REGULATION OF T-CELL-MEDIATED LYMPHOLYSIS BY THE MURINE MAJOR HISTOCOMPATIBILITY COMPLEX

I. Preferential In Vitro Responses to Trinitrophenyl-Modified Self K- and D-Coded Gene Products in Parental and F_1 Hybrid Mouse Strains

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Cell-mediated lympholysis (CML)[1] responses to trinitrophenyl (TNP)-modified autologous cells are controlled by genes mapping in the murine major histocompatibility (H-2) complex at two distinct but interacting levels. First, it was demonstrated that the specificity of the effector cells generated in vitro to TNP-self involved the recognition of K- and D-coded-self structures, as well as the TNP moiety (1, 2). Second, genes mapping in the K and I regions appeared to affect the magnitude of the response by effectors that are specific for H-2D^d-TNP in a manner resembling immune-response genes (3, 4). A number of other examples of bifunctional control of cytotoxic responses by H-2-linked genes have been reported for viral (5–10) and minor transplantation (11) antigens.

One difficulty in investigating the self specificity associated with K- and D-region products is that effector cells generated in vitro against TNP-self also display significant but lower lysis on targets which are not H-2 matched with the stimulating population (1, 12, 13). Therefore, a portion of the cytotoxicity observed on target cells that are K or D matched with the stimulator cells is the result of cross-reactive cytotoxic T lymphocytes (CTL) (12). To more accurately define the K- and D-restricted TNP-self CTL responses, inhibition experiments have been performed at the lytic phase with nonradioactive blocking cells. The results indicate that *H-2^b* strains generate equivalent CTL responses to both K^b- and D^b-TNP antigens, whereas the *H-2^k* and *H-2^a* (3, 4) strains respond preferentially to K^k-TNP. Studies using several *H-2^d* strains demonstrated a marked preference for the generation of D^d-TNP- over K^d-TNP-specific effectors. Furthermore, in experiments using F_1 hybrids, the expected patterns of K-TNP- and D-TNP-CTL generation were not observed. In F_1 combinations in which K^k alleles were present in the responding population and were presented on the TNP-modified stimulating cells, weak or no D-TNP-specific-CTL to H-2D^{k,d,b} antigens were detected. However, if the stimulating cells did not express the k allele, H-2D-TNP-specific-CTL responses were generated by the F_1-responding cells. These results indicate that cytotoxic responses to K-end TNP-self products can control the response to H-2D TNP-self depending on the K-end alleles expressed in

[1] *Abbreviations used in this paper:* CML, cell-mediated lympholysis; CTL, cytotoxic T lymphocytes; H-2, murine major histocompatibility complex; TNBS, trinitrobenzenesulfonate; TNP, trinitrophenyl.

TABLE I

Mouse Strains, H-2 Haplotypes, and Abbreviations Used in This Study

Mouse strain	H-2 haplotype	H-2 regions*									K- and D-region abbreviations
		K	I-A	I-B	I-J	I-E	I-C	S	G	D	
B10.BR, C3H	k	k	k	k	k	k	k	k	k	k	k-k
B10.A	a	k	k	k	k	k	d	d	d	d	k-d
C57BL/10	b	b	b	b	b	b	b	b	b	b	b-b
B10.D2, DBA/2	d	d	d	d	d	d	d	d	d	d	d-d
(B10 × B10.BR)F_1	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b-b k-k
(B10 × B10.A)F_1	b/a	b/k	b/k	b/k	b/k	b/k	b/d	b/d	b/d	b/d	b-b k-d
C3D2F_1	k/d	k/d	k/d	k/d	k/d	k/d	k/d	k/d	k/d	k/d	k-k d-d
A.TH	t2	s	s	s	s	s	s	s	s	d	s-d
A.AL	a1	k	k	k	k	k	k	k	k	d	k-d
A.SW	s	s	s	s	s	s	s	s	s	s	s-s

* Reference 19.

the responding and modified stimulating cells. The mechanisms considered for such immunoregulation include the T-cell repertoire, immunodominance, and suppression, and are discussed in the context of other H-2-restricted cytotoxic systems in which similar results have been observed.

Materials and Methods

Animals. A list of the various strains and their H-2-region alleles is presented in Table I. A.TH, A.AL, and A.SW mice were obtained from the colony of Dr. D. H. Sachs (National Cancer Institute, Bethesda, Md.). The C3H, DBA/2, and (C3H × DBA/2)F_1 (C3D2F_1) strains were obtained from the Animal Production Facility, National Cancer Institute. All C57BL/10 congenic parental and F_1-hybrid mice were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Sensitization Cultures. Spleen cells were aseptically removed, minced, with forceps, and passed through sterile nylon to prepare single-cell suspensions. The cells were washed in Hanks' balanced salt solution (National Institutes of Health Media Unit) and centrifuged for 10 min at 1,000 rpm. Sensitization medium consisted of RPMI-1640 (Microbiological Associates, Walkersville, Md.) supplemented with sodium pyruvate, nonessential amino acids, 10% fetal bovine serum (all reagents are from Microbiological Associates, Walkersville, Md.), glutamine, penicillin-streptomycin (National Institutes of Health Media Unit), and 2-mercaptoethanol. Stimulating populations were freed of erythrocytes by treatment with ammonium chloride-lysing solution (National Institutes of Health Media Unit), and incubated with trinitrobenzenesulfonate (TNBS, Pierce Chemical Co., Rockford, Ill.) for 10 min at 37°C. After three washes in medium supplemented with 5% fetal bovine serum, the cells were irradiated with 2,000 rads (^{137}Cs source, Gammator; Isomedix Inc., Parsippany, N. J.) and 2 × 10^6 cells were added to 5 × 10^6 responding cells in 24 × 16-mm flat-bottom wells (Linbro Chemical Co., Hamden, Conn.). The plates were incubated at 37°C, in 95% air-5% CO_2 atmosphere.

Chromium-Release and Cold Target-Cell Competition Assays. After a 5-d incubation, the effector cells were harvested, washed, and 100 μl were added to microtiter wells (Linbro Chemical Co., Hamden, Conn.). Phytohemagglutinin (PHA)-stimulated (Difco Laboratories, Detroit, Mich.) spleen cells or ascites-passaged tumors were chromium labeled (New England Nuclear, Boston, Mass.), washed three times, and 100 μl were added to the effector cells. The plates were centrifuged for 4 min at 300 rpm, and incubated for 4 h at 37°C, in a 95% air-5% CO_2 atmosphere. After the incubation, the plates were centrifuged for 5 min at 800 rpm, the

supernate was collected with the Titertek Supernatant Collection System (Flow Laboratories, Inc., Rockville, Md.), and counted in a Packard Auto Gamma Scintillation Spectrometer (Packard Instrument Co., Downers Grove, Ill.).

Single-cell suspensions of nonradioactive (cold) blocking cells were prepared from fresh spleens, and erythrocytes were removed with ammonium chloride-lysing solution. Approximately one-half the cells were treated with 10 mM TNBS, washed three times, and adjusted to the desired concentration. 50 μl of these cold targets were incubated with 50 μl of effector cells for 20 min at 37°C. 100 μl of ^{51}Cr-target cells were then added, and the plates were treated as described above.

Results

Selective Cytotoxic Responses to TNP-Modified K- and D-Region Products by Spleen Cells from H-2^{b,k,d} Mouse Strains. To determine whether H-2K-TNP- and/or H-2D-TNP-specific cytotoxic effector cells were generated by spleen cells stimulated with TNBS-modified autologous cells, nonlabeled splenic-blocking cells were mixed with the effector cells and assayed on ^{51}Cr-labeled targets. The results illustrated in Fig. 1 were obtained using effector cells generated by B10 (*H-2^b*), B10.BR (*H-2^k*), and B10.D2 (*H-2^d*) spleen cells. The blocking results shown in Fig. 1 A and B at effector:target cell ratios of 40:1 and 20:1, respectively, indicate that B10 spleen cells respond equally well to TNP-self in association with H-2K- and H-2D-region products. B10-TNP cells (b-b-TNP) gave the best inhibition of lysis, whereas B10.A (5R)-TNP (b-d-TNP) and B10.HTG-TNP (d-b TNP) exhibited equal, but partial, inhibition. In all three cases the inhibition observed was directly proportional to the blocker:effector cell ratio. Unmodified blocking cells from all three strains gave no appreciable level of inhibition, and the low level observed was not proportional to the blocker:effector cell ratio (data shown for unmodified B10 blockers only). Fig. 1 C shows an experiment involving the TNP-self response of B10.BR spleen cells at an effector:target cell ratio of 40:1. In this strain, TNP-modified B10.A (k-d-TNP) spleen cells inhibited the cytotoxic response as well as modified, syngeneic blocking cells (k-k-TNP). However, C3H.OH-TNP (d-k-TNP), which is H-2 matched at D with B10.BR, did not inhibit this response. Similarly, non-H-2-matched B10.D2-TNP (d-d-TNP) and unmodified B10.BR (k-k) blockers did not inhibit the response. Therefore, cytotoxic cells with specificity for *H-2K^k*-TNP, but not for *H-2D^k*-TNP, could be detected in the B10.BR strain. Finally, as shown in Fig. 1 D and E, B10.D2 effector cells were assayed on an *H-2^d* target in the presence of d-d-TNP (B10.D2), k-d-TNP (B10.A), or d-k-TNP (C3H.OH) splenic blocking populations. The d-d-TNP and k-d-TNP blockers inhibited lysis proportional to the number of blocking cells which were added. However, the latter blockers were not as effective as the d-d-TNP blockers. Again, unmodified blockers exhibited no discernible inhibition. The d-k-TNP blockers also displayed no appreciable inhibition. Therefore, only TNP blockers which shared K and D or D, but not K, with the stimulating cells inhibited the lysis by the B10.D2 effectors. Thus, the majority of CTL generated by B10.D2 anti-TNP responders have specificity for *H-2D^d*-TNP antigens.

Selective Cytotoxic Responses to TNP-Modified K- and D-Region Products by Spleen Cells from F_1 Hybrid Mouse Strains. The results of the previous section demonstrated that B10 spleen cells generated effector cells with specificity to TNP in association with both K- and D-region self products, whereas B10.BR and B10.D2 spleen cells generated preferential cytotoxic activity to TNP-self in association with *H-2K^k* and

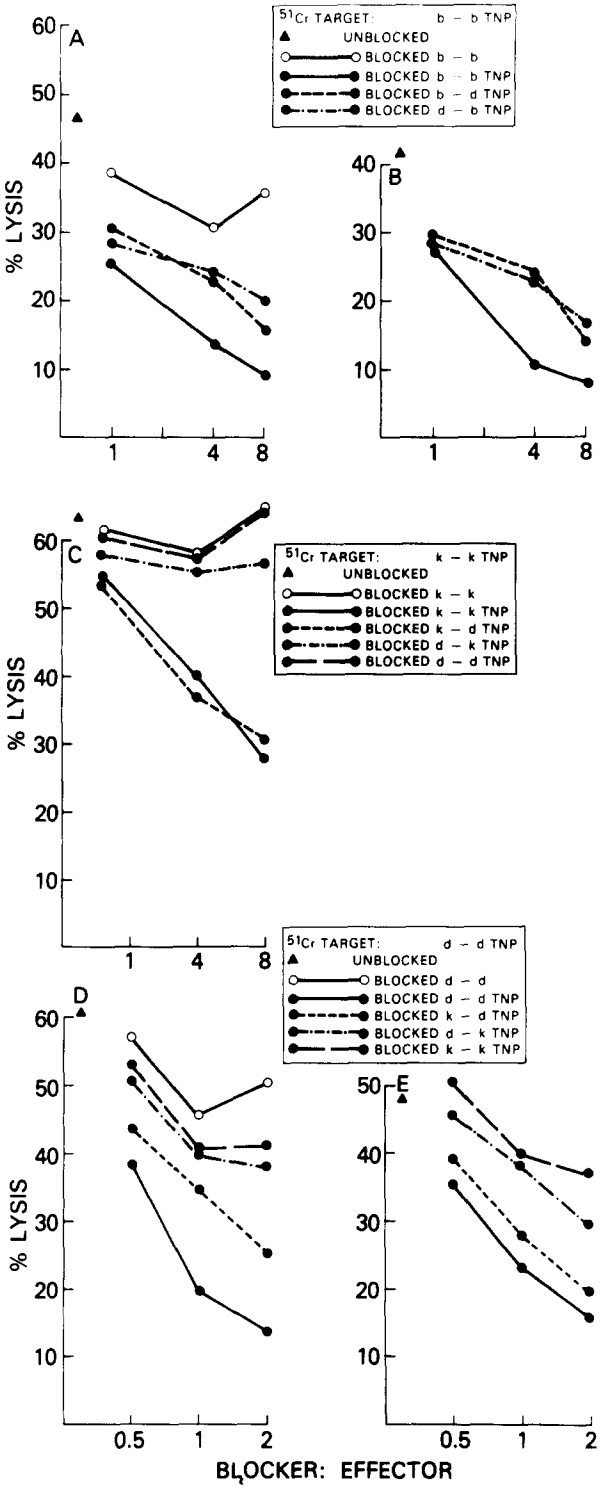

FIG. 1. Inhibition of effector cell activity to TNP-self by TNP-modified and unmodified syngeneic and allogeneic blocking cells. B10($H\text{-}2^b$) anti-B10-TNP effector cells assayed on B10-TNP PHA blast targets at effector:target ratios of 40:1 (1A) and 20:1 (1B). B10.BR ($H\text{-}2^k$) anti B10.BR-TNP effector cells assayed on B10.BR-TNP PHS blast targets at 40:1 (1C). B10.D2 ($H\text{-}2^d$) anti-B10.D2-TNP effector cells assayed on B10.D2-TNP PHA blast targets at 40:1 (1D) and 20:1 (1E). K- and D-region haplotypes of the blocking cells are shown in the insets.

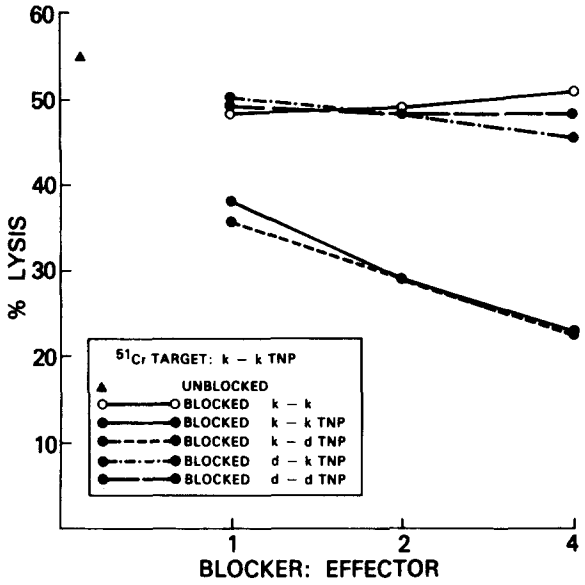

FIG. 2. Inhibition of effector cell activity to TNP-self by TNP-modified and unmodified syngeneic and allogeneic blocking cells. (B10 × B10.BR)F_1 (*H-2*^{b} × *H-2*^{k}) anti-F_1 TNP effector cells assayed on B10.BR-TNP PHA blasts at effector:target ratio of 40:1. K- and D-region haplotypes of the blocking cells are shown in the insets.

H-2D^d, respectively. Experiments were performed using spleen cells from (B10 × B10.BR)F_1 and (B10 × B10.A)F_1 mice to determine whether introduction of the *b* haplotype would increase cytotoxic activity to D^k-TNP or D^d-TNP in F_1 spleen cells, or whether introduction of the *k* haplotype would decrease the cytotoxic activity to D^b-TNP and D^d-TNP by these F_1 lymphocytes.

The results illustrated in Fig. 2 show that when (B10 × B10.BR)F_1 spleen cells were stimulated with TNBS-modified cells and assayed on *H-2*^{k} target cells, the effector cell activity was inhibited only by TNP-modified blockers which expressed the K^k antigens (B10.BR, k-k-TNP, or B10.A, k-d-TNP). However, blocking cells which shared the D^k antigens or those which did not share H-2 antigens with the responding and stimulating cells exhibited no detectable blocking. These results indicated that introduction of the *H-2*^{b} haplotype did not result in the generation of detectable CTL against TNP-self in association with *H-2D*^{k} antigens.

Spleen cells from (B10 × B10.A)F_1 mice were sensitized to TNP-modified autologous cells. The effectors generated were assayed on TNP-modified *H-2*^{a} and *H-2*^{b} tumor targets in the presence of modified blocking cells K, D, or K and D matched with the parental haplotypes. The results in Fig. 3 A illustrate that *H-2*^{b}-TNP (b-b-TNP) blocking cells effectively inhibited the lysis by F_1 effectors when assayed on an *H-2*^{b} target. TNP-modified blocking cells, which shared only the K^b antigens with the stimulating cells, inhibited lysis as well as the parental (*H-2*^{b}) blocking cells. In contrast, blocking cells which shared only the D^b antigens with the stimulating cells were approximately eightfold less effective in blocking than the two former blocking populations. Therefore, in contrast to the results shown in Fig. 1 A and B, the effectors generated by F_1 cells exhibited a preferential response against TNP in association

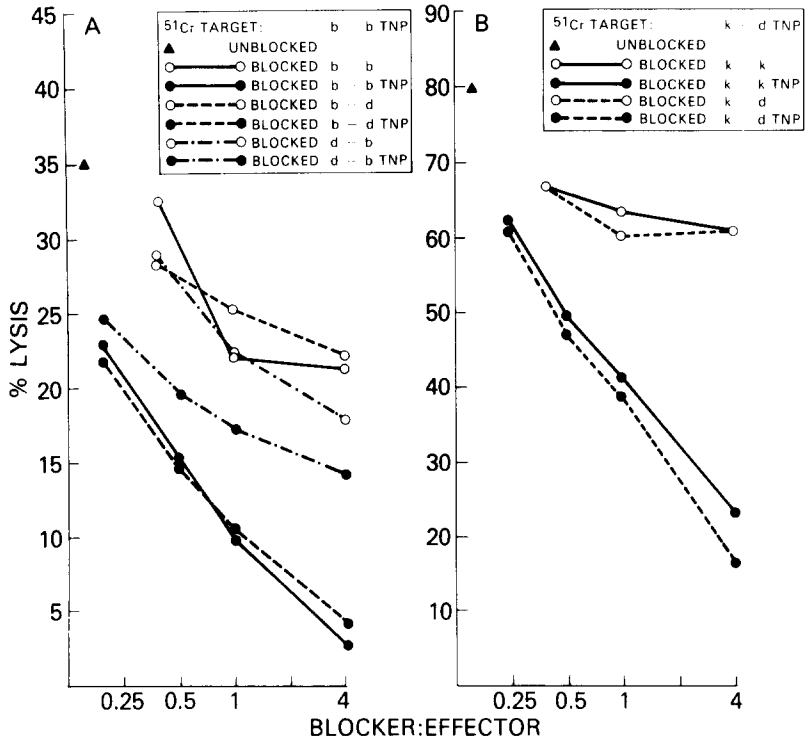

FIG. 3. Inhibition of effector cell activity to TNP-self by TNP-modified and unmodified syngeneic and allogeneic blocking cells. (B10 × B10.A(F_1 (*H-2*^{k} × *H-2*^{a}) anti-F_1 TNP effector cells assayed on B10-TNP (3A) and B10.BR (3B) PHA blast cells at an effector:target ratio of 40:1. K- and D-region haplotypes of the blocking cells are shown in the insets.

with K^b-self. When these (B10 × B10.A)F_1 effectors were assayed on an *H-2*^{a} target, the results were similar to those observed in the previous experiment using (B10 × B10.BR)F_1 effectors (Fig. 2). Modified blocking cells, that were matched at the K region with the stimulating cells, were able to inhibit the lysis as well as modified blockers which shared both the K and D regions, indicating that there was a strong preferential response to K^k-TNP. Therefore, again, introduction of the *H-2*^{b} haplotype failed to result in the generation of a detectable cytotoxic response against D-region self products (in this case, D^d-TNP). In fact, introduction of the *a* haplotype into the (B10 × B10.A)F_1 resulted in an appreciable loss of cytotoxic activity specific for D^b-TNP when F_1 modified stimulators were used.

Spleen cells from *H-2*^{d} mice preferentially responded to TNP-modified D-region self products (Fig. 1 D and E); whereas *H-2*^{k} (Fig. 1 C) and *H-2*^{a} (3, 4) preferentially responded to TNP-modified K-region self products. Therefore, it was of interest to determine whether (*H-2*^{k} × *H-2*^{d})F_1 spleen cells would generate effectors with specificity for D^d-TNP. Spleen cells from C3D2F_1 mice were sensitized against TNP-self. The effectors generated showed a high level of lysis when assayed on *H-2*^{a}-TNP target cells (Fig. 4). This lysis was effectively inhibited by TNP-modified $F_1\left(\frac{k\text{-}k}{d\text{-}d}\text{-TNP}\right)$ and B10.A (k-d-TNP) blocking cells. Furthermore, modified B10.BR (k-k-TNP) blocking cells similarly inhibited lysis. In contrast, modified B10.D2 (d-d-

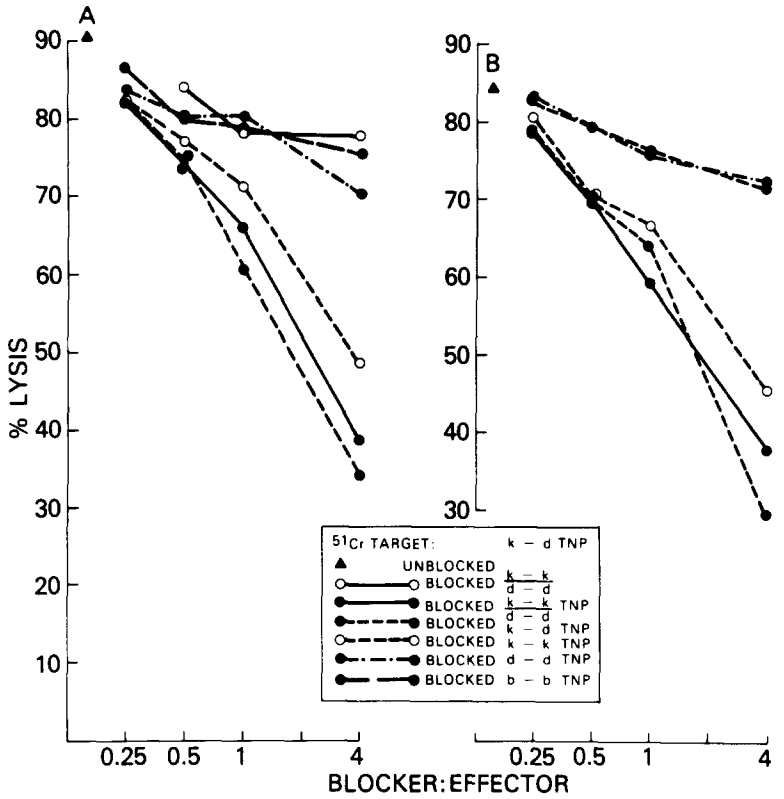

FIG. 4. Inhibition of effector cell activity to TNP-self by TNP-modified and unmodified syngeneic and allogeneic blocking cells. (C3H × DBA/2)F_1 ($H\text{-}2^k \times H\text{-}2^d$) anti-$F_1$ TNP effector cells assayed on B10.A-TNP PHA blast cells at an effector:target ratio of 40:1 (4A) and 20:1 (4B). K- and D-region haplotypes of the blocking cells are shown in the insets.

TNP), B10 (b-b-TNP), and C3H.OH (d-k-TNP) blocking cells failed to inhibit lysis. These results indicated that those modified blocking cells, expressing the K^k antigens, effectively inhibited the cytolysis of the $H\text{-}2^a$-TNP target, whereas the modified blockers which expressed the D^d antigen without K^k were unable to inhibit the cytolysis. Therefore, in this F_1 hybrid, cytotoxic cells with specificity for TNP-modified K^k, but not D^d, self products were detected.

Parental Stimulating Cells Can Induce K- and D-Region TNP-Specific Cytotoxic Responses by F_1 Spleen Cells. Because ($H\text{-}2^k \times H\text{-}2^d$)$F_1$ spleen cells failed to generate detectable levels of TNP-specific D-region lysis when stimulated with modified F_1 spleen cells, parental $H\text{-}2^d$-TNP (DBA/2) modified cells were used to stimulate ($H\text{-}2^k \times H\text{-}2^d$)$F_1$ (C3D2F_1) responding cells (Fig. 5A). The effector cells were assayed on $H\text{-}2^a$-TNP (k-d-TNP) targets in the presence of modified F_1 $\left(\frac{\text{k-k}}{\text{d-d}}\text{-TNP}\right)$, parental (k-k-TNP), or (d-d-TNP), or unrelated (b-b-TNP) blocking cells. Both the modified F_1 and $H\text{-}2^d$ parental blocking cells effectively inhibited the lysis induced by the $H\text{-}2^d$-TNP stimulators. In contrast, when F_1-TNP cells were used to stimulate the F_1 responders (Fig. 5C) parental $H\text{-}2k^k$-TNP and F_1-TNP, but not parental $H\text{-}2^d$-TNP, blocking cells inhibited lysis. Thus, as previously shown (Fig. 4), when modified F_1 cells were

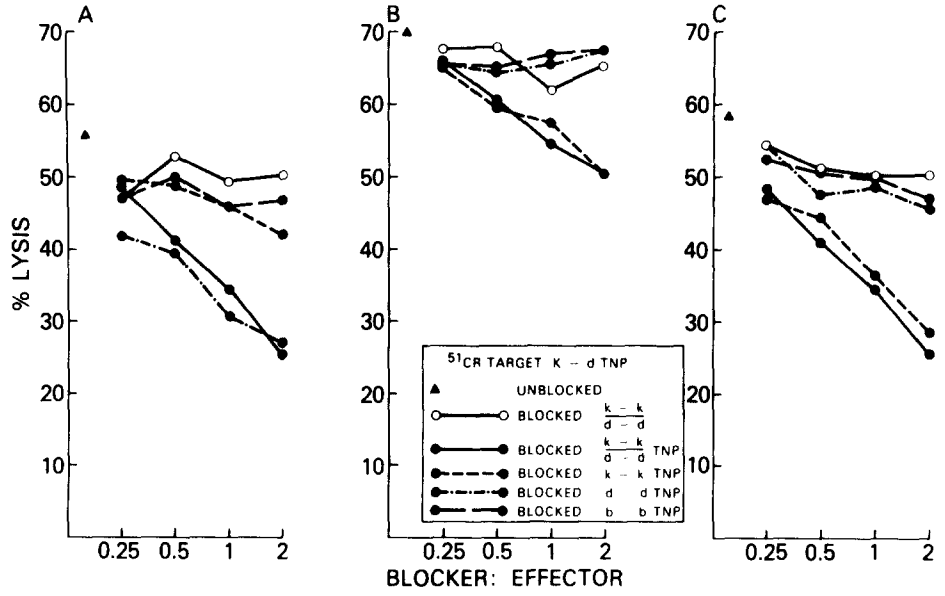

FIG. 5. Inhibition of effector cell activity to TNP-self by TNP-modified and unmodified syngeneic and allogeneic blocking cells. (C3H × DBA/2)F_1 ($H\text{-}2^k \times H\text{-}2^d$) anti DBA/2-TNP ($H\text{-}2^d$) (5A); anti-C3H-TNP ($H\text{-}2^k$) (5B); and anti F_1-TNP ($H\text{-}2^{k/d}$) (5C). Effector cells assayed on B10.A-TNP PHA blast cells at an effector:target ratio of 40:1. K- and D-region haplotypes of the blocking cells are shown in the insets.

used as stimulators, cytotoxic cells with specificity for TNP in association with K, but not D, region self products were generated. However, when modified $H\text{-}2^d$ parental cells were used as stimulators, the F_1 responders generated cytotoxic cells with specificity for TNP in association with D-region-self products. When parental $H\text{-}2^k$-TNP cells were used as the stimulating population (Fig. 5B), the cytotoxic activity assayed against $H\text{-}2^a$-TNP target cells was inhibited by the TNP-modified F_1 and $H\text{-}2^k$ blocking cells, but not by $H\text{-}2^d$- or $H\text{-}2^b$-modified blockers. Therefore, as expected, effector cells with specificity for TNP in association with K^k self products were induced by stimulating the F_1 spleen cells with $H\text{-}2^k$ parental modified cells.

Discussion

Earlier studies concerned with the self specificity of cytotoxic effectors generated in vitro against TNP-modified autologous cells indicated that spleen cells from B10.A mice responded well to K^k-TNP but poorly to D^d-TNP (3, 4). The present study has investigated whether other C57BL/10 congenic mouse strains would also show K- or D-region preferential self recognition for TNP-specific cytotoxic responses. However, because lower but appreciable levels of lysis have been observed on TNP-modified non-H-2-matched targets (1, 12, 13), selective cytotoxic responses against K- and D-modified self products are difficult to evaluate in direct CML assays. Therefore, nonradioactive (cold target) blocking cells were used to specifically inhibit effectors generated against TNP-modified K- or D-region self products. Using this blocking cell approach, three distinct patterns of responses were observed using spleen cells from the three strains studied. Cells from B10 (b-b) donors generated strong and equivalent responses to TNP in association with K^b- and D^b-self products. In contrast,

spleen cells from B10.D2 (d-d) (as well as other *H-2*^{d} strains, R. B. Levy and G. M. Shearer, manuscript in preparation) responded preferentially to D^d-TNP. In contrast to both of the above response patterns, cells from B10.BR (k-k) donors generated strong cytotoxic activity against K^k-TNP, but undetectable activity against D^k-TNP. Taken together, these three patterns of responsiveness illustrate the complex effects of H-2-linked genes in determining the specificity of cytotoxic responses.

F_1 hybrids from these and other inbred strains were studied to determine the effects of these genes on the generation of TNP-specific cytotoxic responses to K- and D-region self products. Introduction of either the *b* or *d* haplotype into a (b × k)F_1, or a (k × d)F_1 did not result in a strong CML response to D^k-TNP. In fact, when the self specificity of the F_1 effectors was tested using the appropriate blockers and targets, the response of (b × a)F_1 to D^b-TNP and the response of (k × d)F_1 to D^d-TNP were undetectable. This was unexpected because both of these responses (D^b-TNP and D^d-TNP) were strong in the respective b-b and d-d parental strains. It was confirmed that the (k × d)F_1 did possess the potential to generate specific cytotoxic effectors against D^d-TNP when stimulated with *H-2*^{d}-TNP parental cells. Therefore, in addition to the presence of D^d-TNP on the stimulating population, the absence of K^k-TNP antigen on these cells was required for the generation of a cytotoxic response to D^d-TNP.

Preferential cytotoxic responses to antigenic determinants in association with K- or D-region self products have been observed in a number of other models including the vaccinia (7–9), Sendai (7), SV-40 (10), murine sarcoma (5), Friend (6), and influenza (9) viruses, as well as the male-specific H-Y antigen (11). Some of the viral models exhibit preferential response patterns which are notably similar to those observed for TNP. These include vaccinia, Sendai, influenza, and SV-40, and their known response patterns are summarized in Table II. The in vitro cytotoxic response of mouse spleen cells against SV-40 virally infected cells resulted in response patterns against K- and D-self products that were almost identical to those observed with TNP-modified cells (Table II) (10). It is noteworthy that in the SV-40 model, spleen cells which possessed the k allele in the *H-2K* region and either the d (B10.A) or b (B10.A [2R], B10.A [4R]) alleles in the *H-2D* region, generated a markedly higher virally restricted response to K^k-self products than to D^d- or D^b-self products (10). These results are identical to those observed in the TNP model in which the presence of K^k antigens in the responding and stimulating cell population is associated with a strong response against K-, but a weak response against D-, region antigens in association with the modifying agent.

The response patterns of inbred mouse strains to vaccinia, Sendai, and influenza virus are also presented in Table II. (Compare all columns, lines 1–5) *H-2*^{k} (line 3), *H-2*^{a} (line 4), and *H-2*^{i4} (line 5) strains all exhibit notably greater cytotoxic responses in association with K^k-self products, than with D^k-, D^d-, or D^b-self products, irrespective of whether the infecting or modifying agent was vaccinia, Sendai, influenza, SV-40, or TNP. One distinction that can be made in this inbred strain comparison is with the influenza response in *H-2*^{b} mice (line 1 of Table II). Unlike the cytotoxic responses to the other four agents, *H-2*^{b} mice generate a preferential influenza response to D^b in association with influenza, i.e., they are unresponsive to influenza in association with K^b (9) (line 1 of Table II). The cytotoxic response patterns to vaccinia, Sendai, and influenza by *H-2*^{d} mice differ from the pattern exhibited by the responses to SV-40

TABLE II

Summary of Cytotoxic Responses to Virally Infected or TNP-Modified Cells with Preferential Reactivity for K- or D-Region Self Products

Responding haplotype of origin				Stimulating haplotype of origin				K- and D-restricted cytotoxic responses against targets infected or modified with:							
								Vaccinia/ Sendai*		Influenza ‡		SV-40§		TNP ‖	
K	I	S	D	K	I	S	D	K	D	K	D	K	D	K	D
b	b	b	b	b	b	b	b	+	+	−	+	+	+	+	+
d	d	d	d	d	d	d	d	+	+	+	+	−	+	±	+
k	k	k	k	k	k	k	k	+	−	+	±	+	−	+	±
k	k/d	d	d	k	k/d	d	d	+	+¶	+	+¶	+	−	+	±
k	k/b	b	b	k	k/b	b	b	+	±	+	+	+	±	+	±
k	k	k	k	k	k	k	k	b^+	b^-					b^+	b^-
b	b	b	b	b	b	b	b								
k	k	k	k	b	b	b	b	N.T.	b^+					b^+	b^+
b	b	b	b												
k	k	k	k	k	k/d	d	b	N.T.	b^-						
b	b	b	b												
k	k/d	d	d	k	k/d	d	d			(+?)				b^+	b^-
b	b	b	b	b	b	b	b								
k	k/d	d	d	k	k/d	d	d	b^-	b^-						
b	b	b	b												
k	k/d	d	d	b	b	b	b	b^+	b^+						
b	b	b	b												
k	k/d	d	d	k	k/b	b	b	b^-	b^-						
b	b	b	b												
k	k	k	k	k	k	k	k								d^-
d	d	d	d	d	d	d	d								
k	k	k	k	k	k	k	k							k^+	
d	d	d	d												
k	k	k	k	d	d	d	d								d^+
d	d	d	d												

Blank spaces indicate that data were not available.

* References 7, 8, and 9.

‡ Reference 9.

§ Reference 10.

‖ References 3, 4, and 13, this manuscript, and unpublished data.

± Indicated that the response associated with the indicated product was estimated to be at least threefold less than that associated with the other self product.

(±) Cytotoxicity was positive on targets which did not discriminate between K and D self products.

¶ Indicates that the response to vaccinia-*H-2D*^{d} was positive, although consistently less than that to vaccinia-*H-2K*^{k} in B10.A mice (Table II, reference 7, and Biddison and Doherty, personal communication). Similar observations have been made for influenza although differences in lysability between k-k and d-k target cells could account for the differences detected in the responses (reference 9, and Biddison and Doherty, personal communication).

and TNP in that mice infected with vaccinia, Sendai, and influenza generated restricted cytotoxic cells to K- and D-region self products. Although the response pattern to influenza differs in *H-2*^{b} mice from the other four agents considered in Table II, the response patterns to influenza are identical in *H-2*^{b} mice to other H-2-restricted models. For example, *H-2*^{b} mice respond to MSV and H-Y in association with D^b- but not K^b-self products (5, 11).

As summarized in Table II (Figs. 2–5), F_1 hybrids between k × b or a × b, as well as k × d, exhibited TNP cytotoxic-response patterns that were identical to those observed by the *H-2*^{k} or *H-2*^{a} parent when F_1-TNP cells were used to stimulate these F_1 responders (lines 6, 9, and 13 of Table II). It is noteworthy that both the (k × b)F_1 and (k × d)F_1 can generate a response to *H-2D*^{b}-TNP and *H-2D*^{d}-TNP, respectively, if stimulated by TNP-modified *H-2*^{b} or *H-2*^{d} parental cells. Similar results have been reported for cytotoxic responses to vaccinia and Sendai viruses by F_1 hybrid mice (7, 8). Thus, the presence of K^k, and I-A^k in both the responding and stimulating cells, is associated with a poor TNP- or viral-specific D-region response. The influence of *H-2*^{k} alleles on D-region responses has been mapped for vaccinia to the left of I-A in the B10.BYR recombinant mouse strain (7). In summary, F_1 hybrids between D-region responder (*H-2*^{b,d}) and nonresponder (*H-2*^{k,a}) strains generate a weak D-region-restricted cytotoxic response in association with TNP, vaccinia, or Sendai.

The similarities of the response patterns observed with respect to K- and D-self products: (*a*) to vaccinia, Sendai, influenza, SV-40, and TNP in *H-2*^{k}, *H-2*^{a}, and *H-2*^{i4} strains; (*b*) to vaccinia, Sendai, SV-40, and TNP in *H-2*^{b} and *H-2*^{d} strains; and (*c*) to influenza, MSV, and H-Y in *H-2*^{b} mice, raise the possibility that common H-2-linked genetic effects can influence preferential responsiveness to K- and D-self products. In some cases, these genetic effects may be independent of the cytotoxic specificity contributed by the infecting virus, modifying agent, or tumor antigen. Control of other CML responses localized to the D region might also be accounted for by regulation with stimulating cells which express K^k. For example, in the F_1 anti-*H-2*^{b} parental CML, (B10 × B10.A)F_1- or (B10 × B10.BR)F_1-responding cells generate *H-2D*^{b}-specific effectors when stimulated with B10 parental (14) or with B10.HTG-stimulating cells. However, much weaker or no responses are detected when these F_1-responding cells are stimulated with B10.A(2R) or B10.A(4R) cells (G. M. Shearer and G. Cudkowicz, unpublished observations, and reference 14). These findings are also consistent with the presence of K^k alleles in the stimulating population affecting an F_1 anti-*H-2D*^{b} parental cytotoxic response.

The mechanism by which the TNP-specific *H-2D*^{k,d,b}-restricted CML response is affected by a simultaneous K^k-TNP response in unknown. Three possibilities which have been considered by Zinkernagel et al. (7, 8) for similar effects in restricted vaccinia and Sendai CTL responses are immunodominance, T-cell-repertoire defects, and suppression. An immunodominant mechanism could be envisioned in which a strong response in association with K^k self recognition could affect the simultaneous generation of TNP-specific effectors restricted to *H-2D*^{k,d,b}. Cytotoxic responses to K^k-TNP are known to be stronger than those to TNP in association with any other K- or D-self allele except for K^s (13). Such an immunodominant mechanism could seem less likely if a strong D-region TNP response could be generated simultaneously with a K-region TNP response comparable in magnitude to that of a K^k-TNP CTL response. In fact, such CML responses have been generated simultaneously to K^s- and D^d-TNP by cultures of A.TH and B10.HTT spleen cells (4). These observations

indicate that: (*a*) a strong K-region TNP-specific response is not necessarily accompanied by a weak D-region TNP CTL response; and (*b*) that D^d-TNP is a strong CML stimulator when the appropriate K- and/or I-region alleles are expressed. Furthermore, reasonably strong D^d- and D^k-TNP responses have been observed when accompanied by *b* or *d* alleles, rather than *k* alleles, in the K and I regions. Therefore, it appears that the absence of *k* alleles, rather than the presence of *s* alleles, in the K or K and I regions, are critical for obtaining D-region TNP-cytotoxic responses. A similar conclusion has been reached in the vaccinia model (7, 8).

An alternate explanation to account for this phenomenon could involve a defect in the T-cell repertoire, i.e., a lack of ability of cells expressing *k* alleles at K or K and I to generate a response to TNP in association with D-region self products. A T-cell-repertoire defect might be expected to result in the absence of any detectable TNP-specific D-region CML response. This was not always the case, however, because low levels of D-region-restricted responses can be detected in the TNP (3, 4) and viral (7) systems. However, these low levels of lysis detected on D-region-matched targets could be a result of cross-reactive lysis which would not necessarily argue against a repertoire defect. A repertoire defect might be expected to be corrected by introduction of a responding haplotype in an F_1 hybrid. Because response potential was introduced into the (k × b)F_1 and (k × d)F_1 hybrids, as illustrated by a D^b-TNP or D^d-TNP response when stimulated with TNP-modified *H-2*^{b} or *H-2*^{d} parental cells, respectively, these findings do not support a defect in the T-cell repertoire as being responsible for the F_1-response patterns.

The effect of the H-2K^k-TNP CML response on *H-2*$D^{k,d,b}$-TNP effector cell activity could also be accounted for by regulatory mechanisms other than immunodominance. One possibility would be that the generation of a *H-2*K^k-restricted cytotoxic response results in suppression of a response restricted to H-2D. The findings presented here, as well as those reported for virus responses, neither support nor exclude a suppressive mechanism. It does appear that abolition of the *H-2*K^k-restricted response to vaccinia or influenza either in radiation chimeras (15) or by negative selection (16) results in an increased CML response to the virus in association with D^b. This would be consistent either with the simultaneous abrogation of a suppressor effect by a K-region-restricted response or with elimination of the effects of immunodominance. Regulatory mechanisms activated by modified-self have been described which appear to suppress antibody responses to TNP (17) and delayed-type hypersensitivity reactions to dinitrochlorobenzene (18). Such a suppressor mechanism would be consistent with the absence of D-region responses in F_1 hybrids which do possess the potential to respond to D-region TNP antigens. Studies are in progress to determine the possible roles of immunodominance, suppression, and repertoire defects in the regulation of TNP CML responses in F_1 and parental mice.

Summary

Spleen cells from *H-2*^{b,k,d} C57Bl/10 congenic mice were sensitized in vitro to trinitrobenzenesulfonate (TNBS)-modified autologous spleen cells. Cold target competition studies at the lytic phase demonstrated three distinct patterns of cytotoxic responsiveness: (*a*) *H-2*^{b} spleen cells generated approximately equivalent CTL responses against K^b and D^b modified self products, (*b*) *H-2*^{d} spleen cells generated preferential responses against D^d modified self products, and (*c*) *H-2*^{k} spleen cells

generated cytotoxic responses which could only be detected against K^k self products in association with TNP.

F_1 spleen cells were sensitized against autologous TNBS-treated cells. The results showed that, although $H\text{-}2^b$ parental cells generated approximately equivalent K^b-TNP- and D^b-TNP-specific CTL, the presence of the $H\text{-}2^b$ haplotype did not result in the generation of (*a*) D^k-TNP CTL response by ($H\text{-}2^b \times H\text{-}2^k$) spleen cells, nor (*b*) a D^b CTL response by ($H\text{-}2^b \times H\text{-}2^a$) F_1 spleen cells. Additionally, ($H\text{-}2^d \times H\text{-}2^k$) F_1 cells failed to generate detectable D^d-TNP-specific CTL, although $H\text{-}2^d$ parental cells generated D-regional-specific CTL. The findings demonstrated that these F_1 response patterns paralleled those of the $H\text{-}2^k$ and $H\text{-}2^a$ parents, i.e. weak or no D-region TNP-specific CTL were induced.

Because ($H\text{-}2^d \times H\text{-}2^a$) F_1 responders stimulated with $H\text{-}2^d$ TNBS-treated cells did generate good D^d TNP responses, the results illustrated that the presence of responder genes was not sufficient to result in a D-region TNP CML. It is suggested that the absence of K^k alleles on the stimulating population is necessary for the generation of D-region TNP CTL in these F_1's. Mechanisms which could account for these response patterns in parental F_1 mice are discussed including immunodominance, suppression, T-cell response, and Ir-gene defects.

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