in the generation of trinitrophenyl (TNP)-specific cytotoxic T lymphocytes (CTL). It was demonstrated that there exists a stringent requirement for the recognition of host thymic-type Ia determinants, but there exists only a preference for host thymic-type H-2K/D determinants. Indeed, once the stringent requirement for recognition of host Ia determinants was fulfilled, anti-TNP CTL were generated in response to TNP-modified stimulators that ex-

pressed either donor-type or host-type H-2K/D determinants. The CTL that were generated in response to TNP-modified donor-type stimulators were shown to be specific for TNP and restricted to the non-thymic H-2K/D determinants of the chimeric donor. Thus, these results demonstrate in a single immune response that the thymic hypothesis accurately predicts the self-specificity expressed by Ia-restricted T cells, but does not fully account for the self-specificity expressed by H-2K/D-restricted T cells. These results are consistent with the concept that H-2K/D-restricted T cells, but not Ia-restricted T cells, can differentiate into functional competence either intra-thymically or extra-thymically.

The present results are also informative for understanding the cellular interactions that are required for the generation of antigen-specific CTL responses. The Ia-restricted T cells that are required for the generation of H-2K/D-restricted anti-TNP CTL were shown to be helper T (T_H) cells since (a) like T_H cells functioning in antibody responses, they were specific for Ia determinants expressed by accessory cells, and (b) their function could be replaced by either TNP-primed, irradiated T_H cells or by nonspecific soluble helper factors. It was also shown that the T-T cell interaction between Ia-restricted T_H cells and H-2K/D-restricted precursor CTL (pCTL) is not Ia restricted. Rather, the results demonstrate that the generation of anti-TNP CTL responses involve two parallel sets of major histocompatibility complex-restricted cell interactions, an Ia-restricted T_H -accessory cell interaction required for T_H cell activation, and an H-2K/D-restricted pCTL-stimulator cell interaction required for pCTL stimulation. The interaction between activated T_H cells and stimulated pCTL is mediated, at least in part, by nonspecific soluble helper factors.

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