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EDITORIAL

The H-Y Antigen

The H-Y antigen is a male-specific minor (*i.e.* non-MHC) histocompatibility antigen which was first described in 1955, following the observation that females of some inbred mouse strains rejected skin grafts from otherwise syngeneic males.[1] The term H-Y antigen reflects the widely held belief that the antigen is coded by a gene on the Y chromosome, and conceptually this represents the simplest approach. Nevertheless evidence obtained from a recently isolated cDNA clone[2] suggests that the structural gene for H-Y may be autosomally coded, its expression being contingent on the presence of a gene on the Y chromosome, with possible regulatory genes on other chromosomes along the lines previously postulated.[3] It has been clearly established that the expression of the antigen is not dependent on the presence of male hormones.[4] While the genetic processes which lead to the expression of the H-Y antigen may be complex, nevertheless male-female incompatibility within an inbred laboratory strain provides a grafting situation which for all practical purposes involves a single histocompatibility antigenic difference between donor and host, and which is equivalent to a naturally occurring congenic donor-host pair.

The demonstration of the mouse H-Y antigen in 1955 was soon followed by reports of a similar antigenic system in rats.[5,6] For several years the experimental approach to the H-Y antigen relied on transplantation experiments. Since it is difficult to identify the relatively weak H-Y antigen in the presence of other histoincompatibility, experiments were largely restricted to mice and rats among which inbred strains are widely available. It was established that with one possible exception,[7] the H-Y antigen in the mouse appears to have no alleles, *i.e.,* the antigen is the same in all the strains of mice studied, and also in wild mice.[8,12] A similar situation applies among inbred rat strains.[9] The apparent absence of polymorphism at the H-Y locus contrast with the polymorphism of the major histocompatibility antigens and at least some of the minor histocompatibility antigens.[10,11]

Minor histocompatibility antigens readily evoke T cell responses. Thus, the H-Y antigen has not been difficult to identify *in vivo* using transplantation techniques nor *in vitro* using cytotoxic T cells.[11,12] Since minor histocompatibility antigens are not very efficient provokers of B cell responses,[11] it is not surprising that antibodies to the H-Y antigen have generally been difficult to raise by conventional techniques, nor that H-Y serology dates only from 1971.[13] Once H-Y antibodies became available it soon became apparent that not only was the H-Y antigen similar within a given species, but also that it was similar or cross reactive between different species.[8] Indeed the H-Y antigen was present in normal males of all the mammalian species studied. While it characterises males among mammals, it characterised females among birds, fish and some amphibia.[8] In these latter species the female is the heterogametic sex (*i.e.* having dissimilar sex chromosomes). While this extensive evolutionary conservation of the antigen implies a very basic function, the exact nature of this has yet to be established with certainty. The possible functions of the H-Y antigen are discussed below.

Researchers studying the H-Y

antigen have viewed it from several quite different angles. From the transplantation point of view, it is much less obtrusive than the MHC antigens or many of the other minor histocompatibility antigens. Intrastrain male-female incompatibility has however provided a prototype weak histocompatibility system, and in this context studies on the cytotoxic and helper T cell responses to the H-Y antigen have supplied a wealth of information on the phenomenon of MHC restriction.[12] There is nevertheless still a great deal to be learned about the immunological processes which make up the chronic graft rejection reaction evoked by H-Y incompatibility. The reactivity to the H-Y antigen exhibited by female hosts varies widely between different strains among inbred mice and rats. In mice the rejection of H-Y incompatible skin grafts is largely confined to female mice of the H-2^b haplotype, female hosts of most other H-2 haplotypes being non-reactive and accepting otherwise syngeneic male skin for over 100 days.[12] At least two Ir genes, linked respectively to H-2 and H-3,[12] control reactivity in the mouse. Inbred rats behave slightly differently from inbred mice in their reaction to H-Y incompatible skin grafts. Females of all the rat strains so far examined reject intrastrain male skin grafts, although rejection is apt to be very protracted, and often takes over 100 days.[9,14] The rejection process is initiated quite early but may take months for its completion.[14,15] An interesting feature of the rejection of male skin grafts by female rats is the pronounced intragraft epidermal and dermal hyperplasia,[15] which seems to be a consequence of the immune response and whose possible significance is discussed below.

Another aspect of research on the H-Y antigen has a purely practical aim -- to sex embryos and to identify and separate X and Y bearing sperm. Since the antigen is similar in all mammalian species, methodology suitable for one species is theoretically applicable to others. The potential agricultural usefulness of H-Y antisera has been apparent since the first antibodies were raised in the early 1970s, and has been regularly, although perhaps unintentionally, underlined by the widespread use of the mouse sperm cytotoxicity test.[8,13] This test identifies H-Y antibody by its capacity to kill (in the presence of complement) those sperm which express H-Y and which are the putatively Y chromosome-bearing sperm. While the idea of sexing sperm is simple, the execution has so far fallen well short of expectations. Unfortunately H-Y serology has always been beset with technical difficulties. Most of the antisera, and even some monoclonal antibodies, have had low titres and low affinity. Antisera used at low dilutions are prone to react non-specifically, and it may be difficult to absorb out unwanted antibodies without also reducing the already low titre of H-Y antibodies. The difficulties of raising antibodies by conventional methods are compounded by the unsatisfactory nature of some of the assays. Many of the tests used for identifying the antigen on the cell surface not only have considerable "background noise", but are also difficult to interpret, so that the difference between putatively positive experimental results and negative controls tends to be small. Attempts to identify H-Y antigen on the cell surface have in some laboratories been superseded by ELISA tests using secreted H-Y antigen, in the form of Daudi culture supernatant[16] (Daudi is a human Burkitt lymphoma line and the cells secrete H-Y antigen into the medium), or bovine testicular extract[17] – the Sertoli cells in the testis produce large quantities of H-Y antigen. These tests in our hands have been much easier to interpret than tests using whole cells. The optimum reagent for ELISA tests may well turn out to be pure H-Y antigen-unfortunately this is not yet readily available in sufficient quantities.

These improved assays together with newer immunisation schedules for raising H-Y antisera promise some progress in this hitherto difficult area. In order to avoid the production of unwanted antibodies which subsequently require absorption, H-Y antisera are ideally raised within inbred strains. Most of the early work on H-Y antisera used inbred mice. Rats may however prove to be a more propitious species than mice for this purpose. It has recently been shown that high titre H-Y antibodies can regularly be raised in females of several inbred rat strains by the intrasplenic implantation of small portions of otherwise syngeneic male skin.[18] Such antisera need to be absorbed by female cells from the host strain in order to remove autoantibodies, which are quite commonly produced during H-Y immunisation schedules. In our experience antibody titres of 1:800 to 1:2000 after absorption with female cells can be raised quite regularly by this method.[14,18] While monoclonal antibodies may ultimately prove to be the optimum reagents, very large numbers of hybridomas need to be screened to obtain antibodies which do not also react with female cells; additionally, high affinity antibodies are rare among the male specific-antibodies. Affinity-purified polyclonal antisera are substantially cheaper and easier to prepare, and the multiple antibodies contained therein may together bind more efficiently than single antibodies.

The most challenging aspect of the work on H-Y remains the elucidation of its function. The H-Y gene product was originally held to be the sex determining gene, *i.e.* to be the testis determining gene in mammals.[19] This interpretation has attracted increasing criticism,[3,20] although the evidence refuting it has been

derived largely from animals with anomalies of sexual development, and may not be completely relevant to the normal situation.[21] Other evidence, which stems from the study of sex reversed mice, suggests that the H-Y antigen has an important role in spermatogenesis.[22] Quite apart from the genetic evidence which supports this contention, the large quantities of H-Y antigen on the Sertoli cells which envelop the maturing sperm also hint at a function for H-Y in spermatogenesis.[23] In addition to the above postulated functions of the H-Y antigen, it is also possible to adduce evidence which suggests a growth regulatory function. This idea is prompted by several observations:

(i) The very early (pre-implantation embryos[24,25]) and nearly ubiqitous[8] expression of the antigen is hard to reconcile with the above postulated functions of H-Y. It seems inherently unlikely that male and female embryos need to differ in any attribute at this stage except perhaps in their growth rate. Mammalian male foetuses grow faster than female foetuses and are on average heavier at birth (although it remains to be established whether the growth rate is faster throughout intrauterine life).

(ii) The male gonad in at least some (? all) mammalian species grows faster than the female gonad well before there is any histological evidence of the definitive sex.[26] Mittwoch has suggested that maleness may be the outcome of nothing more than the faster gonodal growth rate of males (this triggers the production of Sertoli cells and so ensures testicular differentation).[26] She suggests that there may be no need to postulate a sex determining gene on the Y chromosome other than the DNA sequences which enhance the growth of somatic cells in the gonodal rudiment.

(iii) The rejection of H-Y incompatible skin grafts is associated with an impressive hyperplasia of the graft epithelium and the dermal fibroblasts, this reaction apparently being a consequence of the immune reaction to the H-Y antigen.[15] If H-Y were a growth regulator, an immune response directed against it (and triggered by the artifactual grafting situation) might conceivably stimulate hyperplasia of the graft cells in much the same way as thyroid stimulating auto-antibodies cause hyperthyroidism by combining with the thyrotrophin receptor on thyroid epithelium.[27]

(iv) A growth regulator might theoretically function as an oncogene if it underwent a translocation.[28] Such an occurrence might underlie the high incidence of some otherwise very rare ovarian tumours (gonadoblastomas and 'dysgerminomas) in those female patients with XY dysgenesis who express the H-Y antigen but not in those without it.[29-31]

Male-female incompatibility as seen from the transplantation point of view provides a deceptively simple model system, in relation to which an impressive number of questions remain to be answered. How many genes contribute to the normal functioning of the system? On which chromosomes are they located? Does secreted H-Y antigen have the same function as cell membrane H-Y antigen? Is the transplantation antigen the same as the serologically identifiable antigen? Why do auto-antibodies so frequently result from H-Y immunisation schedules? If H-Y is a hormone, what is its receptor? How are the receptors distributed? Or is cell membrane H-Y itself a receptor? And what does the antigen do in non-mammalian species? This somewhat insignificant transplantation antigen provides a meeting point for disciplines as diverse as immunology, embryology, genetics, histology and pathology, as well as providing a point of entry to the practicalities of agriculture. Therein lies its fascination.

Barbara F. Heslop
Mark P. Bradley
Margaret A. Baird
Department of Surgery,
University of Otago Medical School,
Dunedin, New Zealand

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