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Absence of any male-specific antigen recognized by T lymphocytes in X/X*Sxr′* male mice

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SUMMARY

Previous work has established that whereas X/X mice carrying the sex-reversing chromosomal fragment *Sxr* are positive for the male-specific transplantation antigen, H-Y, X/X mice carrying the variant *Sxr′*, although they too develop as phenotypic males, are H-Y negative. In this paper we show that X/X*Sxr′* male mice do not express any male-specific antigen that can induce skin-graft rejection.

INTRODUCTION

X/X*Sxr* (sex-reversed) mice are phenotypically male because a fragment of the mouse Y chromosome is attached to one of their X chromosomes (Cattanach, Pollard & Hawkes, 1971; Singh & Jones, 1982; McLaren & Monk, 1982). This *Sxr* fragment carries genetic information controlling not only the development of the fetal gonad as a testis rather than as an ovary, but also the expression of the male-specific H-Y transplantation antigen (Eichwald & Silmser, 1955). X/X*Sxr* males have been shown to express H-Y antigen both by skin grafting and by *in vitro* tests using H-Y-specific cytotoxic T cells and T-cell clones (Bennett *et al.*, 1977; Simpson *et al.*, 1981, 1986).

A variant *Sxr* fragment, termed Sxr′, was detected by McLaren *et al.* (1984). X/X*Sxr′* mice still developed as phenotypic males, but they no longer expressed H-Y antigen. Their spleen cells were no longer recognized as targets by H-Y-specific cytotoxic T cells and T-cell clones (McLaren *et al.*, 1984; Simpson *et al.*, 1986), while X/Y male skin was rejected by X/X*Sxr′* males and immunization of X/X females with X/X*Sxr′* male spleen cells failed to elicit a subsequent second-set response to a syngeneic X/Y male skin graft (Simpson *et al.*, 1986).

These results showed that X/X*Sxr′* males lack the antigenic determinant or determinants recognized as H-Y by H-2-compatible T cells. They could not reveal whether the transition from *Sxr* to *Sxr′* was associated with a change of antigenic type, so that X/X*Sxr′* male mice might carry a mutant male-specific antigen no longer recognized by H-Y-specific T cells, or whether X/X*Sxr′* males failed to express any antigen that can be recognized by T cells. We have sought to resolve this question by grafting X/X*Sxr′* male skin on to X/X females. This approach has required repeated back-crossing of the initially outbred *Sxr′* stock on to the C57BL/6 inbred strain, in order to eliminate as far as possible other irrelevant minor histocompatibility differences between graft donors and hosts.

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MATERIALS AND METHODS

Skin graft recipients were female mice from the C57BL/6Mcl inbred strain. X/X*Sxr′* male donors were from the fifth (Experiment 1) and seventh (Experiment 2) generation of repeated crossing of carrier males with C57BL/6Mcl females, while X/X*Sxr* male donors (Experiment 2) were from the sixth and seventh generation of a similar back-crossing programme. All mice were at least 8 weeks old at the beginning of each experiment, and were bred and maintained at the MRC Mammalian Development Unit, London.

In order to confirm the H-2 and H-Y status of skin-graft donors, partial splenectomies were performed before the donor mice were killed, and the spleen cells typed blind using H-2 and H-Y-specific T-cell clones and cytotoxicity testing as described by Simpson *et al.* (1986). All proved to be H-2^b homozygotes, of the expected H-Y type (i.e. X/X females and X/X*Sxr′* males were H-Y negative, X/X*Sxr* males and X/Y males were H-Y positive). In Experiment 2 in which second-set grafting was used, four recipients per donor were immunized 13 days before skin grafting with an i.p. dose of 10^7 spleen cells from mice of the same karyotype as that of the donor subsequently used for skin grafting. The second-set grafts in Experiment 1 followed immunization by the first set of grafts. In order to minimize the effects of possible residual heterozygosity for minor histocompatibility antigens other than H-Y, each recipient mouse received spleen cells for immunization from a different donor mouse than that used as the subsequent source of tail skin.

Skin grafting was carried out by the method of Billingham & Medawar (1951), using one graft from each donor on to each of

Table 1. Results of skin grafting C57BL/6 female mice with skin from X/Y, X/X*Sxr* and X/X*Sxr′* male and X/X female backcrossed mice

Donor	Survival time (days) on C57BL/6 female recipients	
	Primary grafts	Secondary grafts
Exp. 1		
XY♂	> 100, 35, 56	29, > 50*
XY♂	> 100, 59, 35, 44	29, 29, 20*
XY♂	> 100, 59, 44, 31	> 50, 29, 13
XX*Sxr′*♂	> 100 (× 3)	> 50 (× 3)*
XX*Sxr′*♂	> 100 (× 4)	> 50 (× 4)
XX*Sxr′*♂	> 100 (× 2), 56	> 50 (× 2), 29*
XX♀	> 100 (× 4)	> 50 (× 2)
XX♀	> 100 (× 4)	> 50 (× 4)
XX♀	> 100 (× 4)	> 50 (× 4)
Exp. 2		
XX*Sxr*♂		15 (× 4)
XX*Sxr*♂	ND	15 (× 3)*
XX*Sxr*♂		13 (× 2)*
XX*Sxr′*♂	ND	> 50 (× 4)
XX*Sxr′*♂		> 50 (× 3)*
XX♀		> 50 (× 4)
XX♀	ND	> 50 (× 4)
XX♀		> 50 (× 4)

* One or two mice in each of these groups died of anaesthesia during grafting.

ND, not done.

Table 2. Survival rates of donor skin of various sex chromosome constitutions grafted on to otherwise syngeneic female recipients

Exp.	Donor	Survival of first-set grafts beyond 100 days	Survival of second-set grafts beyond 100 days
1	XY♂	4/12	3/9
	XX*Sxr′*♂	9/10	9/10
	XX♀	12/12	10/10
2	XX*Sxr*♂	—	0/9
	XX*Sxr′*♂	—	7/7
	XX♀	—	12/12

four recipient females. Grafts were examined for a period of 100 days (first set grafts) or 50 days (second set grafts). Graft survival was assessed without knowledge of the genotype of the donors.

RESULTS

Skin was grafted from X/Y♂, X/X*Sxr*♂, X/X*Sxr′*♂ and X/X♀ donors on to C57BL/6 X/X♀ recipients. Tables 1 and 2 show the results of the rejection patterns of first-set (Exp. 1) and second-set (Exps 1 and 2) skin grafts. Immunization for second-set rejection was either by a skin graft placed 6 months previously (Exp. 1) or by i.p. spleen cell injection 13 days previously. This difference in mode and timing of immunization and the more advanced age of the recipients at the time of second-set grafting in Exp. 1, account for the more rapid tempo of second-set graft rejection in the second experiment. It is clear that both XY male and XX*Sxr* male grafts were recognized as foreign by C57BL/6 female recipients and rejected, although the first-set rejection times in Exp. 1 are rather scattered, and there were some non-rejector recipients. This is not unusual for first-set rejection of grafts carrying single minor transplantation antigens, including H-Y (Bailey, 1975; Simpson *et al.*, 1984). Second-set graft rejection of XY and XX*Sxr* male grafts was rapid, especially in Exp. 2. In Exp. 1 there were three non-rejector recipients, which had possibly been made tolerant to H-Y as a result of the first graft. Again, this is not an uncommon finding for H-Y. In contrast, there was no evidence that recipients of skin from either XX females or XX*Sxr′* males could recognize any foreign transplantation antigen on this tissue, even after immunization. The single recipient in Exp. 1 that rejected a primary graft from an XX*Sxr′* male also rejected a second-set graft. This rejection may have been directed at other minor transplantation antigens still segregating in the sixth backcross generation of donors.

DISCUSSION

We conclude from these results that X/X*Sxr′* male mice do not express any male-specific antigen recognized by the T cells involved in skin-graft rejection, since there was no significant difference between the fate of X/X*Sxr′*♂ skin grafts and that of X/X grafts.

These experiments were designed to test whether X/X*Sxr*^{1} male mice express an altered H-Y antigen. The mutation resulting in the derivation of *Sxr′* from *Sxr* could conceivably have changed the specificity of the H-Y antigen, such that it could not be recognized by H-Y-specific cytotoxic T cells or T-cell clones (McLaren *et al.*, 1984). Our previous grafting experiments established only that X/X*Sxr′* male mice recognized H-Y antigen on XY male tissue (Simpson *et al.*, 1986): they did not preclude that such mice expressed some altered antigenic product. The results of the experiments reported here establish that X/X*Sxr′* males do not express any male-specific transplantation antigen. This suggests that the *Hya* locus, rather than being modified by the *Sxr* → *Sxr′* transition, has been lost (for example by unequal exchange during meiosis).

We do not yet know whether or not X/X*Sxr′* male mice express any serologically detectable male antigens, that is, any antigens recognized by B cells (Silvers, Gasser & Eicher, 1982).

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