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H-Y ANTIGEN AND SEX DETERMINATION

To the Editor: Rosenfeld et al. [1] presented an interesting case report on an abnormal phenotypic female who was evaluated for the presence of the H-Y antigen. This paper, and similar papers published on the same subject, raise several issues which I believe need further discussion.

The first issue deals with how the various authors determine whether H-Y antigen is present or absent in a given individual, and whether a quantitative difference in a given case is significant. In Rosenfeld et al., the authors conclude that "all three assays indicated decreased absorption of H-Y antiserum by the subject's cells as compared to normal male control cells. This implies reduced expression of H-Y antigen on both leukocytes and fibroblasts of the patient." Whether the antigen is reduced in amount or is completely absent is critically important. Although the PA-SRBC test suggested that the subject's cells were indistinguishable from those of a normal female, the sperm cytotoxicity test indicated that the subject may in fact possess the antigen. After absorption of antiserum by normal male cells, 28% of mouse sperm cells were killed; after absorption by normal female cells, 41% of the target cells were killed; and after absorption by the patient's cells, 35% of the target cells were killed. The corresponding values in the mixed hemadsorption-hybrid antibody test were 62%, 76%, and 71%, respectively. Since the conclusions of previous papers in this area depended upon establishing the significance of small differences [2–7], the method of significance testing is crucial. For example, the amount of H-Y antigen in this phenotypic female appears to be comparable to the amount detected in an XX phenotypic male in an earlier study [2].

To understand whether differences in this test are significant, it would be of great interest to know how much variation is to be expected among individuals of the same genotype. Have any normal females ever been identified who have as much H-Y antigen as some normal males (i.e., do the ranges overlap)? In a study on sex-reversed mice, approximately 30% of the spermatozoa of one XY,*Sxr*/– male were killed, while 50% of the spermatozoa were killed from a second mouse of the same genotype [3]. Since the amount of sperm killed after absorption by normal females differed by only 13% (41%–28%) in the Rosenfeld et al. study [1], sampling variation becomes an especially important issue.

Second, isn't the H-Y theory of sex determination severely damaged if the patient described by Rosenfeld et al. possesses the antigen, even at a reduced level? Previous discussions of the H-Y theory of sex determination would lead one to believe that the presence of H-Y is all that should be required for at least some expression of maleness [8–11]. This patient's having normal female genitalia as well as a normal uterus and

fallopian tubes and no sign of maleness would not be expected in an individual who possesses what is "ostensibly the product of Y-chromosomal testis-determining genes" [1]. Although one could argue that this patient lacks the receptor for H-Y [12], this is presumably an extremely rare event unrelated to H-Y genotype, since normal females are expected to have the receptor for H-Y on their ovaries [8]. Since this patient also has reduced expression of H-Y, it does not seem likely that two independent, extremely rare events would occur in the same individual.

The last issue deals with how the authors' proposal of a "family of testis-determinants" [1] is related to the H-Y antigen as it was originally defined. The term H-Y was originally assigned to the mouse transplantation antigen which stimulated the rejection of male skin grafts by females of the same strain [13]. As far as I am aware, it has never been shown that the antigen detected in the sperm cytotoxicity assay, for example, is the same as the antigen which causes skin graft rejection. In fact, a mutant male mouse lacking the transplantation antigen, but possessing the serologically detectable antigen, has been reported [14]. If the proposal is now being made that sex determination is controlled by a family of Y-linked genes, on what basis are these genes being referred to as H-Y? The term "H-Y" has a very specific meaning, and it ought not be used to refer to every antigen present in males that is not present in females. Although female-antimale sera undoubtedly detect multiple specificities, it is possible that none of these are H-Y as defined by the criterion of transplantation. The various descriptions of the H-Y theory of sex determination strongly imply that the transplantation antigen is the inducer of the male phenotype [8–11]. Rosenfeld et al. reinforce this impression by stating: "Recent reports have established that a genetic locus on the Y chromosome controls expression of H-Y antigen, a cell surface component responsible for rejection of male skin grafts by syngeneic females." Although sex differentiation is most likely controlled by a family of Y-linked genes, isn't the evidence rather incomplete that H-Y is a member of this family?

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RESPONSE I TO GASSER'S LETTER

To the Editor: Many of Dr. Gasser's points are well taken. Nevertheless, it seems that to attack the concept of H-Y antigen as the testis-organizer at its crudest form is a little unfair. (1) The quantitation of H-Y antigen levels can be done by graduated absorption tests [1]. However, since such a series of detailed studies quickly exhausts a year's supply of H-Y antibody, the exact quantitation is impractical for the clinical study of individual patients. (2) Implicit in the finding that the H-Y antigen (+) XX male condition can be inherited as an autosomal recessive trait as in the goat [2] is the fact that H-Y antigen levels below the critical threshold don't interfere with ovarian organogenesis, as mothers of these XX billy goats are obligatory heterozygotes, expressing at most half the H-Y antigen level of their XX sons. (3) The XX male condition and XX true hermaphroditism can run in the same family; for example, an H-Y antigen (+) bitch who produced XX sons was herself a true hermaphrodite endowed with a pair of ovotestes [3], thereby showing that a somewhat subnormal H-Y antigen level is barely sufficient for the testicular organogenesis and that H-Y antigen (+) fertile females can be true hermaphrodites. (4) A reduced H-Y antigen level and a mutational defect of the gonad-specific H-Y antigen receptor are not likely to be found together in the same individual. However, it should be remembered that the customary way of proving the presence of a mutationally defective enzyme is to use the antibody raised against the wild-type enzyme. By the same token, H-Y antibody, raised against the wild-type H-Y antigen, is likely to detect a defective H-Y antigen that has lost its receptor-binding activity, and, therefore, its testis-organizing capacity. In fact, such an in vitro mutation of human H-Y antigen has already been reported [4].

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