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Evidence for the presence of testicular tissue and *Sxs* antigen in the absence of Y-derived sequences

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Summary

Eleven XX males and seven XX true hermaphrodites have been tested for the presence of Y-derived DNA sequences using six different probes. All eleven XX males were positive with at least one of the probes but none of the seven true hermaphrodites could be shown to possess any Y-DNA. Using a new sensitive test for serologically sex-specific (*Sxs*) antigen, we found that, despite their apparent lack of Y-DNA, the XX true hermaphrodites were positive for their expression of the *Sxs* antigen.

Key words: H-Y antigen, *Sxs* antigen, Y-DNA, XX males, XX hermaphrodites, human.

Introduction

There is now good evidence that a gene or genes on the human Y chromosome long arm (Yq) controls the expression of the mammalian male-specific H-Y antigen (Eichwald & Silmser, 1955; Billingham & Silvers, 1960) as defined by MHC-restricted cytotoxic T lymphocytes (CTLs) *in vitro* (Simpson *et al.* 1987). Although there is no molecular evidence to show that this H-Y antigen is the same as the one defined by classical skin grafting (Eichwald & Silmser, 1955), strong circumstantial evidence suggests that it is (Simpson *et al.* 1986; Simpson *et al.* 1987; Wiberg & Lattermann, 1987).

In contrast, most of the available evidence now favours an autosomal location (Lau, Chan, Kan & Goldberg, 1986) of genes coding for a serologically detectable sex-specific antigen (Goldberg *et al.* 1971), which some authors have named SDM (Silvers, Gasser & Eicher, 1982). The above evidence suggests that the H-Y antigen and the SDM antigen are not the same molecule, unless different epitopes of that molecule are recognized by the different immunological assays (reviewed in Wiberg, 1987).

In this paper, we will restrict our discussion on these antigens to the 'SDM' antigen which, however, we will term the *Sxs* antigen (Wiberg, 1987) because it is also present in the female of certain species with female heterogamety (Wachtel, 1983; Bradley, Ebensperger & Wiberg, 1987). (*Sxs* stands for serologically sex-specific.)

Until quite recently, *Sxs* was usually assayed using complement-dependent cytotoxicity tests. Anti-*Sxs* antisera were raised in females of inbred rodents, usually by six, weekly, intraperitoneal injections of syngeneic male spleen cells. A common experience in all labs working with *Sxs* was that the titres of *Sxs* antisera were extremely low; usually one eighth or less. Furthermore, because of the presence of unspecific antibodies in the polyclonal antisera, there was always some absorption by female cells. In other words, in mammals, there is no evidence proving that the female lacks the *Sxs* antigen altogether. In fact, present evidence indicating that the structural genes for the *Sxs* antigen are on human chromosome 6 (Lau *et al.* 1986) suggests some very low gene activity in the mammalian female. To summarize, *Sxs* serology has been rather tricky and time-consuming, and there are a number of contradictory findings reported in the literature.

Recently, a method to raise high-titre anti-*Sxs* antisera in the BN and HS rats by transplanting small pieces of syngeneic male skin directly into the spleens of females has been described (Bradley & Heslop, 1985). Maximum anti-male activity of antisera was reproducibly scored after 2–3 weeks, using a urease–ELISA (Bradley & Heslop, 1984). Together with Bradley we have further developed and standardized this urease–ELISA for the *Sxs* antigen, and the

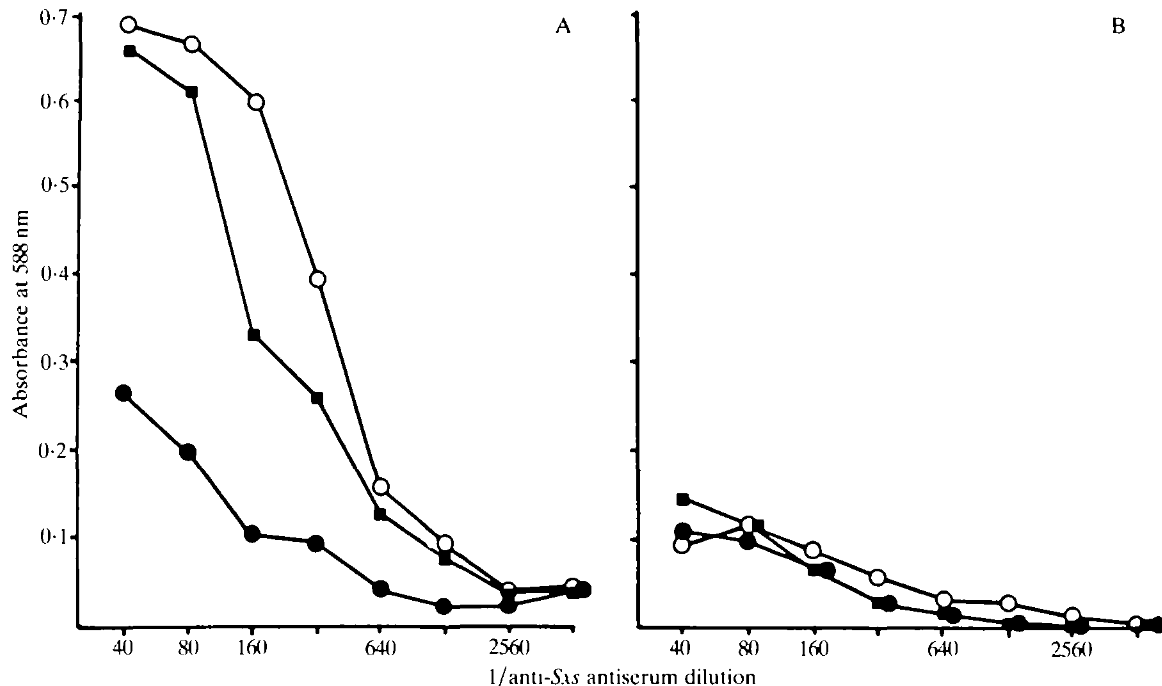

Fig. 1. Urease–ELISA of anti-*Sxs* antiserum absorbed with chicken ovary (●), or testis (■) homogenates, and unabsorbed (○) antiserum. The solid phase (A) is bovine testes extract and (B) bovine ovary extract. Statistics. (A) H_O1: ♀ = ♂; $P<0{\cdot}05$; H_O2: unabsorbed = ♂; $P>0{\cdot}22$. (B) H_O: ♀ = ♂; $P>0{\cdot}4$.

method has been described in detail elsewhere (Bradley *et al.* 1987). Briefly, the antigen (rat or bovine testes extract) is used as the solid phase. 600 ng–1 μg of testes extract proteins are plated out in each well of an immunoplate which is then reacted with serially diluted, absorbed and unabsorbed, anti-*Sxs* antiserum from the rat, followed by anti-rat urease-conjugated IgG as the second antibody. A control immunoplate, where the solid phase is ovary extract proteins, is processed in parallel. The substrate contains urea and bromocresol purple. Since the urease coupled to the second antibody hydrolyses the urea into carbon dioxide and ammonia, a positive reaction causes a pH shift which is visualized by a vivid colour change from yellow to purple, and thus the reaction can be read objectively in a spectrophotometer.

A typical example of the urease–ELISA is shown in Fig. 1. When anti-*Sxs* antiserum was absorbed with testes or ovary of the chicken, the bird testis did not significantly absorb the antiserum. In contrast, the bird ovary strongly absorbed the anti-*Sxs* antiserum. From these results one may conclude that the bird ovary carries the *Sxs* antigen, while the bird testis does not. It can also be concluded from the figure that the bovine ovary does not carry the *Sxs* antigen.

We felt it necessary to go into some detail about the *Sxs* methodology because it is of relevance to our further discussion of recent findings, obtained in our laboratories in Freiburg, on the aetiology of XX males and XX true hermaphrodites in man.

An individual having gonads, or parts of gonads, representing both sexes, i.e. testis and ovary, is classified as a true hermaphrodite. If only testicular tissue is found in an XX individual, he is classified as an XX male. 70–80 % of XX males investigated for the presence or absence of Y-specific DNA in their genomes have been found to carry such DNA (e.g. Vergnaud *et al.* 1986; Affara *et al.* 1986). The other 20–30 % of XX males may, or may not, lack Y-specific DNA altogether and, as far as we know, gonadal histology has not been carried out in this latter group of XX males. We will return to this problem.

In contrast to XX males, none of the few XX true hermaphrodites studied has been found to carry Y-specific DNA (Vergnaud *et al.* 1986). Using six different DNA probes, we investigated seven XX true hermaphrodites (two of them being related), eleven XX males, and one case of 45,X mixed gonadal dysgenesis. The results are shown in Table 1. All eleven XX males were positive for at least one of the probes, while none of the seven XX true hermaphrodites was positive for any of the probes. The XO mixed gonadal dysgenesis case was also negative for all probes. The fact that all eleven XX males typed positive with the Fr 80-II probe, while all seven XX true hermaphrodites typed negative for Fr 80-II (and all other probes as well), offers a strong statistical argument to imply a difference in the aetiology of XX males as well, and XX true hermaphrodites. Interestingly, one of the XX true hermaphrodites

Table 1. *Testing for Y-specific DNA in human XX males, XX true hermaphrodites and a case of 45,X mixed gonadal dysgenesis*

Probe	Chromosomal localization of probe	Y-specific hybridization signal in XX♂ (n = 11)	XX⚥ (n = 7)	XO⚥ (n = 1)
Fr 80-II	Yp, Xq	11	0	0
47z	Yp, Xq	8	0	0
50f2	Yp, Yq, A	7	0	0
Fr 35-II	Yp	7	0	0
pJA 1143 (DYZ1)	Yq	0	0	0
pHY2.1 (DYZ2)	Yq	0	0	0

All data from: Waibel *et al.* (1987).

reported here was originally incorrectly classified as an XX male. It is possible that this may be the case with some other XX males reported in the literature to lack Y-specific sequences. We therefore hypothesize that all XX true hermaphrodites lack Y/specific sequences, but that all XX males carry such sequences. Because part of the gonads of XX true hermaphrodites constitutes testicular tissue, the hypothesis that they lack Y-specific sequences leads to the postulate that Y-linked TDF is not required for the development of testicular tissue.

Determination of the *Sxs* states of the seven XX true hermaphrodites, and the single case of XO mixed gonadal dysgenesis (using the urease–ELISA and the Raji cell cytotoxicity test), revealed that all of these individuals carried the *Sxs* antigen (Waibel *et al.* 1987). This is compatible with recent findings already mentioned, that genes responsible for *Sxs* activity map to chromosome 6 of man (Lau *et al.* 1986). These *Sxs* genes are probably controlled by factors on the Y- and X-chromosomes (cf. Wolf, 1978, 1979) and, when mutated, as may be the case in the XX true hermaphrodites, they may become constitutively expressed (Waibel *et al.* 1987). The *Sxs* controlling gene on the Y chromosome, which may or may not be identical with TDF, is required for the development of a normal, fertile testis in mammals.

The results obtained in our laboratories and presented at this meeting have been described in detail elsewhere (Waibel *et al.* 1987).

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