A Gene Controlling H-Y Antigen on the X Chromosome

Tentative Assignment by Deletion Mapping to Xp223

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Summary. The existence of a strict correlation between presence of testicular tissue and presence of H-Y antigen in mammals and man leads to the conclusion that H-Y antigen is an essential differentiation factor in testicular morphogenesis. Presence of low titers of this differentiation antigen even in fertile females indicates that its morphogenetic effect depends on a threshold. Here, studies on H-Y antigen in female individuals with various deletions of the X-chromosome are reported. It turns out that deletion of Xp results in the synthesis of reduced amounts of H-Y antigen, while deletion of Xq does not. In a fertile female with only Xp223 deleted due to an X/Y translocation, including the distal Yq, presence of a reduced H-Y titer allows for the tentative assignment of a controlling gene repressing the H-Y structural gene. From the cases studied, it follows that the H-Y structural gene is autosomal and under the control of X- and Y-linked genes. The conception emerges that interaction between X- and Y-linked genes or their products results in variation of the H-Y antigen titer. The fate of the indifferent gonadal anlage to differentiate into the male or the female direction will depend on the titer of H-Y antigen reached by the action or interaction of the controlling genes involved.

Introduction

H-Y antigen was shown to be present in the human male, but not in the human female (Wachtel et al., 1974; Ohno, 1976). Furthermore, studies on patients with disorders of sexual development revealed that, without exception, H-Y antigen is detected even if only traces of testicular tissue are demonstrable by gonad histology (e.g. Wachtel et al., 1977; Wolf, 1978). Thus, there is a strict correlation between the presence of testicular histology and the presence of the antigen. On the other hand, exceptional female individuals occur who exhibit H-Y antigen without demonstrable testicular tissue. This does not refer to the testicular feminization syndrome, shown to be H-Y positive in the presence of functional testes (Koo et al., 1977). However, in XY gonadal dysgenesis, the majority of patients are H-Y positive, a finding which is explained by the assumption of a defect of the gonad-specific H-Y antigen receptor (Wolf, 1979). Apart from these syndromes which can be interpreted on the conventional basis that presence of H-Y antigen depends on the presence of the Y chromosome or relevant segments of it, there are a number of female individuals reported in the literature who turned out to be H-Y positive without detectable Y-chromosome. This does not refer to the testicular feminization syndrome shown to be H-Y positive in the presence of functional testes (Koo et al., 1977). However, in XY gonadal dysgenesis, the majority of patients are H-Y positive, a finding which is explained by the assumption of a defect of the gonad-specific H-Y antigen receptor (Wolf, 1979). Apart from these syndromes which can be interpreted on the conventional basis that presence of H-Y antigen depends on the presence of the Y chromosome or relevant segments of it, there are a number of female individuals reported in the literature who turned out to be H-Y positive without detectable Y-chromosome or a part of it. There are some examples in the collection of cases studied by Breg et al. (1979), including a number of patients with Turner syndrome. In all instances, these authors assume the presence in one or the other form, of Y-chromosome material in the karyotype, without the possibility to demonstrate it. Even in fertile 46,XX females, H-Y antigen was found to be present, though with reduced titer (de la Chapelle et al., 1978). Here again, translocation to an autosome of some genes of a multigen family present normally on the Y-chromosome is assumed to have taken place. — In contrast to these considerations, one of us favoured a
model ascribing mere regulatory functions to the Y-linked gene or genes involved in the H-Y antigen system. In this model, an autosomal localization of the H-Y structural gene, and a regulatory interaction between Y- and X-linked genes responsible for the expression of the autosomal structural H-Y gene was assumed (Wolf, 1978).

Under this hypothesis, the postulate of Y-chromosome material present in one or the other undetectable form somewhere in the karyotype becomes superfluous.

The present study was prompted by the detection of H-Y antigen in a fertile female with an X/Y translocation, resulting in deletion of Xp223, and presence of the distal long arm including the fluorescent segment of the Y-chromosome (Tiepolo et al., 1977).

Patients

No. 91 (F.M.). Karyotype 46.X,t(X;Y)(Xqter→Xp22.2::Yq11→Yqter). Fertile normal female, 28 years, monosomic for Xp22.3. Mother of a son (No. 90) nullisomic for this band who has genital malformations. For further details, see Tiepolo et al. (1977,1980).

No. 153 (B.R.). Karyotype 46.X,t(X;Y)(Xqter→Xp11.2::Yq11→Yqter). Female, 26 years, with secondary amenorrhoea and short stature. For more details, see Hecht et al. (1980).

No. 198 (M.A.). Karyotype 46.X,derX(Xpter→Xq22::13q32→13qter)mat, consistently found in all 46 blood cells analyzed. Female, 24 years, with secondary amenorrhoea.

No. 199 (C.V.). Karyotype 46.X,del(X)(p11), consistently found in all 73 blood cells analyzed. Female, 15 years, with short stature (129.5 cm) and some other features of Turner syndrome. Familial study of the Xg blood group not informative.

No. 202 (F.B.). Mosaic karyotype 46.X,del(X)(q21)/45,X in the proportions 13/1/25 in blood. Female, 20 years, with primary amenorrhoea and gonadal dysgenesis. Familial study of the Xg blood group not informative.

No. 206 (M.O.). Karyotype 46.X,del(X)(q13), consistently found in more than 200 blood cells analyzed. Female, 16 years, with short stature, primary amenorrhoea and gonadal streaks.

Methods

Antiserum to H-Y antigen was raised in isogenic Lewis rats by six weekly intraperitoneal injections into female animals of 20×10⁶ male spleen cells each. Blood was taken one week after the last immunization, the serum was inactivated at 56°C for 30 min and absorbed 1:1 with AB-human female erythrocytes. Rabbit complement was selected according to low cytotoxicity on target cells (Raji cells, see below).

H-Y antigen in the blood samples of patients and normal male and female controls was detected by two different test procedures.

1. A cytotoxic test followed essentially the method of Fellous et al. (1978), using the human male Burkitt lymphoma cell line "Raji" as targets. However, a modification was introduced in the absorption procedure with the blood samples to be tested. When we detected that male granulocytes and erythrocytes do absorb anti-H-Y antiserum, while lymphocytes do not or only to a small extent (apparently only the minor fraction consisting of B-lymphocytes absorbs the antiserum), we no longer used just the buffy coat for absorption. Instead, of each blood sample the granulocytes and erythrocytes, respectively were isolated, and used separately for independent determinations of H-Y antigen.

A detailed description of these methods and results will be published elsewhere (Mayeroviá, 1980).

2. The finding that erythrocytes can be used to test for absorption of anti-H-Y antiserum, prompted us to develop a method employing hemolysis as parameter to discriminate between different amounts of H-Y antigen present on the cell surface. In this method, the isolated erythrocyte fraction to be tested is exposed to antiserum in the presence of complement, and the eventual hemolytic effect is determined spectrophotometrically. The result is expressed in mg% of hemoglobin. The method will be published elsewhere in detail (Hecht et al., 1980).

Results

After absorption of anti-H-Y antiserum with blood cell fractions of normal female control samples, cytotoxicity is somewhat lower than with unabsorbed antiserum; this effect may be due to unspecific absorption. In male controls, some residual cytotoxicity remains, partially at least because the antiserum concentration surmounts the binding capacity of the respective cell samples; but it cannot be excluded that there is some variation in the H-Y antigen titer in the normal male. Apart from these variations, the sex difference of antiserum cytotoxicity after absorption is far from overlapping under appropriate test conditions, though also varying to a considerable degree depending on these conditions. Thus, in the experiments taking a favourable course, titers deviating from the controls can be definitely identified. It should be clear, however, that quantification is hardly possible in the cytotoxicity test.

In contrast, the haemolysis method opens the possibility for quantification, and it is at least in part a matter of standardization of the test procedure, how small deviations from the control range will be detected.

Using the cytotoxicity test, the findings in the patients studied here as compared to controls are depicted in Figs. 1–3. It is seen that the X/Y-translocation cases both (Nos. 91 and 153), clearly deviate from the female and male controls, showing an intermediary course of cytotoxicity at various antiserum dilutions (Figs. 1 and 2). Thus, it is to be concluded that they are H-Y positive, but with a reduced titer. In the Xp− case (No. 199), H-Y antigen activity even falls into the male control range (Fig. 3) which was not quite so in the haemolysis test (see below). In contrast, the two