Brief Definitive Report

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 × 10⁷ 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 ⁵¹Cr-labeled blasts induced by lipopolysaccharide (LPS) (2, 5).

Chimeras. Chimeras were prepared by injecting lethally (880 R) irradiated female (CBA/J x C57B1/6)F₁ hybrids with anti-e-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 × 10⁸ 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 C57B1/6 bone marrow cells, respectively, into lethally irradiated female (CBA/J x C57B1/6)F₁ hybrids. Lymphoid cell chimerism in the spleen was tested 3 mo later, and ranged in the different chimeras from 20 to 30% C57B1/6 cells, and from 70 to 80% CBA/J cells. Persisting F₁ 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 ⁵¹Cr. H-2<sup>+</sup>-restricted cytotoxic T cells were obtained
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 × CBA)F\(_1\) 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 × C57Bl/6)F\(_1\) 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 × CBA/J)F\(_1\) 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
Figs. 2 and 3. Stimulation of H-2-restricted cytotoxic effector cells from primed female (CBA/J × C57Bl/6)F₁ hybrids by C57Bl/6 δ (bδ), CBA/J δ (kδ), (CBA/J × C57Bl/6)F₁ δ (k × bδ), and by a mixture of kδ and bδ (kδ bδ), or kδ and bδ (kδ bδ) cells from either normal or chimeric mice.

As shown in Fig. 3, XX H-2b T cells from chimeras could be sensitized to lyse XY H-2k targets. The same could not be demonstrated with XX H-2b T cells itself does not mount an anti-Y response. However, XY H-2b 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-2b or H-2k male cells (Fig. 3), while responding to allogeneic cells (not shown).
FIG. 4. Lysis of XX and XY H-2b and XY H-2k targets by XX H-2b TDL negatively selected to H-2k alloantigens (○), or XX H-2b T cells (©) stimulated by XY (CBA/J × C57BL/6)F1 cells. The mice had been primed in vivo with XY (CBA/J × C57BL/6)F1 cells. Thoracic duct lymphocytes (TDL) were collected between 24 and 48 h after injection of XX F1 cells. The TDL were then cultured with XY (CBA/J × C57BL/6)F1 stimulators.

As shown in Fig. 4, the TDL could be activated to lyse XY H-2b targets, but not XY H-2k targets. In control cultures, XY-primed H-2b T cells were stimulated with XY (CBA/J × C57BL/6)F1 cells. These cells lysed XY H-2b targets and, more effectively, allogeneic XY H-2k 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 C57BL/6 mice were primed in vivo with XY (CBA/J + C57BL/6)F1 cells, and injected intravenously 14 days later with irradiated (1,000 R) XX (CBA/J × C57BL/6)F1 cells, to recruit T cells reactive to H-2k alloantigens to the spleen (6). Thoracic duct lymphocytes (TDL) were collected between 24 and 48 h after injection of XX F1 cells. The TDL were then cultured with XY (CBA/J × C57BL/6)F1 stimulators.

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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