Immunogenetics 17 523–531, 1983

Immuno-genetics
© Springer-Verlag 1983

The Recognition of Abnormal Sex Chromosome Constitution by HLA-Restricted Anti-H-Y Cytotoxic T Cells and Antibody

Els Goulmy[1], Aad van Leeuwen[1], Els Blokland[1], Eva S Sachs[2], and Joep P M Geraedts[3]

[1] Department of Immunohaematology, University Hospital Leiden, The Netherlands
[2] Department of Clinical Genetics and Department of Pediatrics, University Hospital and Sophia Childrens Hospital, Rotterdam, The Netherlands
[3] Department of Human Genetics, State University Leiden, The Netherlands

Abstract. Using the cell-mediated lympholysis (CML) technique, we studied lymphocytes of six individuals with discrepancies between the karyotypic and phenotypic sex Two sets of cytotoxic T cells (CTLs) obtained from two multitransfused female aplastic anemia patients were used as typing reagents These cells were previously shown to kill allogeneic target cells from HLA-A2- or B7-positive male donors An antiserum obtained from one of the patients likewise killed HLA-A2 male lymphocytes The six patients studied were selected for the required antigens Positive reactions were obtained with lymphocytes from a 46,XY woman with pure gonadal dysgenesis and a 45,XO male Target cells of the mother of the latter patient were also lysed One individual with a 45,XO/46,X,del(Y)? karyotype was weakly positive, while three 46,XX males were completely negative The reactivity of the HLA-A2-restricted H-Y-specific antibody showed the same discriminatory patterns The results obtained by the *HLA*-restricted CTLs as well as by the antiserum did not correlate with the presence of testes as is the case in a different test system for the serologically detectable male (SDM) antigen in man On the other hand, there was a correlation with the presence of cytologically detectable Y-chromosome material in five of the six individuals studied The HLA-restricted CTLs and the antibody might recognize the classical transplantation antigen H-Y

Introduction

In mammals the bipotential gonads differentiate into testes in the presence of a Y chromosome regardless of the number of X chromosomes Thereafter, a male phenotype will result from the androgen that is produced by the testes The male

Address for offprint requests Dr E Goulmy Department of Immunohaematology, University Hospital, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

determination therefore depends on the presence of one or more genes on the Y chromosome The translocation mechanism of chromosome material carrying the male determinant segment is most important in explaining the rarely observed exceptions of the above-mentioned association between the presence of a Y chromosome and initiation of testes development (Wachtel et al 1976)

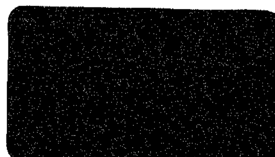
Since H-Y antigen is detected in all patients with testes (even if these are rudimentary, small, or azoospermic), this plasma membrane component is thought to be responsible for male development It has been suggested that the production of the H-Y antigen is under the control of X- and Y-linked genes and that the development of male or female characteristics is dependent on a threshold value, above which male development occurs (Wolf et al 1980)

The H-Y antigen was first discovered to be a transplantation antigen by male-to-female skin graft rejection in histocompatible inbred strains of mice (Eichwald and Silmser 1955) Later it was shown that an interstrain variation of this type of rejection, under control of the *H-2* complex exists in the mouse (Bailey 1971) The ability of female mice to reject syngeneic male skin is determined by genes coded by the *H-2* complex and by non-*H-2* genes (Gasser and Shreffler 1974)

Using an in vitro cytotoxicity test, Goldberg and co-workers (1973) demonstrated specific anti-H-Y immune spleen cells that were obtained from female mice during rejection of male grafts Subsequently, Gordon and co-workers (1975) described that the in vitro cell-mediated cytotoxic responses to the male-specific (H-Y) antigen were found to be *H-2* restricted Different T-cell subpopulations participating in the male-specific H-Y antigen immune response are controlled by *H-2*-linked *Ir* genes and by non-*H-2*-linked genes (Hurme et al 1977, 1978, von Boehmer and Haas 1979, Lieuw and Simpson 1980, Fierz et al 1982)

There is growing evidence that the classical male-specific transplantation antigen H-Y, as defined by graft rejection, is similar to the H-Y antigen as recognized by different in vitro assays (see above), nevertheless clearly distinct from the serologically detectable male (SDM) H-Y antigen (Melvold et al 1977, Simpson et al 1982, Silvers et al 1982)

In 1977 Goulmy and co-workers demonstrated, analogous to the mouse system, H-Y-specific, HLA-restricted cytotoxic T cells (CTLs) in man A female patient suffering from aplastic anemia received a bone marrow graft from her HLA-identical brother There was a temporary take, but the graft was rejected after 20 days Rejection was accompanied by a spontaneous recovery of the patients' bone marrow hemopoietic function, thus allowing further investigations When we used the cell-mediated lympholysis (CML) technique, it appeared that peripheral blood lymphocytes (PBLs) from this multitransfused female aplastic anemia patient were able to show HLA-A2-restricted, anti-male cytotoxicity Later it was demonstrated that the serum from the same patient contained an HLA-A2-restricted anti-male antibody activity (van Leeuwen et al 1979) Subsequently, another example of HLA-restricted, anti-male cytotoxicity was observed The lymphocytes of this patient showed cytotoxicity against HLA-A2-positive as well as HLA-B7-positive male cells (Goulmy et al 1979) Nevertheless, two normal XX females were marginally positive in the cytotoxicity and the antibody test system We assumed that the antigen recognized by the CTLs and the antibody is most probably identical with the classical transplantation antigen H-Y



The present study was prompted by this consideration. We studied the HLA-restricted cytotoxicity (using the male-specific CTLs and antibody) on the lymphocytes from six individuals with a discrepancy between the karyotypic and phenotypic sex.

Materials and Methods

Patient 1. A 17 year-old phenotypic male was mentally retarded, 160 cm tall and weighed 45 kg. He had a mongoloid appearance with a large mouth, bilateral narrow meatus acusticus externi, abnormal auricles, long ear lobes, synophrys, and webbing of the neck. He had short, curved fingers and abnormally implanted thumbs. The external genitalia were normal. Furthermore he suffered from hypothyreoidea due to Hashimoto's disease and had some hearing problems. The karyotype of 200 cells (from lymphocyte and fibroblast cultures) appeared to be 45 XO with no remnant of a Y chromosome and no signs of translocation (after Q banding). Both parents had normal chromosomes.

Patient 2. This 25 year-old phenotypic female was presented with primary amenorrhea and had normal external genitalia and infantile hypoplastic internal genitalia. A diagnosis of pure gonadal dysgenesis (PGD) was made. On the left there was a cyst with no functioning ovarian tissue, the right streak gonad measuring 10 by 4 mm was used for chromosome analysis. Chromosome studies revealed only normal male (46,XY) karyotypes in lymphocytes (15 cells) and gonadal tissue (68 cells). The buccal smear was X-chromatin negative and Y-chromatin positive.

Patient 3. A 36-year-old man had a normal male stature and external genitalia. The volumes of his testes were 5 and 7 ml. His primary infertility was due to azoospermia. The karyotype appeared to be 46,XX in repeated cultures of peripheral blood as well as gonadal tissue. A buccal smear was X-chromatin positive and Y chromatin negative.

Patient 4. A 20-year-old male was referred for chromosome analysis because of sterility and suspicion of Klinefelter syndrome. A 46,XX karyotype, with no evidence of Y chromosome material was obtained in the peripheral blood. A buccal smear was also characteristic for this karyotypic variant of the Klinefelter syndrome: it was X-chromatin positive and Y-chromatin negative.

Patient 5. This 1-year old boy was prematurely born after cesarean section and had severe hyaline membrane disease neonatally. He had horseshoe kidneys (with normal renal function), growth retardation and ambiguous genitalia. The penis measured 2 cm, with normal corpora cavernosa, there was an extreme hypospadias and an incomplete fusion of an empty scrotum. Examination of the internal genitalia revealed that both gonads contained exclusively testicular tissue (histologically verified), also remnants of tubae and fimbriae and a vagina like structure were in the cavum Douglasi, ending in the distal part of the urethra. There was a normal testosterone production and peripheral responsiveness. X chromatin investigation of the buccal smear was negative, while chromosome studies of the peripheral blood lymphocytes revealed a 45,XO karyotype in 10 cells and 46 X,del(Y)? karyotype in 18 cells. The presumed deleted Y chromosome measured about 2/5 the size of the long arm of chromosome 22 and showed no intense fluorescence after Q-banding. All gonadal cultures revealed only the cell line with 46 chromosomes. Both parents had normal chromosomes with a clearly fluorescent distal part of the Y in the father.

Patient 6. This 11-year-old male was referred for diagnostic analysis and eventual therapy for this ambiguous external genitalia. There was a pubertairy penis with small, retractile testes, which were not studied histologically. Furthermore there was a penoscrotal hypospadias, a bifid scrotum. Laparotomy revealed a vagina ending about 4 cm from the urethral meatus in the urethra on which the seminal vesicles and vasa deferentes were visible. Chromosome analysis of lymphocytes and cultured biopsies taken from scrotum skin, and vagina revealed a 46,XX pattern with no evidence of X-Y translocation.

Detection of HLA-A, B, C and DR antigens. Typing for HLA-A, B and C antigens was performed with standard lymphocytotoxicity techniques (van Rood 1974). Typing for HLA DR antigens was performed with the two color fluorescence complement dependent cytotoxicity test (van Rood et al. 1976).

CML assay The CML assay has been described in detail previously (Goulmy 1982) Briefly, cytotoxicity was measured using an isotope release assay The target cells (in this particular study, the lymphocytes of patients 1 to 6 described above, the mother of patient 1, and control cells) were incubated with phytohemagglutinin (PHA) for 72 h and thereafter labeled with 100 Ci ^{51}Cr for 1 h at 37 °C The control group consisted of 100 randomly chosen normal males and females

The CTLs, as described below, were collected after 6 days in vitro sensitization and mixed with the target cells at varying CTL target ratios in round-bottomed microtiter plates and thereafter incubated for 4 h at 37 °C After incubation, the supernatants were removed and counted Spontaneous release values were derived from those wells with target cells incubated with culture medium alone Maximum release values were obtained from Zaponin (Zaponin, Counter Electronics Ltd) distilled water lysis of the target cells The percentage lysis was calculated using the following formula

$$\frac{\text{Experimental mean cpm} - \text{Spontaneous release mean cpm}}{\text{Maximum release mean cpm} - \text{Spontaneous release mean cpm}} \times 100 = \%\ \text{Lysis}$$

Standard errors of the mean of triplicate determinations had to be less than 5% The results were expressed on a scale on which the spontaneous release value was set at 0% and the maximum release at 100% Spontaneous release, which is determined in wells with assay medium alone, normally amounts to 15–25% of the maximum release values When only one CTL to target ratio was used, percentages lysis equal or below 10% were considered as negative, 11–15% as weakly positive, 16–40% as positive and greater than 40% as strongly positive The culture medium was RPMI 1640, supplemented with 3mM glutamine, 100 IU penicillin/ml, 100 µg streptomycin/ml and 20% heat-inactivated pooled human AB serum from healthy male donors

CTLs PBLs of two female aplastic anemia patients have been used throughout this study (Goulmy et al 1977, 1979)

HLA-A2-restricted anti-H-Y CTLs (CTLs 1) CTLs 1 have been derived from a multitransfused woman (patient 1) suffering from aplastic anemia in partial remission (HLA-phenotypes A2, Bw44, B40, Cw3, Cw5, DR4, DRw6) This patient received a bone marrow graft from an HLA-identical male sibling donor, which was subsequently rejected We have previously shown (Goulmy et al 1977) that her cells (after a 6-day in vitro sensitization period against irradiated PBLs from an HLA-A, B, C and DR identical but mixed lymphocyte reaction positive unrelated male donor) (Goulmy et al 1978) were able to show preferential lysis This lysis was directed to all male target cells carrying the HLA-A2 antigen Some killing was also directed against two HLA-A2 female target cells, but this was at a considerable lower level than that directed against male cells

HLA-A2 and -B7 restricted anti H-Y CTLs (CTLs 2) CTLs 2 have been derived from another multitransfused female aplastic anemia patient (patient 2, HLA-phenotypes A2, A28, B7, Bw62, Cw3, DR1, DR2) After in vitro sensitization (Goulmy et al 1979) CTLs, which specifically lysed HLA-A2 and HLA-B7 male target cells, were generated

Control CTLs Alloimmune CTLs specific for HLA-A2 and HLA-B7 were generated between unrelated responder/stimulator combinations differing only for HLA-A2 and HLA-B7 antigen, respectively

HLA-A2 restricted H-Y-specific antibody In previous studies of female aplastic anemia patient 1 (whose lymphocytes generate anti-H-Y HLA-A2-restricted cytotoxicity), we demonstrated the presence of an IgM antibody in her serum that reacted only with HLA-A2-positive male cells The specificity of this antiserum (designated serum R) was virtually indistinguishable from that of the anti-H-Y cytotoxic T lymphocytes obtained from the same patient (van Leeuwen et al 1979) It was found that antibodies, when tested in the complement-dependent cytotoxicity test, reacted only with some of the cells stained with anti-Ig fluorescein isothiocyanate (B cells) Serologic studies with this HLA-A2-restricted, H-Y-specific antibody have been carried out using an indirect immunofluorescence method (van Leeuwen et al 1979)

Results

Table 1 shows the results of the HLA typing of all subjects studied Our CTLs and antibody reagents showed HLA-restricted specificity and therefore the presence of HLA-A2 or HLA-B7 antigen, or both, was a prerequisite for the inclusion of our patients' cases in this study Patient 2 and the mother of patient 1 had both HLA antigens The lymphocytes of the subjects were used as target cells in the CML assay and in addition tested against the H-Y-specific antibody

Table 2 shows the percentages cytotoxicity obtained by (a) control CTLs, (b) CTLs 1 or 2, or both and (c) antibody

Positive anti-H-Y reactions were obtained using the lymphocytes of patient 1, his mother, and patient 2 as target cells A borderline reaction was obtained when target cells of patient 5 were tested against the HLA-restricted cytotoxic effector cells The lymphocytes of all males with a 46, XX karyotype showed no CML sensitivity The absence of lysis in some patients is not due to a defect on the target cell level, since the lymphocytes of all patients studied showed a normal sensitivity to lysis by anti-HLA-A2/B7 CTLs (i e , the control CTLs).

Six of eight subjects have also been studied with serum R using the complement-dependent cytotoxicity test As already mentioned (see *Materials and Methods*), the reactivity of this antiserum appeared to be directed mainly against male cells providing that these cells carry the HLA-A2 antigen The percentages of positive cells obtained with this antiserum are given in Table 2 The reactivity pattern of serum R shows an excellent correlation with the results obtained with the anti-H-Y CTLs 1 and 2

Discussion

The application of a direct cellular cytotoxicity assay that we used in this study might be a new tool for characterizing sex chromosome abnormalities. When this test was first applied, it was shown that target cells of all normal male individuals with the appropriate HLA haplotypes (A2 or B7 positive, or both) were lysed by the cytotoxic effector cells of both aplastic anemia patients.

The similarity of the results in the CML test and the complement-dependent cytotoxicity test, as demonstrated earlier (van Leeuwen et al 1979) and shown in Table 2, raises in principle the possibility of using the HLA-restricted H-Y-specific

Table 1. HLA phenotypes of all patients studied

Patient	HLA phenotypes
1	A1, Bw35, Cw4, w6 / A2, Bw44, Cw5, w4
Mother of patient 1	A2, Bw44, Cw5, w4 / Aw24, B7, w6
2	A2, A3, B7, B27, Cw2, w4, w6
3	A3, Aw32, B7, Bw60, Cw3, w6
4	A2, Aw24, B8, Bw60, Cw3, w6
5	A1 A2, B8, Bw60, Cw3, w6
6	A2, Aw33, Bw51, B14, w4, w6

Table 2 Reactivity patterns of the HLA A2 and B7 restricted anti H Y CTLs and HLA A2 restricted anti H Y serum

Patient	Karyotype	Phenotype	HLA A2/B7‡	Percentages specific lysis			
				Control CTLs	H Y CTLs*		A2 H Y antiserum†
					A2	A2/B7	
1	45 XO	Male	+/−	49	31		40
Mother‖	46 XX	Female	+/+	78	28	12	54
2	46 XY	Female	+/+	78	86	85	40
3	46 XX	Male	−/+	43		− 2	
4	46 XX	Male	+/−	44	3		8
5	45 XO/46 X del(Y)?	Male	+/−	85	10	12	14
6	46 XX	Male	+/−	40	1		

* The control values included in this study are (a) A2 and B7 positive normal male cells 81 (±5%) and 73% (±5%) respectively (b) A2 and B7 negative normal male cells A2 and B7 positive normal female less than 2%

Mean percentages of cytotoxicity of the antiserum against control cells included in this study are (a) A2 positive normal male cells 40% (±5%) (b) A2 negative normal male cells and A2 positive normal female cells 4° (±2%) The control AB serum value varied between 4 and 7%

‡ Presence (+) or absence (−) of the required HLA A2 or HLA B7 antigen or both

‖ Mother of patient 1

antibody for further investigations in this field Unfortunately, the antibody activity of serum from this patient declined A consistent decline of the HLA-A2-restricted anti-H-Y reactive CTLs was also observed, however, it was possible to recall the H-Y-specific effector cells by an in vitro stimulation (Goulmy et al 1978)

In previous studies, we observed extra reactivities in two phenotypically and karyotypically normal women from a randomly selected panel of more than 50 normal women tested (Goulmy et al 1977) Since we raised our HLA-restricted anti-H-Y CTLs in in vivo sensitized patients, it is likely that small numbers of T-cell clones might be directed against other minor histocompatibility antigens Recently, we were able to demonstrate that cloned cultures specific for HLA-A2-positive male target cells could be derived from expanded CTL lines expressing the original lytic capacity (Goulmy et al 1980) Nevertheless, we were so far not able to detect CTL clones that were specifically directed against determinants present on the lymphocytes of the two (above described) normal women This suggests in itself that presumable small numbers of T-cell clones are present that are directed against other minor histocompatibility antigens

The most unexpected finding in this study was the observation that all 3 XX males (patients 3, 4, and 6) are completely negative in our test system because it has been observed that cells from XX males do express SDM antigen (Wachtel et al 1976)

The fact that the lymphocytes from patient 2 with PGD and a 46, XY karyotype were lysed to the same extent as control cells from normal male individuals is not conclusive It has been shown that individuals with this condition can be either

SDM negative (Ghosh et al 1978, Wolf 1979) or positive (Dorus et al 1977, Wolf 1979, Wachtel et al 1980) The latter situation is explained by the lack of H-Y antigen receptors on the indifferentiated gonadal cells (Wolf 1979, Wachtel et al 1980) It is more difficult to draw conclusions from our findings on the remaining subjects Patient 5 was very weakly positive This might be due to the observed mosaicism If the 45,XO cells were lacking the H-Y-related antigen, a substantial decrease of the lysis value should be expected The cell line with the presumptive deleted Y chromosome must be held responsible for the weak expression of the H-Y-related antigen An alternative explanation is that the weak expression of the antigen is related to the young age of the patient To test this, cord blood was taken from 17 randomly chosen HLA-A2-positive newborn males The expression of the male-specific antigen in these neonates was not different from that in adult males (data not shown) Therefore, the alternative explanation can be ruled out

Finally, patient 1 was positive but not as strongly as the normal male controls This phenotypic male had a 45,XO karyotype At least three different causes for the development of a male phenotype in the absence of Y chromosome have been suggested (1) One possible cause is *undetected mosaicism* Although 200 cells from four different cell types were lacking a Y chromosome, it is possible that the zygote started off with a normal male karyotype and the Y chromosome was lost subsequent to the development of the testes (2) *Gene mutation* is another possible cause for this development There is no evidence available to prove or disprove this alternative (3) Still another possible cause is *translocation of Y-chromosome material* Since no structural chromosome abnormality could be detected in the proband and both parents had normal karyotypes, no cytogenetic evidence is available supporting this hypothesis However, the results of the cytotoxicity test can be explained by an undetected X-Y translocation, present in the mother and the son, who were both positive Nonrandom inactivation of the translocation chromosome in the mother would result in a fertile female Transmission of the abnormal X chromosome to the son could give rise to male development due to the presence of male determining gene(s) of Y-chromosome origin Furthermore, the clinical findings in the proband are similar to those reported in a case of X-Y translocation (Pfeiffer 1980) and regarded as manifestations of monosomy for the distal part of the short arm of the X chromosome

At this moment it is difficult to give a complete explanation for our observations It is likely that we have detected an H-Y antigen that is different from the SDM antigen In this respect it is interesting that Melvold and co-workers (1977) and Simpson and co-workers (1982) have suggested that the H-Y system may consist of two antigens The first author based his hypothesis on the finding of a 39,XO male mouse among the offspring of an X-irradiated father With serologic tests it was SDM antigen positive, whereas the H-Y histocompatibility antigen could not be demonstrated using skin graft tests Additionally, Simpson and co-workers (1982) demonstrated that XO mice made excellent in vitro anti-H-Y CTL responses, suggesting absence of the H-Y transplantation antigen

At present, some of the patients reported in this study are being analyzed for the presence of SDM antigen by rodent antisera These results together with the data described here will provide us with more definitive answers in the recognition and existence of one or more human male antigens

Acknowledgments The authors thank the following physicians whose patients were included in the investigations Drs H L Haak, H H H Kanhai, and B M Goslings, Leiden, Dr J Steenbergen, Amersfoort, Drs J M de Wit, A C v d Heijden, S L S Drop, and G J Bruining, Rotterdam, and Dr D J Maingay, Bussum This work has been made possible by repeated blood donations of Ms Reefhuis, Ms Wamelink, and Mr van Delft We also thank Dr E Simpson for critical comments, Ms H M Emanuelson-Oostvriesland, and Ms C Davijt for layout and typing of the manuscript In part supported by the Dutch Foundation for Medical Research (FUNGO), which is subsidized by the Dutch Foundation for the Advancement of Pure Research (ZWO), the J A Cohen Institute for Radiopathology and Radiation Protection (IRS)

References

Bailey, D W Allelic form of a gene controlling the female immune response to the male antigen in mice *Transplantation 11* 426–428, 1971

Dorus, E, Amarose, A P, Koo, G C, Wachtel, S S Clinical, pathologic, and genetic findings in a case of 46,XY pure gonadal dysgenesis (Swyer's syndrome) *Am J Obstet Gynecol 127* 829–831, 1977

Eichwald, E J and Silmser, C R Communication *Transplant Bull 2* 148–149, 1955

Fierz, W, Brenan, M, Mullbacher, A, and Simpson, E Non-*H-2* and *H 2*-linked immune response genes control the cytotoxic T-cell response to H-Y *Immunogenetics 15* 261–270, 1982

Gasser, D L and Shreffler, D C Genetic control of the immune response in mice V Minor genes involved in the response to H-Y and Ea-1 antigens *Immunogenetics 1* 133–140, 1978

Ghosh, S N, Shah, P N, and Gharpure, H M Absence of H-Y antigen in XY females with dysgenetic gonads *Nature 276* 180–181, 1978

Goldberg, E G, Shen, F W, and Tokuda, S Detection of H-Y (male) antigen on mouse lymph node cells by the cell to cell cytotoxicity test *Transplantation 15* 334–336, 1973

Gordon, R D, Simpson, E, and Samelson, L E In vitro cell mediated immune responses to the male specific (H-Y) antigen in mice *J Exp Med 142* 1108–1120, 1975

Goulmy, E HLA-A, -B restriction of cytotoxic T cells *In* S Ferrone and B G Solheim (eds) *HLA Typing Methodology and in Clinical Aspects* Vol 2, pp 105–122 CRC press, New York, 1982

Goulmy, E, Termijtelen, A, Bradley, B A, and van Rood, J J Y-antigen killing by T cells of women is restricted by HLA *Nature 266* 544–545, 1977

Goulmy, E, Bradley, B A, Lansbergen, Q, and van Rood, J J The importance of H-Y incompatibility in human organ transplantation *Transplantation 25* 315–319, 1978

Goulmy, E, Hamilton, J D, and Bradley, B A Anti-self HLA may be clonally expressed *J Exp Med 149* 545–550, 1979

Goulmy, E, Blokland, E, van Rood, J J, Charmot, D, Malissen B and Mawas, C Production, expansion and clonal analysis of T cells with specific HLA-restricted male lysis *J Exp Med 132* 182S–190S, 1980

Hurme, M, Hetherington, C M, Chandler, P R, Gordon, R D, and Simpson, E Cytotoxic T cell responses to H-Y Ir genes and associative antigens map in H2 *Immunogenetics 5* 453–459, 1977

Hurme, M, Hetherington, C M, Chandler, C M, and Simpson, E Cytotoxic T-cell responses to H Y mapping of the Ir genes *J Exp Med 147* 758–767, 1978

Lieuw, F Y, and Simpson, E Delayed-type hypersensitivity response to H-Y characterization and mapping of *Ir* genes *Immunogenetics 11* 255–266, 1980

Melvold, R W, Kohn, H I, Yerganian, G, and Fawcett, D W Evidence suggesting the existence of two H-Y antigens in the mouse *Immunogenetics 5* 33–41 1977

Pfeiffer, R A Observations in a case of an X/Y translocation t(X,Y) (p22, q11), in a mother and son *Cytogenet Cell Genet 26* 150–157, 1980

Silvers, W K, Gasser, D L, Eicher, E M H-Y antigen serologically detectable male antigen and sex determination *Cell 28* 439–440, 1982

Simpson, E, McLaren, A, and Chandler, P Evidence for two male antigens in mice *Immunogenetics 15* 609–614 1982

van Leeuwen, A Goulmy, E, and van Rood J J Major histocompatibility complex-restricted antibody reactivity mainly, but not exclusively directed against cells from male donors *J Exp Med 150* 1075–1083, 1979

van Rood J J Microlymphocytotoxicity test Manual of tissue typing techniques *In* J G Ray, D B Hare P D Pederson, and D E Kayhae (eds) pp 75–76 Bethesda, National Institutes of Health, Bethesda Maryland 1974

van Rood J J, van Leeuwen, A, and Ploem, J S Simultaneous detection of two cell populations by two colour fluorescence and application to the recognition of B cell determinants *Nature (Lond) 262* 795–797, 1976

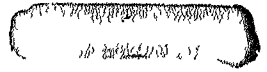
von Boehmer, H and Haas, W Distinct Ir genes for helper and killer cells in the cytotoxic response to H-Y antigen *J Exp Med 150* 1134–1142, 1979

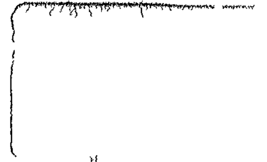
Wachtel, S S, Koo, G C, Breg, W R Thaler, H T, Dillard, G M, Rosenthal, I M, Dosik, H, Gerald, P S Saenger P, New, M, Lieber, E, and Miller O J Serological detection of a Y linked gene in XX males and XX true hermaphrodites *N Engl J Med 295* 750–754, 1976

Wachtel, S S, Koo, G C, de la Chapelle, A Kallio, H, Heyman, J H, and Miller, O J H-Y antigen in 46 XY gonadal dysgenesis *Hum Genet 54* 25–30, 1980

Wolf, U XY gonadal dysgenesis and the H Y antigen *Hum Genet 47* 269–277 1979

Wolf U, Muller, U, and Ohno, S H Y antigen and sex determination in mammals *In* M D Bennet, M Bobrow, and G Hewitt, (eds) *Chromosomes Today 7*, pp 258–270, George Allen & Unwin, London, 1980





Received November 12, 1982 revised version received January 31, 1983