The Role of H-Y Antigen in Gonadal Differentiation and Anomalous Sexual Development

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Gonadal differentiation involves the organization of indifferent gonads containing primordial germ cells into either seminiferous tubules or primary follicles. This process, which in the human becomes evident in the 7th week of gestation, appears to be determined by the presence or absence of a Y chromosome. The indifferent gonad differentiates into a testis in the presence of a Y chromosome and into an ovary if a Y chromosome is lacking. The number of X-chromosomes is irrelevant to testicular or ovarian differentiation, although it does affect the later maturation and function of the gonads. The genes on the Y chromosome exert their influence on gonadal differentiation through the intermediary of a male determining factor which has now been identified as the H-Y antigen. The correlation between gonadal development and the presence of a Y chromosome has exceptions, examples of which are 46 XX males and 46 XY females. Testicular differentiation appears to correlate better with the presence of H-Y antigen. This paper reviews the H-Y antigen in various anomalies of sexual development and the light it has shed on the mechanism of gonadal differentiation.

Discovery of the H-Y Antigen

The H-Y antigen was first recognised as a transplantation antigen in mice. Highly inbred strains of mice are genetically identical so that skin grafts transplanted between members of the same inbred strain should be accepted. It was noted, however, that in such animals slow graft rejection occurred in male to female grafts but not in grafts between males or between females or from females to males[6]. This implied the existence of male-specific transplantation

using a double - breasting technique. If there is a complete absence of the left diaphragm, a flap of the lower rib-cage, connected by the intercostals, is fashioned and is sutured to the thin rim of diaphragm on the posterior wall.

Post-Operative Care

Intermittent positive pressure ventilation is usually required for the first few hours after operation and moreover in those cases where hypoplasia of the lung is marked. In these high pressure ventilation is usually required and ventilation-induced pneumothorax is a common complication. Regular post-operative chest X-Rays are thus required to assess reinflation of the lung and also the possibility of contralateral pneumothorax. The timing for removal of the infant from the ventilator is vital and in general the return to spontaneous breathing with well maintained blood gases is the principal indication for extubation. Meanwhile continuous gastric aspiration and intravenous therapy are essential till the infant is well enough to resume oral feeding.

Sequelae in Surviving, Post-Operative Cases

The most common complications in the early post-operative period are related to pulmonary function and have already been outlined. Other complications may arise from the variety of associated anomalies affecting the heart and large vessels. In general infants who require prolonged ventilation are those who have marked hypoplasia of the lungs, having therefore a smaller chance for survival. The overall mortality is in the region of 65%. The long term morbidity results from other congenital anomalies frequently associated.

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antigen which was presumed to be determined by a Y-linked gene. Since the histocompatability antigens in mice had already been labelled H-1, H-2, H-3, etc., the new antigen was named H-Y.

At the time of its discovery the role of the H-Y antigen in sex determination had not been recognized. However, it was noted that an identical or closely similar antigen was also detectable in all the other mammalian and non-mammalian species which had been studied. The strict phylogenetic conservatism of a sex-specific antigen throughout vertebrates from frog to man suggested that the H-Y antigen could have a fundamental sex-associated role and might even be the *male determining factor by* which the Y chromosome controlled gonadal development[21]. Solid support for this hypothesis was soon forthcoming from studies of the H-Y antigen in a wide variety of anomalies of sexual development in man and animals.

Detection of H-Y Action

The H-Y antigen is found in all male tissues associated with the plasma membrane of cells. It is most readily demonstrable on spermatozoa which are quickly immobilized and killed when exposed to specific antiserum containing H-Y antibodies. This forms the basis of the sperm cytotoxicity test which can be used for the detection of H-Y antigen on any other cells including leucocytes, liver, spleen, kidney, brain and gonadal cells. The cells to be tested are exposed to H-Y antiserum in appropriate dilution before reacting it with sperm. If the cells have H-Y antigen, they will absorb H-Y antibody which will no longer be available for reaction with the sperm and consequently a smaller proportion of sperms will be killed. Dead spermatozoa can easily be visualised and counted microscopically.

The H-Y antigen can also be detected using fluorescent-labelled antibodies and other immunological techniques.

Location of the H-Y Gene

There is plenty of evidence that the structural gene for the H-Y antigen is located on the Y chromosome. The H-Y antigen is always present in 46 XY males. Increased amounts are detectable in males with two Y chromosomes (eg. XYY and XXYY) indicating a gene dosage effect[22]. Normal levels of H-Y antigen are found in males with a non-fluorescent Y chromosome, in whom the brilliantly fluorescent distal segment of the Y chromosome is missing but male sexual development and function are completely normal. Moreover, studies of H-Y antigen in individuals with various structural abnormalities, including deletions or isochromosomes of the short or long arms of the Y chromosome, indicated that the H-Y locus was situated in the pericentric region of the Y chromosomes and probably on its short arm[14]. Anomalous situations including the presence of H-Y antigen in XX males or XX true hermaphrodites and its absence in some cases of 46 XY females with ovarian dysgenesis have been very difficult to explain, but have nevertheless provided important insights into the mechanisms of action of H-Y antigen.

H-Y Antigen in XX Males - Inducer for Testicular Development

The clinical condition in 46 XX males resembles that in the Klinefelter Syndrome (47 XXY); in both conditions the testes are small, spermatogenesis is impaired and the genitalia are normal male. The proposed theories attempting to explain the testicular development and consequent male phenotypes in XX males have postulated the presence of occult Y chromosome material. In fact, with refined cytogenetic techniques, Y chromosome material has been detected either translocated on to an X chromosome or an autosome or as occult mosaicism in some tissues or in a small proportion of cells.

Although some cases of XX males still defy cytological detection of some form of Y chromosome material, H-Y antigen has so far been detected in every case, although it may be reduced in amount in some individuals[23]. In certain animals, particularly rodents, *XX sex reversal* is fairly common and in some cases appears to be transmitted as an autosomal dominant condition: XX or XO males carrying the autosomal dominant gene, Sxr (sex-reversed) are sterile males and express H-Y antigen; XY animals transmit the gene without being adversely affected by its presence[2]. This autosomal gene could represent translocation of a minute, cytologically undetectable fragment of the Y-chromosome.

Much more intriguing, however, is the situation in polled (or hornless) goats of Saanan breed which produced XX phenotypic males manifesting H-Y antigen as a condition following an autosomal recessive mode of inheritance. It is manifested in XX animals, homozygous for the abnormal gene and transmitted by the parents both of which are heterozygous carriers of the abnormal gene without affecting their fertility[18] [25]. A remarkably similar situation has also been reported in humans in a family with three XX-males and a pedigree strongly suggestive of autosomal recessive inheritance[5]. Such a mode of inheritance is not convincingly explainable as the basis of translocated Y chromosome material and it is likely that H-Y antigen expression and testicular differentiation can be invoked by autosomal recessive genes.

The H-Y Antigen in XY Females

There are two broad categories of 46 XY phenotypic females - the testicular feminization syndrome and XY gonadal dysgenesis. In the testicular feminization syndrome, testes, XY chromosomes and H-Y antigen are invariably present but the external genitalia are undoubtedly female. This condition, which is also found in rodents, is caused by an X-linked mutant gene conferring tissue insensitivity

to androgens[1 15]. Early testicular differentiation is not affected.

In XY gonadal dysgenesis, the affected individuals lack testes and are phenotypically female with dysgenetic or streak gonads. There are two distinct types of this condition with different aetiologies depending on whether the subject is H-Y negative or positive.

XY Gonadal Dysgenesis without H-Y Expression - Evidence for an X-linked Repressor Gene

The easiest way to explain absence of H-Y antigen expression in the presence of a Y chromosome would be deletion or suppression of the H-Y locus. Although the condition is rare in humans much valuable information has been derived from the curious situation in the Scandinavian wood lemming in which females outnumber males and about one half of the females have XY chromosomes but are phenotypically normal and fertile females[10]. Genetic studies have shown that in these animals there is an X-linked gene which blocks synthesis of H-Y antigen and testicular differentiation by repressing the H-Y locus[11 24].

A similar X-linked repressor gene may also account for the reported cases of XY gonadal dysgenesis without H-Y expression in humans. In fact, several cases of familial XY gonadal dysgenesis compatible with X-linked inheritance have been documented[7 12 13 19]. Although XY females in the wood lemming are fertile, the condition is still basically similar to that in humans. In the wood lemming with, in effect, only one X-chromosome, as in other rodents with XO sex chromosomes, the gonads develop into normal ovaries containing follicles, but their fertile life span is reduced. Similarly, in human females with XY gonadal dysgenesis, or XO Turner Syndrome, ovarian and follicular development are normal in foetal life but rapidly degenerate thereafter[3]. Streak gonads lacking ovarian follicles are the only reamains by the time of puberty.

XY Gonadal Dysgenesis with H-Y Antigen Expression - Evidence for Specific H-Y Receptors.

This condition is highly paradoxical because testicular failure occurs in spite of a normal Y chromosome and H-Y antigen expression. It could be explained if one postulates the existence of specific gonadal H-Y receptors, which, in this case fail to bind with the H-Y antigen. Nevertheless, H-Y antigen is found on all male tissue cells bound to the plasma membrane. This introduces a new concept in H-Y antigen expression: the H-Y locus directs the synthesis of H-Y antigen which then binds to the plasma membrane of cells. There are two H-Y antigen binding sites:

a) the non-specific membrane anchorage site which is present on all tissues, and,

b) the specific gonadal receptor.

It is the binding of H-Y antigen to the specific receptor on the gonad which is responsible for inducing testicular differentiation[16] and it is precisely this process which fails in H-Y positive ovarian dysgenesis. Specific gonadal receptors for H-Y are found in the gonads of either sex and will bind with H-Y antigen to induce testicular differentiation whether this is produced endogenously by the cells themselves or is available from an exogenous source. This concept has been validated by *in vitro* experimentation[17 27] which is summarised below.

If XY foetal testicular cells are dissociated to form a cell suspension and then cultured under appropriate conditions, they will reaggregate to form long twisting tubular structures closely resembling seminiferous tubules. Similarly, dissociated foetal ovarian cells reaggregate to form spherical structures, resembling ovarian follicles.

It is possible to remove cell surface antigens from dissociated cells by a process termed *lyostripping*. Dissociated foetal testicular cells which have been lyostripped of their H-Y antigen will not aggregate into tubules but will form spherical follicular structures instead. Conversely, dissociated foetal ovary cells, if cultured in a medium rich in exogenous H-Y antigen, will bind the H-Y antigen and reaggregate into seminiferous tubule-like structures instead of follicles.

True Hermaphroditism — H-Y Antigen Expression and Partial Receptor Failure

True hermaphroditism is characterised by the coexistence of testicular and ovarian tissues and is usually accompanied by ambiguous genitalia. Over 80% of true hermaphrodites have 46 XX karyotypes but serologically they express H-Y antigen, though possibly in reduced amounts. In this respect, they resemble XX males and the arguments relating to testicular development and the presence of occult Y chromosome material also apply. The new problem that arises here is that part of the gonad develops into an ovary in spite of H-Y antigen expression. This problem is similar to that of H-Y antigen positive ovarian dysgenesis and it therefore appears that receptor failure on part of the gonad is responsible for the ovarian development. In fact, it has been shown[26] that the testicular portion of an ovotestis was H-Y antigen positive, while the ovarian portion was H-Y antigen negative. However, it remains a puzzling problem as to why receptor failure should involve only some of the cells.

Conclusion - Mechanism of Gonadal Differentiation

The evidence which has accrued from the study of the H-Y antigen in rare and frequently exotic anomalies of sexual development has considerably enhanced our understanding of the mechanism of gonadal differentiation, which is illustrated in Fig. 1.

It appears that the H-Y antigen, is in fact the *testis determining factor*, controlled by the H-Y locus in the pericentric region of the Y chromosome. However, its expression is also dependant on other factors which are controlled by autosomal or X-linked genes. The H-Y antigen, whose synthesis is regulated by the H-Y locus becomes attached to the plasma membrane of all cells. Binding to a specific receptor site on the gonadal cells is necessary for the H-Y antigen to exert its inductive influence on testicular differentiation. Ovarian differentiation requires only the presence of one X chromosome and the absence of H-Y antigen. Further testicular and ovarian development are influenced by the sex chromosome complement because spermatogenesis cannot proceed in a germ cell line which has two X chromosomes and follicular degeneration occurs if a second X chromosome is lacking.

Development of the accessory genital organs depends on factors secreted by a functioning testis - Müllerian inhibiting factor, Wolffian duct stimulating factor and androgens.

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