

ROLE OF SELF AND FOREIGN ANTIGENIC DETERMINANTS IN ALLOGENEIC AND SELF-RESTRICTED CYTOTOXIC T CELL RECOGNITION

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The strongest primary cytotoxic responses in all species are induced by allogeneic histocompatibility antigens. This finding, which is associated with a relatively high frequency of alloreactive precursor cells (1–10%) (1, 2), has been difficult to understand within the context of the host's autologous immunological environment, including the demonstration of major histocompatibility complex (MHC)¹-restricted cytotoxicity (3, 4). It has recently been reported that alloantigen-stimulated cytotoxic lymphocytes lyse autologous cells treated with trinitrobenzene sulfonic acid (TNBS) (5). These results have raised the possibility that alloantigens can resemble self determinants that are associated with certain foreign (X) antigens (5). Thus, cytotoxic T lymphocytes (CTL) directed against alloantigens may be a manifestation of the host's ability to respond to one or more self plus X antigens. If such a proposal were correct, two important questions to be addressed to further analyze this hypothesis are: (a) whether alloantigen-activated CTL can lyse self targets recognized in association with haptenic or viral determinants other than trinitrophenyl (TNP), and, if so, whether distinct populations of allo-induced CTL can distinguish between self plus TNP and self plus X target cells; and (b) can potent effector cell activity induced against non-H-2 antigens recognize self target cells modified with TNP? Our results indicate that alloantigen-activated CTL can lyse autologous, fluorescein isothiocyanate (FITC)-conjugated target cells, and, in addition, that distinct clones of these effector cells lyse self plus TNP and self plus FITC target cells. The present results also illustrate that, in contrast to these hapten-self targets, male target cells expressing the H-Y antigen are not lysed by such allogeneic effector cells. Furthermore, the present study demonstrates that anti-H-Y CTL populations with quantitatively equal or greater activity than alloantigen-activated effector cell populations do not lyse self targets modified with TNP. These findings are discussed in the context of the self and X antigenic determinants involved in allogeneic and self-restricted CTL models.

Materials and Methods

Animals

The C3H/HeN and DBA/2 strains were obtained from the Animal Production Facility, National Cancer Institute, Bethesda, Md. All B10 congenic mice were purchased from The Jackson Laboratory, Bar Harbor, Maine

¹ *Abbreviations used in this paper.* ACK, ammonium chloride-lysing solution; BSS, balanced salt solution, Con A, concanavalin A; CTL, cytotoxic T lymphocyte(s); FITC, fluorescein isothiocyanate; MHC, major histocompatibility complex; TNBS, trinitrobenzene sulfonic acid, TNP, trinitrophenyl, X, foreign.

In Vivo Immunizations

Allogeneic. Mice were injected intraperitoneally with varying numbers of EL4 (*H-2^b*) lymphoma cells, which had been maintained by serial passage in syngeneic C57BL/10 mice. All inoculations were performed in a vol of 1.0 ml, in a solution of phosphate-buffered saline (National Institutes of Health Media Unit). Mice were sacrificed from 3 to 6 wk after injection.

Male Antigen. Female C57BL/10 mice were injected with 10^7 C57BL/10 male spleen cells that had been freed of erythrocytes by treatment with ammonium chloride-lysing solution (ACK) (National Institutes of Health Media Unit). All inoculations were administered intraperitoneally, in a vol of 1.0 ml of phosphate-buffered saline. Mice were sacrificed from 3 to 9 wk after injection.

Conjugation of Spleen Cells with TNBS and FITC

Spleens were minced, passed through sterile nylon, and washed in a balanced salt solution (BSS) (National Institutes of Health Media Unit). The pellet was treated with ACK to remove erythrocytes and washed with BSS. Solutions of TNBS (Pierce Chemical Co., Rockford, Ill.) and FITC (Sigma Chemical Co., St. Louis, Mo.) were prepared in pH 7.4 and 9.0 phosphate-buffered saline, respectively. After centrifugation, the washed cells were resuspended in the appropriate hapten solution and incubated at 37°C (TNBS: 5 mM, 10 min; and FITC: 200 µg/ml, 15 min) in a humidified, air atmosphere. After the reactions, the cells were washed three times in BSS with 5% fetal bovine serum (Microbiological Associates, Walkersville, Md.) and counted for use.

Sensitization Cultures

Cytotoxic effector cells were generated in 24- × 16-mm flat-bottom wells (Linbro Chemical Co., Hamden, Conn.) as described in detail elsewhere (6). Briefly, responding spleen cells (5×10^6) were cocultured with 2,000-rad-irradiated (^{137}Cs source; Gammator; Isomedix Inc., Parsippany, N. J.) allogeneic (2×10^6) or male (4×10^6) stimulating cells that had been freed of erythrocytes by ACK treatment. The plates were incubated at 37°C, in 95% air-5% CO₂ atmosphere for 5 d.

Chromium Release and Cold Target-Cell Competition Assays

Effector cells were harvested, washed, resuspended at the desired concentration and 100 µl were added to microtiter wells (Linbro Chemical Co.). Phytohemagglutinin- (Difco Laboratories, Detroit, Mich.) or concanavalin A (Con A)- (Sigma Chemical Co.) stimulated spleen cells (6) were chromium labeled (New England Nuclear, Boston, Mass.), washed three times, and 100 µl were mixed with the effector cells at the appropriate titrations. The plates were centrifuged for 4 min at 300 rpm, and incubated for 4 h at 37°C, in a 95% air-5% CO₂ 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., Inc., Downers Grove, Ill.).

Single-cell suspensions of nonradioactive (cold) blocking cells were prepared from fresh spleens, and erythrocytes were removed with ACK. Approximately one-third of the cells were haptenated as described above, washed three times, and adjusted to the desired concentration. 50 µl of these cold targets was incubated with 50 µl of effector cells for 20 min at 37°C. 100 µl of ^{51}Cr -target cells was then added, and the plates were treated as described above. The percentage of lysis and standard error of the mean were calculated based on triplicate samples as previously described (6). Standard errors never exceeded 4.5% and are presented in certain figures to enable more accurate comparison between experimental groups.

Results

Primary In Vitro Alloantigen-induced CTL Lyse Self Target Cells Conjugated with TNP or FITC. Allogeneic CTL generated from unprimed spleen cells after in vitro sensitization cultures were tested on TNBS-treated self target cells. The results in Fig. 1A and B illustrate that C3H anti-DBA/2 effector cells can lyse C3H-TNP target cells

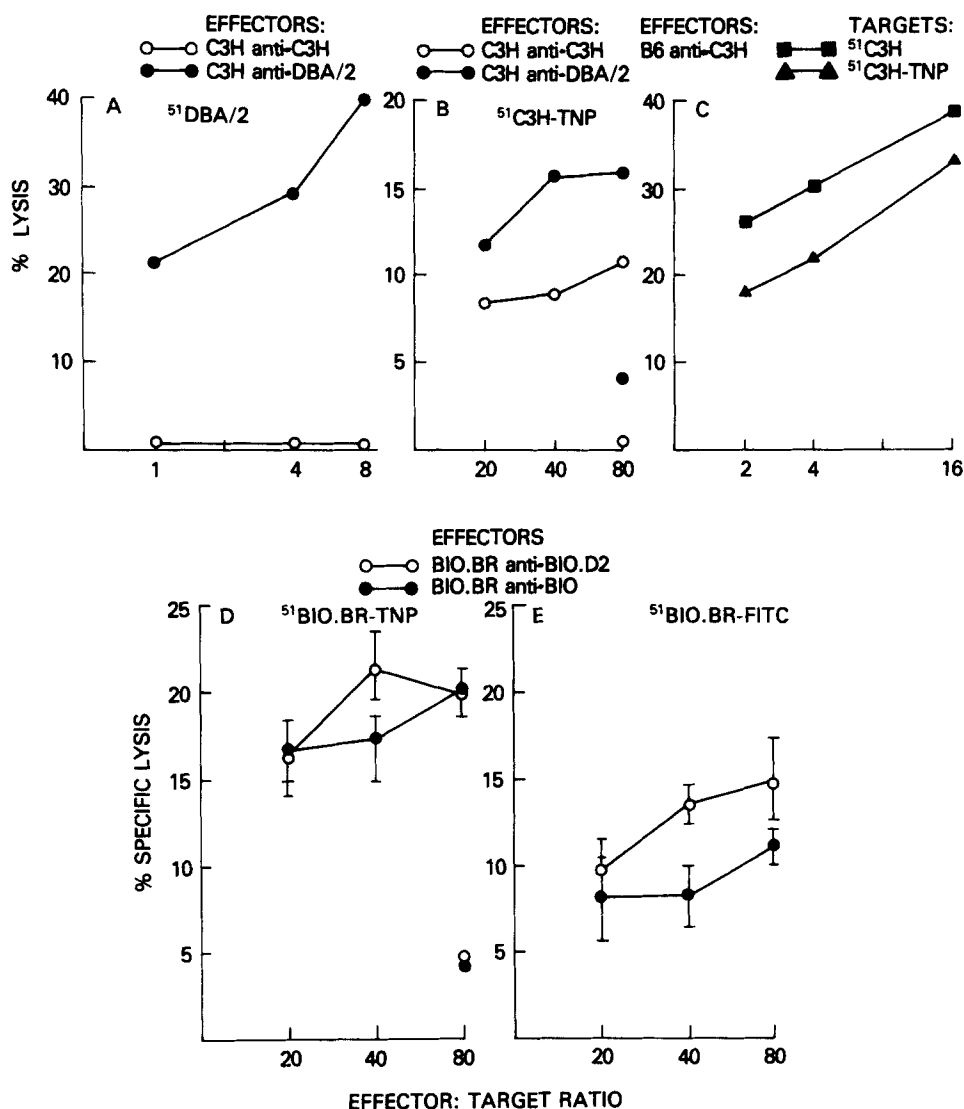


FIG. 1. C3H anti-DBA/2 effector cells assayed against DBA/2 (A) and C3H-TNP (B) PHA-stimulated target cells. (B) ○ and ● represent the percent lysis, respectively, by these effector populations against C3H unmodified target cells at an effector:target ratio of 80:1. Spontaneous release: DBA/2, 23.9%; C3H-TNP, 23.3%; C3H, 23.9%. B10.BR effector cells assayed against PHA blast B10.BR-TNP (D) and B10.BR-FITC (E) target cells. Hapten conjugation was performed as described in Materials and Methods. ○ and ● represent percent lysis, respectively, by these effector populations on B10.BR unmodified targets at an effector:target ratio of 80:1. B10.BR anti-B10.BR cultures mediated 2.2% and -6.2% lysis on B10.BR-TNP and B10.BR-FITC targets, respectively. Spontaneous release: B10.BR-TNP, 34.4%; B10.BR-FITC, 38.5%.

but not uncoupled target cells at high effector:target ratios as previously reported (5). It should be noted that in numerous experiments cultures of nonantigen-stimulated effector cells (i.e., C3H anti-C3H) often show cytotoxic activity on self target cells modified with TNP (Fig. 1 B) (7). This activity was always less than that from alloantigen-activated cultures, but obviously complicates the interpretation of the

results. Notably, these control cultures do not exhibit activity when the responding cells are taken from alloantigen-primed mice. In all subsequent experiments, the percentage of lysis by these control cultures is not shown, but it has been subtracted from the lysis mediated by stimulated cultures for the sake of clarity.

An important control that also must be addressed in this model is the lytic susceptibility of modified vs. unmodified target cells. As shown in Fig. 1 C, allogeneic effector cells generated against $H-2^k$ alloantigens did not lyse C3H-TNP target cells more efficiently than C3H target cells. A difference in target cell susceptibility is, therefore, not likely to account for the difference of C3H anti-DBA/2 lysis of C3H-TNP vs. C3H target cells (Fig. 1 B).

The results of a separate experiment (Fig. 1 D and E) demonstrate again that alloantigen-stimulated CTL cultures ($H-2^k$ anti- $H-2^d$ and $H-2^k$ anti- $H-2^b$) can lyse TNP-conjugated self targets (Fig. 1 D), and, in addition, these cultures also exhibit activity against FITC-conjugated self targets (Fig. 1 E). As was consistently observed in our experiments, (a) the lysis detected on FITC-self targets was less than that displayed on TNP-self targets, and (b) no consistent difference in the quantity of lysis observed on hapten-self targets was induced by different H-2 allogeneic stimulating cell populations. These results thus demonstrate that alloantigen-induced effector cells can cross-react with self-target cells associated with an X determinant other than TNP.

Secondary Cultures of Alloantigen-stimulated CTL Mediate Enhanced Lysis of Self Target Cells Conjugated with TNP or FITC. To enhance the lysis detectable on hapten-conjugated self target cells, mice were inoculated with allogeneic tumor cells several weeks before in vitro sensitization. The results in Fig. 2 A illustrate that C3H spleen cells from mice injected with EL4 ($H-2^b$) lymphoma cells generated markedly greater cytotoxicity against B10 ($H-2^b$) target cells than a primary C3H anti-B10 effector population. When the secondary CTL populations were assayed on TNP- (Fig. 2 C) and FITC-conjugated (Fig. 2 D) C3H target cells, significantly greater lysis was detected compared with the primary CTL population. Again, no activity was observed on unmodified C3H target cells (Fig. 2 C).

Effector cells generated against $H-2^k$ alloantigens lysed C3H-TNP and C3H-FITC target cells to essentially the same extent as C3H target cells (Fig. 2 B). These findings again indicate that the lysis mediated by C3H anti-B10 CTL against C3H-TNP and C3H-FITC target cells is not the result of their increased susceptibility to lysis and, similarly, would not explain the consistently greater lysis observed against TNP-self compared with FITC-self targets. Therefore, the results of Fig. 2 demonstrate that enhanced allogeneic CTL activity as detected on the appropriate allogeneic target was accompanied by enhanced CTL activity detected on hapten-modified self targets.

Cold Target Competition Analysis Demonstrates Separate Alloantigen-induced Cytotoxic Populations That Recognize TNP-Self and FITC Target Cells. Because secondary cultures of alloantigen-primed spleen cells were found to mediate 25–40% specific lysis against TNP-self and FITC-self target cells, these effectors were examined in cold target competition experiments. A representative experiment is illustrated in Fig. 3. Spleen cells from C3H ($H-2^k$) mice were primed and restimulated against $H-2^b$ alloantigens, and assayed on $H-2^b$, $H-2^k$ -TNP, and $H-2^k$ -FITC target cells. As shown in Fig. 3 A–C, when compared with a primary in vitro activated CTL population, the primed effector population exhibited markedly greater cytotoxicity detected on $H-2^b$ targets

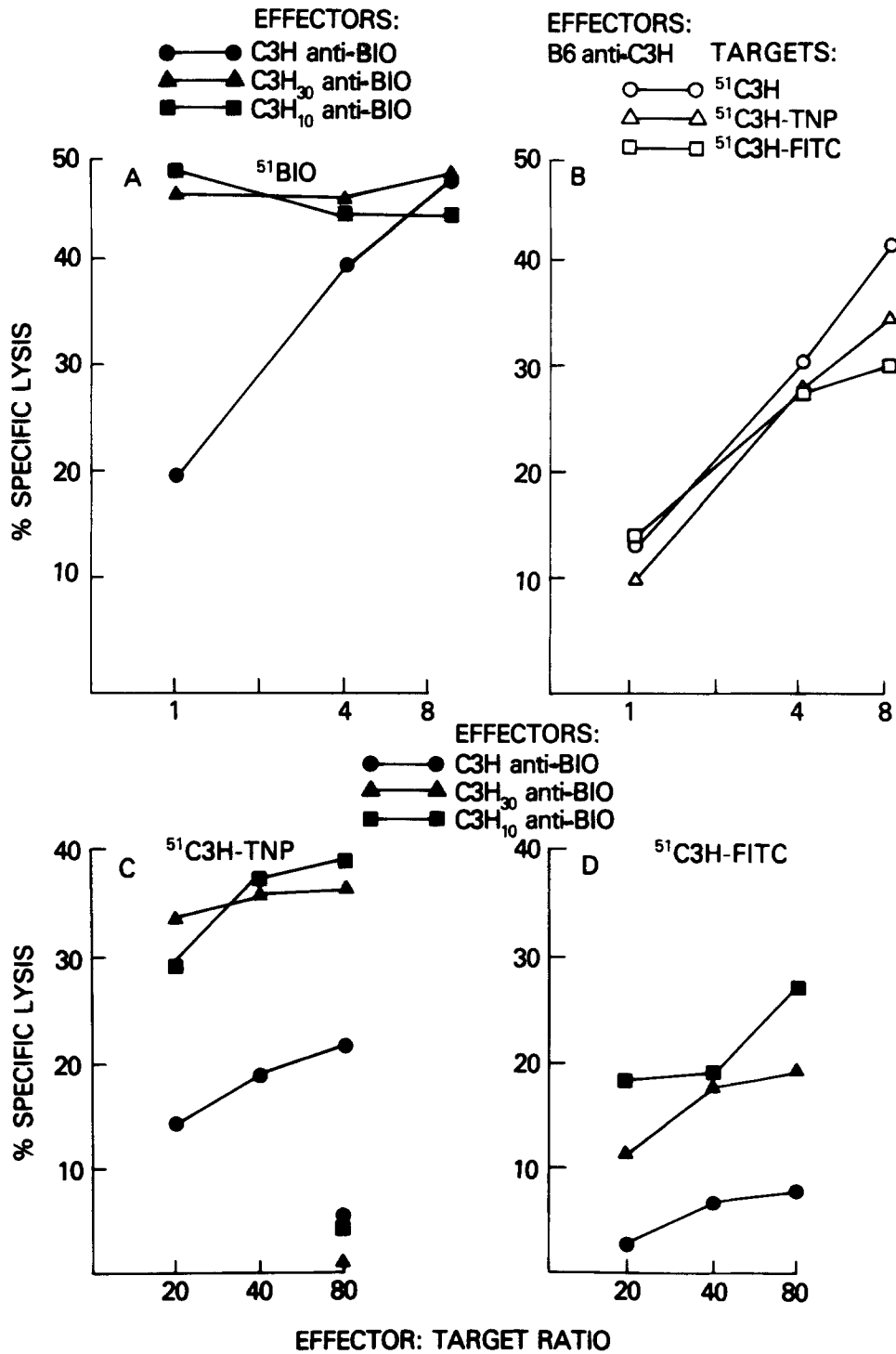


FIG 2 Spleen cells from normal (○) or allogeneic (*H-2^b*) primed (▲, injected with 30×10^6 , ■ injected with 10×10^6) C3H mice were stimulated with B10 (*H-2^b*) spleen cells and assayed on B10 (*H-2^b*) (A), C3H-TNP (C), and C3H-FITC (D) PHA-stimulated target cells (C) ●, ▲, and ■ represent lysis by the three effector populations on unmodified C3H targets at 80:1 C3H anti-C3H cultures from the three responding populations mediated from -5.7 to 9.7% lysis at 80:1 on C3H-TNP and C3H-FITC targets. Spontaneous release: C3H, 21.8%, C3H-TNP, 26.6%, C3H-FITC, 27.5%, B10, 23%

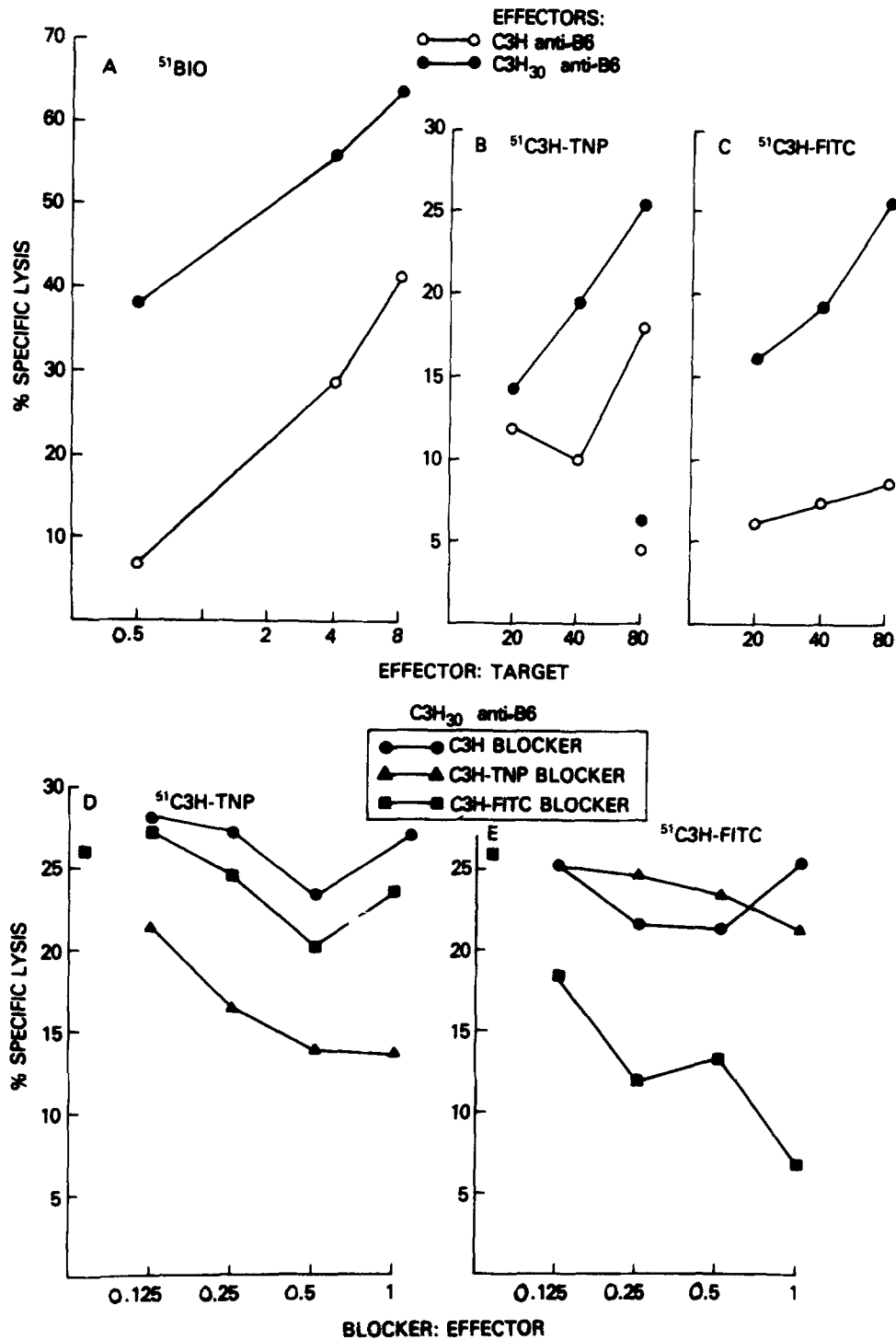


FIG. 3. Spleen cells from normal (○) or allogeneic ($H-2^b$) primed (●) C3H mice were stimulated with B6 ($H-2^b$) spleen cells and assayed on B10 ($H-2^b$) (A), C3H-TNP (B and D), and C3H-FITC (C and E) PHA-stimulated target cells (B) ○ and ● represent lysis by these effector populations on unmodified C3H target cells at 80:1. In D and E, ■ represents the lysis by primed C3H anti- $H-2^b$ effectors on C3H-TNP and C3H-FITC at an effector target ratio of 80:1, in the absence of blocking cells. Cold target blocking populations were added at the indicated blocker effector ratio as described in Materials and Methods (D and E). C3H-anti-C3H cultures from the responding populations mediated from -5.2 to 8.1% lysis at 80:1 on C3H-TNP and C3H-FITC targets. Spontaneous release: C3H, 30.9%, C3H-TNP, 30.0%, C3H-FITC, 33.0%, B10, 22.2%.

(Fig. 3A) as well as significantly increased lysis on $H-2^k$ -TNP (Fig. 3B) and $H-2^k$ -FITC (Fig. 3C) target cells. These primed effector cells were simultaneously assayed on these latter two targets in the presence of nonradioactively labeled (cold) targets. The results of Fig. 3D and E clearly demonstrate that cold $H-2^k$ -TNP blockers inhibit cytotoxicity detected on $H-2^k$ -TNP but not $H-2^k$ -FITC-self (radioactively labeled) target cells. Reciprocally, $H-2^k$ -FITC blockers only inhibit lysis against C3H targets conjugated with FITC but not TNP. These findings indicate that the allogeneic CTL population that is able to lyse self-TNP targets is not the same population that is able to lyse self-FITC target cells.

Can Antigens Other Than Allogeneic H-2 Determinants Induce CTL That Lyse Hapten-conjugated Self Target Cells? Because allogeneic H-2 antigen-induced CTL lyse hapten-modified self target cells, it was important to investigate whether CTL activated by different antigens could also mediate this lytic response. The results presented in Figs. 4 and 5 illustrate two independent experiments comparing the ability of allogeneic H-2 antigen-induced and H-Y antigen-induced CTL populations to lyse their specific targets as well as a common self target conjugated with TNP. The experiment shown in Fig. 4 demonstrates that (a) B10 female mice primed and restimulated against B10 male spleen cells generate a strong cytotoxic response assayed on B10 male target cells (Fig. 4B), (b) there is no difference in the ability of this primed B10 female population, compared with an unprimed population, to generate alloantigen-specific CTL (Fig. 4A); (c) an extensive effector:target titration indicates a comparison between the allogeneic and H-Y antigen-induced CTL responses by primed (against B10 male cells) B10 female responders that little, if any, quantitative difference can be detected (Fig. 4C); and (d) when the latter two effector populations are assayed on self- (B10 female) TNP (or FITC [data not shown]) target cells, only the alloantigen-induced effector population mediates cytotoxicity (Fig. 4D).

The data from a similar experiment are presented in Fig. 5. The results again indicate that B10 female responding cells primed against B10 male cells generate a strong cytotoxic response after in vitro stimulation with male cells (Fig. 5B), and, additionally, that this response is not detected against a non- $H-2^b$ (i.e., $H-2^d$) male target (Fig. 5A). Fig. 5C indicates that, in this experiment, the H-Y-specific CTL response was actually stronger than the allogeneic response by B10 female H-Y-primed responding cells. However, as previously observed (Fig. 4D), when these two effector populations were tested on self-TNP target cells, only the allogeneic effector population showed significant cytotoxicity (Fig. 5D). Although this B10 female allogeneic effector population lysed self-TNP target cells, the data in Fig. 5B indicate that this same population did not contain effector cells that recognized B10 (i.e., self) H-2 determinants in association with the male antigen.

Discussion

The results of the present study have analyzed autologous cross-reactive lysis by antigen-stimulated cytotoxic lymphocytes. Our data: (a) verify the findings of earlier reports that allogeneically stimulated effector populations can lyse TNP-conjugated self target cells (5, 8–10), but demonstrate that these allogeneic effectors do not lyse self targets expressing the H-Y antigen; (b) indicate that allogeneic populations of effectors can also lyse self targets conjugated with another noncross-reacting hapten (FITC); (c) illustrate that in allogeneically induced CTL cultures, separate subpopu-

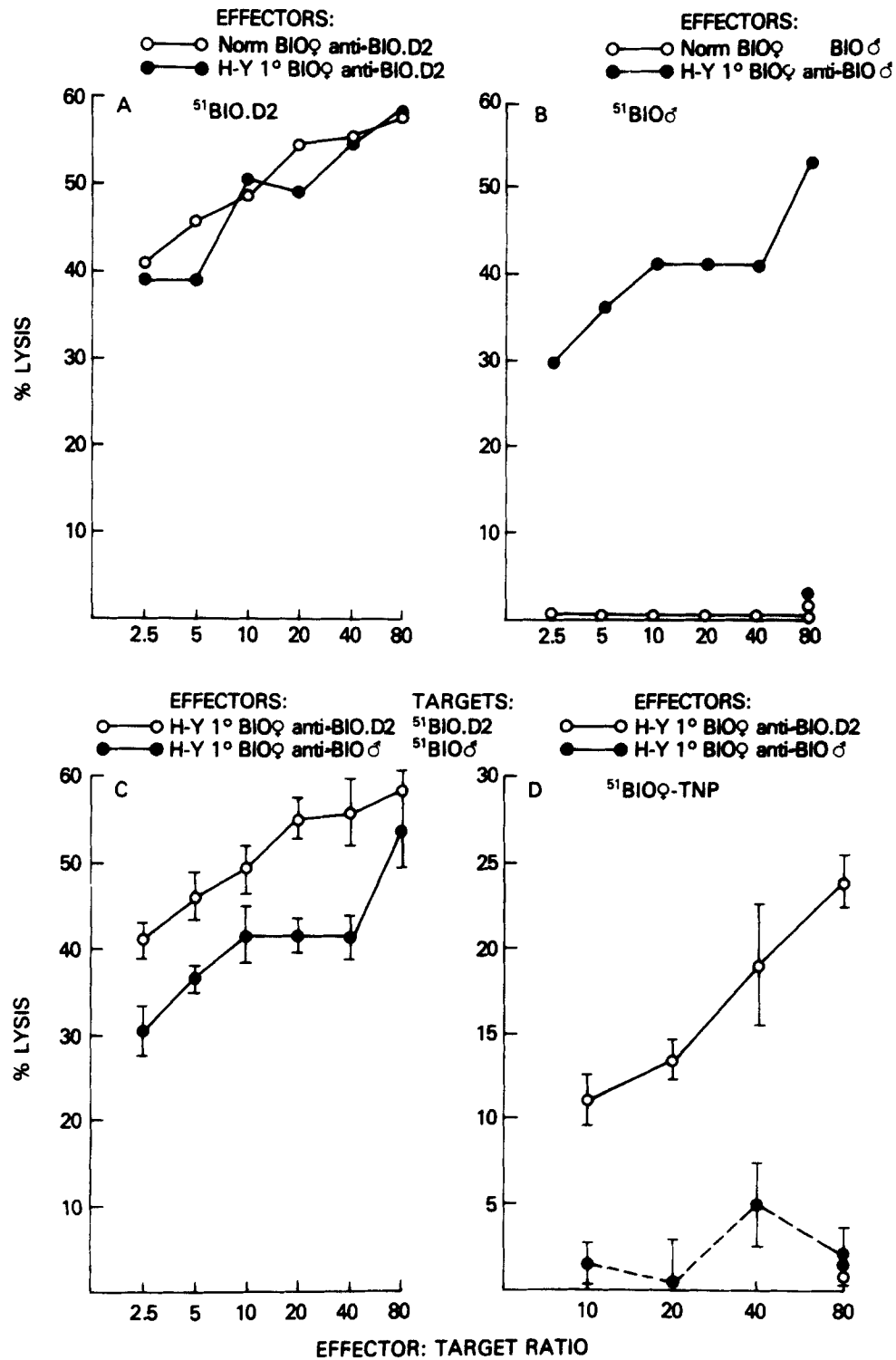


FIG. 4 Spleen cells from normal (○) or H-Y-primed (●) B10 female mice were stimulated with B10 male (B) and B10 D2 male (A) spleen cells, and assayed on B10 male (B) and B10.D2 male (A) Con A-stimulated target cells (B) ○ and ● represent the percent lysis by the normal and H-Y-primed effector populations against B10 female target cells at 80:1. Both effector cell populations generated from H-Y-primed mice (○, anti-B10.D2; ●, anti-B male) were plotted for comparison against their specific target cells in Fig. 4C. In Fig 4D, these effector cell populations were assayed on B10-TNP female target cells (D) ○ and ● represent the percent lysis by these effector populations on unmodified B10 female target cells at 80:1. Spontaneous release: B10 female, 38.3%, B10-TNP female, 40.4%; B10 male, 32.6%, B10.D2 male, 33.5% 1°, primary

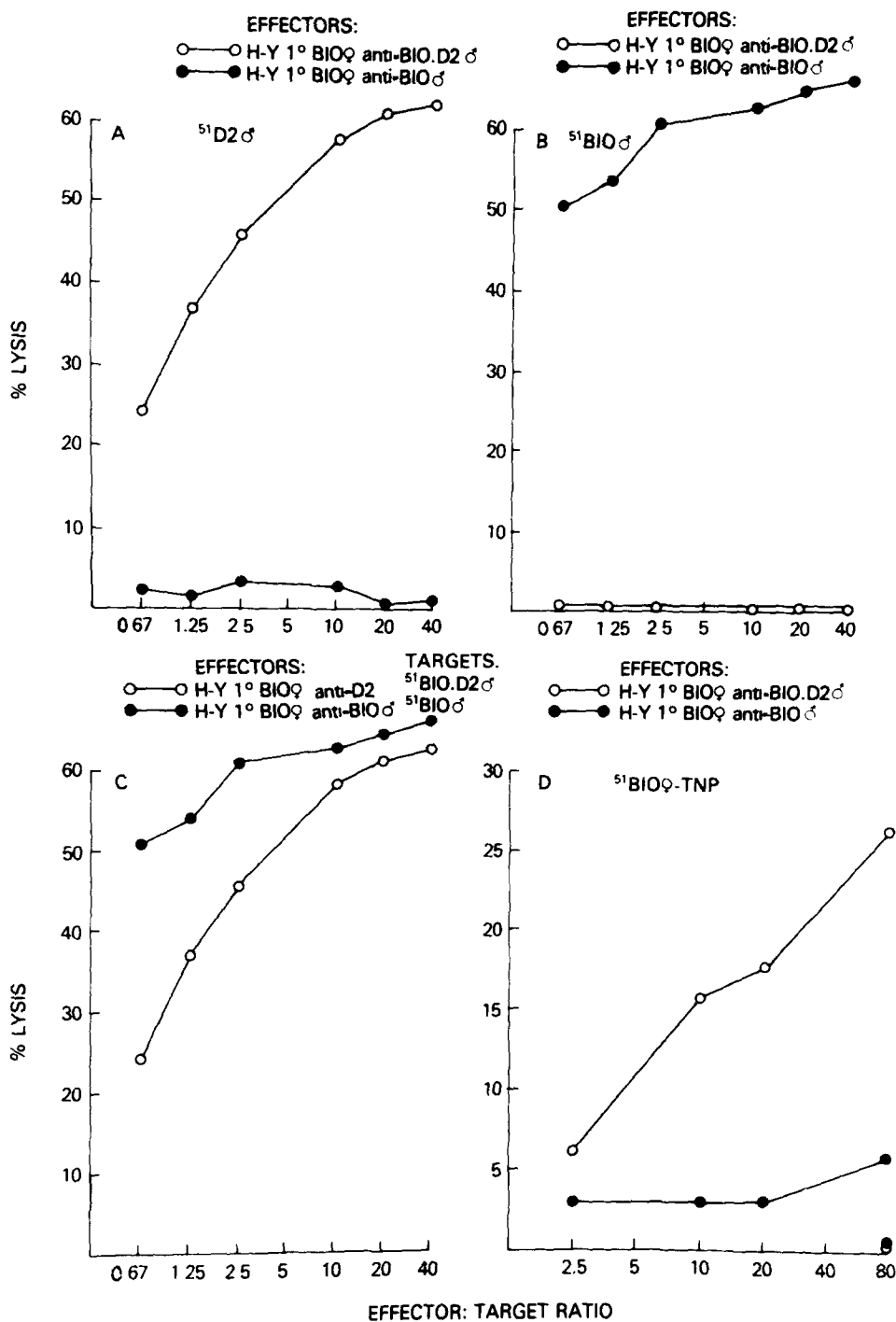


FIG. 5. Spleen cells from H-Y-primed B10 female mice were stimulated with B10 D2 male (○) and B10 male (●) spleen cells and assayed on B10 D2 (A), B10 male (B), and B10-TNP female (D) Con A-stimulated target cells. In Fig 5C, both effector cell populations were plotted for comparison against their specific target cells. (D) ○ and ● represent the percent lysis by these effector populations against unmodified B10 female target cells at 80:1. Spontaneous release: B10 female, 22.0%, B10-TNP female, 18.5%, B10 male, 22.5%, B10 D2 male, 19.6%. 1°, primary.

lations of effectors lyse TNP- and FITC-conjugated self targets; (d) demonstrate that secondary allogeneic H-2 CTL cultures mediate enhanced lytic activity against hapten-conjugated self targets, as well as against the specific H-2 alloantigens used for priming and restimulation; and (e) indicate that CTL cultures against the H-Y antigen (with CTL activity equal to or greater than that of allogeneic cultures) do not contain effectors that cross-react with hapten-conjugated self targets.

Previous studies have reported that TNP-modified self targets could be lysed by alloantigen-induced cytotoxic cells (5, 8, 10). The results of the present report have now identified a second X determinant, FITC, which also renders self target cells susceptible to allo-induced CTL lysis. Because cytotoxic cells generated in primary cultures against TNP- or FITC-conjugated autologous cells only lysed targets associated with the stimulating hapten (11, 12), the results suggested that different populations of alloantigen-induced CTL may have been involved in the recognition of self-TNP and self-FITC target cells. Cold target blocking studies (Fig. 3D and E) confirmed this hypothesis, thus demonstrating that the CTL clones that mediated lysis against TNP-self targets were not responsible for the lysis of FITC-self targets. Thus, alloantigen-activated CTL cultures appear to be composed of multiple cytotoxic components (13, 14) of which specific subpopulations are able to recognize different self plus haptenated target cells. These findings, therefore, demonstrate that this response is immunologically specific, and is not a result of an artifact induced by hapten conjugation to the target cells. At least three X antigens (listed in the present report and elsewhere [8]), all of which are haptens, have now been shown to serve as self plus X targets for distinct populations of allogeneically stimulated CTL. It is, therefore, important to determine whether other types of antigens that have been shown to be involved in MHC-restricted CTL can also serve as targets recognized in association with self determinants for allogeneically stimulated effectors. The results of Fig. 5B demonstrate that B10 female allogeneic CTL populations do not contain effector cells that recognize B10 male self plus H-Y antigens, although these effectors exhibited strong cytotoxic activity when assayed on TNP-conjugated B10 female targets. Therefore, although both haptens and the H-Y antigen can be recognized in association with self H-2 determinants by autologous CTL, our results with allogeneic CTL indicate that a difference probably exists in the way in which chemical haptens and minor antigens, such as the H-Y antigen, interact with H-2 products. It should be noted, however, that a study concerning minor antigen-induced BALB/c CTL showed that such effector cells were capable of lysing (BALB/c \times C57BL/6) F_1 target cells (15). It was suggested that such cross-reactions were the result of the creation of hybrid alloantigens as a result of an interaction of H-2- and non-H-2-coded determinants (15). It should be noted that no examples have been reported thus far concerning the question of whether such effectors can lyse MHC-coded self determinants plus virus. A recent study has demonstrated that human CTL stimulated with a pool of allogeneic stimulator cells lysed autologous lymphoblastoid cell lines but not autologous normal cells (16).

Recent studies from this and other laboratories have reported that *in vivo* priming with autologous TNP-conjugated cells does not result in an increased proportion of H-2-restricted (vs. nonrestricted) cytotoxicity (17, 18) after restimulation with TNP-self stimulators. The results in the present report have shown that *in vivo* alloantigen-primed and restimulated effector populations exhibit markedly enhanced autologous

cross-reactive lytic activity in addition to an enhanced allogeneic cytotoxic response. Such findings raise questions concerning the immunological significance of the expansion of self plus X reactive clones by alloantigens or X immunogens that cross-react with these alloantigens. The potential relevance of this observation can be considered for (a) autoimmunity, (b) protection against pathogens, and (c) surveillance against spontaneously arising neoplasms. In the context of autoimmunity, it is worth noting that this autologous cross-reactive lysis on TNP-self generated in primary cultures is strongest with spleen cells from NZB mice (R. B. Levy and G. M. Shearer. Unpublished observations.). This enhanced level of activity could be a result of a lack of regulation of the self plus X CTL repertoire, and, in this context, it may be noteworthy that NZB mice have been shown to lack suppressor cells (19).

To further analyze the significance of allogeneic H-2 antigens in this autologous cross-reactive model, it was important to ask whether any effector cell population, i.e., one directed against a different antigenic system, could also cross-react with hapten-modified self target cells. The H-Y CTL model is a well-defined system that permits consistent generation of an MHC-restricted effector population involving the recognition of the male antigen, as well as self-MHC determinants (20). In addition to the known specificity of these effector cells, another advantage of this model is the strength of the cytotoxic response that can be generated. The data presented in Figs. 4 and 5 demonstrate that an anti-H-Y effector population with equal or greater lytic activity than an allogeneically induced CTL population from the same responding pool did not recognize hapten-conjugated self target cells. Therefore, cytotoxic responses that result in the generation of effector cells that lyse hapten-conjugated targets appears, in addition to the magnitude of the response, to be dependent upon the specificity of these effectors (see below). Preliminary results obtained with xenogeneically induced CTL suggest that the recognition of xenoantigens is not sufficient to result in the activation of clones of effectors that recognize hapten-conjugated self targets (R. B. Levy, W. E. Biddison, and G. M. Shearer. Unpublished results.). Our studies therefore provide the first indication that allogeneic H-2 antigens may be unique in their ability to induce autologous cross-reactive lysis.

An important question that arises from the above observations is whether only certain H-2 allogeneic differences can generate cytotoxic effectors that cross-react with hapten-conjugated self targets. The results of Fig. 1 D and E (as well as our unpublished data) have indicated thus far that all H-2 haplotypes used in allogeneic stimulations generated approximately equivalent levels of autologous cross-reactive lysis. These findings suggest that probably all H-2 haplotypes possess antigens that can cross-react with hapten-conjugated self target cells. Cold target blocking studies (5) also indicated that at least some of the antigens of one H-2 haplotype that induce this cross-reaction are not shared by a second (i.e., different) H-2 haplotype. Additionally, effector cells generated specifically against H-2K or H-2D products (R. B. Levy, P. E. Gillheany, and G. M. Shearer. Unpublished observations.) as well as H-2L (10) products can also mediate lysis against TNP-self targets. This last result was particularly important, because, in these studies, the hapten-conjugated self target population did not express the H-2L products, thus indicating that CTL can be induced by the products of one locus but recognize the products of another in association with the hapten being used (10). Recent investigations concerning the amino acid sequencing of H-2 molecules have indicated marked homology among the

H-2K, H-2D, and H-2L allelic products of different haplotypes (21, 22). The results demonstrating autologous cross-reactive CTL also suggest that the H-2 molecules of different haplotypes are similar enough to induce CTL that can recognize self products in the presence of an X antigenic determinant. Therefore, it is possible that the regions of sequence homology within H-2 may be involved in these allogeneic self plus X cross-reactions.

The differences in the lysis of haptens plus self vs. H-Y plus self by allogeneic CTL could be a result of the covalent coupling of the hapten to multiple sites on the H-2 molecule (i.e., via lysine residues), whereas minor antigens and many viruses may associate in a different manner with H-2. In such a model, these multiple sites of interaction would include three types of determinants that potentially function as self-recognition structures for CTL: (a) common determinants expressed by all haplotypes within the species; (b) shared determinants expressed by some but not all haplotypes within the species; and (c) determinants unique to a particular haplotype. In this context, it may be significant that the CTL response generated against the H-Y antigen is restricted to lyse only H-2-matched target cells (20). In contrast, the CTL response generated against TNP consists of effector populations, all of which lyse H-2-matched, and some of which lyse H-2-nonmatched, TNP-conjugated targets (17, 23). It is possible that the TNP effectors that lyse the H-2-matched TNP-conjugated targets include separate populations that recognize the common, shared, and unique determinants as self, whereas those effectors that lyse H-2-nonmatched TNP-conjugated targets recognize the common and/or shared determinants on the H-2 molecule as self. In this model, allogeneic CTL against H-2 gene products would also recognize unique, shared, and common H-2 determinants. However, the unique determinant would be recognized as X, whereas the common and/or shared determinants would be recognized as self. This model is also consistent with the observations that (a) human anti-TNP-self CTL do not lyse TNP-conjugated murine targets, although they lyse HLA-mismatched TNP-conjugated human targets (24), and (b) CTL against xenogeneic antigens have thus far not been found to lyse TNP-conjugated self targets. Thus, it is possible that those clones of effectors generated by stimulation with alloantigens that lyse hapten-conjugated self targets and those generated against hapten-self that lyse H-2-mismatched hapten-conjugated targets recognize the same common and/or shared H-2 determinants as self.

Summary

Murine spleen cells were sensitized *in vitro* to H-2 disparate allogeneic spleen cells and assayed on syngeneic target cells conjugated with the trinitrophenyl (TNP)-self or the fluorescein isothiocyanate (FITC)-self haptens, or on syngeneic target cells expressing the male H-Y antigen (H-Y self). The results indicated that allo-induced cytotoxic T lymphocytes (CTL) contained effectors that lysed both hapten-self but not H-Y-self targets. Furthermore, it was demonstrated that separate populations of these allogeneic CTL were responsible for the lysis of TNP-self and FITC-self targets. This study also showed that cytotoxic effectors generated against the H-Y antigen with lytic activity equal to or greater than that of an allogeneically induced CTL response were unable to lyse hapten-self targets. These findings provide the first evidence that H-2 alloantigens may be unique in their ability to induce effectors that lyse hapten-conjugated autologous targets. These observations are discussed with

respect to the self and foreign antigenic determinants involved in allogeneic and self-restricted CTL models.

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References

- 1 Skinner, M. A., and J Marbrook 1976 An estimation of the frequency of precursor cells which generate cytotoxic lymphocytes *J. Exp. Med.* **143**:1562
- 2 Lindahl, K. F., and D. B. Wilson. 1977. Histocompatibility antigen-activated cytotoxic T lymphocytes. II Estimates of the frequency and specificity of precursors. *J. Exp. Med.* **145**: 508.
- 3 Shearer, G. M., T G Rehn, and C. A. Garbarino 1975 Cell-mediated lympholysis to trinitrophenyl-modified autologous lymphocytes. Effector cell specificity to modified cell surface components controlled by the *H-2K* and *H-2D* serological regions of the murine major histocompatibility complex. *J Exp Med.* **141**:1348.
- 4 Zinkernagel, R M , and P C Doherty. 1975. *H-2* compatibility requirement for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus Different cytotoxic T-cell specificities are associated with structures coded for in *H-2K* or *H-2D* *J. Exp. Med.* **141**:1427
- 5 Lemonnier, F, S. J. Burakoff, R. N Germain, and B Benacerraf. 1977. Cytotoxic T lymphocytes specific for allogeneic stimulator cells cross-react with chemically modified syngeneic cells. *Proc Natl Acad. Sci U S A* **74**:1229
- 6 Levy, R B , and G M. Shearer 1979. Regulation of T-cell-mediated cytotoxicity by the murine major histocompatibility complex I. Preferential in vitro responses to trinitrophenyl-modified self K- and D-coded gene products in parental and F₁ hybrid mouse strains *J. Exp. Med* **149**:1379
- 7 Levy, R B , G M Shearer, K J. Kim, and R M Asofsky 1979 Xenogeneic serum-induced murine cytotoxic cells. I. The generation of effector-components specific for self and allogeneic target cells. *Cell Immunol* **48**:276.
8. Lindahl, K F 1979 Antigen Recognition by Cytotoxic T-Lymphocytes in Natural and Induced Cell-Mediated Cytotoxicity G Riethmüller, P. Wernet, and G Cudkovicz, editors 122
- 9 Burakoff, S J., R Finberg, L Glimcher, F. Lemonnier, B Benacerraf, and H. Cantor. 1978. The biologic significance of alloreactivity. The ontogeny of T-cell sets specific for alloantigens or modified self antigens *J Exp Med.* **148**:1414
- 10 Levy, R B., and T H Hansen 1980. Functional studies of the products of the H-2L locus *Immunogenetics* **10**:7
- 11 Starzinski-Powitz, A , K. Pfizenmaier, M Rollinghoff, and H Wagner 1976 In vivo sensitization of T-cells to hapten-conjugated syngeneic structures of major histocompatibility complex I Effect of in vitro culture upon generation of cytotoxic T-lymphocytes *Eur J Immunol* **6**:799.
- 12 Gilheany, P., P. K Arora, R B Levy, and G. M Shearer. H-2 linked genetic control of murine cell-mediated lympholysis to autologous cells modified with high and low concentrations of fluorescein isothiocyanate *Cell Immunol.* In press.
13. Vazquez, A , C Néauport-Sautés, and A. Senik. 1980. Separate cytotoxic T lymphocyte subsets recognize the different H-2 specificities. *J. Exp. Med.* **151**:773
- 14 Lucas, C J., R. B Levy, and G M Shearer Cytotoxic responses against alloantigens

- exhibit preferential effector cell activity for H-2K or H-2D region products, similar to that of H-2 restricted cytotoxic responses. *J. Immunol.* In press.
- 15 Bevan, J. M. 1975 Interaction antigens detected by cytotoxic T cells with the major histocompatibility complex as modifier. *Nature (Lond.)*. **256**:419.
 - 16 Zarling, J. M., and F. Bach. 1979. Continuous culture of T-cells cytotoxic for autologous human leukemia cells. *Nature (Lond.)*. **280**:685.
 - 17 Fujiwara, H., R. B. Levy, and G. M. Shearer. Studies on in vivo priming of the trinitrophenyl-reactive cytotoxic effector cell system. III The effects of priming and the involvement of helper cells in the generation of restricted and non-restricted cytotoxic responses. *Eur. J. Immunol.* In press
 - 18 Finberg, R., M. I. Greene, B. Benacerraf, and S. J. Burakoff. 1979. The cytotoxic T-lymphocyte response to trinitrophenyl-modified syngeneic cells. I. Evidence for antigen-specific helper T-cells. *J. Immunol.* **123**:1205.
 - 19 Gerber, N. L., A. Hardin, T. M. Chused, and A. D. Steinberg. 1974 Loss with age in NZB/w mice of thymic suppressor cells in the graft vs host reaction. *J. Immunol.* **113**:1618.
 - 20 Gordon, R. D., E. Simpson, and L. E. Samelson. 1975. In vitro cell-mediated immune responses to the male specific (H-Y) antigen in mice. *J. Exp. Med.* **142**:1108.
 - 21 Uehara, H., B. M. Ewenstein, J. M. Martinko, S. G. Nathenson, and J. E. Coligan 1980 Primary structure of murine histocompatibility complex antigens. amino acid sequence of the amino-terminal one hundred and seventy-three residues of the H-2K^b glycoprotein. *Biochemistry* **19**:306
 - 22 Coligan, J. E., T. J. Kindt, R. Nairn, S. G. Nathenson, D. H. Sachs, and T. H. Hansen 1980. Primary structural studies of an H-2L molecule confirm that it is a unique gene product with homology to H-2K and H-2D antigens. *Proc. Natl. Acad. Sci U S A* **77**:1134.
 - 23 Burakoff, S. J., R. N. Germain, and B. Benacerraf. 1976. Cross-reactive lysis of trinitrophenyl (TNP)-derivatized H-2 incompatible target cells by cytolytic T-lymphocytes generated against syngeneic TNP spleen cells. *J. Exp. Med* **144**:1609.
 - 24 Shaw, S., D. L. Nelson, and G. M. Shearer. 1978. Human cytotoxic response in vitro to trinitrophenyl-modified autologous cells. I T-cell recognition of TNP in association with widely shared animals *J. Immunol* **121**:281.