

CROSS-REACTIVE LYSIS OF TRINITROPHENYL
(TNP)-DERIVATIZED *H-2* INCOMPATIBLE TARGET
CELLS BY CYTOLYTIC T LYMPHOCYTES
GENERATED AGAINST SYNGENEIC TNP SPLEEN CELLS*

By STEVEN J. BURAKOFF, RONALD N. GERMAIN, AND BARUJ BENACERRAF

(From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115)

Cytolytic T lymphocytes (CTL)¹ can be generated in the mouse in response to appropriately "modified" syngeneic cells. Such CTL responses have been documented using virus-infected (1, 2), chemically derivatized (3-8) and malignantly transformed (9, 11) stimulator cells, as well as with *H-2* identical responder and stimulator cells differing only at minor histocompatibility (*H*) loci (12, 13). All such CTL have been reported to lyse only target cells that possess both the appropriate "modifier" and the same *H-2 K* and/or *D* loci as the effector CTL. The *H-2* restrictions for cytolysis in these systems appeared absolute in most studies and two general hypotheses have been proposed to account for this phenomenon (1). (a) The dual (immunological-physiological) interaction model postulates that CTL possess two functionally distinct receptor sites, both necessary for lysis. One recognizes the antigenic determinants of the viruses, chemicals, or minor H antigens and the other, coded for by the *K* or *D* region of the *H-2* complex of the CTL, is able to specifically and preferentially interact with the identical *H-2* product on the target cell. (b) The altered-self hypothesis on the other hand proposes the immunological recognition by the CTL of new antigenic determinants created by the interaction of the modifier with a *D* or *K* locus product of the target cells. This hypothesis postulates further that the immunological dictionary of these CTL is generally restricted to the recognition of *K* and *D* products in their native or modified forms.

Experiments using F₁ CTL against modified parental cells have led several authors to favor the altered-self hypothesis (1, 3, 5, 11, 12). Earlier studies from our laboratory with the trinitrophenyl (TNP) model are also consistent with the altered-self hypothesis (8). We observed that effectors generated to TNP syngeneic lymphoid cells could lyse TNP targets differing at *H-2* from both effector and stimulator, although less efficiently than syngeneic targets. Such a finding would make the simple form of a dual recognition hypothesis unlikely, since no opportunity for like-like *H-2* interaction is available under these circumstances. In addition, it was demonstrated that antisera against the *H-2D* or *K* products

* Supported by NIH grants CA-14723 and CA-09130.

¹ Abbreviations used in this paper: B6, C57BL/6; CRBC, chicken red blood cells; CTL, cytolytic T lymphocytes; SMEM-10, minimal essential medium supplemented with fetal calf serum and amino acids; TNP, trinitrophenyl; θ , Thy 1.2.

on such TNP-modified allogeneic targets could inhibit cytolysis while antisera to *H-2* specificities of the effector cells could not. Finally, several reports have shown that the lymphocyte populations of radiation chimeras unable to respond to the host *H-2* haplotype can nevertheless be immunized in vitro by chemically or virally modified cells bearing the tolerated *H-2* haplotype, and that the immunized CTL lyse preferentially these cells in comparison to similarly modified target cells syngeneic to the killer cells (14-16). These experiments are evidence against a requirement for like-like *H-2* interactions between CTL and target.

In this study, the phenomenon of cross-reactive lysis of TNP-conjugated target cells has been investigated further. Also, the specificity of inhibition of lysis by anti-*H-2* antisera was also reinvestigated. We have documented the CTL nature of the effector cells mediating lysis of TNP targets differing from the effector at *H-2* and we have demonstrated that antisera directed only to *K* and *D* products, but not antisera against *Ia* or other cell surface markers, are able to inhibit such cytolysis in a specific manner.

Materials and Methods

Mice. 6- to 12-wk-old male and female mice of the following strains were employed for these studies: A/J (*H-2^a*), B10.BR (*H-2^b*), BALB/c (*H-2^d*), C57BL/6 [B6] (*H-2^b*), and DBA/1 (*H-2^q*). All were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Tumors. The P815 (*H-2^d*) mastocytoma, and L1210 (*H-2^d*) leukemia were maintained in DBA/2 (*H-2^d*) mice. The EL4 (*H-2^b*) leukemia was maintained in B6 mice. These tumors were carried in ascites form using biweekly intraperitoneal transfer.

Antisera. Alloantisera were raised by four to eight intraperitoneal injections of either tumor or lymphoid cells. The donor-recipient combinations used and the specificity of each serum are given in the legend to Table III. The anti-*H-2* and anti-*Ia* sera were gifts of Dr. Martin Dorf, Harvard Medical School, Boston, Mass. Anti-L1210 serum was the gift of Dr. Robert Humphreys, University of Massachusetts Medical School, Worcester, Mass.

Generation of Effector Cells. The method used to generate cytotoxic effector cells to TNP-derivatized stimulator cells has been described previously (8). Briefly, 7×10^6 normal spleen cells were cultured at 37°C in 16-mm Linbro tissue culture wells (Linbro Chemical Co., New Haven, Conn.) together with 3×10^6 TNP-derivatized, irradiated spleen cells for 5 days and then assayed.

Anti-Thy 1.2 (θ) plus Complement (C) Treatment. The preparation, use, and specificity of anti- θ serum were the same as previously reported except that DNase was eliminated from the medium (17).

Trinitrophenylation of Chicken Red Blood Cells. Fresh chicken red blood cells (CRBC) were washed three times with Eagle's minimum essential medium and 0.3 ml packed cells suspended in 2 ml of cacodylate buffer (0.28 M) containing 40 mg trinitrobenzene sulfonic acid. Cells were incubated 10 min at 25°C, then washed five times with MEM and resuspended to the appropriate concentration in minimal essential medium supplemented with fetal calf serum and amino acids (SMEM-10).

⁵¹Cr-Release Assay. The ⁵¹Cr-release assay used in this study and the method of antisera inhibition have been previously described in detail (8). In some experiments, ⁵¹Cr-labeled spleen targets were treated like effector cells with anti- θ serum plus C to enrich *Ia*-bearing B lymphocytes and then TNP derivatized as described (8). In those experiments involving competitive inhibition by unlabeled target cells, to 50 μ l of ⁵¹Cr targets were added various numbers of unlabeled cells in 50 μ l of SMEM-10, just before the addition of effector cells in a 100 μ l vol. Therefore, all cultures contained a total of 200 μ l. These cultures were incubated for 4 h at 37°C, the supernates harvested, and the percent specific release calculated as detailed elsewhere (8). All measurements were performed in duplicate or triplicate. The standard error of the mean was always <2-3%, and is omitted from the tables and figures for clarity. Differences of >5% are almost invariably significant at $P < 0.05$.

Regression Analysis. Linear regression analysis of data and the Pearson's product moment correlation were determined using a Wang 760 computer program (Wang Laboratories, Inc., Tewksbury, Mass.).

Results

Cytotoxic Effector Cells Generated to TNP-Derivatized Syngeneic Stimulators Lyse H-2 Compatible and H-2 Incompatible TNP-Derivatized Targets. In Table I it can be seen that B6 spleen cells ($H-2^b$) sensitized to TNP-derivatized B6 stimulators (B6-TNP) lysed the EL4-TNP ($H-2^b$) tumor target or B6-TNP spleen target most effectively, but also demonstrated significant cytotoxicity on the $H-2$ incompatible tumor target P815-TNP ($H-2^d$) and the A/J-TNP ($H-2^a$), B10.BR-TNP ($H-2^k$), and DBA/1-TNP ($H-2^g$) spleen targets. This cytolysis of TNP-derivatized targets differing from the effectors at $H-2$ is termed cross-reactive lysis.

In order to assess the relative effectiveness of cross-reactive as compared to $H-2$ identical cell lysis, effector cells were tested on both $H-2$ identical and $H-2$ different TNP targets at several effector/target (E/T) ratios. Semilogarithmic plots were constructed of "percent specific ^{51}Cr release" vs. E/T ratio of B6 anti-B6-TNP effectors on $H-2$ identical ($H-2^b$) or $H-2$ different ($H-2^a$) TNP spleen targets. By extrapolating the straight lines to equal percent release, an estimate of relative effector activity was obtained. The efficiency of lysis of the $H-2$ identical target was approximately five times that of lysis of the $H-2$ different target. In several comparisons of this type, cross-reactive lysis usually was 15-25% of syngeneic lysis.

Cross-Reactive Cytotoxicity Correlates with the Extent of Cytotoxicity on the Syngeneic Target. Values for cross-reactive and syngeneic target cell lysis mediated by the same effector cell pool were obtained from six to nine separate experiments and the data plotted as shown in Figs. 1 and 2. As can be seen in Fig. 1, when B6 effectors generated to B6-TNP were assayed on EL4-TNP ($H-2$ identical targets) or P815-TNP ($H-2$ different targets), the data showed a direct correlation between the lysis on EL4-TNP and P815-TNP and fit a straight line with $r = 0.96$. For a similar comparison of BALB/c anti-BALB/c-TNP effectors on the same two tumor targets, r was 0.93. Excellent direct correlation between syngeneic and cross-reactive lysis was also observed using spleen cell targets. In Fig. 2, B6 anti-B6-TNP effectors were assayed on B6-TNP and BALB/c-TNP targets. The data on cytolysis again fit a straight line, with $r = 0.89$. The reciprocal analysis of BALB/c anti-BALB/c-TNP on the same two spleen targets gave $r = 0.78$.

Cross-Reactive Cytotoxicity is Mediated by Cytolytic T Lymphocytes. Effector cells stimulated with TNP-derivatized syngeneic spleen cells were treated with anti- θ antiserum plus C just before assaying for cytotoxic activity. As has been previously reported (4) and is shown in Table II, lysis of $H-2$ identical TNP-targets was virtually abolished by such treatment of effectors. The results were the same for cross-reactive lysis. Table II shows that cross-reactive lysis by B6 anti-B6-TNP or BALB/c anti-BALB/c-TNP effectors on B10.BR-TNP targets is reduced to background by anti- θ plus C. Similarly, both syngeneic and cross-reactive lysis of L1210-TNP, a tumor target, are also eliminated by removing

TABLE I
Cytolysis of Tumor and Spleen Targets by Effectors Generated against TNP-Derivatized Syngeneic Stimulators*

| Exp. no. | Responder | Stimulator | Target‡ | | | |
|----------|----------------------------|------------|---------------------------------|------|-----------------------------------|--------|
| | | | P815-TNP (H-2 ^d) | P815 | EL4-TNP (H-2 ^b) | EL4 |
| 1 | BALB/c (H-2 ^d) | BALB/c-TNP | 44.5 | 0 | 20.7 | 2.8 |
| | B6 (H-2 ^b) | B6-TNP | 14.0 | 4.5 | 31.2 | 0 |
| | | | B6-TNP | B6 | A/J-TNP | A/J |
| 2 | B6 | B6-TNP | 57.2 | 0 | 20.0 | 4.6 |
| | A/J (H-2 ^a) | A/J-TNP | 10.0 | 0 | 34.3 | 0 |
| | | | B6-TNP | B6 | B10.BR-TNP (H-2 ^k) | B10.BR |
| 3 | B6 | B6-TNP | 55.3 | 0 | 19.4 | 0 |
| | | | B6-TNP | | DBA/1-TNP (H-2 ^g) | |
| 4 | B6 | B6-TNP | 75.1 | | 58.8 | |

* Values are in percent specific release of ⁵¹Cr.

‡ Effector to target ratio was 40:1. Spontaneous release from P815 and P815-TNP was 10-18%; for EL4 and EL4-TNP, 18-26%; 31-39% for B6 and B6-TNP; 28-31% for A/J and A/J-TNP; B10.BR-TNP and B10.BR is 36-39%; and 46% for DBA/1-TNP.

θ -bearing cells from the effector population. Treatment with anti- θ alone augmented killing slightly, while C alone had no effect.

Alloantisera Directed to Target H-2 Specificities Inhibit Cross-Reactive Cytolysis. Schmitt-Verhulst et al. (3), and ourselves (8), have reported that alloantisera directed to the gene products of the *K* and *D* loci of the *H-2* complex of the target cell inhibit cytolysis of the target by effector cells generated against TNP-derivatized spleen cells that are *H-2* identical to the target. We have also demonstrated that alloantisera directed to target *H-2* specificities inhibit cross-reactive lysis (8). However, evidence of the unique inhibitory capacity of antisera specific for *H-2D* and/or *K* products as opposed to other cell surface antigens was deemed necessary. The inhibitory properties of two antisera directed against membrane specificities other than *H-2D* and *H-2K* were investigated. Freund et al., reported recently that the DBA/2 leukemia L1210 (*H-2^d*) possesses a high concentration of a cell surface antigen which elicits a strong antibody response in the *H-2* identical C57BL/Ks (*H-2^d*) strain of mice.² The antigen detected by anti-L1210 antisera is also present in significant amounts on normal B lymphocytes. This antigen, since it elicits antibodies in mice identical

² Freund, J. G., A. Ahmed, R. E. Budd, M. E. Dorf, K. W. Sell, W. E. Vannier, and R. E. Humphreys. 1976. The L1210 leukemia cell bears a B-lymphocyte-specific, non-*H-2*-linked alloantigen. *J. Immunol. In press.*

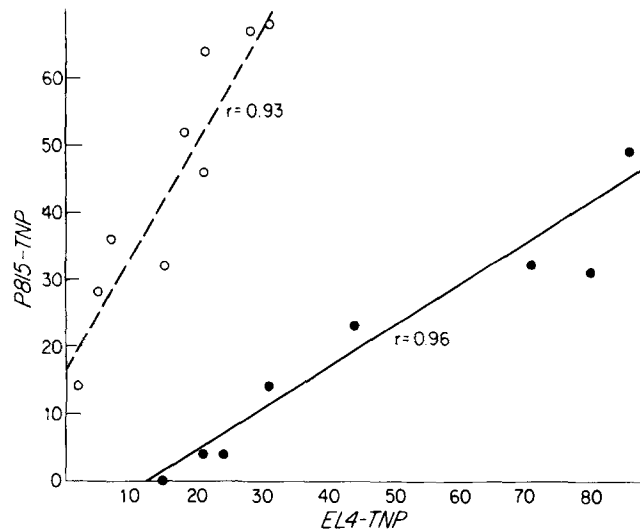


FIG. 1. Correlation of the extent of cytotoxicity on *H-2* identical and *H-2* different TNP tumor targets expressed as percent specific release of ^{51}Cr . B6 (*H-2^b*) anti-B6-TNP (closed circles) and BALB/c (*H-2^d*) anti-BALB/c-TNP (open circles) effectors were assayed simultaneously on EL4-TNP (*H-2^b*) and P815-TNP (*H-2^d*) targets. The correlation coefficient was determined and the data points were fitted to a straight line by linear regression analysis. Each point represents a separate experiment and the assays were performed at an effector to target ratio of 40:1.

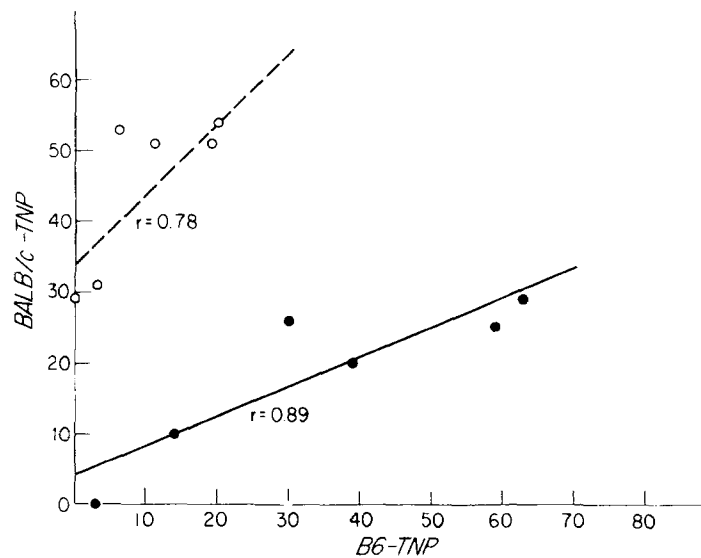


FIG. 2. Correlation of the extent of cytotoxicity on *H-2* identical and *H-2* different TNP spleen targets expressed as percent specific release of ^{51}Cr . B6 (*H-2^b*) anti-B6-TNP (closed circles) and BALB/c anti-BALB/c-TNP (open circles) effectors were assayed simultaneously on B6-TNP and BALB/c-TNP targets. The correlation coefficient was determined and the data points were fitted to a straight line by linear regression analysis. Each point represents a separate experiment and the assays were performed at an effector to target ratio of 40:1.

TABLE II
Sensitivity of Effector cells to Anti- θ Plus C Treatment

| Exp. no. | Responder | Stimulator | Effector Cell Treatment | Target* | % Specific Release of ^{51}Cr |
|----------|--------------------|------------|-------------------------|-----------------------|--|
| 1 | B10.BR ($H-2^k$) | B10.BR-TNP | None | B10.BR-TNP | 68.5 |
| | B10.BR | B10.BR-TNP | $\alpha \theta$ Plus C | B10.BR-TNP | 11.9 |
| 2 | B6 ($H-2^b$) | B6-TNP | None | B10.BR-TNP | 12.7 |
| | B6 | B6-TNP | $\alpha \theta$ Plus C | B10.BR-TNP | 1.7 |
| | BALB/c ($H-2^d$) | BALB/c-TNP | None | B10.BR-TNP | 11.0 |
| | BALB/c | BALB/c-TNP | $\alpha \theta$ Plus C | B10.BR-TNP | 0.4 |
| 3 | BALB/c | BALB/c-TNP | None | L1210-TNP ($H-2^d$) | 38.6 |
| | BALB/c | BALB/c-TNP | $\alpha \theta$ Plus C | L1210-TNP | 2.4 |
| | B6 | B6-TNP | None | L1210-TNP | 21.5 |
| | B6 | B6-TNP | $\alpha \theta$ Plus C | L1210-TNP | 0.5 |

* B10.BR-TNP spleen cells were treated with anti- θ antiserum plus C and the remaining viable cells were used as target cells. Spontaneous release of L1210 targets was 9–16% and B10.BR-TNP targets was 33–40%. Effector to target ratio was 40:1.

with the tumor at $H-2$, is not coded for by the $H-2$ complex. Such a potent anti-L1210 antiserum was used to determine if antibodies to non- $H-2$ cell surface markers on target cells could inhibit lysis. Table III shows that neither syngeneic nor more importantly cross-reactive lysis can be inhibited by an antiserum to the L1210 non- $H-2$ alloantigen, even though this antiserum had a greater C-dependent lytic titer on L1210 cells than the anti- $H-2D^d$ antiserum endowed with inhibitory properties.

The specificity of inhibition of syngeneic and cross-reactive lysis by alloantisera to $H-2 D$ and K was also confirmed by attempting to inhibit lysis using an antiserum to other $H-2$ products, namely Ia antigens. To insure maximal representation of Ia on target cells, B10.BR spleen cells were treated with anti- θ serum plus C and only the remaining viable cells used as targets. After such treatment 80–90% of the viable cells were surface immunoglobulin and Ia^k positive. Table III documents that cross-reactive lysis of such "B-lymphocyte" B10.BR-TNP targets by BALB/c anti-BALB/c-TNP or B6 anti-B6-TNP effectors was not inhibited by an antiserum to the I-region gene products of the $H-2^k$ haplotype of the B10.BR-TNP target, in contrast to the inhibitory properties of an antiserum to the $H-2^k D$ and K antigens with equivalent cytotoxic titer.

Failure to Detect TNP-Specific CTL Using TNP-CRBC as Inhibitors of Cytolysis. The ability of anti- $H-2 D$ or K antisera to inhibit cross-reactive lysis indicated a probable functional role for the D and K gene products in cytolysis. However, such data did not preclude the possibility, suggested by Dennert and Hatlen, that such cross-reactive killers were primarily "hapten specific," i.e. recognized the TNP determinant via their antigen receptors, and that the $H-2 D$ or K restrictions concerned other features of the cell-cell interaction necessary

TABLE III
*Inhibition of Cytolysis by Antisera Directed to Cell Surface antigens of the Targets**

| Exp. no. | Responder | Stimulator | Target | Control‡ | α -L1210 | α -H-2D ^d |
|----------|-----------|------------|-------------|----------|---------------------------|--|
| 1 | BALB/c | BALB/c-TNP | L1210-TNP | 16.5 | 20.9 | 4.7 |
| | B6 | B6-TNP | L1210-TNP | 11.1 | 15.9 | 7.2 |
| | B10.BR | B10.BR-TNP | L1210-TNP | 30.7 | 33.7 | 9.9 |
| | | | | Control | α -Ia ^k | α -H-2K ^k D ^k |
| 2 | B10.BR | B10.BR-TNP | B10.BR-TNP§ | 61.5 | 43.3 | 7.9 |
| 3 | BALB/c | BALB/c-TNP | B10.BR-TNP | 24.6 | 19.6 | 5.1 |
| | B6 | B6-TNP | B10.BR-TNP | 22.6 | 21.7 | 0 |

* Values are in percent specific release of ⁵¹Cr.

‡ Control consisted of either MEM or NMS at a final dilution of 1:12. Anti-L1210 antiserum is C57BL/Ks (H-2^d) anti-L1210 (H-2^d); with a lytic titer of 1/960; anti-Ia^k is A.TH anti-A.TL with a lytic titer of 1/1,280; anti-H-2D^d is (B10.LP.R III)F₁ anti-18R with a lytic titer of 1/2,560; and anti-H-2K^kD^k is (B10 × A.TH)F₁ anti-B10.BR with a lytic titer of 1/1,280. All antisera are at a final dilution of 1:12.

§ B10.BR-TNP spleen cells were treated with anti- θ antiserum plus C and the remaining viable cells were used as targets. 80-90% of the remaining spleen cells were lysed by anti-Ia^k antiserum plus C. Spontaneous release of L1210-TNP is 16 and 49% for B10.BR-TNP. Effector to target ratio was 40:1, except for B10.BR effectors tested on B10.BR-TNP target, where the ratio was 5:1.

for lysis (6, 7). To evaluate this possibility, attempts were made to inhibit lysis of TNP targets using unlabeled TNP-CRBC as potential competitors for TNP-specific receptor binding in the ⁵¹Cr-release assay. TNP-CRBC were derivatized by Dennert's method (6) and various concentrations of these cells were added to effectors and ⁵¹Cr-labeled targets. No inhibition of syngeneic or cross-reactive lysis of TNP derivatized at ratios up to and including 120 TNP-CRBC:1 ⁵¹Cr target cell was observed. A ratio of 240:1 nonspecifically inhibited allogeneic killing of non-TNP-derivatized targets and therefore was not informative.

Inhibition of Cytolysis by Competition with Cold Spleen Targets. Cold target competition experiments with unlabeled spleen cells were performed to determine whether the CTL-mediated lysis of H-2 different TNP-targets belong to the same set of CTL as those mediating lysis of syngeneic TNP targets, or whether they were totally distinct effectors. Fig. 3 demonstrates that the cytolysis of BALB/c-TNP ⁵¹Cr-labeled targets by BALB/c effectors can only be inhibited by the presence of TNP-derivatized nonradiolabeled BALB/c spleen cells. B6 effectors assayed on ⁵¹Cr-labeled B6-TNP targets can also be inhibited only by TNP-derivatized non-⁵¹Cr-labeled spleen cells that are syngeneic to the target cell.

Cross-reactive cytolysis of B6 effectors generated to B6-TNP stimulators and assayed on BALB/c-TNP ⁵¹Cr targets, however, can be inhibited similarly by BALB/c-TNP and B6-TNP unlabeled targets. Data are given for a ratio of 40 unlabeled spleen cells:1 ⁵¹Cr-labeled spleen cell, but qualitatively identical results were obtained at a 20:1 ratio. These findings indicate that the same CTL population responsible for the lysis of H-2 syngeneic TNP targets also lyse H-2 different TNP targets.

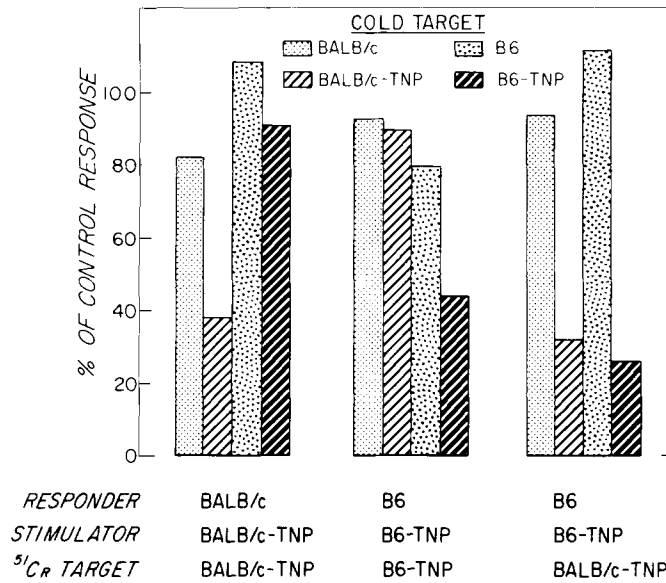


FIG. 3. Inhibition of cytolysis by competition with nonradiolabeled spleen targets. Effectors were assayed in the absence of nonradiolabeled spleen cells (control response) or in the presence of a ratio of 40 nonradiolabeled spleen cells: 1 ⁵¹Cr-labeled spleen target. The control response for BALB/c anti-BALB/c-TNP assayed on BALB/c-TNP was 27% specific release, 63% for B6 anti-B6-TNP assayed on B6-TNP, and 37% for B6 anti-B6-TNP assayed on BALB/c-TNP. Effector to ⁵¹Cr target ratio was 40:1. Spontaneous release ranged between 39 and 48%.

Discussion

Normal spleen cells, when cultured with irradiated TNP-derivatized syngeneic spleen cells, develop cytotoxic effectors that lyse most effectively a TNP-derivatized target that is *H-2* compatible with the effector; these cytolytic effectors, however, also lyse to a lesser extent TNP tumor and TNP spleen targets that do not share products of the *H-2 K* or *D* loci with the effector. Cross-reactive lysis correlates directly with the degree of cytolysis seen on the TNP-derivatized syngeneic target, it appears to be mediated by θ -bearing cells and it is inhibited by antisera to the *K* and/or *D* loci of the target cell and not by several other antisera to non-*D* or non-*K* surface antigens. Nonradiolabeled TNP-derivatized lymphoid cells syngeneic to either the stimulator or the target are able to competitively inhibit cross-reactive lysis, while TNP-CRBC are unable to specifically inhibit cytolysis even at a 120:1 ratio to the target cells.

The high positive correlation noted between lysis of *H-2* identical and *H-2* nonidentical target cells mediated by the same effector population indicates that cross-reactive lysis is not an independent, randomly occurring event. Shearer et al. (4) have observed that effector cells sensitized with TNP-modified autologous spleen cells demonstrated a significant degree of lysis of non-*H-2*-matched TNP-modified tumor but not lymphoid targets. They suggest that this cross-reactivity is due to TNP-modified tumor targets being more sensitive to nonspecific lysis by cytotoxic effector cells. We have noted cross-reactive lysis on TNP-derivatized spleen as well as on TNP tumor targets. The fact that this cross-reactivity is

inhibited only by antisera directed to the *H-2 K* and *D* gene products makes it very improbable that we are simply observing nonspecific lysis. The hypothesis that "weak" B6 effectors to unmodified *H-2^d* alloantigens are generated in our cultures, which are detected on more "lysable" BALB/c-TNP targets in preference to BALB/c targets, is also unlikely to be valid, since competitive inhibition should then occur with unlabeled BALB/c cells as well as with BALB/c-TNP spleen cells. No such inhibition was observed.

It is not clear why cross-reactive lysis has not been reported by others. The quantitative relationships between syngeneic and cross-reactive lysis demonstrated in this study may account for the absence of such killing in most previous experiments with the TNP model, since the level of syngeneic lysis in those studies rarely exceeded 35%, the minimum level needed to see cross-reactive killing. In addition, we have noted (unpublished observations) that CTL from mice bearing certain *H-2* haplotypes fail to show cross-reactive lysis even though syngeneic lysis is greater than 80%. Thus, the particular mouse strain investigated may be critically responsible for the differences. It should be noted, however, that in several virus models investigated using some of the same strains as reported here, e.g. BALB/c, little or no cross-reactive lysis was noted even with specific release exceeding 70% on *H-2* identical targets (18). The reason for this marked difference between viral and chemical systems is not apparent but may relate to the possibly more extensive modifications of the *H-2 K* or *D* products of the target cells induced by TNP conjugation compared to interaction with viral products.

The evidence supporting the relevance of blocking data with specific anti-*H-2* antisera to the analysis of the functional role of *H-2 D* and *K* products in CTL target interaction is substantial. Previous reports in allogeneic systems as well as in the TNP model clearly show that only antisera directed to gene products of the *H-2* complex that can be shown by genetic analysis to be important in CTL-target interaction are able to inhibit cytolysis (3, 8, 19). Antisera against Ia antigens do not block cytolysis by CTL specific for modified syngeneic targets (3). This result is not unexpected since *I* region products have not been found to be involved in the specificity of CTL against modified syngeneic targets. We have extended these observations to cross-reactive lysis and shown that an antiserum to a cell-surface alloantigen distinct from *H-2* products fails to inhibit such lysis, whereas an anti-*H-2D^d* antiserum is inhibitory. Similarly, an anti-Ia^k antiserum shows no effect on B6 anti-B6-TNP CTL-mediated lysis of B10.BR-TNP cells while an anti-*H-2K^kD^k* antiserum is inhibitory. Such results make it highly improbable that blocking of CTL by anti-*H-2* antisera represent a non-specific effect on the target membrane, reducing target lysability, or alternatively that such inhibition results from nonspecific steric hindrance of CTL-target interaction due to large amounts of unrelated antibody present on the target cell membrane. These and previous data on alloantisera inhibition of cross-reactive lysis also demonstrate that the antisera inhibit by acting on the target cells and not the CTL, since antisera against effector CTL cannot block cross-reactive lysis (8).

As stated in the introduction, two models have been proposed to explain the *H-2* restriction of effector-target interaction for effectors generated to modified syngeneic cells: the dual recognition model and the altered-self hypothesis. The

dual recognition hypothesis as presently stated cannot account adequately for our observations of cytolysis between *H-2* incompatible effector and target cells, nor for the observations of others with radiation chimeras (14-16). In these chimeras, cytotoxic effectors could be induced against virally infected or TNP-modified *H-2* incompatible tolerated target cells. In both situations, cytolysis is observed with effector and target cells differing at *H-2 D* and *K*, precluding a strict requirement for *H-2 D* or *K* identity for cell-cell interaction. The possibility that much less efficient, but still detectable, "physiologic interaction" could occur between effector *H-2* recognition structures and target cell allogeneic *H-2* markers is made unlikely by the finding that in the chimeric systems killing of *H-2* different targets matched to the stimulator cells is considerably greater than killing of targets matched to the effector *H-2* (14-16). If "cross-reactive" physiologic interaction were the explanation for the apparent violation of the *H-2* identity rule, killing of the latter targets should at least equal that of the *H-2* different targets. Since the opposite is found, the cross-reactivity observed in our experiments is consistent with the altered-self hypothesis, i.e., the formation of new antigenic determinants involving TNP modification of *H-2 D* or *K*. Effector cells generated to TNP-derivatized syngeneic cells would recognize shared determinants resulting from TNP derivatization of *H-2* different targets. If cross-reactivity occurs for this reason (i.e., shared antigens), one would predict that unlabeled TNP spleen cells syngeneic to the stimulator as well as the non-*H-2*-matched target would competitively inhibit cross-reactive lysis. This was shown to be the case. ⁵¹Cr release by B6 effectors generated to B6-TNP stimulators and assayed on BALB/c-TNP targets was inhibited equally well by B6-TNP and BALB/c-TNP spleen cells. Thus, the data suggest that the antigens recognized on the BALB/c-TNP targets were the same or similar to the antigens recognized on the B6-TNP targets (and stimulators), since B6-TNP could competitively inhibit this cross-reactive lysis. This would imply that a subset of the clones that lyse the *H-2* compatible targets constitute the subpopulation of CTL that lyses the *H-2* incompatible targets. Since allogeneic TNP cold targets do not inhibit lysis of syngeneic TNP targets, this subset is most likely a small fraction of the total CTL population (i.e., 15-25%).

In conclusion, it would appear that our data on cross-reactive lysis of TNP-conjugated targets is most consistent with the altered-self hypothesis. To account for our data and that involving chimeras, the dual (immunological-physiological) interaction model would require the effector cell to bear, besides an antigen-specific receptor, a surface component that could interact with histoincompatible target cells, a possibility for which little supporting data exists.

Summary

Normal spleen cells, when cultured with irradiated trinitrophenyl (TNP)-derivatized syngeneic spleen cells, develop cytotoxic effectors that lyse most effectively a TNP-derivatized target that is *H-2* compatible with the effector. However, these effectors also lyse to a lesser extent TNP tumor and TNP spleen targets that are *H-2* incompatible. This cross-reactive lysis correlates with the degree of cytolysis seen on the TNP-derivatized syngeneic target; it appears to

be mediated by Thy 1.2-bearing cells and is inhibited by antisera to the *K* and/or *D* loci of the target cell and not by antisera to non-*K* or non-*D* surface antigens. Nonradiolabeled TNP-derivatized lymphoid cells syngeneic to either the stimulator or the target are able to competitively inhibit cross-reactive lysis, while TNP chicken red blood cells are unable to specifically inhibit lysis. These data on cross-reactive lysis of TNP-conjugated targets are most consistent with the altered-self hypothesis.

We wish to thank Dr. Martin Dorf for his valuable advice and contribution of antisera; and Mr. Sheldon Ratnofsky for his excellent technical assistance.

Received for publication 22 July 1976.

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