Generation of Cytotoxic Lymphocytes in Mixed Lymphocyte Reactions II. Importance of Private and Public H-2 Alloantigens on the Expression of Cytotoxicity

James Forman and Göran Möller

Division of Immunobiology, Karolinska Institutet, Wallenberg Laboratory, Lilla Freskati, 104 05 Stockholm 50, Sweden.

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Abstract

MLC were established to test for the generation of specific cytotoxic effector cells in CML. The target cell used to assay for CML in the five combinations tested was of a different H-2 haplotype from the stimulating cell population. Cytotoxicity was observed against this target only when it shared private alloantigens (antigens that are specific for the H-2D and H-2K region of different H-2 haplotypes) with the stimulating cell population. Very weak or no cytotoxicity was found when such alloantigens were not shared, although cross-reactive public H-2 specificities were. These findings indicate that T cells display a cytotoxic potential against private H-2 antigens in a primary response in vitro and are not capable of responding to public H-2 specificities to the same level.

Introduction

The proliferative response in the mixed lymphocyte culture (MLC) and specificity of effector cells generated in cell-mediated lymphocytotoxicity (CML) are known to be governed by different regions of the major histocompatibility gene complex.

BSS = balanced salt solution
CML = cell-mediated lympholysis
GPC = guinea pig complement
$^{125}$IuR = $^{125}$I-ido-deoxyuridine
MLC = mixed lymphocyte culture
SE = standard error

$^1$Present address: Department of Microbiology, University of Texas Southwestern Medical School, Dallas, Texas 75235.
In a preceding communication we demonstrated that the cytotoxicity observed in CML required binding between target cells and effector lymphocytes (Forman and Möller 1973). The specificity of the cytotoxic effect appeared to be the consequence of binding between specific receptors on the killer cells and the antigenic determinants of the targets. That cytotoxic effector cells obtained after specific immunization have receptors for target cell antigens has clearly been shown by the ability of killer cells to be absorbed specifically onto target cell monolayers (Golstein et al. 1971, Berke and Levey 1972). The cytotoxic effect on corresponding target antigens is increased for such absorbed cells, while that of nonadherent cells has been shown to be decreased.

Target cell antigens to which cytotoxic effector cells have specificity for are controlled by two regions termed $H-2D$ and $H-2K$ located at the extreme end of the $H-2$ gene complex (Klein and Shreffler 1972). Using appropriate antisera, the antigens coded for by these regions can be shown to fall into two groups, public and private. Private $H-2$ antigens represent individual specificities of the $H-2D$ and $H-2K$ locus of the $H-2$ complex and thus each $H-2$ haplotype has two sets of private specificities. In contrast, public antigens are shared widely among various $H-2$ haplotypes (Snell et al. 1971, Klein and Shreffler 1971).

In the experiments reported in this paper, the cytotoxicity of effector cells generated in MLC has been assayed against target cells sharing or not sharing private $H-2$ specificities. The evidence obtained indicates that the cytotoxic cells can kill very weak or target cells that share antigens with the sensitizing cells. No cytotoxicity was observed when target cells did not share private specificities but did share some public specificities.

**Materials and Methods**

**Animals.** Mice of the following inbred strains and their $F_1$ hybrids were used: A, CBA, DBA/2, C57BL, (A × CBA)$F_1$, (A × C57BL)$F_1$, (C57BL × CBA)$F_1$, (C57BL × DBA/2)$F_1$, and (DBA/2 × C3H)$F_1$. All mice were matched according to age and sex in individual experiments.

**Target Cell Tumor.** The YAC ($H-2^a$) Moloney virus-induced ascites leukemia was obtained from the Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden. The tumor cells were labeled with $^{125}$I-iododeoxyuridine ($^{125}$IUdR), as described previously (Forman and Britton 1973).

**Isotope.** $^{125}$IUdR and $^3$H-thymidine were obtained from Amersham Searle, Buckinghamshire, England.

**Immunizations.** Mice were immunized by one intraperitoneal inoculation of $10^7$ viable nucleated spleen cells. Four and 7 days later, the animals were sacrificed and their spleens excised using a sterile technique. Spleens from groups of animals were pooled, a single cell suspension made, and cell concentrations were determined on the basis of viable nucleated cells.

**Mixed Lymphocyte Cultures for the Generation of Cytotoxic Cells.** Spleens were removed from mice and cell suspensions were prepared in a balanced salt solu-
tion (BSS). After the cells were washed, they were resuspended in a minimal essential medium, with supplements (Mishell and Dutton 1966), including 10% fetal bovine serum. The concentration of viable cells was adjusted to $10^6$ ml. Mixed cultures were obtained by adding 0.5 ml of parental and semisyngeneic cell suspensions to 35 mm Petri dishes (A/S Nunc, Roskilde, Denmark). Control nonmixed cultures consisted of 1 ml of an individual suspension. Each group contained triplicate cultures, and the dishes were kept in an atmosphere of nitrogen (83%), oxygen (7%), and carbon dioxide (10%). Each day the dishes were fed with a nutritional cocktail (Britton 1972).

**Cytotoxic Assay.** At varying times after the MLC was started, labeled YAC cells (usually $10^5$) were added to the cultures in a volume of 0.1 ml. The cultures were incubated with the target cells for 16 hours. After that time, cultures were harvested and the amount of $^{125}$I released from tumor cells was determined as described previously (Forman and Britton 1973). Spleens taken from mice that had been immunized in vivo were added to the Petri dishes together with the YAC cells and incubated and harvested as described for MLC cultures.

**Mixed Lymphocyte Culture for Determination of DNA Synthesis.** Cultures consisting of 1 ml of media with $2 	imes 10^6$ viable cells containing equal numbers from two different strains or unmixed controls were placed in 12 x 75 mm plastic tissue culture tubes (No. 2058 Falcon Plastics, Los Angeles, Calif.). Triplicate cultures were incubated at 37°C in an atmosphere of 10% CO$_2$.

After 72 hours, 2 µCi of $^3$H-thymidine were added to the cultures, and 24 hours later the cultures were harvested by pouring them onto filters in a “manifold” multiple sample collector (Millipore Corp., Bedford, Mass.). The cells were washed successively by 10 ml of BSS, 10 ml of ice-cold 5% trichloroacetic acid and 2 ml of ethanol. The filters were placed into scintillation vials and left to dry overnight. Ten milliliters of a toluene scintillation fluid were added to each vial and the samples counted in a liquid scintillation spectrometer.

**Preparation of Nonadherent Cells and Supernatants from MLC.** To obtain nonadherent cells, MLC were cultured for 5 days. After that time, the nonadherent cells from individual Petri dishes were poured into new dishes and then tested for cytotoxic activity against YAC target cells.

Supernatants were obtained from MLC by pooling cultures within each group after 5 days of incubation, centrifuging for 10 minutes at 1500 rpm, and then carefully removing the supernatant fluid. The supernatant was tested immediately for its cytotoxic effect against YAC.

In some cultures fresh spleen cells taken from C57BL mice were added to cultures containing MLC supernatants and YAC cells. The fresh spleen cells were suspended in BSS at $100 	imes 10^6$ cells per milliliter and 0.1 ml of this suspension was then added to each culture.

**Antibody Titrations.** Mice received four weekly inoculations of allogeneic spleen cells and were bled 1 week after the last injection. Sera were titered against both YAC tumor cells and strain A lymph node lymphocytes in the presence of guinea pig complement (GPC) preabsorbed with agarose. Target cells were adjusted to a concentration of $10^7$ cells per milliliter and 0.1 ml of the suspension was added to tubes containing equal volumes of antiserum and GPC (diluted 1/3). After 1 hour at 37°C, viability of the cells was determined by trypan blue staining.
Results

Characteristics of Cytotoxicity Induced in CML Against a Target Cell of a Different H-2 Haplotype from the Stimulator. We have previously shown that the cytotoxic effect in CML in our system peaks between days 4 and 6 after the cultures are started (Forman and Möller 1973). Similar results were also found in combinations where target cells (YAC) were not identical to the stimulator in the MLC but shared some H-2 alloantigens with the sensitizing spleen cell population. In Fig. 1 some of these reactions are presented. CBA (H-2k) lymphocytes mixed with (A X CBA)F1 cells were sensitized to (H-2a) antigens and, as expected, expressed cytotoxicity when tested against YAC (H-2a) targets. Strain A (H-2a) cells sensitized to (A X CBA)F1 cells were not cytotoxic to YAC targets. In three other combinations, C57BL or CBA cells were presumably sensitized to H-2 antigens shared by different H-2 haplotypes with target YAC cells (Klein and Shreffler 1971). However, strong cytotoxicity was observed in only two of the combinations.

When varying numbers of H-2a (YAC) cells were added as targets to CBA anti (A X CBA)F1 (H-2k anti H-2a) MLC or to C57BL anti (C57BL X CBA)F1 (H-2b anti H-2k) MLC to assay for CML, the amount of isotope released was similar in both groups (Fig. 2). Thus, with respect to this parameter, the cytotoxic effect is similar regardless of whether the target shares the same H-2 haplotype as the stimulator. The

Fig. 1. Per cent release of $^{125}$I ± SE from $^{125}$IUdR labeled YAC cells added to cultures between 4 and 6 days after their start. • = A + (A X CBA)F1; • CBA + (A X CBA)F1; □ = CBA + (CBA X C57BL)F1; ○ = C57BL + (CBA X C57BL)F1; ★ = C57BL + (C57BL X DBA/2)F1. Per cent release of $^{125}$I from nonmixed cultures on day 4 was 15.1 ± 0.9.
cytotoxicity of C57BL anti (C57BL × CBA)F₁ cells against YAC was not due to non-specific effects, since we have demonstrated that this does not occur in the assay system (Forman and Möller 1973).

Characteristics of Cytotoxicity Induced by In Vivo Sensitization. C57BL mice received 10⁷ (C57BL × CBA)F₁ spleen cells intraperitoneally, while CBA mice received a similar number of (A × CBA)F₁ cells. Four and 7 days later, the spleens were removed from recipient mice, pooled within the groups, and tested at varying effector-to-target cell ratios for cytotoxicity against YAC.

The isotope release curve obtained by plotting data from the killing of YAC target cells by C57BL spleen cells sensitized against (CBA × C57BL)F₁ cells was similar to that obtained with CBA spleen cells sensitized against (A × CBA)F₁ cells (Fig. 3). Thus, data on in vivo sensitized cells indicates that the specificity of the cytotoxic effect displayed by anti H-2ᵃ and H-2ᵏ cytotoxic cells against H-2ᵃ targets is similar.

Ability of Supernatants and Nonadherent Cells from MLC to Mediate Killing. While cytotoxicity in CML may be mediated by thymus-derived (T) cells (Häyry et al. 1972), it is possible that targets can be killed by other cytotoxic effector mechanisms, such as antibody-induced, cell-mediated cytotoxicity (Perlmann et al. 1972) or macrophage mediated cytotoxicity (Evans and Alexander 1972). Supernatants from 5-day MLCs were added to YAC target cells alone or with 10⁷ unsensitized C57BL spleen cells to test for the presence of antibody capable of transforming B cells or macro-

![Graph](image)

**Fig. 2.** Per cent release of ¹²⁵I ± SE from ¹²⁵IUDR labeled YAC cells added to day 5 MLC. ▲ = A + (A × CBA)F₁; ○ = CBA + (A × CBA)F₁; ○ = C57BL + (C57BL × CBA)F₁. Per cent release of ¹²⁵I from nonmixed cultures when 10⁵ YAC cells were added was 19.8 ± 1.5.
phages into cytotoxic cells. In agreement with our previous findings (Forman and Möller 1973), these supernatants had no activity themselves nor did they induce fresh spleen cells to exert cytotoxicity when tested against YAC targets (Fig. 4). When non-adherent supernatant lymphocytes were tested, their cytotoxic effect was similar to that of the unseparated MLC effector cell population, further indicating that macrophages are not active in these cultures.

Expression of Cytotoxicity Against Targets Sharing Some Alloantigenic Specificities with the Sensitizing Cell Population. In Table 1 two experiments are presented in which several MLC combinations were tested for their cytotoxicity against YAC cells. In these experiments, YAC had either the same or a different H-2 haplotype from the stimulator cell of the MLC.

MLCs were incubated for 5 days. At that time, $10^5$ labeled YAC cells were added to the cultures and the amount of isotope released was determined 16 hours later. Before the labeled target cells were added, viable cells were counted in these cultures to ascertain whether cell survival was the same between groups. In general, after 5 days of culture, there were $2.3 \times 10^6$ viable cells per dish, without significant

![Graph](image-url)

Fig. 3. Index of isotope release from labeled YAC cells mixed with spleen cells from mice 4 and 7 days after priming and tested at various effector to target cell ratios. • = CBA immunized with $(A \times CBA)F_1$ cells; ▲ = C57BL immunized with $(C57BL \times CBA)F_1$ cells. - - - - tested 4 days after host priming; - - - - tested 7 days after host priming.

Cytotoxic index =

\[
\frac{\text{Percent } ^{125}\text{I release immune cells} - \% ^{125}\text{I release nonimmune cells}}{\text{Percent } ^{125}\text{I release immune cells}} \times 100
\]
variation between groups. Therefore, the effector-to-target cell ratio was approximately 25:1 in these experiments.

We found that in certain combinations there was strong cytotoxicity against YAC, while in others there was very weak or no cytotoxicity. Thus, in experiment 2 (Table 1), when YAC was the target in the MLC combinations C57BL anti (CBA X C57B1) (H-2\textsuperscript{b} anti H-2\textsuperscript{k}) and DBA/2 anti (C3H X DBA/2) (H-2\textsuperscript{d} anti H-2\textsuperscript{k}) and DBA/2 anti (C3H X DBA/2) (H-2\textsuperscript{d} and H-2\textsuperscript{k}), there was as much cytotoxicity observed as when YAC shared the same H-2 haplotype with the stimulator; i.e., in C57BL anti (A X C57BL) (H-2\textsuperscript{b} anti H-2\textsuperscript{a}) and CBA anti (A X CBA) (H-2\textsuperscript{k} anti H-2\textsuperscript{a}) MLCs. On the other hand, CBA anti (C57BL X CBA) (H-2\textsuperscript{k} anti H-2\textsuperscript{b}) and DBA/2 anti (C57BL X DBA/2) (H-2\textsuperscript{d} anti H-2\textsuperscript{b}) induced little or no cytotoxicity against YAC. It should also be noted that in the system we used, there was significant DNA synthesis in all mixed cultures (Experiment 1, Table 1).

All of these experiments are summarized in Table 2. The data have been arranged into two groups. The first group includes experiments in which there was strong cytotoxicity so that the net killing — i.e., isotope release in mixed cultures minus isotope release in nonmixed cultures — was greater than 40%. The second group includes experiments in which cytotoxicity was very weak and the net killing was less than 15%. There were no experiments in which net killing fell between these values. Using this arrangement, three combinations were found in which there was strong cyto-

![Fig. 4. Per cent release of $^{125}$I $\pm$ SE from $^{125}$IUdR labeled YAC cells added to day 5 MLC. Solid bars = activity of whole cultures; open bars = activity of nonadherent cells; vertical-lined bars = activity of supernatants; horizontal lined bars = activity of supernatants mixed with $10^7$ nonimmune C57BL spleen cells.](image-url)
### Table 1. Ability of Effector Cells Generated in MLC to be Cytotoxic to YAC Target Cells

| Exp. No. | Responding Cell | Stimulating Cell | Per cent Release $^{125}\text{I} \pm \text{SE}$ in Nonmixed Cultures | Per cent Release $^{125}\text{I} \pm \text{SE}$ in Mixed Lymphocyte Cultures | Net % Release (Mixed-Nonmixed) | Incorporation of $^{3}\text{H}-\text{Td}$ as Measured by cpm ± SE in Mixed Lymphocyte Cultures | Factor of Stimulation $^b$ |
|----------|-----------------|------------------|-------------------------------------------------|-------------------------------------------------|--------------------------------|---------------------------------------------------------------------|------------------|
| 1 $^a$   | A               | (A × CBA)$F_1$   | 15.1 ± 0.9                                       | 15.4 ± 1.3                                       | 0.3                            | 16,448 ± 692 $^c$                                                   | 1.60             |
|          | CBA             | (A × CBA)$F_1$   | 82.2 ± 1.3                                       | 67.1                                            |                                | 15,405 ± 589                                                       | 1.50             |
|          | CBA             | (CBA × C57BL)$F_1$ | 23.5 ± 2.6                                      | 8.4                                            |                                | 22,221 ± 610                                                        | 2.17             |
|          | C57BL           | (CBA × C57BL)$F_1$ | 95.7 ± 1.0                                      | 80.6                                            |                                | 23,058 ± 1889                                                      | 2.25             |
|          | C57BL           | (C57BL × DBA/2)$F_1$ | 93.1 ± 0.9                                      | 78.0                                            |                                | 21,143 ± 1340                                                      | 2.06             |
| 2        | A               | (A × CBA)$F_1$   | 42.7 ± 6.0                                       | 39.6 ± 1.8                                       | −3.1                           |                                                                    |                  |
|          | A               | (A × C57BL)$F_1$ | 45.7 ± 2.5                                       | 3.0                                            |                                |                                                                    |                  |
|          | C57BL           | (A × C57BL)$F_1$ | 94.7 ± 0.2                                       | 52.0                                            |                                |                                                                    |                  |
|          | CBA             | (A × CBA)$F_1$   | 85.3 ± 3.4                                       | 42.6                                            |                                |                                                                    |                  |
|          | C57BL           | (C57BL × CBA)$F_1$ | 94.9 ± 1.2                                      | 52.2                                            |                                |                                                                    |                  |
|          | CBA             | (C57BL × CBA)$F_1$ | 52.6 ± 2.1                                      | 9.9                                            |                                |                                                                    |                  |
|          | DBA/2           | (C3H × DBA/2)$F_1$ | 87.5 ± 4.1                                      | 44.8                                            |                                |                                                                    |                  |
|          | DBA/2           | (C57BL × DBA/2)$F_1$ | 41.5 ± 6.2                                      | −1.2                                            |                                |                                                                    |                  |

$^a$Taken from Fig. 1  
$^b$cpm in mixed cultures  
$^c$cpm ± SE from nonmixed cultures was 10,261 ± 369.
toxicity and two were found in which killing was weak or absent. In the two combinations where cytotoxicity was not observed, private H-2 antigens were not shared between the stimulator and YAC targets, although sharing occurred with respect to public H-2 antigens (Table 3 and Discussion).

Results of Titrating Antisera Against YAC and A Strain Lymph Node Cells. Although there should be public H-2 specificities on YAC target cells to which effector cells could be potentially sensitized in two MLC combinations (H-2\(^k\) anti H-2\(^b\) and H-2\(^d\) anti H-2\(^b\)), we detected no cytotoxic effect (Tables 2 and 3). To determine whether YAC maintained these public specificities of the H-2\(^a\) haplotype, we used sera from hyperimmune mice and tested the ability of these antisera to kill YAC targets in the presence of complement. Thus, sera from CBA (H-2\(^k\)) and DBA/2 (H-2\(^d\)) mice immunized with C57BL (H-2\(^b\)) spleen cells were cytotoxic to both YAC and A strain lymph node cells in the presence of GPC (Fig. 5). The results indicate that these public specificities are maintained by YAC cells.

Discussion

The H-2 complex is a multiple gene region that includes the two loci H-2\(D\) and H2\(K\), which code for serologically detectable specificities. Thorsby (1971), Snell et al. (1971) and Klein and Shreffler (1972) have suggested that H-2 alloantigens can be grouped into public and private antigens similar to that for HL-A. Each H-2 haplotype would have private specificity (ies) controlled by the H-2\(D\) and H-2\(K\) locus. Other alloantigens detected are termed public and represent specificities shared widely between alleles (Snell et al. 1971, Klein and Shreffler 1971). Evidence supporting the hypothesis that private H-2\(K\) and H-2\(D\) specificities are independent gene products was obtained by Cullen et al. (1972) who demonstrated (by independently immunoprecipitating membrane products solubilized by detergents with monospecific antisera) that determinants representative of the four loci in F\(_1\) hybrid mice are on different mole-

| Responding Cell | Stimulating F\(_1\) Cells         | No. of Experiments | No. of Experiments in Which Net Killing Was > 40% | No. of Experiments in Which Net Killing Was < 15% |
|-----------------|----------------------------------|--------------------|-----------------------------------------------|-----------------------------------------------|
| C57BL           | (C57BL x CBA)\(F_1\)            | 3                  | 3                                             | 0                                             |
| C57BL           | (C57BL x DBA/2)\(F_1\)          | 2                  | 2                                             | 0                                             |
| DBA/2           | (DBA/2 x C3H)\(F_1\)            | 3                  | 3                                             | 0                                             |
| DBA/2           | (C57BL x DBA/2)\(F_1\)          | 2                  | 0                                             | 2                                             |
| CBA             | (C57BL x CBA)\(F_1\)            | 4                  | 0                                             | 4                                             |
cules. Additional evidence supporting this interpretation also has been reported by Neauport-Sautes et al. (1973) who found that private specificities cap independently.

At least some public H-2 specificities have been found on the same molecules as private antigens (Cullen and Nathenson 1971). They may either be a part of the private H-2 alloantigenic determinant or they may be located at another site on the molecule. Evidence in favor of the latter possibility was obtained by Pancake and Nathenson (1973) who, by chemically modifying lysine residues, were able to selectively decrease the binding capacity of a private determinant for antibody while not affecting the public determinant.

How H-2 antigenic specificities are recognized by the antigen-reactive cell (ARC) in these systems is not known. Brondz (1972) has suggested that ARC are polyclonal with respect to receptors and recognize a group of individual (both public and private) specificities "en bloc." Target cell killing, according to Brondz, is then dependent on the ability of the killer cell to recognize this arrangement of specificities on the target cell.

If T cells are the ARC and cytotoxic effectors in CML (Häyry et al. 1972), their specificity may be such that they are only able to recognize or bind private specificities. Thus killing would be determined by whether or not target cells share a private specificity with the sensitizing cell.

In Table 3, we have presented a list of private specificities for the H-2 haplotypes, as suggested by Snell et al. (1971) and Klein and Shreffler (1971), together with our data on CML. In the five combinations that we tested, it was found that a YAC cell was killed when and only when it shared at least one private specificity with the sensitizing cell. No, or weak, cytotoxicity was seen in the two combinations in which YAC did not share a private specificity with the stimulator in the MLC. It should be noted that the H-2a haplotype is believed to be the result of a crossover in a (H-2a × H-2k)F₁ mouse. Thus, this recombinant would share the H-2D private specificity with

![Fig. 5](image-url)  
**Fig. 5.** Ability of CBA anti-C57BL and DBA/2 anti-C57BL hyperimmune sera to be cytotoxic to YAC and A-strain lymph node cells in the presence of GPC⁺. Sera were tested from CBA mice immunized with C57BL cells --- , or DBA/2 mice immunized with C57BL cells - - -, and tested against lymph node ● or YAC cells ○. Per cent dead cells in the absence of antiserum for lymph node cells was 18% and for YAC was 0%.
Table 3. Relationship of Private and Public H-2 Specificities to Killing in CML

| Responding Cell | Stimulating Cell       | Private Specificities of Stimulating Cell<sup>a, b</sup> | Private Specificities Shared by YAC Target | No. of Public Specificities Shared by Target Cells (YAC) and Stimulator But Not Shared by Responding Parental Cell | Total No. of Public Specificities on Stimulating Cell Not Shared by Responding Parental Cell<sup>a</sup> | Cytotoxicity Observed |
|-----------------|------------------------|----------------------------------------------------------|-------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------|----------------------|
| C57BL           | (C57BL × CBA)F<sub>1</sub> | 32 H-2D, 23 H-2K                                        |                                           | C57BL (C57BL × CBA)F<sub>1</sub>                                                             | 3/3                                            | yes                  |
| H-2<sup>b</sup> | H-2<sup>b</sup>/H-2<sup>k</sup> |                                                          |                                           |                                                                                                |                                                |                      |
| C57BL           | (CBA × DBA/2)F<sub>1</sub> | 4 H-2D, 31 H-2K                                        | 4 H-2D, 23 H-2K                          | C57BL (C57BL × DBA/2)F<sub>1</sub>                                                            | 6/7                                            | yes                  |
| H-2<sup>b</sup> | H-2<sup>b</sup>/H-2<sup>d</sup> |                                                          |                                           |                                                                                                |                                                |                      |
| DBA/2           | (DBA/2 × C3H)F<sub>1</sub> | 32 H-2D, 23 H-2K                                        |                                           | DBA/2 (DBA/2 × C3H)F<sub>1</sub>                                                             | 2/2                                            | yes                  |
| H-2<sup>d</sup> | H-2<sup>d</sup>/H-2<sup>k</sup> |                                                          |                                           |                                                                                                |                                                |                      |
| DBA/2           | (DBA/2 × C57BL)F<sub>1</sub> | 2 H-2D, 33 H-2K                                        |                                           | DBA/2 (DBA/2 × C57BL)F<sub>1</sub>                                                            | 1/1                                            | no                   |
| H-2<sup>d</sup> | H-2<sup>d</sup>/H-2<sup>b</sup> |                                                          |                                           |                                                                                                |                                                |                      |
| CBA             | (C57BL × CBA)F<sub>1</sub> | 2 H-2D, 33 H-2K                                        |                                           | CBA (C57BL × CBA)F<sub>1</sub>                                                               | 6/6                                            | no                   |
| H-2<sup>k</sup> | H-2<sup>b</sup>/H-2<sup>k</sup> |                                                          |                                           |                                                                                                |                                                |                      |

<sup>a</sup>See Snell et al. (1971) and Klein and Shreffler (1971)

<sup>b</sup>Only H-2 specificities allogeneic to responder are listed.
Table 4. Relationship of Shared Private and Public H-2 Specificities Between Stimulating and Target Cells and Reactivity in In Vitro and In Vivo Test Systems

| Responding Cell Haplotype | Stimulating Cell Haplotype | Private Specificities of Stimulating Cells, a, b | Haplotype of Target | Private Specificities Shared by Target and Stimulating Cell |
|---------------------------|-----------------------------|-----------------------------------------------|---------------------|----------------------------------------------------------|
| H-2k                      | H-2k/H-2b                   | 2 33                                          | H-2d                | -                                                        |
| H-2k                      | H-2d                        | 4 31                                          | H-2b                | -                                                        |
| H-2d                      | H-2b                        | 2 33                                          | H-2a, H-2k          | -                                                        |
| H-2k                      | H-2d                        | 4 31                                          | H-2b, H-2a          | 4                                                        |
| H-2b                      | H-2d                        | 4 31                                          | H-2k                | -                                                        |
| H-2k                      | H-2b                        | 2 33                                          | H-2a                | -                                                        |

aSee Snell et al. (1971) and Klein and Shreffler (1971).
bOnly H-2 specificities allogeneic to responder are listed.

the H-2d haplotype and the H-2K private specificity with the H-2k haplotype (Snell et al. 1953).

Further, there is data from several other laboratories, using different assay systems both in vivo and in vitro where the same interpretation can be made. In Table 4 we have listed a series of reports in which tests against targets of varying H-2 haplotypes served as controls for the specificity of the cytotoxic effect. In all of these experiments, effector function was seen only against targets when private specificities were shared by the sensitizing and target cells.

At present it is not clear why cytotoxicity is not observed when target cells only share public specificities with the sensitizing cells. It is possible that T cells lack receptors or respond poorly to public antigens, whereas B cells respond more readily to these determinants. It is also conceivable that public determinants are not immunodominant molecules and that the major activity in a primary response to H-2 alloantigens is against private (dominant) antigens. Since public antigens are detected with hyperimmune antisera (Snell et al. 1971), multiple sensitizations of T cells may demonstrate that they can also be sensitized against public specificities. Finally, public
| No. of Public Specificities Shared by Target and Stimulator But Not Shared by Responding Cell | Total No. of Public Specificities on Stimulating Cell Not Shared by Responding Cell | Effector Function Observed | Remarks and References |
|---|---|---|---|
| 6/6  | No | In vivo activated thoracic duct cells tested against targets in vitro (Sprent and Miller 1971). |
| 6/11 | No | In vitro sensitized cells tested against targets in vitro (Wagner and Feldmann 1972). |
| 1/1  | No | Ability of monolayers to absorb cytotoxic effector cells (Berks and Levey 1972). |
| 1/2  | No | |
| 6/11 | No | Effector cells sensitized in vitro and tested in vitro (Wagner et al. 1972). |
| 10/11| Yes| |
| 2/7  | No | In vivo sensitized cells tested against target cells in vivo (Forman et al. 1972). |
| 6/6  | No | Homing of labeled sensitized lymphocytes to skin grafts (Lance and Cooper 1972). |

antigens may be more hidden in the membrane and less susceptible to the cytotoxic effector cell. Since these antigens combine with antibody and are on the same molecule as private H-2 antigens (Cullen and Nathenson 1971), this would imply that the inability of T cells to display cytotoxic activity in CML is at the level of the T-cell receptor's ability to bind to the antigen on the target itself.

There is evidence that antibody reactive against public antigens can play a role in graft rejection in vivo. An example of this can be cited from the studies of Brondz (1972). He found that H-2d recipients (B10.D2) immunized with H-2b (C57B1/10) cells not only rapidly reject C57BL/10 skin grafts, but that under certain immunizing conditions they also would reject in an accelerated fashion third-party H-2a (B10.A) grafts that do not share private specificities with the sensitizing tissue (Klein and Shreffler 1971). He also observed that he could transfer the specific immunity against the C57BL/10 skin to secondary B10.D2 recipients with immune lymphocytes, while he was unable to transfer the accelerated rejection of the third party H-2a graft. However, when immune serum was transferred, he did observe accelerated rejection of the B10.A graft. This finding would seem to be consistent with antibody, probably in
collaboration with other effector cells (Perlmann and Holm 1969), as the mechanism of accelerated graft rejection. Klein and Murphy (1973) have also reported that mice of one H-2 haplotype sensitized with cells from a second H-2 haplotype and challenged with skin from a third H-2 haplotype sharing only public antigens with the sensitizing haplotype reject such skin in an accelerated fashion. In addition, Phillips et al. (1973) have observed some cross-reactivity in CML in one combination where private specificities were not shared between the stimulator and target. However, this effect was of a much smaller magnitude than the cytotoxic effect against the specific target.

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