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Major histocompatibility complex-linked immune-responsiveness is acquired by lymphocytes of low-responder mice differentiating in thymus of high-responder mice

(generation of diversity/chimera/T-cell receptor)

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ABSTRACT **Female murine T cells can respond to the Y antigen of male cells by generating cytotoxic T-killer lymphocytes. Responsiveness is linked to several *H-2* genes. Two types of low responders can be distinguished: the B10.A(5R) (*H-2*^{15}) strain, a low responder because it lacks Y-specific precursor T cells able to differentiate into cytotoxic T-killer cells; and the CBA/J (*H-2*^{k}) strain, a low responder because it lacks Y-specific T-helper cells able to support differentiation of T-killer cell precursors. B10.A(5R) stem cells differentiating in an x-irradiated (CBA/J × C57BL/6) (*H-2*^{k} × *H-2*^{b})F_1 host respond to Y antigen by generating T-killer cells whereas CBA/J stem cells do not. The results are consistent with the hypothesis that diversity of T-cell receptors is generated by somatic mutation of germ-line genes encoding specificity for self-*H-2*. A detailed account of this hypothesis is presented.**

Jerne has proposed (1) that the germ cells of an animal carry a set of genes encoding the combining sites of lymphocyte receptors directed against a complete set of certain major histocompatibility complex (MHC)-encoded antigens of the species to which the animal belongs. One pair of these germ-line genes is expressed on a lymphocyte at an early stage of differentiation. Mutants that recognize foreign antigens arise in lymphocyte clones expressing germ-line genes coding for receptors directed against MHC gene products of the individual itself. The primary lymphoid organs (e.g., the thymus) are viewed as mutant breeding organs.

Recent experiments indicate that cells expressing receptors for MHC antigens are selected by these MHC antigens as expressed on nonlymphoid tissue of the thymus (2). The selected cells may acquire specificity for other antigens while retaining specificity for the selecting MHC antigens—i.e., a T lymphocyte expresses two classes of receptors, one recognizing a MHC antigen and a second recognizing a different antigen.

The MHC antigens encoded by the genome of an animal determine which of its receptor-encoding germ-line genes will be available for mutation. The selection of these mutated genes leads to the generation of a diversity of receptors of the second class that recognize many but not all antigens.

The hypothesis predicts that MHC-linked responsiveness can be acquired by low-responder lymphocytes differentiating in the thymus of high-responder strains. Results consistent with this prediction are described in this report.

MATERIAL AND METHODS

Mice. B10.A(5R) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred at the Basel Institute for Immunology. CBA/J, C57BL/6, and their F_1 hybrids were obtained from the Institut für Biologische-Medizinische Forschung AG (Füllinsdorf, Switzerland). The MHC haplotypes of the mice are given in Table 1, according to David (3).

Chimeras. Chimeras were prepared by injecting lethally irradiated female recipients with anti-Thy 1.2-treated female bone marrow cells from various strains (4). Chimeras prepared by injecting CBA/J bone marrow cells into (CBA/J × C57BL/6)F_1 recipients will be referred to as *kkk* → (*kkk* × *bbb*)F_1 chimeras (see Table 1). In all chimeras described, more than 90% of lymphoid cells were donor-derived as shown by anti-H-2 antibody-mediated cytotoxicity. Two months after bone marrow reconstitution, the chimeras were immunized against Y antigen by intravenous injection of 2×10^7 (*kkk* × *bbb*)F_1 male cells that had been x-irradiated [2000 R (5.2 C/kg)].

Cytotoxic Anti-Male Responses. Between 2 and 8 weeks after priming, spleen cells were cultured for 5 days with x-irradiated male cells and cytotoxic tests were performed on ^{51}Cr-labeled lipopolysaccharide-induced blasts as described (4, 5).

RESULTS

For simplicity, we use the notation given in Table 1 to identify the mouse strains used.

Low-Responder T-Cells Can Acquire Responsiveness in a Chimera. The interaction of murine T cells with other cells is restricted by MHC gene products: T-killer cells are restricted by *K* and *D* region gene products (6, 7) and T-helper cells, by *I* region products (8, 9). Virus-specific and hapten-specific T-killer cells from a (*bbb*) mouse lyse targets expressing either K^b or D^b antigens equally well (10, 11). T-killer cells specific for Y antigen (the test system used in our experiments) from a (*bbb*) mouse lyse only male targets expressing D^b antigens (5, 12). This implies that, on T-killer cells from *bbb* mice, the expression of receptors recognizing Y antigen is linked to the expression of receptors recognizing D^b antigen.

It has been impossible to obtain killer cells recognizing Y antigen in association with either K^b or D^d antigens in all strain combinations tested so far (5, 12). The *b*Ⓑ*d* mouse expressing K^b and D^d is therefore a low responder. The ⓀkⓀ mouse is also a low responder even though T-killer cells lysing male targets expressing either K^k or D^k antigens can be obtained from appropriate hybrids such as (ⓀkⓀ × *b*Ⓑ*b*Ⓑ)F_1 (5, 12). Because ⓀkⓀ mice possess permissive (encircled K^k and D^k) antigens for Y-specific T-killer cells, their low responsiveness is not due

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Abbreviations: MHC, major histocompatibility complex; R, receptor.

Table 1. MHC haplotypes of mice used

Strains	Haplotype	*H-2* regions									Responder to Y antigen	Notation used in text*
		K	*A*	*B*	*J*	*E*	*C*	*S*	*G*	*D*		
C57BL/6	*H-2^b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	+	*bbb*
CBA/J	*H-2^k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	−	*kkk*
B10.A(5R)	*H-2^{i5}*	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*	*d*	−	*bbd*

* To avoid too cumbersome a notation in the text of this paper, for simplicity we refer to *H-2^b* mice expressing K^b, IA^b, and D^b antigens as *bbb*, to *H-2^k* mice as *kkk*, and to *H-2^{i5}* mice as *bbd* mice. F_1 hybrids of *H-2^b* and *H-2^k* mice are referred to as (*bbb* × *kkk*)F_1 mice. The generation of Y-antigen specific T-killer cell precursors requires the presence, in the thymus, of D^b, K^k, or D^k antigen. The generation of Y-antigen specific T-helper cells requires the presence, in the thymus, of *IA^b* antigen. In the text, these "permissive" MHC alleles have been encircled—e.g., *b*ⓑ*b*, ⓚ*k*ⓚ, and *b*ⓑ*d*.

to the absence of Y-specific T-killer cell precursors. Recent experiments by Zinkernagel *et al.* (13) suggest that T-helper cells interact with T-killer cells or their precursors expressing appropriate *I* region products. Thus, ⓚ*k*ⓚ mice may be low responders because they express nonpermissive I$_a^k$ antigens for Y-specific T-helper cells. One would therefore predict that lymphocyte stem cells from low-responder ⓚ*k*ⓚ and *b*ⓑ*d* mice differentiating in an x-irradiated (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 host would behave differently in their response to Y antigen. ⓚ*k*ⓚ T cells from such chimeras should still be unresponsive, because of the lack of Y-specific T-helper cells able to interact with Y-specific T-killer cells expressing nonpermissive I$_a^k$ antigens. On the other hand, *b*ⓑ*d* cells should be responsive, because *b*ⓑ*d* T-killer cell precursors expressing permissive I$_a^b$ antigens for Y-specific T-helper cells and recognizing Y antigen in association with permissive D^b, K^k, and D^k antigens should be generated in the thymus of the recipient.

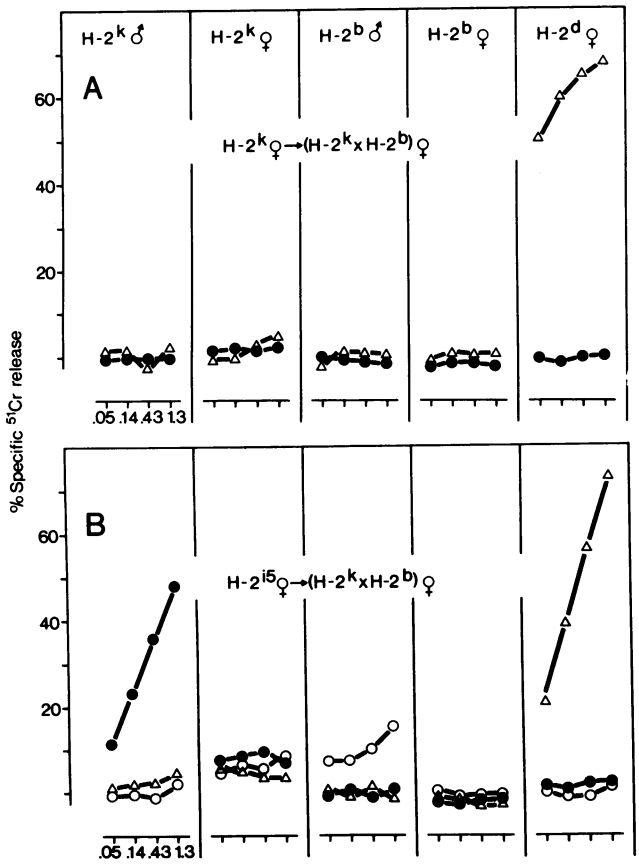

FIG. 1. Cytotoxic responses of T cells from different chimeras against male and female targets of various strains. (*A*) ♀ⓚ*k*ⓚ → ♀(ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 chimeras were immunized *in vivo* with ♂ (*kkk* × *bbb*)F_1 cells and restimulated *in vitro* with either ♂ (*kkk* × *bbb*)F_1 (●—●) or ♀ *ddd* (△—△) cells. (*B*) ♀ *b*ⓑ*d* → ♀ (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 chimeras were immunized *in vivo* with ♂ (*kkk* × *bbb*)F_1 cells and restimulated *in vitro* with ♂ *kkk* (●—●), ♂ *bbb* (○—○), or ♀ *ddd* (△—△) cells. Numbers on the abscissa indicate the number of responder cells (×10^6) cultured on day 0, the descendants of which are killer cells tested on 2 × 10^4 target cells (as indicated) on day 5. Both experiments were repeated twice with cells from other chimeras with similar results. (In this figure, the haplotype notation of Table 1 is used.)

As shown in Fig. 1, results consistent with this prediction were obtained. Female ⓚ*k*ⓚ T cells derived from chimeras ⓚ*k*ⓚ → (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 could not be induced to lyse male targets (Fig. 1*A*). In contrast, female *b*ⓑ*d* T cells derived from chimeras *b*ⓑ*d* → (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 lysed male ⓚ*k*ⓚ as well as male *b*ⓑ*b* targets (Fig. 1*B*). T cells from both chimeras responded equally well to allogeneic *ddd* cells. Female *kkk* or *bbb* targets were not lysed. The fact that *b*ⓑ*d* T-killer cells from chimeras lysed male ⓚ*k*ⓚ targets better than male *b*ⓑ*b* targets is in accordance with data by Simpson and Gordon (12) showing that mice immunized with male (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 cells usually respond better to male ⓚ*k*ⓚ cells than to male *b*ⓑ*b* cells.

Female Responder (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 Lymphocytes, Differentiating in Low-Responder ⓚ*k*ⓚ Parental Thymus, Are Unresponsive to Male Cells. Low responsiveness in the ⓚ*k*ⓚ strain is apparently due to the absence of Y-antigen specific T-helper cells. T cells from a (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 responder → ⓚ*k*ⓚ low responder chimera should only be selected for interaction with nonpermissive I$_a^k$ antigens and consequently not possess Y-antigen specific T-helper cells. Fig. 2 shows that female T cells from such chimeras indeed failed

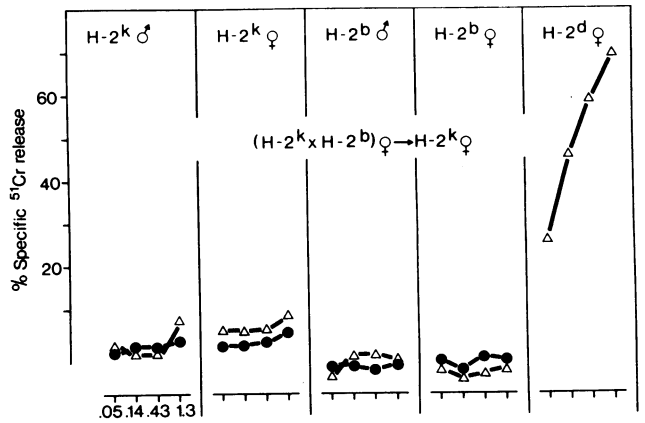

FIG. 2. Cytotoxic responses of T cells from ♀ (ⓚ*k*ⓚ × *b*ⓑ*b*)F_1 → ♀ (ⓚ*k*ⓚ) chimeras against male and female targets of various strains. The chimeras were immunized with ♂ (*kkk* × *bbb*) F_1 cells and restimulated *in vitro* with either ♂ (*kkk* × *bbb*)F_1 (●—●) or ♀ (*ddd*) (△—△) cells. For further details see Fig. 1. The experiment was repeated with cells from another chimera with similar results. (In this figure, the haplotype notation of Table 1 is used.)

to respond to male (k)k(k) cells or male b(b)(b) cells but responded well to allogeneic *ddd* cells.

DISCUSSION

The experiments described here are consistent with Jerne's hypothesis (1) that T cells are selected in the thymus according to their germ-line encoded specificity for H-2 antigens of the species and that diversity is generated by somatic mutation of germ-line genes expressing specificity for H-2 antigens of the individual itself.

For interpretation of the restriction of T-cell interactions with other cells and of other recent findings (2, 14), this hypothesis needs to be augmented by the following rules:

(*i*) A T lymphocyte expresses two classes of receptors, R_0 and R_1, recognizing two different antigens. As a rule, both R_0 and R_1 must recognize antigen displayed on the surface of another cell for cell interactions to occur.

(*ii*) At an initial stage of differentiation, R_0 and R_1 possess identical combining sites. T-helper cell precursors recognize a complete set of certain *I*-region products of the species, whereas T-killer cells recognize a complete set of certain *D*- and *K*-region products of the species.

(*iii*) MHC gene products on thymus epithelium interact with T-cell receptors that recognize these self-antigens. This leads to exhaustive proliferation and to the selection of T cells that express an unaltered R_0 receptor together with a mutant R_1 receptor.

(*iv*) Each of the selected T-helper cells is equipped with two classes of receptors: R_0 recognizing I_a and R_1 recognizing some other antigen. In this way, T-helper cell precursors recognizing a given thymic *I*-region antigen will give rise to a population of T-helper cells whose R_1 receptors recognize a set of foreign antigens. This set does not encompass all foreign antigens, because some may not be recognized by any of the occurring R_1 mutants.

(*v*) Likewise, T-killer cell precursors recognizing a given thymic *K*- or *D*-region antigen will give rise to a population of potential T-killer cells. Each of the cells of this population is equipped with two classes of receptors: R_0 recognizing K and R_1 recognizing an incomplete set of other antigens; or R_0 recognizing D and R_1 recognizing another incomplete set of antigens.

(*vi*) In normal animals, T cells and thymus epithelial cells will have identical genomes and therefore identical MHC encoded antigens. In chimeras the thymus epithelium of recipient animals may possess MHC alleles that differ from the MHC alleles of the lymphocytes arising from the bone marrow stem cells of the donor. The sets of antigens recognized by the R_0 and R_1 receptors of T cells in chimeras will be determined by the MHC of the recipient thymus, and not by the MHC of the donor T cells themselves.

(*vii*) Differentiation of executive lymphocytes (i.e., T-killer cells and antibody-secreting B cells) is supported by T-helper cells (except for allogeneic killer cells and T-independent antigens). These T-helper cells are restricted by MHC: their R_0 receptors must recognize that I_a antigen of the cell with which they interact.

(*viii*) The first requirement for obtaining T-killer cells in a chimera is, therefore, that the *I* allele expressed by the recipient thymus must be identical to the *I* allele expressed on the T-cell precursors of the donor.

(*ix*) With respect to a given foreign antigen, the second requirement is that the R_1 receptor initially recognizing this I product can mutate to a receptor recognizing the given antigen.

(*x*) And, as the third requirement, the T-killer cell precursor whose R_0 receptor recognizes a K or D product must be able, by mutation, to develop an R_1 receptor recognizing the given antigen.

Thus, in order to obtain responsiveness to Y antigen in a chimera, the recipient thymus as well as the donor lymphocytes must express I_a^b antigens. For generation of Y-antigen specific T-killer cell precursors, the thymus must express permissive K or D antigens. We were not able to detect Y-specific killing in mice unless they expressed I_a^b antigens. This is in contrast to findings of Simpson and Gordon (12) demonstrating Y-specific killing in various mice (especially F_1 hybrids) not expressing I_a^b antigens. We have reason to believe that in some of these latter experiments the generation of Y-specific killers was independent of Y-specific T-helper cells.

Our experiments on Y-antigen specific T-cells are in good agreement with data on rejection of male skin grafts: all mouse strains that rapidly reject male skin grafts express I^b antigens (15–17). Also, the b(b)d strain expressing nonpermissive K and D antigens for Y-antigen specific T-killer cells rapidly rejects male skin grafts. We conclude therefore that, in mice that are unable to generate cytotoxic T lymphocytes, I^b restricted T cells reject male skin grafts. This is not surprising because *I* restricted T cells can cause delayed type hypersensitivity, an inflammatory reaction in skin (18). In all other mouse strains tested, there is either no or only late skin graft rejection (15–17), and in our experiments there was no significant induction of cytotoxic T cells. This means that Y-specific T-killer cell precursors cannot mediate rapid male skin graft rejection without Y-antigen specific T-helper cells.

The hypothesis states that, at an initial stage of differentiation, R_0 and R_1 express the same specificity, encoded by a *V* gene or a pair of *V* genes of a certain set. Only those cells in which the R_1 class of receptors mutates are selected for maturation.

T-killer cells and T-helper cells must use a different set of germ-line *V* genes. The occurrence of shared *V* gene allotypes and idiotypes on T- and B-cell receptors suggests that B cells and T cells make use of the same sets of *V* genes (19–21). The repertoire of mature B cells, however, appears to be different from that of T cells because no clear *H-2* linkage of B-cell specificity has been documented. The somatic generation of diversity in B cells may therefore require a selection mechanism different from that operating for T cells.

MHC polymorphism increases the total diversity of T-cell receptors in a population of animals of the same species. All individuals of a species therefore would not be vulnerable to the same calamity. It might be disadvantageous for every individual to express this total diversity because of certain MHC-linked diseases.

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