

Brief Definitive Report

SELECTIVE RESPONSE TO H-Y ANTIGEN BY F₁ FEMALE MICE SENSITIZED TO F₁ MALE CELLS

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In vitro T-cell-mediated cytotoxic responses to H-Y antigen require co-recognition of H-Y and H-2 antigens on target cells (1). F₁ females sensitized to male cells of either parental H-2 haplotype will only lyse male cells expressing at least part (H-2K or H-2D) of that parental H-2 haplotype and will not lyse allogeneic male cells or male cells of the other parental H-2 haplotype (2, 3). Thus, (CBA × B10)F₁ females¹ primed in vivo and challenged in vitro in mixed lymphocyte culture (MLC) to CBA male cells will lyse CBA but not B10 male target cells. This restricted F₁ anti-parent response requiring co-recognition of non-H-2 and H-2 antigens has been demonstrated in a number of experimental systems including cell-mediated cytotoxic responses to viruses, hapten-modified cells, minor histocompatibility antigens, and tumor cells (4-8). We report here that F₁ females which can respond to male cells of each parent, when sensitized with F₁ male cells, make a preferential response and lyse male cells of only one parental H-2 haplotype. It is suggested that this may represent an effect of antigen presentation under immune response (*I*r) gene control, or an effect of suppressor genes which may also be located in the major histocompatibility gene complex (MHC).

Materials and Methods

Animals. All mice used in these experiments were obtained from the breeding unit of the Animal Division of the Clinical Research Centre, Harrow, England.

In Vivo Sensitization. Female mice were primed to H-Y antigen by the intraperitoneal injection of 1×10^7 viable male spleen cells 2 wk to 4 mo before use.

In Vitro Sensitization and Microcytotoxicity Assay. The materials and methods for in vitro sensitization (MLC) and microcytotoxicity assay have been previously described (9). Briefly, for MLC, spleen cells from primed females were adjusted to 5×10^6 /ml in RPMI 1640 medium with 10% fetal calf serum (FCS) and co-cultured at 37°C in a humidified 10% CO₂ atmosphere with an equal number of irradiated male spleen cells. After 5 days, responder cells for assay were harvested as a source of cytotoxic attacking cells, adjusted to 2×10^6 /ml in Eagle's minimal essential medium with 10% FCS, and twofold serial dilutions were performed. 0.2 ml of each attacking cell suspension was added to 0.3 ml capacity wells of an assay plate, allowing three

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¹ Throughout this paper, maternal parents are listed first describing the origins of F₁ animals. Thus (CBA × B10)F₁ means (CBA female × B10 male)F₁.

TABLE I
Selective Responses to H-Y Antigen by (CBA × B10)F₁ Females

Responder female	Male cells (antigen)		Corrected % lysis of target cells (A:T = 4:1)			
	In vivo	In vitro	CBA male	B10 male	(CBA × B10)F ₁	
					Male	Female
(CBA × B10)F ₁	(CBA × B10)F ₁	(CBA × B10)F ₁	14.96 ± 1.10	0.97 ± 0.40	12.43 ± 0.52	-2.53 ± 1.49
(B10 × CBA)F ₁	(B10 × CBA)F ₁	(B10 × CBA)F ₁	17.16 ± 2.44	0.47 ± 3.36	17.06 ± 1.75*	3.44 ± 0.95
(CBA × B10)F ₁	(CBA × B10)F ₁	CBA	15.08 ± 0.37	-2.89 ± 0.40	10.61 ± 0.21	-1.74 ± 0.77
(CBA × B10)F ₁	(CBA × B10)F ₁	B10	0.61 ± 0.42	1.38 ± 0.73	-1.13 ± 0.21	-1.05 ± 0.59
(CBA × B10)F ₁	CBA	(CBA × B10)F ₁	33.20 ± 0.62	-4.26 ± 0.37	25.57 ± 1.01	-1.86 ± 0.69
(CBA × B10)F ₁	B10	(CBA × B10)F ₁	0.16 ± 1.22	21.92 ± 1.59	15.47 ± 1.63	-1.03 ± 1.63

F₁ females were primed in vivo and challenged in vitro in MLC with the male cells shown and assayed for 3 h against 1×10^5 ⁵¹Cr-labeled target cells at A:T = 1:1, 2:1, 4:1, and 8:1. Corrected % lysis is the percent lysis of target cells ± SE as determined from a four point linear regression fit. Background (spontaneous) release was less than 20%. Maternal parents are listed first in describing the origins of F₁ mice [e.g., (CBA × B10)F₁ means (CBA female × B10 male)F₁].

* Lysis of (B10 × CBA)F₁ male was 16.47 ± 2.41.

replicates for each attacking cell to target cell ratio (A:T). 0.05 ml (1×10^5) of ⁵¹Cr-labeled concanavalin A blast spleen target cells was also dispensed into each well. Maximum lysis was determined by adding 0.05 ml target cells and 0.2 ml 5% Triton to a set of wells. Spontaneous release (background) was measured from target cells incubated with medium alone. After 5 min centrifugation at 500 rpm, plates were incubated for 3 h at 37°C in 10% humidified CO₂. Plates were then centrifuged at 1,000 rpm for 10 min and 0.1-ml samples harvested for gamma counting. The corrected percent lysis was computed according to the formula of Wunderlich et al. (10).

Results and Discussion

(CBA × B10)F₁ females, primed with male cells of either parental haplotype (CBA or B10) and challenged in MLC with (CBA × B10)F₁ male cells, will lyse male cells of the priming parental *H-2* haplotype or F₁ male cells, but not male cells of the other parental *H-2* haplotype. This is shown in lines 5 and 6 of Table I. This confirms that (CBA × B10)F₁ male stimulating and target cells express H-Y antigen in association with both *H-2^k* and *H-2^b* parental haplotypes. However, as shown in line 1, Table I (CBA × B10)F₁ females, primed in vivo and challenged in MLC with (CBA × B10)F₁ male cells, lyse CBA male and F₁ male target cells, but not B10 male cells. This preferential response to H-Y in association with the *H-2^k* haplotype is established during priming in vivo: this is shown by the failure of F₁ females primed in vivo with F₁ male cells to respond when subsequently challenged in vitro with the "nonpreferred" parental (B10) male cells (lines 3 and 4, Table I). As mentioned above, F₁ females primed in vivo with B10 male cells and challenged in MLC with F₁ male cells, will lyse B10 and F₁ male targets (line 6, Table I).

We have studied whether or not the preferential response of F₁ females primed and challenged with F₁ male cells is an effect of the direction of inheritance of either H-Y or MHC genes. However, the results are the same whether CBA males and B10 females, or B10 males and CBA females are used as the parental pairs for responder F₁ females, F₁ stimulator cells, or F₁ target cells. One example of these results is given in line 2, Table I. (B10 × CBA)F₁ females, primed and challenged with (B10 × CBA)F₁ male cells, lyse CBA, (CBA × B10)F₁, and (B10 × CBA)F₁ but not B10 male target cells. Thus, preferential responses to H-Y by F₁ females cannot be explained on the basis of inheritance of H-Y and MHC genes from parental lines.

TABLE II
Selective Responses to H-Y Antigen by (BALB/c × B10)F₁ Females

Responder female	Male cells (antigen)		Corrected % lysis of target cells (A:T = 4:1)			
	In vivo	In vitro	BALB/c male	B10 male	(BALB/c × B10)F ₁	
					Male	Female
(BALB/c × B10)F ₁	(BALB/c × B10)F ₁	(BALB/c × B10)F ₁	8.87 ± 0.41	35.62 ± 2.38	28.94 ± 0.73	2.24 ± 0.05
(BALB/c × B10)F ₁	(BALB/c × B10)F ₁	BALB/c	4.16 ± 0.71	-0.76 ± 0.38	2.91 ± 0.34	-0.04 ± 0.53
(BALB/c × B10)F ₁	(BALB/c × B10)F ₁	B10	0.47 ± 0.71	24.60 ± 2.11	17.43 ± 1.72	-0.15 ± 0.52
(BALB/c × B10)F ₁	BALB/c	(BALB/c × B10)F ₁	15.52 ± 1.01	1.64 ± 0.81	8.38 ± 0.27	0.60 ± 0.32
(BALB/c × B10)F ₁	B10	(BALB/c × B10)F ₁	2.50 ± 0.30	43.19 ± 0.32	25.74 ± 0.50	1.64 ± 0.81

F₁ females were primed in vivo and challenged in vitro with the male cells shown and assayed for 3 h against 1×10^5 ⁵¹Cr-labeled target cells at A:T = 1:1, 2:1, 4:1, and 8:1. Corrected % lysis is the percent lysis of target cells ± SE as determined from a four point linear regression fit. Background (spontaneous) release was less than 20%. Maternal parents are listed first in describing the origins of F₁ mice [e.g., (BALB/c × B10)F₁, means (BALB/c female × B10 male)F₁].

Table II demonstrates that a similar result is found when (BALB/c × B10)F₁ females are primed and challenged with (BALB/c × B10)F₁ male cells. In this case the response is toward H-Y in association with the *H-2^b* haplotype, since (BALB/c × B10)F₁ females sensitized to F₁ male cells will lyse B10 and F₁ male target cells, but not BALB/c male target cells (line 1, Table II). The data in lines 4 and 5, Table II, again demonstrate that H-Y is present in association with both *H-2^b* and *H-2^d* on (BALB/c × B10)F₁ male stimulating cells and target cells and, together with the data in lines 2 and 3, confirms that the preferential response to F₁ females to the *H-2^b* is a restriction of in vivo primary sensitization rather than the secondary MLC.

Thus, we have here demonstrated that an in vitro assay of secondary cell-mediated cytotoxic responses to H-Y, *H-2^k* shows a stronger association with H-Y than *H-2^b*, and *H-2^b* shows a stronger association than *H-2^d*. Skin grafts from *H-2^k* males have been shown to be a stronger stimulus than grafts from *H-2^b* males on congenic (*H-2^k* × *H-2^b*)F₁ females (11). This supports our observation that H-Y on an *H-2^k* background shows greater antigenicity than H-Y on an *H-2^b* background during in vivo sensitization. The basis for these preferential responses is not yet clear. Although cell-mediated responses to H-Y and other families of antigens require co-recognition of MHC gene products, it has not been established whether this represents an immune response by a T lymphocyte with a single receptor for a biochemically modified ("altered self") MHC product, or a T cell with two receptors requiring dual recognition of H-Y and *H-2* gene products. It seems unlikely to us that *H-2* gene products are biochemically altered by minor histocompatibility antigens such as H-Y and recent data on the nature of the T-cell receptor based on the study of T-cell idiotype has been interpreted as favoring a two receptor model (12). However, more data is needed to settle this most important fundamental question about T lymphocytes.

We have recently reported that cytotoxic T-cell responses to H-Y are under the control of *Ir* genes probably mapping in the MHC (13).² This is in agreement with earlier observations on skin graft rejection of syngeneic male skin being under the control of *Ir* genes in the MHC (14). *Ir* gene products could affect the

² Hurme, M., C. M. Hetherington, P. R. Chandler, R. D. Gordon, and E. Simpson. 1977. Manuscript submitted for publication.

response in several ways. One involves antigen presentation, i.e. that the product of the *Ir* gene(s) constituting the responder in question, for example a (CBA × B10) F_1 female, could associate best (e.g., by higher affinity) with H-Y antigen, linked with *H-2D^k* antigen(s) rather than with H-Y antigen linked to *H-2D^b* antigen(s). This association could be by the production of soluble factors required for antigen presentation by macrophages or other cells, or by some other mechanism (15). Thus, the ability to respond would reside not only in the *Ir* gene(s) itself but in H-Y being presented in association with appropriate *K* and *D* antigens. The preferential response of an F_1 to one parental haplotype may be a result of relative strengths or affinities of H-Y and *H-2K* or *D* antigens. However, it is possible, although no data yet exists to support the notion, that suppressor genes also mapping in the MHC selectively suppress the response to H-Y in association with a particular haplotype.

The development of cytotoxic T-cell responses to H-Y and the ability of females to reject syngeneic male skin is positively correlated (13, 14). It is therefore pertinent to compare our results on preferential anti-H-Y cytotoxic T-cell responses in F_1 females primed with F_1 male cells with the very interesting results obtained by skin grafting various F_1 female mice with parental male skin reported recently (16). Using the time of skin graft rejection as the criterion, these authors found a hierarchy of antigenicity with *H-2^k* associated H-Y being stronger than *H-2^b* or *H-2^d*. However, their parental H-Y donors were in most cases *H-2* recombinant strains, with *K* and *D* ends of the MHC derived from different haplotypes. Thus, when B10.A (*kkkddd*) and B10.A(5R) (*bbbddd*) were compared, the greater immunogenicity of B10.A was attributed to the *K^k* of B10.A, and the weak immunogenicity of B10.A(5R) to *K^b*. Our mapping data would suggest that H-Y does not associate with *D^d* or *K^b*, and we would expect B10.A(5R) to be nonimmunogenic for this reason. One further example is their comparison of B10.A(2R) (*kkkddb*) and B10 (*bbbbbb*) in which they found that B10.A(2R) were more immunogenic, and this was also attributed to *K^k*; however, from our data it can be seen that B10.A(2R) presents two H-Y associable antigens, *K^k* and *D^b*, whereas B10 presents only *D^b*. Another factor which should also be taken into consideration is the presence of the H-Y *Ir* gene(s) in the MHC of several nonresponder haplotypes (13).² This has been implied from the finding of responder F_1 mice, derived from mating of parental strains of which neither is a responder. It is therefore possible that the response of any F_1 is modified by the interaction between two or more H-Y *Ir* genes inherited from each parental haplotype. An apparent difference of antigenicity of H-Y in association with different *H-2* haplotypes could thus be an expression of *Ir* gene interaction. We are in broad agreement with the findings of Kralova and Demant (16) inasmuch as our F_1 cytotoxicity data would suggest a hierarchy of association, *H-2^k* being stronger than *H-2^b* which in turn is stronger than *H-2^d*, but we would attempt to interpret them in the light of the *H-2 K* and/or *D* end mapping of H-Y responses of the different haplotypes, i.e. the affinity of H-Y with these *H-2 K/D* products, and perhaps also in the light of *Ir* gene complementation.

Summary

T-cell-mediated cytotoxic responses to H-Y antigen require co-recognition of

H-Y and H-2 gene products. F₁ male stimulating cells and target cells express H-Y antigen in association with both parental H-2 haplotypes. However, F₁ females primed in vivo and challenged in vitro with F₁ male cells lyse male target cells of F₁ and only one parental H-2 haplotype. Thus, (CBA × B10)F₁ females sensitized to (CBA × B10)F₁ male cells lyse (CBA × B10)F₁ and CBA but not B10 male target cells, and (BALB/c × B10)F₁ females sensitized to (BALB/c × B10)F₁ male cells will lyse (BALB/c × B10)F₁ and B10 but not BALB/c male target cells. It is suggested that this may represent an effect of immune response or suppressor genes mapping in the major histocompatibility gene complex which regulate responsiveness to H-Y antigen.

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References

- 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.
- Gordon, R. D., and E. Simpson. 1976. H-2 restricted T-cell cytotoxic responses to H-Y antigen. In Proceedings of the Tenth Leukocyte Culture Conference. V. P. Eijssvoegel, D. Roos, and W. P. Zeijlemaker, editors. Academic Press, Inc., New York. 521.
- Gordon, R. D., B. Mathieson, L. E. Samelson, E. A. Boyse, and E. Simpson. 1976. The effect of allogeneic presensitization on H-Y graft survival and in vitro cell-mediated responses to H-Y antigen. *J. Exp. Med.* 144:810.
- Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel. 1976. Specificity of virus-immune T-cells for H-2K or H-2D compatible interactions: implications for H-antigen diversity. *Transplant. Rev.* 29:89.
- Shearer, G. M., T. G. Rehn, and A. Schmitt-Verhulst. 1976. Role of the murine major histocompatibility complex in the specificity of in vitro T-cell mediated lympholysis against chemically-modified autologous lymphocytes. *Transplant. Rev.* 29:222.
- Bevan, M. J. 1975. Interaction of antigens detected by cytotoxic T-cells with the major histocompatibility complex as modifier. *Nature (Lond.)* 256:419.
- Bevan, M. J. 1975. The major histocompatibility complex determines susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. *J. Exp. Med.* 142:1349.
- Schrader, J. W., and G. M. Edelman. 1976. Participation of the H-2 antigens of tumor cells in their lysis by syngeneic T cells. *J. Exp. Med.* 143:601.
- Simpson, E., R. Gordon, M. Taylor, J. Mertin, and P. Chandler. 1975. *Eur. J. Immunol.* 5:451.
- Wunderlich, J. R., G. N. Rogentine, and R. A. Yankee. 1972. Rapid *in vitro* detection of cellular immunity in man against freshly explanted allogeneic cells. *Transplantation (Baltimore)* 8:861.
- Wachtel, S. S., D. L. Gasser, and W. K. Silvers. 1973. Male-specific antigen: modification of potency by the H-2 locus in mice. *Science (Wash. D. C.)* 181:862.
- Janeway, C. A., H. Wigzell, and H. Binz. 1977. Hypothesis: two different V_H gene products make up the T-cell receptors. *Scand. J. Immunol.* 5:993.
- Gordon, R. D., and E. Simpson. 1977. Immune response gene control of cytotoxic T-cell responses to H-Y. *Transplant. Proc.* 9:885.
- Bailey, D. W., and J. Hoste. 1971. A gene governing the female immune response to the male antigen in the mouse. *Transplantation (Baltimore)* 11:404.
- Mozes, E. 1975. Review: expression of immune response (*Ir*) genes in T and B cells. *Immunogenetics.* 2:397.
- Kralova, J., and P. Demant. 1976. Expression of the H-Y antigen on thymus cells and skin: differential genetic control linked to the K end of H-2. *Immunogenetics.* 3:583.