H-Y Antigen: Expression in Human Subjects with the Testicular Feminization Syndrome

Abstract. *Androgen-insensitive subjects with a 46,XY karyotype develop as phenotypic females despite presence of testes. The white blood cells of these females type H-Y antigen-positive indicate that expression of the H-Y cell surface component is androgen-independent.*

In humans, as in mice and rats, there is a form of male pseudohermaphroditism known as "testicular feminization syndrome" (TFS). In mice this condition is due to an X-linked mutation called *Tfm*, which leads to deficiency of the nuclear cytosol androgen receptor (*1*). Androgen-insensitivity in man is also an X-linked trait (*2*). Affected individuals have a karyotype indistinguishable from that of normal males (46,XY in man), and under the influence of the Y-chromosome their gonads differentiate as testes. Nevertheless, since these individuals cannot respond to testosterone, they develop as phenotypic females. But the fetal testes of these females apparently secrete Mullerian inhibiting factor; development of the fallopian tubes, uterus, and the upper third of the vagina is suppressed (*3*).

Masculinization of androgen-dependent sex organs fails to occur in patients with TFS despite blood production rate and serum levels of testosterone that are equivalent to those found in normal males (*4, 5*). Conversion of testosterone to dihydrotestosterone also appears to be normal (*5*) (Table 1). Thus patients with TFS are resistant not only to testosterone but also to its immediate derivative 5α-dihydrotestosterone (*6*), which is necessary for orderly differentiation of external male genitalia during fetal life (*7*).

Because their cells are androgen-insensitive, patients with TFS are excellent subjects for the study of histocompatibility-Y (H-Y) antigen expression and its relation to male hormone. H-Y antigen was discovered with the observation that female mice reject skin grafts from males of the same inbred strain (*8*). Although there are indications that the *H-Y* gene is on the Y-chromosome, there are also data which favor the argument that H-Y antigen expression is governed by an autosomal or X-linked gene which depends for its expression on a male hormonal environment. Indeed some controversy lingers as to the requirement of a male environment for cells to sustain expression of H-Y on their membranes, and several experiments involving male $\leftarrow\rightarrow$ female transplantation or hormonal manipulation (or both) have given conflicting results (*9*). However, the development in our laboratory of routine serological assays for H-Y antigen has provided a rigorous approach to questions involving the expression of this cell surface component. Thus, for example, we have shown that the male environment can exert at most a quantitative rather than qualitative influence, because male cells resident in male-to-female radiation chimeras continue to express H-Y antigen (*10*).

One of the most important findings of H-Y serology is that H-Y antigen has been phylogenetically conserved, occurring in the heterogametic sex of all vertebrate species so far examined, including amphibians, birds, and mammals (*11*). This conservation signifies a vital function.

Our hypothesis regarding the nature of this function is that the cell surface component recognized serologically as H-Y antigen governs development of the initially indifferent embryonic gonad toward the male or testicular mode in species in which the male is the heterogametic sex (XY) (*12*). (The testis then produces androgen, which effects virilization of the accessory glands and ducts.) According to this hypothesis, H-Y antigen expression should always be associated with the presence of at least rudimentary testes in mammals, regardless of phenotype or karyotype. Indeed, XX mice carrying the autosomal dominant *Sxr* ("sex-reversed") develop as males; and these animals, like human XX males and XX true hermaphrodites, are H-Y$^+$ (*13*), implying presence in the genome of *H-Y* and testis-determining (Y-derived) genes, which we propose are identical (*9, 13*).

In mice, *Tfm*/Y females are H-Y$^+$. Thus expression of H-Y is not testosterone-dependent in this species (*14*). We have now studied cells from human cases of TFS, and our results confirm the findings in *Tfm* mice. The basis of the test with human subjects is to assay the capacity of their peripheral blood leukocytes to absorb activity from mouse H-Y antiserum, which is then reacted with mouse sperm in the complement-dependent cytotoxicity assay, the standard serological test for H-Y antigen. The necessary controls are provided by leukocytes from normal male and female human donors (*9*).

Table 2 shows that leukocytes from human subjects with TFS, like leukocytes from normal 46,XY males, absorb H-Y antibody and must therefore be H-Y$^+$. Because the cells of patients exhibiting TFS cannot bind testosterone, or alternatively, because they display abnormal re-

Table 1. Androgen dynamics in case 5 (age 20 years, before castration).

Item	Patient	Normal male	Normal female
Serum testosterone (ng/dl) (*16*)	594	546 ± 278*	39 ± 21*
Blood testosterone production (μg/day) (*4*)	4000	5780 ± 1420*	316 ± 101*
Conversion of testosterone to dihydrotestosterone (%) (*17*)	8.3	4.6 to 6.4†	3.2 to 4.2†

*Mean ± standard deviation. †Range.

Table 2. Tests for H-Y antigen were performed according to described procedures (*9*). Briefly, mouse H-Y antiserums were selected and pooled, and the pools were divided into four parts. One part was unabsorbed and the other three parts were absorbed with white blood cells from normal females and males, and from patients with TFS, respectively. Positive absorption (indicating presence of H-Y antigen on the absorbing cells) was manifested as a decrease in reactivity of absorbed serums, that is, a decrease in number of mouse sperm killed in the cytotoxicity test (see *9*).

Subject	Buccal smear: Barr bodies/ cells studied	Metaphases studied (No.)	Cells karyotyped (No.)	Karyotype	H-Y antigen
Normal males	0			46,XY	+
Normal females	≥ 20%			46,XX	−
TFS*					
Case 1	0/200	20	6	46,XY	+
Case 2	0/300	31	4	46,XY	+
Case 3		50	7	46,XY	+
Case 4	0/100	16	1	46,XY	+
Case 5		23	3	46,XY	+
Case 6	0/100	65	2	46,XY	+

*Phenotypic females with testicular feminization syndrome. Patients 2 to 6 were castrated prior to H-Y typing.

ceptor-chromatin interaction or abnormal androgen-mediated transcription (*15*), it cannot be claimed that retention of the H-Y$^+$ cellular phenotype depends on testosterone or on any male characteristic that is secondary to any action of testosterone.

These findings are consistent with our hypothesis that H-Y is the primary determinant of male gonadal sex in XY-male species. This conclusion has practical as well as theoretical implications, for it bears on the ultimate value of H-Y typing as a diagnostic measure in the appraisal of cases of abnormal sexual development in man.

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Spinal Neurons Project to the Dorsal Column Nuclei of Rhesus Monkeys

Abstract. *Cells of origin of ascending nonprimary afferents to the dorsal column nuclei of rhesus monkeys have been identified in the spinal cord by the retrograde transport of horseradish peroxidase. These neurons are mainly located in lamina IV and medially in more ventral laminae of the dorsal horn on the side ipsilateral to the medullary injection. Large neurons in the ventral horn ("spinal border cells") also appear to project to the ipsilateral dorsal medulla. The dorsal column nuclei of a primate thus are the recipient not only of ascending dorsal root fibers but also of a more complexly integrated spinal input.*

Traditional concepts concerning the organization and function of ascending sensory systems in the mammalian spinal cord are founded upon a dichotomy between the anterolateral and the dorsal column–medial lemniscal systems. The anterolateral ascending system comprises spinothalamic fibers which, in primates, originate mainly from neurons in the dorsal horn (*1*), ascend in the contralateral anterolateral funiculus of the spinal cord, and terminate in several thalamic nuclei (*2*). In the dorsal columns (or dorsal funiculi) ascend uncrossed fibers which originate from dorsal root ganglion cells and terminate in the dorsal column nuclei (DCN, including gracile and cuneate) in the caudal medulla oblongata. Fibers from cells in these nuclei cross, form the medial lemniscus, and also terminate in the thalamus (*2*). Transmission of different sensory modalities is usually attributed to these two major pathways, although some overlap seems to exist in the role that these two systems play in the mediation of somatic sensibility (*3*).

Recent anatomical and electrophysiological data have shown that the dorsal column nuclei of higher mammals are the target not only of dorsal root afferents, but also of fibers arising from the spinal gray matter and ascending in the dorsal and dorsolateral funiculi (*4*). In the present study, cells of origin of these ascending fibers have been visualized in the monkey by means of the retrograde transport of horseradish peroxidase (HRP) (*5*). Like spinothalamic cells in the same species, spino-DCN cells are mainly located in the dorsal horn; therefore, the spinothalamic tract and the dorsal column nuclei–medial lemniscus pathway convey ascending information relayed by cells which are accessible to activation by similar peripheral input.

In six adult rhesus monkeys, 30 percent HRP (Sigma type VI or Boeringher) was injected either unilaterally or bilaterally into the dorsal medulla. All animals were anesthetized with Nembutal (40 mg/kg, intraperitoneally) and were held in the frame of a stereotaxic apparatus. After removal of a small portion of the occipital bone and incision of the dura mater, part of the posterior cerebellum was aspirated. By this approach the dorsal aspect of the DCN throughout the rostrocaudal extent was exposed. Single or multiple injections totaling 0.05 to 0.5 μl per side were made with a CR700-20 Hamilton syringe fitted with a 26S gauge needle (outer diameter 0.5 mm, inner diameter 0.37 mm). After 48 to 72 hours all animals were perfused with 0.9 percent saline, followed by a double aldehyde mixture (0.5 percent paraformaldehyde and 2.5 percent glutaraldehyde in 0.1*M* phosphate buffer at *p*H 7.2). Immediately after perfusion the brain and spinal cord were removed and washed for at least 24 hours in 0.1*M* phosphate buffer at *p*H 7.2 to which 30 percent sucrose was added. Serial frozen 40-μm sections through the medulla and spinal cord were incubated for 30 minutes at room temperature in a 0.05 percent solution of 3,3′-diaminobenzidine-tetrahydrochloride in tris-HCl buffer (*p*H 7.6) to which freshly prepared 1 percent hydrogen peroxide was added (*6*). All sections were screened under dark- or bright-field illumination, and the locations of labeled cells were plotted on tracing paper with an electronic pantograph. The injection sites were reconstructed from serial transverse sections through the medulla with the aid of an overhead projector and of microscopic observation.

The extent of the HRP infiltration into the dorsal medulla and the number of labeled neurons in the spinal cord varied according to the total volume injected. In four cases in which the focus of the HRP infiltration was within the boundaries of the DCN, the majority of the labeled neurons were in the dorsal horn of both sides (after bilateral injections) or of the side ipsilateral to the injected medulla (after unilateral injection).

The results in one case with a unilateral injection are shown in Fig. 1. On the side ipsilateral to the injected medulla labeled cells in the upper three cervical segments were concentrated in the medial part of the dorsal horn and in the intermediate zone. Throughout the brachial and lum-