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Mapping the H-Y gene

ELIZABETH SIMPSON[1], PHILLIP CHANDLER[1], ANNE McLAREN[2], ELS GOULMY[3], CHRISTINE M. DISTECHE[4], DAVID C. PAGE[5] and MALCOLM A. FERGUSON-SMITH[6]

[1]*Transplantation Biology Section, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK*
[2]*MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK*
[3]*Dept Immunohaematology & Blood Bank AZL, University Hospital Leiden, The Netherlands*
[4]*Dept Pathology, University of Washington, Seattle, Washington 98195, USA*
[5]*Whitehead Institute, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA*
[6]*Duncan Guthrie Institute of Medical Genetics, University of Glasgow, Glasgow G3 8SJ, UK*

Summary

This paper uses cytotoxic and proliferative T cell clones specific for H-Y and restricted by MHC molecules to type mice and humans inheriting incomplete portions of the Y chromosome. The data have allowed us to map the H-Y antigen gene *Hya* in mouse to a position closely linked with, but separable from, *Tdy* on the *Sxr* fragment and thus presumably to a position of the normal mouse Y chromosome near the centromere. The human H-Y gene maps between deletion intervals 4B and 7, separate from *TDF* which is on interval 1. We are currently testing cells from a number of additional patients who have inherited different portions of the Y chromosome to pinpoint the mapping more closely. It is of interest that in mouse a Y-linked gene controlling spermatogenesis (*Spy*) maps near *Hya* on the *Sxr* fragment: they could be the same or closely linked genes. In man, a gene controlling spermatogenesis maps to Yq and the data so far do not exclude that it could be coincident with the H-Y gene.

Key words: H-Y gene, cytotoxic T cell, sex-reversed mice, sex-reversed humans.

Introduction: T cell recognition of H-Y

The male-specific transplantation antigen, H-Y, is controlled by a gene located on the Y chromosome in both humans and mice. H-Y is a member of a family of minor histocompatibility (H) antigens, each characterized by their ability to stimulate certain immune responses of T lymphocytes (Loveland & Simpson, 1986). At one time, the examination of H-Y expression was limited to grafting experiments but since the advent of methods for generating specific cytotoxic and proliferative T cell responses *in vitro* and of maintaining these as cloned lines following the introduction of T cell growth factors, H-Y expression can be tested *in vitro* as well (Simpson, McLaren, Chandler & Tomonari, 1984; Simpson *et al.* 1987). This approach has been particularly useful for examining the H-Y phenotype of individuals from outbred populations who are not so amenable to the grafting approach. One constraint on such *in vitro* testing with H-Y-specific T cells is the need to identify the major histocompatibility complex (MHC: HLA in man, H-2 in mouse) alleles of the individual to be typed, since the recognition of H-Y, like other minor H antigens, is MHC restricted (Simpson & Gordon, 1977). T cells recognize H-Y only when it is associated with a particular self-MHC allele, so an appropriate panel of H-Y-specific T cells in necessary to H-Y type individuals of different MHC allotypes.

H-Y expression in sex-reversed mice

H-Y typing of mice is simpler than that of man, because of the ease of preparing H-Y-specific T cells restricted by all of the common H-2 haplotypes, using inbred mouse strains (Simpson, 1982). Female mice of inbred strains of appropriate H-2 type can be selected for immunization with H-2 compatible male cells and from these either *in vitro* bulk cultures of cytotoxic T cells or T cells cloned from these can be prepared for H-Y phenotyping the mice of interest. Examples of the MHC restriction and H-Y specificity of cytotoxic T cells from mixed lymphocyte cultures (MLC) of C57BL/10 (H-2^b) and C57BL/10×

CBA)F_1 (H-$2^{b/k}$) females immunized with (C57BL/10(H-2^b) and CBA (H-2^k) male cells, respectively, are given in Table 1 (Simpson, 1982). Table 2 shows the MHC restriction and H-Y specificity of proliferative T cell clones isolated from similar MLC using spleen cells from C57BL/6 (H-2^b) and C3H (H-2^k) female mice immunized with syngeneic male cells (Simpson, 1985). H-Y-specific cytotoxic T cells and clones were used to type cells from a panel of mice carrying the sex-reversing mutation *Sxr* (Table 3). These include XX*Sxr* males and T16HX*Sxr* females carrying the T16H, X-autosome translocation, which is invariably active, so that the X*Sxr* of paternal origin is inactive. This permits the female development of these individuals, since *Sxr* is presumably inactive in the majority of cells, at least during gonadogenesis (McLaren & Monk, 1982). The results in Table 3 indicate that each of the XX*Sxr* and XY males were H-Y positive with the cytotoxic T cells and T cell clones appropriate for their H-2 haplotype. These mice are from a noninbred colony in which H-2^k and H-2^b are segregating. Each of the XX females is H-Y negative, whilst of the nine T16HX*Sxr* females, eight are clearly H-Y positive, indicating that in adult life, at least, the gene controlling expression of the H-Y antigen, *Hya*, on *Sxr* is expressed in some spleen cells. The ninth mouse, number 39, was phenotypically H-Y negative: she was subsequently progeny tested (T16H*Sxr* females, unlike XX*Sxr* males, are fertile) and since all of the non-XY progeny inheriting her *Sxr* were H-Y negative, it was clear that a mutation had altered her *Sxr* fragment. This variant is now designated *Sxr'* (McLaren *et al.* 1984). XO*Sxr'* male mice are also H-Y negative when tested by T cells *in vitro* so that XX*Sxr'* and T16HX*Sxr'* mice are not H-Y negative merely because Sxr' in them is inactivated

Table 1. *H-Y responses in H-2^b homozygotes and H-$2^{b/k}$ heterozygotes*

Responder female	H-2 K	A	E	D	Priming and boosting antigen	Target cell	H-2 K	A	E	D	Corrected* % lysis	Restricting specificity for H-Y recognition
B10	b	b	(b)	b	B10♂	B10♂	b	b	(b)	b	*33·3*	
						B10♀	b	b	(b)	b	2·5	
						C3H♂	k	k	k	k	7·3	
						C3H.SW♂	b	b	(b)	b	*38·5*	H-2D^b
						B10.A(2R)♂	k	k	k	b	*30·6*	
						B10.A(2R)♀	k	k	k	b	2·2	
						B10.A(5R)♂	b	b	b	d	3·9	
(B10 × CBA)F_1	b	b	(b)	b/	CBA♂	CBA♂	k	k	k	k	*31·1*	
	k	k	k	k		CBA♀	k	k	k	k	2·4	
						B10.A♂	k	k	k	d	4·6	H-2D^k
						C3H.OH♂	d	d	d	k	*35·1*	
						B10♂	b	b	b	b	1·2	

* Per cent specific lysis of target cells at A:T/4:1 as determined from a four-point regression curve

Table 2. *Proliferative responses of H-Y-specific T cell clones*

Stimulating cells (KID)		Clone (origin and restriction specificity) 2-1-1(B6.A^h)	10-2(B6.D^h)	C-3(C3H.D^k)
None		199	541	
C57BL/6♂	bbb	*26637*	*241455*	
C57BL/6♀	bbb	389	1988	
B10.A(5R)♂	bbd	*31085*	3558°	
B10.A(4R)♂	kkb	219		
B10 A(2R)♂	kkb		*270175*	
bm12♂	bb*b	177	*172956*†	
bm14♂	bbb*		5025	
CBA♂	kkk			*108970*
CBA♀	kkk			557
C3H OH♂	ddk			*176428*
B10.A♂	kkd			648

° Data from a separate experiment in which the stimulation by C57BL/6♂ was 65434 and medium only was 1575
† From a separate experiment in which addition of C57BL/6♂ gave 287737 cts min^{-1} and medium alone gave 1910
Data from Tomonari (1983).

(Simpson, 1986). XX*Sxr'* and T16HX*Sxr'* mice are also H-Y negative when tested for its presence by transplantation, arguing for the identity of H-Y detected by these two methods, one *in vitro* and one *in vivo*, and for the absence of H-Y antigen from all cells in the body (Simpson *et al.* 1986). *Sxr'* has lost *Hya* or the ability to express this gene, but still causes sex reversal in XX*Sxr'* males, therefore the Y-chromosome-associated testis-determining gene *Tdy* on *Sxr* is clearly separated from *Hya* by this mutation, although the two genes are closely linked on *Sxr* and therefore presumably on the portion of the normal Y chromosome, close to the centromere, where *Tdy* and *Hya* are normally located (Simpson, 1986). Another mutation which provides evidence for the linkage of *Tdy* and *Hya* is Y* described by Eicher & Washburn (1986). Y* is apparently a rearranged Y chromosome in which the pairing region is located close to the centromere: amongst the sperm generated by carrier males is an X^Y, bearing a paternal X to which the greater part of the Y is attached. The XX^Y mice created by the fertilization of a normal X-bearing ovum with such a sperm are H-Y positive and phenotypically male, with aspermatogenic testes (like XX*Sxr*: Simpson *et al.* 1983).

H-Y expressed in sex-reversed humans

The investigation of the position of the human H-Y gene on the Y chromosome has produced findings which are in parallel with those of mice, since they clearly separate the testis-determining factor, *TDF*, from the H-Y gene, but in man the linkage between these two genes, unlike mouse, is not at all close (Simpson *et al.* 1987).

H-Y typing in man is possible because of the isolation of T cell clones specific for H-Y from transfused spontaneously recovered female aplastic anaemia patients (Goulmy, 1985). Clones currently available are either HLA-A2 or HLA-B7 restricted, so this limits our ability to type cells from individuals carrying one or both of these alleles; fortunately, this includes more than 50 % of the population. For the localization of the H-Y gene in man, potentially informative patients are those who have inherited a partly deleted paternal Y chromosome or a translocated Y chromosome fragment. Such patients are in two phenotypic categories: XX males and XY females. The six males described here have inherited variable portions of Yp whilst the two females possess Yq and a variable portion of the Yp. Table 4 shows the results of HLA and H-Y typing lymphoblastoid B

Table 3. *H-Y typing by CML and proliferation of H-Y specific-clones of normal mice and of mice of both sex phenotypes carrying* Sxr

Cells added from mouse	Proliferation of H-Y specific clone (restriction specificity)			CML typing with		H-2* type	H-Y† type
	C-3(D^k)	10-2(D^b)	2-1(A^b)	anti-H-Y^k	anti-H-Y^b		
None	1016	301	765				
30 XX♀	2174	1078	197	0·9	1·1	k	–
32	929	1312	489	−1·2	2·0	k	–
33	1487	552	245	−14·4	−7·7	k	–
34	591	649	3932	3·0	1·7	k	–
4 T16HX*Sxr*♀	*26406*	651	3252	*20·0*	8·5	k	+
13	*58269*	379	3086	*30·6*	6·4	k	+
35	*66208*	828	518	*26·4*	8·4	k	+
36	*42014*	1531	2526	*23·0*	−1·1	k	+
37	*46783*	586	2053	*23·3*	5·8	k	+
38	*64640*	1255	1160	*29·4*	2·0	k	+
39	1904	1648	1685	1·3	1·9	k	–
40	*61249*	1613	4149	*25·2*	3·1	k	+
41	*47145*	778	7653	*25·8*	6·2	k	+
42 XX*Sxr*♂	*82529*	1229	704	ND	ND	k	+
43	*40797*	341	225	ND	ND	k	+
47	899	*22400*	ND	−0·7	*12·4*	b	+
31 XY♂	549	*32610*	*30472*	−3·7	*29·4*	b	+
45	*3178*	1451	579	*29·3*	2·7	k	+
46	*12092*	635	30	*16·9*	−1·1	k	+

For method of proliferation see legend for Table 2.
CML: % cytotoxicity at A:T/10:1 from 12-point regression analysis.
* H-2 type established with allospecific cytotoxic T cells.
† Summary of H-Y typing with H-Y specific cytotoxic T cells and H-Y-specific proliferative clones.

cell lines from these patients and appropriate A2- and B7-positive normal male and female controls, with cytotoxic T cells. It is important to confirm serological HLA typing with T cells, since variants of A2 and B7 exist which are not distinguishable serologically but which cannot be recognized by allospecific or MHC-restricted T cells (Horai, von der Poel & Goulmy, 1982). A negative H-Y typing can thus only be interpreted as such in face of a positive allotyping for the restriction element with T cells (A2 or B7 in the case of individuals shown in Table 4).

The deletion map shown in Fig. 1 is based on Vergnaud *et al.* (1986), Disteche *et al.* (1986) and Page (1986) and includes the summarized H-Y results of Table 4 as well as unpublished data on class I XX males. Since six class 3 males were H-Y negative it is clear that the gene for H-Y does not map to deletion interval 1–3 on Yp (*TDF* is in interval 1, see also Affara *et al.* 1986). Likewise the gene for H-Y is excluded from interval 4A, since the class 2 XY female is H-Y positive and lacks this portion of Yp. The H-Y gene thus maps between intervals 4B and 7, far from *TDF* in interval 1.

Conclusion

In summary, these data, using cytotoxic and proliferative T cell clones specific for H-Y and restricted by MHC molecules to type mice and humans inheriting incomplete portions of the Y chromosome, have allowed us to map the H-Y antigen gene *Hya* in mouse to a position closely linked with, but separable from, *Tdy* on the *Sxr* fragment and thus presumably to a portion of the normal mouse Y chromosome near the centromere. The human H-Y gene maps between deletion intervals 4B and 7, separate from *TDF* which is on interval 1. We are currently testing cells from a

Table 4. *HLA and H-Y typing of B cell lines from XX males, XY females and normal controls*

Exp.	Karyotype/ sex	Individual	HLA* serology A	HLA* serology B	αA-2†	αH-Y/A-2†	H-Y phenotype
1	XX♂	RH	2,3	21,40	*18*	9	–
	XX♂	JT	*2*	44,45	*24*	3	–
	XX♂	LGL 105	2,3	35,44	*13*	4	–
	XY♂	Normal male	1,2	8	*20*	*38*	+
	XX♀	Normal female	2,11	8,44	*17*	8	–
2	XX♂	WB	2,9	17,18	*37*	0	–
	XY♂	Normal male	1,2	8	*25*	*17*	+
	XX♀	Normal female	2,11	8,44	*17*	3	–
					αB7	αH-Y/B7	
3	XX♂	WHT 950	1,3	7	*76*	9	–
	XX♂	JM	3,28	7	*62*	1	–
	XY♂	Normal male	9	7,44	ND	*40*	+
	XX♀	Normal female	3,24	7	*54*	0	–
4	XY♀	WHT1003 (case 1)	3	7,13	*55*	*70*	+
	XY♀	WHT 715 (case 2)	3	7	*57*	*69*	+
	XY♂	Father of case 2	28,3	7,40	*52*	*61*	+
	XX♀	Mother of case 2	29	7	*36*	6	—

* HLA serology performed by Lorna Kennedy at ICRF, Lincoln's Inn Fields or Donald Palmer of Dept Immunology, RPMS
† Per cent specific lysis of target cells at A·T/10·1 as determined from a 6-point regression curve.
Modified from table 1, Simpson *et al.* 1987

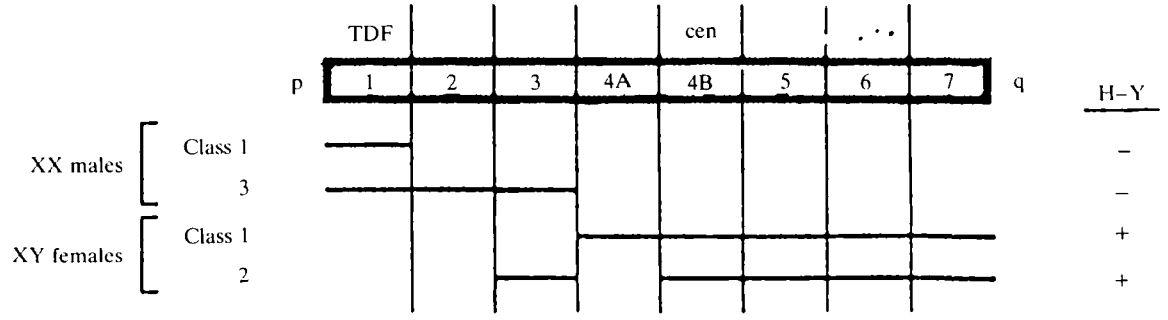

Fig. 1. 8-interval deletion map of the human Y chromosome (based on Page, 1986).

number of additional patients who have inherited different portions of the Y chromosome to pinpoint the mapping more closely. It is of interest that in mouse a Y-linked gene, *Spy*, controlling spermatogenesis maps near *Hya* (Burgoyne, Levy & McLaren, 1986; for discussion see Burgoyne, this symposium) on the *Sxr* fragment: they could be the same or closely linked genes. In man, a gene controlling spermatogenesis maps to Yq (Tieopolo & Zuffardi, 1976), and the data so far do not exclude the possibility that it could be coincident with the H-Y gene.

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