CYTOTOXIC T-CELL RESPONSES TO H-Y: CORRELATION WITH THE REJECTION OF SYNGENEIC MALE SKIN GRAFTS

By M. HURME,* P. R. CHANDLER, C. M. HETHERINGTON, AND ELIZABETH SIMPSON

(From the Transplantation Biology Section, and the Division of Comparative Medicine, Clinical Research Centre, Harrow, HA1 3UJ England)

Work done in this laboratory (1–6) has shown that the anti-H-Y cytotoxic response in mice is regulated by two or three genes in the *H-2* complex: one of these genes is located in the *H-2K* or *H-2D* region, and its products serve as the associative structure for the H-Y antigen in the induction and effector phase of T-cell cytotoxicity. Of the other types of regulatory genes, *Ir* genes, the dominant gene of the $H\text{-}2^b$ haplotype maps in the *IA* region, whereas the complementary genes of other haplotypes map in the *IC* region. The presence of these *Ir* genes is also necessary for anti-H-Y cytotoxicity to occur.

The rejection of syngeneic male skin grafts by females of various mouse strains has been extensively studied (7), and it has been shown that the ability of females to reject syngeneic male skin is largely determined by the *H-2* complex (8, 9), but in some cases non-H-2 genes may have an effect on rejection (8, 10, 11). Mapping data obtained by using inbred recombinant mouse strains indicate that genes causing rapid rejection of syngeneic male skin grafts by female mice localize near the K-end of the $H\text{-}2^b$ haplotype (12). Our findings on the *Ir* gene regulation of the anti-H-Y cytotoxic cell formation now make the comparison of these two phenomena possible.

Materials and Methods

Mice. All mice used were obtained from the breeding unit of the Division of Comparative Medicine at the Clinical Research Centre, Harrow, England. Their *H-2* haplotypes are indicated in Table I.

Skin Grafting. Skin grafting was done as described by Billingham and Medawar (13). Full-thickness skin grafts, $\cong 0.5 \times 1.0$ cm from the tail, were grafted onto the side of the thorax of the recipient, and protected for 9–11 days by a plaster bandage. Rejection was scored macroscopically three times a week for 100 days. The results are expressed as a median survival time of the graft (rejection in 50% of mice in a group). The statistical significance was determined by a computerized log rank test (14).

Results

To compare the rejection of syngeneic male skin grafts with the induction of anti-H-Y cytotoxic cell formation, we used the previously described methods to examine the ability of various inbred mouse strains and their F_1 hybrids to

* Recipient of a European Molecular Biology Organization Fellowship. Present address: Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland.

TABLE I
H-2 Haplotypes of Mice Used

Strains	*H-2* Regions								
	K	A	B	J	E	C	S	G	D
C57BL/10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
B10.G	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*
CBA, B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*
BALB/c, B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
B10.A(2R)	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*b*
B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.A(5R)	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*	*d*
A, B10.A	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*d*
C3H.OH	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*k*
HTI	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*d*
B10.HTG	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*b*
A.TL	*s*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*d*

mount an anti-H-Y cytotoxic response (1–6). In brief, female mice were primed with male spleen cells intraperitoneally, or with a male skin graft, and spleen cells from these mice were then restimulated in vitro in mixed lymphocyte culture with the male spleen cells from the strain used for priming. Priming with spleen cells or by skin grafting has always given identical results and, in fact, there is evidence that in skin grafting, the passenger leukocytes are responsible for the priming of the recipient (15). In Tables II–IV, the capacity of cytotoxic cell production is indicated as positive or negative. The criteria for a positive result are set out in the accompanying paper (6) and are shown in detail in that paper.

Female mice of various inbred strains were first grafted with syngeneic male skin. Early rejection was associated with the *H-2*^{b} haplotype: in B10 and in the recombinant strains having a part of the *H-2*^{b} haplotype (B10.A(4R), B10.A(5R) and HTI), the median survival time of the graft varied from 21 to 65 days (Table II), but the differences between these strains were not statistically significant. In all the other strains, only one or two grafts were rejected in 100 days (in every group, $P < 0.01$ when compared to B10.A(5R) group). We used B10.HTG mice instead of HTG, and the absence of rejection in these mice confirms the findings of Gasser and Shreffler (11) that the male skin graft rejection by HTG females is caused by genes outside *H-2*. Of those mouse strains able to reject 50% of skin grafts in less than 65 days, only B10 mice were able to mount a secondary cytotoxic reaction against syngeneic male cells in vitro. Of the strains unable to reject grafts, only C3H.OH was able to produce cytotoxic cells.

In studies on the dominance of the genes causing rapid skin graft rejection (Table III), it was found that (B10 × BALB/c)F_1 females rejected male skin grafts from either parental strain, and this rejection was accompanied by the ability to mount a cytotoxic response. Similar results have been obtained for example with (B10 × CBA)F_1 females (data not shown). The dominance of *H-2*^{b} gene(s) in skin graft rejection was also seen in the experiments with (HTI × B10.A(2R))F_1 and (BALB/c × B10.A(5R))F_1 females, but in contrast to the

TABLE II

Syngeneic Male Skin Graft Rejection by Female Mice of Various Inbred Strains

Recipient ♀	Donor ♂	No. in group	Median survival time	Anti-H-Y cytotoxicity
			days	
B10	B10	11	40	+
CBA	CBA	15	>100	−
BALB/c	BALB/c	14	>100	−
B10.S	B10.S	11	>100	−
B10.G	B10.G	11	>100	−
B10.BR	B10.BR	10	>100	−
B10.D2	B10.D2	10	>100	−
B10.A	B10.A	14	>100	−
B10.A(2R)	B10.A(2R)	10	>100	−
B10.A(4R)	B10.A(4R)	13	65	−
B10.A(5R)	B10.A(5R)	10	21	−
HTI	HTI	13	49	−
A.TL	A.TL	10	>100	−
C3H.OH	C3H.OH	19	>100	+
B10.HTG	B10.HTG	7	>100	−

TABLE III

Rejection of Parental Male Skin Graft by F_1 Hybrid Females Derived from Matings with One Parent Carrying All or Part of the H-2^b Haplotype

Recipient ♀	Donor ♂	No. in group	Median survival time	Anti-H-Y cytotoxicity
			days	
(B10 × BALB/c)F_1	B10	12	53	+
(B10 × BALB/c)F_1	BALB/c	12	41	+
(B10 × B10.D2)F_1	B10	6	42	+
(B10 × B10.D2)F_1	B10.D2	6	47	+
(HTI × B10.A(2R))F_1	HTI	10	53	−
(HTI × B10.A(2R))F_1	B10.A(2R)	8	41	+
(BALB/c × B10.A(5R))F_1	BALB/c	9	33	+
(BALB/c × B10.A(5R))F_1	B10.A(5R)	9	28	−
(B10 × A.TL)F_1	B10	8	47	+
(B10 × A.TL)F_1	A.TL	6	>100	−

results with (B10 × BALB/c)F_1 mice, no cytotoxic response could be mounted after graftings with HTI and B10.A(5R) male skin, respectively. Concomitant absence of skin graft rejection and cytotoxic cell production was noticed in (B10 × A.TL)F_1 females grafted with A.TL male skin.

The grafting results with F_1 hybrids derived from matings of strains not involving the *H-2^b* haplotype and unable to reject male skin grafts were clear: the male skin grafts from either parental strain were not rejected regardless of their capacity to produce cytotoxic cells (Table IV).

Using B10 background H-2 congenic mouse strains, some of the strain combinations using noncongenic strains shown in Tables III and IV were tested for both graft rejection and generation of cytotoxic anti-H-Y responses. The

TABLE IV
Rejection of Parental Male Skin Grafts by F_1 Hybrid Females Derived from Matings of Two Nonresponder Strains

Recipient ♀	Donor ♂	No. in group	Median survival time	Anti-H-Y cytotoxicity
			days	
(CBA × BALB/c)F_1	CBA	10	>100	+
(CBA × BALB/c)F_1	BALB/c	10	>100	−
(B10.BR × B10.D2)F_1	B10.BR	8	>100	+
(B10.BR × B10.D2)F_1	B10.D2	7	>100	−
(CBA × A)F_1	CBA	11	>100	+
(CBA × A)F_1	A	9	>100	−
(CBA × B10.S)F_1	CBA	10	>100	+
(CBA × B10.S)F_1	B10.S	9	>100	+
(B10.A(2R) × B10.S)F_1	B10.A(2R)	14	>100	−
(B10.A(2R) × B10.S)F_1	B10.S	12	>100	−
(B10.G × B10.S)F_1	B10.G	13	>100	−
(B10.G × B10.S)F_1	B10.S	15	>100	+

results were always concordant with those shown, and they indicate that non-H-2 genes were not involved.

Discussion

The results show that the genes enabling rapid rejection of syngeneic male skin grafts by female mice are not the same as those that enable the production of H-2-restricted anti-H-Y cytotoxic cells. Thus, by using suitable recombinant mouse strains and F_1 hybrids, skin graft rejection without concomitant production of anti-H-Y cytotoxic cell formation has been obtained and vice versa.

Table V shows a summary of these results. Only mice with all or part of the *H-2^b* haplotype could reject grafts. The common part of the *H-2^b* haploytpe in mice able to reject male skin grafts is the *IB* region. Regardless of the presence of *IB^b* in all of these strains, the variations in graft survival time indicate that other parts of the *H-2* may have a regulatory effect. These results are in agreement with those of Bailey (12), but Bailey's orthotopic tailskin grafting method is more sensitive. His graft survival times were thus much shorter than ours, and some of our nonrejector strains could be classified as slow rejectors. B10.A(5R) and HTI strains also have the *IA^b* region which contains the *Ir* gene(s) for anti-H-Y cytotoxic cell production (6), but both strains lack the proper associative antigen for the induction of a cytotoxic response (we have never obtained an anti-H-Y response associated with *H-2K^b*- or *H-2D^d*-region products). B10.A(4R), having IA^k and IB^b, are able to reject syngeneic male skin grafts due to the presence of IB^b, but they are unable to produce cytotoxic cells due to the absence of IA^b, regardless of the presence of appropriate associative antigens (H-2D^b and H-2K^k). This confirms a different localization of those *Ir* genes controlling skin graft rejection and those enabling cytotoxic cell production in mice carrying the *H-2^b* haplotype. Both of these genes show dominant character, but the cytotoxic response needs the proper associative antigen to occur, thus, for example (B10 × BALB/c)F_1 females are able to reject

TABLE V

*Mouse Strains and F_1 Hybrids with Negative Correlation between Capacity to Produce an Anti-H-Y Cytotoxic Response and the Rejection of Male Skin Grafts by Female Mice**

Recipient ♀	Donor ♂
Skin graft rejection without cytotoxic cell production	
B10.A(4R)	B10.A(4R)
B10.A(5R)	B10.A(5R)
HTI	HTI
(HTI × B10.A(2R))F_1	HTI
(BALB/c × B10.A(5R))F_1	B10.A(5R)
Cytotoxic cell production without skin graft rejection	
C3H.OH	C3H.OH
(CBA × BALB/c)F_1	CBA
(B10.BR × B10.D2)F_1	B10.BR
(CBA × A)F_1	CBA
(CBA × B10.S)F_1	CBA
(CBA × B10.S)F_1	B10.S
(B10.G × B10.S)F_1	B10.S

* For data, see Tables II–IV.

BALB/c male skin and to mount an H-2K^d-associated anti-H-Y cytotoxic response. The lack of dominance of genes causing rapid skin graft rejection was noticed in (B10 × A.TL)F_1 females: they did not reject skin grafts from A.TL males. It is noteworthy that A.TL mice, having H-2K^s and H-2D^d with which H-Y antigen cannot be associated in the cytotoxic reaction (5), were not able to induce a cytotoxic response. So in this case, there is a concomitant absence of cytotoxic cell formation and skin graft rejection. This was also true of (B10 × A.TH)F_1 females, which did not reject male skin from A.TH (H-2K^s, H-2D^d) mice (data not shown).

If the *H-2^b* genes causing rapid skin graft rejection were absent and the cytotoxic cells were produced by the interaction of complementary *Ir* genes (H-2^k/H-2^d, H-2^k/H-2^s, and H-2^q/H-2^s complementations), male skin grafts were not rejected. C3H.OH females grafted with syngeneic male skin were able to produce a cytotoxic response but failed to reject the graft. The possible mechanisms in cytotoxic cell formation in this strain are discussed in the accompanying paper (6). Those F_1 hybrids mentioned in Table IV were also regrafted, but second-set rejection was not observed (data not shown).

In conclusion, it seems that H-2-restricted anti-H-Y cytotoxic T cells are neither necessary nor sufficient for the rejection of syngeneic male skin grafts, and that these two phenomena are even regulated by different genes. This was a highly unexpected finding because in allograft rejection the cytotoxic T cells are of prime importance, although the actual rejection phase is probably not an autonomous T-cell function (16, 17). So we must assume that syngeneic male skin graft rejection is mediated by a different mechanism which is not detectable in vitro using the chromium release assay, and that anti-H-Y cytotoxic T cells have no in vivo significance. One possibility is that H-2-restricted cytotoxic T

cells are not directed against the correct antigen, and, in fact, there is recent evidence that there are two H-Y antigens. Melvold et al. (18) found a mutant (C57BL/6 × BALB/c)F_1 male mouse lacking the H-Y antigen on the basis of skin graft testing, but the serological tests for H-Y antigen were positive. This mouse, from an X-irradiated father, had 39 chromosomes and the *Y* chromosome was probably absent (a part of the *Y* chromosome was probably translocated to an autosome). They suggested the existence of two H-Y antigens: H-Y1, responsible for male-female graft incompatibility (part of the *Y* chromosome coding for this antigen was missing in their mouse), and H-Y2, which is defined by conventional serological means. This could explain why strains both which do and which do not reject syngeneic male skin grafts may make an antibody response against H-Y (19), implying that anti-H-Y1 and anti-H-Y2 responses are under separate *Ir* gene control. If we use this hypothesis in explaining our data, we must again assume that anti-H-Y1 response is not mediated by cytotoxic T cells (i.e., is not detectable by chromium release assay) and that we have been actually measuring the cytotoxicity against H-Y2. Anti-H-Y2 cytotoxicity might not be able to cause skin graft rejection on the basis of different concentrations of H-Y1 and H-Y2 antigens on lymphocytes and epidermal cells.

Kralova and Demant (20) have suggested that the *H-2* complex also regulates the expression of the H-Y antigen, and the effect of these genes (mapping to the left of IC) may be different on thymus and skin cells. For example, in B10.A(5R) strain, the H-Y antigenicity would be strong on thymus cells but weak on skin cells. Their results show that these genes in the $H\text{-}2^k$ haplotype determine a strong H-Y antigenicity on the skin, whereas the $H\text{-}2^b$ haplotype is associated with low antigenicity. In our experiments, these factors seem to have no effect on skin graft rejection: (HTI × B10.A(2R))F_1 females rejected B10.A(2R) and HTI male skin at the same rate (Table III). To rule out the possibility that the lack of anti-H-Y cytotoxic response in strains capable of graft rejection (e.g. B10.A(5R)) was due to low H-Y antigenicity on lymphocytes (normal targets in the ^{51}Cr-release assay), we used syngenic male ^{51}Cr-labeled epidermal cells as targets, but these results were also negative (unpublished observations).

Whatever the rejection mechanism of syngeneic male graft is, it is evident that the H-Y antigen has some significance in clinical transplantations. It has been reported that female recipients of male kidney grafts survived longer than did female recipients of female grafts (21). This might be attributed to anti-H-Y antibody enhancement. But there are other studies which show that the H-Y antigen is deleterious for graft survival. Goulmy et al. (22) found a patient with aplastic anemia who rejected a bone marrow graft from her HLA-identical brother, and her lymphocytes were cytotoxic to male cells, but restricted by an HLA product in a manner analogous to the mouse system.

Summary

The ability of female mice to rapidly reject syngeneic male skin grafts is largely determined by dominant genes in the *IB* region of the $H\text{-}2^b$ haplotype, whereas the ability to produce anti-H-Y cytotoxic cells is determined by a dominant gene in the *IA* region of the $H\text{-}2^b$ haplotype, or by complementary genes in the *IC* region of some other haplotypes. Thus, it seems that H-2-

restricted anti-H-Y cytotoxic T cells are not responsible for the rejection of syngeneic male skin grafts.

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References

1. 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.
2. Gordon, R. D., B. J. 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.
3. Gordon, R. D., and E. Simpson. 1977. Immune response gene control of cytotoxic T cell response to H-Y. *Transplant. Proc.* 9:885.
4. Simpson, E., and R. D. Gordon. 1977. Responsiveness to H-Y antigen, Ir gene complementation and target cell specificity. *Immunological Rev.* 35:59.
5. Hurme, M., C. M. Hetherington, P. R. Chandler, and E. Simpson. 1977. Cytotoxic T cell responses to H-Y: Ir genes and associative antigens map in H-2. *Immunogenetics.* 5:453.
6. Hurme, M., C. M. Hetherington, P. R. Chandler, and E. Simpson. 1977. Cytotoxic T-cell responses to H-Y: mapping of the Ir genes. *J. Exp. Med.* 147:000.
7. Gasser, D. L., and W. K. Silvers. 1972. Genetics and immunology of sex-linked antigens. *Adv. Immunol.* 15:215.
8. Gasser, D. L., and W. K. Silvers. 1971. Genetic control of the immune response in mice. III. An association between H-2 type and reaction to H-Y. *J. Immunol.* 106:875.
9. Bailey, D. W., and J. Hoste, 1971. A gene governing the female immune response to the male antigen in mice. *Transplantation (Baltimore).* 11:404.
10. Gasser, D. L., and W. K. Silvers. 1971. The genetic basis of male skin rejection in mice. *Transplantation (Baltimore).* 12:412.
11. Gasser, D. L., and D. C. Shreffler. 1974. Genetic control of the immune response in mice. V. Minor genes involved in the response to H-Y and Ea-1 antigens. *Immunogenetics.* 1:133.
12. Bailey, D. W. 1971. Allelic forms of a gene controlling the female immune response to the male antigen in mice. *Transplantation (Baltimore).* 11:426.
13. Billingham, R., and P. B. Medawar. 1951. The technique of free skin grafting in mammals. *J. Exp. Biol.* 28:385.
14. Peto, R., M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto, and P. G. Smith. 1977. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br. J. Cancer* 35:1.
15. Steinmuller, D., and E. A. Hart. 1971. Passenger leucocytes and induction of allograft immunity. *Transplant. Proc.* 3:673.
16. Cerottini, J. C., and K. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. *Adv. Immunol.* 18:67.
17. Häyry, P. 1976. Problems and prospects in surgical immunology. *Med. Biol. (Helsinki).* 54:1.
18. Melvold, R. W., H. I. Kohn, G. Yerganian, and D. W. Fawcett. 1977. Evidence suggesting the existence of two H-Y antigens in the mouse. *Immunogenetics.* 5:33.
19. Goldberg, E., E. A. Boyse, M. Scheid, and D. Bennet. 1972. Production of H-Y antibody by female mice that fail to reject male skin. *Nat. New Biol.* 238:55.

20. Kralova, J., and P. Demant. 1976. Expression of the H-Y antigen on thymus cells and skin: differential genetic control linked to K end of H-2. *Immunogenetics*. 3:583.
21. Oliver, R. T. D. 1974. Possible evidence for Y-linked histocompatibility antigen in man. *Eur. J. Immunol.* 4:519.
22. Goulmy, E., A. Termijtelen, B. A. Bradley, and J. J. van Rood. 1977. Y antigen killing by T cells of women is restricted by HLA. *Nature* (*Lond.*). 266:544.