

CYTOTOXIC T CELLS RECOGNIZE MALE ANTIGEN
AND H-2 AS DISTINCT ENTITIES

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Female mice of some strains are capable of mounting cytotoxic T-cell responses to male cells. The genes determining responsiveness are *H-2*-linked (1, 2). Such cytotoxic T cells discriminate between male targets expressing different *H-2* antigens, i.e. they are restricted by *H-2* (1, 2). The effector cells may either recognize the Y-antigen and *H-2* as two distinct entities, or they may recognize new antigenic determinants formed when the Y-antigen is associated with *H-2*.

In line with the second alternative, it has been proposed that *H-2* antigens are the anchorage site for Y-antigens: to explain virilization of XX bovine freemartin gonads, Ohno and Martin (3, 4) assumed that XY cells from the bull twin disseminate Y-antigen, coating the majority of XX cells.

We report herein tests for the presence of male-specific determinants on XX cells from XX/XY hemopoietic chimeras, and on the reactivity of male or female T cells against *H-2*-different male cells.

Materials and Methods

Cytotoxic Anti-Male Responses. Mice were immunized by intravenous injection of 2×10^7 XY spleen cells irradiated with 2,200 R. From these mice, spleen cells were prepared 2-20 wk later, and cultured for 5 days with irradiated XY spleen cells (2). Cytotoxic tests were performed on ^{51}Cr -labeled blasts induced by lipopolysaccharide (LPS) (2, 5).

Chimeras. Chimeras were prepared by injecting lethally (880 R) irradiated female (CBA/J \times C57Bl/6) F_1 hybrids with anti- θ -treated male or female bone marrow cells from one or both parental strains (5). Lymphoid cell chimerism was checked by using cytotoxic anti-*H-2* sera (5). Some of the chimeras were immunized with male cells 3 mo after bone marrow reconstitution.

Depletion of Alloreactive T Cells. Recirculating lymphocytes specifically depleted of alloreactive T cells were obtained by the method of Sprent and Miller (6). Allogeneic spleen cells were irradiated with 1,000 R, cultured for 5 h in vitro, and then 4×10^8 cells were injected intravenously. Thoracic duct lymphocytes were collected 24-48 h after injection.

Results

XX Cells from XX/XY Chimeras Are Not Lysed by Male-Specific Effector Cells. XX/XY hemopoietic chimeras were produced by injecting equal numbers of XX or XY CBA/J and XY or XX C57Bl/6 bone marrow cells, respectively, into lethally irradiated female (CBA/J \times C57Bl/6) F_1 hybrids. Lymphoid cell chimerism in the spleen was tested 3 mo later, and ranged in the different chimeras from 20 to 30% C57Bl/6 cells, and from 70 to 80% CBA/J cells. Persisting F_1 hybrid cells could not be demonstrated.

It was then tested whether only XY or also XX cells from such chimeras could be lysed by *H-2*-restricted cytotoxic lymphocytes recognizing Y-antigens. Targets were prepared by stimulating spleen cells from XX/XY chimeras with LPS and labeling the cells with ^{51}Cr . *H-2^b*-restricted cytotoxic T cells were obtained

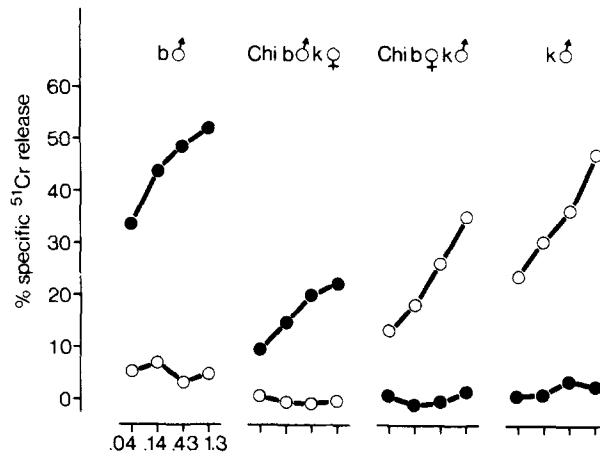


FIG. 1. Lysis of C57Bl/6 ♂ (b♂), CBA/J ♂ (k♂), and b♂ k♀ or b♀ k♂ targets from chimeric mice by anti-male killer cells restricted to H-2^b (●) or to H-2^k (○). The abscissa shows the numbers ($\times 10^6$) of female responder cells cultured on day 0, the descendants of which are the cytotoxic cells producing lysis on day 5 of culture.

from cultures of female C57Bl/6 cells stimulated with H-2^b male cells, H-2^k-restricted effector cells from cultures of female (C57Bl/6 \times CBA)F₁ cells stimulated with male CBA/J cells. (CBA/J mice cannot be sensitized to kill XY H-2^k cells). The two effector cell populations were then tested on various targets. As shown in Fig. 1, H-2^b-restricted killer cells lysed only targets containing XY H-2^b, but not targets containing XX H-2^b and XY H-2^k cells. The reciprocal result was obtained with H-2^k-restricted effector cells. Thus, no Y-antigens were detected on XX cells in XX/XY hemopoietic chimeras. The fact that XY hemopoietic cells from XX recipient mice were lysed, indicates that the Y-antigen recognized by cytotoxic lymphocytes was an endogenous product of the target cells.

The Ability of Cells from XX/XY Chimeras to Induce Cytotoxic Responses. Results like those described in the preceding section were obtained when the stimulatory capacity of cells from XX/XY chimeras was tested. Female (CBA/J \times C57Bl/6)F₁ cells primed in vivo with either CBA/J or C57Bl/6 XY cells were mixed in equal proportions. Such cells stimulated with XY H-2^k or XY H-2^b cells produced cytotoxic effector cells restricted to H-2^k or H-2^b, respectively (Fig. 2). A mixture of XY H-2^b and XX H-2^k cells from either chimeric or normal mice induced male-specific effector cells restricted to H-2^b, whereas a mixture of XX H-2^b and XY H-2^k cells stimulated an H-2^k-restricted response (Fig. 2), indicating once again that Y-antigen was not recognized on female cells.

The Cytotoxic Response of Female or Male H-2^b T Cells to Syngeneic or Allogeneic Male Cells. XX H-2^b T cells from a chimera, produced by injecting XX C57Bl/6 bone marrow into lethally X-irradiated XX (C57Bl/6 \times CBA/J)F₁ hybrids, can be sensitized to kill both H-2^b and H-2^k XY targets (Fig. 3). Thus, as previously reported for 2,4,6-trinitrophenyl (TNP) (7, 8) and for viral antigens (8, 9), the response to male antigens in chimeras can be restricted to both H-2^b of the responder and H-2^k of the recipient strain, even though the H-2^k strain

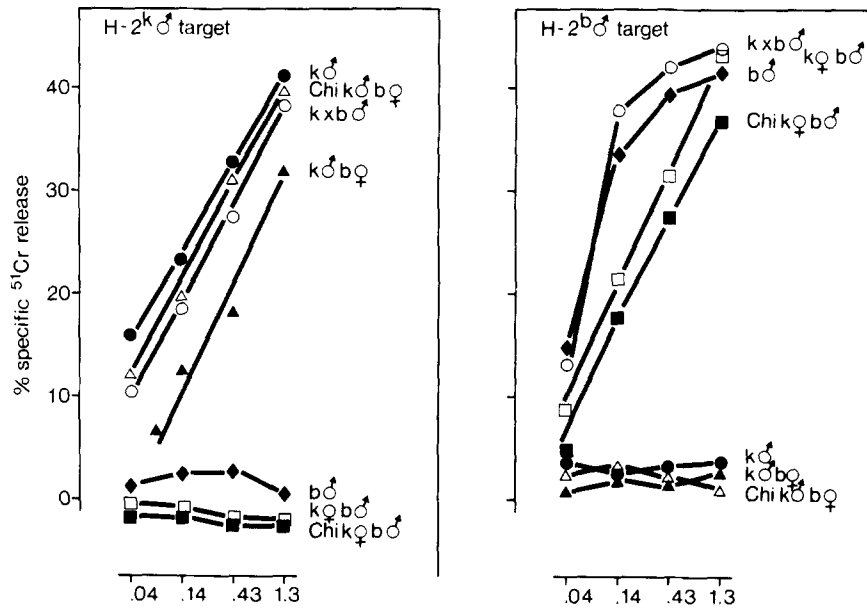


FIG. 2. Stimulation of *H-2*-restricted cytotoxic effector cells from primed female (CBA/J × C57Bl/6)_F₁ hybrids by C57Bl/6 ♂ (b♂), CBA/J ♂ (k♂), (CBA/J × C57Bl/6)_F₁ ♂ (k × b♂), and by a mixture of k♂ and b♀ (k♂ b♀), or k♀ and b♂ (k♀ b♂) cells from either normal or chimeric mice.

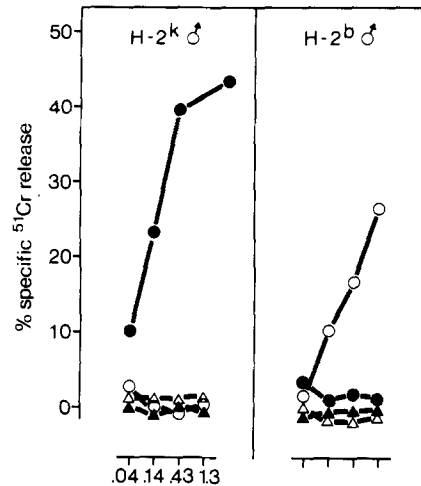


FIG. 3. Lysis of male *H-2*^k or *H-2*^b targets by T cells from C57Bl/6 ♀ → (CBA/J × C57Bl/6)_F₁ ♀ (circles) or C57Bl/6 ♂ → (CBA/J × C57Bl/6)_F₁ ♀ chimeras (triangles) stimulated in vitro by either C57Bl/6 ♂ (○, △) or by CBA/J ♂ (●, ▲) stimulators.

itself does not mount an anti-Y response. However, XY *H-2*^b T cells from a chimera, produced by injecting C57Bl/6 XY bone marrow into XX (C57Bl/6 × CBA/J)_F₁ recipients, cannot be educated to kill either *H-2*^b or *H-2*^k male cells (Fig. 3), while responding to allogeneic cells (not shown).

As shown in Fig. 3, XX *H-2*^b T cells from chimeras could be sensitized to lyse XY *H-2*^k targets. The same could not be demonstrated with XX *H-2*^b T cells

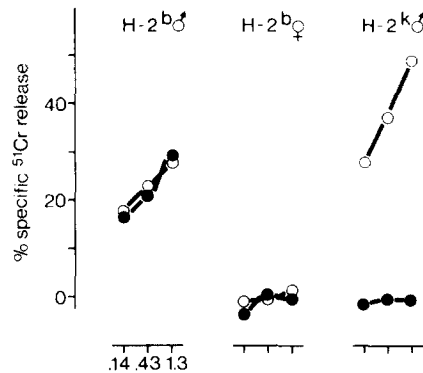


FIG. 4. Lysis of XX and XY $H-2^b$ and XY $H-2^k$ targets by XX $H-2^b$ TDL negatively selected to $H-2^k$ alloantigens (●), or XX $H-2^b$ T cells (○) stimulated by XY (CBA/J \times C57Bl/6) F_1 cells. The mice had been primed in vivo with XY (CBA/J \times C57Bl/6) F_1 cells.

depleted of alloreactive T cells from normal mice. For this experiment, XX C57Bl/6 mice were primed in vivo with XY (CBA/J + C57Bl/6) F_1 cells, and injected intravenously 14 days later with irradiated (1,000 R) XX (CBA/J \times C57Bl/6) F_1 cells, to recruit T cells reactive to $H-2^k$ alloantigens to the spleen (6). Thoracic duct lymphocytes (TDL) were collected between 24 and 48 h after injection of XX F_1 cells. The TDL were then cultured with XY (CBA/J \times C57Bl/6) F_1 stimulators.

As shown in Fig. 4, the TDL could be activated to lyse XY $H-2^b$ targets, but not XY $H-2^k$ targets. In control cultures, XY-primed $H-2^b$ T cells were stimulated with XY (CBA/J \times C57Bl/6) F_1 cells. These cells lysed XY $H-2^b$ targets and, more effectively, allogeneic XY $H-2^k$ as well as XX target cells (not shown).

Discussion

The experiments reported here do not support the idea that male antigens associated with $H-2$ form new antigenic determinants recognized by T cells. XX cells from XX/XY hemopoietic chimeras do not take up male antigens disseminated by XX cells in a way which would allow them to serve as stimulators or targets for male-specific killer cells in vitro. Nor is it likely that new antigenic determinants are formed when male antigens and $H-2$ are produced and expressed by the same cell. In that case, $H-2^b$ male cells tolerant to $H-2^k$ antigens should, like $H-2^b$ female T cells, be sensitized to lyse male $H-2^k$ cells. Our experiments do not entirely rule out the possibility that complexes of disseminated male antigens and $H-2$ exist on stimulator and target cells in quantities below the level of detection, but sufficient to induce tolerance. Even if nonreactivity of XY T cells to allogeneic XY cells could be explained in this fashion, there is no explanation why XX $H-2^b$ T cells negatively selected to $H-2^k$ alloantigens should not be able to recognize and react to new antigens formed by Y-antigens and $H-2^k$ alloantigen. (The negative result of this experiment is in contrast to the findings of Wilson et al. [10] that negatively selected T cells could be activated to kill allogeneic TNP-coupled target cells. The discrepancy would suggest that T cells can be activated to certain haptens coupled directly to alloantigens).

Our experiments imply that T cells distinguish between male cells expressing

different H-2 antigens, and that they do this by recognizing H-2 and the Y-antigen as distinct entities.

But this still leaves open the role of the two entities in activating cytotoxic cells. The fact that XX T cells from a chimera but not those from a normal mouse can respond to Y-antigens on allogeneic cells suggests that T cells in a chimera acquire the potential of recognizing Y-antigens in association with allogeneic H-2. Recent experiments by Zinkernagel et al. (11) indicate that in the thymus of a chimera, T cells do not only become tolerant to H-2 (5), but they also learn which H-2 type to use in interaction with other cells.

Summary

XX cells from XX/XY hemopoietic chimeras do not express male determinants in a way to render them either stimulators or targets for male-specific cytotoxic lymphocytes. XX- but not XY-responder T cells from chimeras can be activated to lyse allogeneic male target cells; T cells from normal XX mice depleted of alloreactive T cells, however, cannot be sensitized to lyse allogeneic XY targets. The results imply that T cells recognize the Y-antigen and H-2 as distinct entities, and that in chimeras, they acquire the potential to react against allogeneic XY cells.

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References

1. Simpson, E., and R. D. Gordon. 1977. Responsiveness to H-Y antigen, Ir gene complementation and target cell specificity. *Immunol. Rev.* 35:59.
2. von Boehmer, H., C. G. Fathman, and W. Haas. 1977. H-2 gene complementation in cytotoxic T cell responses of female against male cells. *Eur. J. Immunol.* 7:443.
3. Ohno, S., and C. L. Christian. 1976. Hormone-like role of H-Y antigen in bovine freemartin gonad. *Nature (Lond.)*. 261:597.
4. Ohno, S. 1976. A hormone-like action of H-Y antigen and gonadal development of XY/XX mosaic males and hermaphrodites. *Hum. Genet.* 35:21.
5. von Boehmer, H., J. Sprent, and M. Nabholz. 1975. Tolerance to histocompatibility determinants in tetraparental bone marrow chimeras. *J. Exp. Med.* 141:322.
6. Sprent, J., and J. F. A. P. Miller. 1976. Effect of recent antigen priming on adoptive immune responses. III. Antigen-induced selective recruitment of subsets of recirculating lymphocytes reactive to H-2 determinants. *J. Exp. Med.* 143:585.
7. von Boehmer, H., and W. Haas. 1976. Cytotoxic T lymphocytes recognize allogeneic tolerated TNP-conjugated cells. *Nature (Lond.)*. 261:241.
8. Pfizenmaier, K., A. Starzinski-Powitz, H. Rodt, M. Röllinghoff, and H. Wagner. 1976. Virus and trinitrophenol hapten-specific T-cell-mediated cytotoxicity against H-2 incompatible target cells. *J. Exp. Med.* 143:999.
9. Zinkernagel, R. M. 1976. Virus-specific T cell mediated cytotoxicity across the H-2 barrier to virus altered alloantigen. *Nature (Lond.)*. 251:547.
10. Wilson, D. B., K. F. Lindahl, D. H. Wilson, and J. Sprent. 1977. The generation of killer cells to trinitrophenyl-modified allogeneic targets by lymphocyte populations negatively selected to strong alloantigens. *J. Exp. Med.* 146:361.
11. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. Klein, and J. Klein. 1977. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* 147:882.