dopaminergic NSB will support intracranial self-stimulation and may therefore play important roles in reinforcement (*16*). It is therefore possible that the learning deficits observed after lesions of these projections may be due to an inability of these animals to be reinforced by correct responses.

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Sex-Associated Antigens in Mice and Rats

Silvers and Yang (*1*) and Wachtel *et al.* (*2*) report on the potency and homology of male-specific antigens as shown by immunization or skin grafting of female recipients by male donors. Readers uninitiated to the field of transplantation immunogenetics may be left with several misleading impressions from the evidence presented. The finding that male lymphoid cells from some but not other strains of rats sensitize C57BL/6 female mice against subsequent C57BL/6 male skin grafts suggests molecular similarity or cross-reactivity between certain male antigens of rats and mice. Actually, male cells from only four of nine rat strains tested were effective in sensitizing female mice against male skin grafts. Moreover, the sensitization achieved was modest and highly variable. Despite this seemingly strong evidence of heterogeneity of male-specific antigens and of responsiveness to such antigens among rats and mice, Silvers and Yang imply that a single Y chromosome determined antigen is involved and is identical in all strains of rats and mice (*1*). Only the conclusion that these mammalian species may share similar H-Y or other male-specific antigens is justified by the evidence.

Wachtel *et al.* (*2*) found that (B10 × B10.BR)F_1 females rejected B10.BR ($H\text{-}2^k$) male grafts faster than ($H\text{-}2^b$) grafts, while F_1 hybrid male grafts yielded an intermediate median survival time. This confirms earlier reports (*3*) that the *H-2* locus itself or closely linked genes moderately influence the degree of responsiveness to male-specific antigen (or antigens). Wachtel *et al.* conclude that the potency or "immunogenicity of H-Y is influenced by the *H-2* locus," but this interpretation is questionable because the congenic strains tested display multiple genetic differences in the H-2 region. The C57BL/10 and B10.BR strains carry different alleles at the *Ir-1* and *Ss-Slp* loci and have significantly different serum complement levels (*4*). Moreover, the *Slp* locus governs a serum globulin antigen restricted to male mice. Much evidence favors the conclusion that *H-2* or associated *Ir* (immune response) genes regulate recipient responsiveness to particular immunogens (*5*). The molecular structure of the H-Y immunogen should remain unchanged, unless antigenic modulation occurs in the foreign environment of the recipient.

The major objection to the male → female experimental design employed by Silvers and colleagues is that one could be dealing with an autosomal gene (or genes) with products sex-limited to males. Moreover, the disparate hormonal environment of males and females affects immune responsiveness as well as gene expression (*6*). Females generally give more vigorous immune responses than males. Actually, male specific cellular antigens of both autosomal and H-Y origins may exist (*7*), a supposition supported by the recent finding of a mouse sperm-specific antigen governed by a *T*-locus gene (*8*). Use of the parental → reciprocal F_1 hybrid male design not only avoids objections to the male → female design, but provides a test for allelism and relative immunogenicity of both *H-X* and *H-Y*. With this approach, we repeatedly found both allelism and nonidentity of products of *H-X* and of *H-Y* loci in mice (*7*, *9*) and in rats (*10*). In our most extensive study in mice (*7*), different X-linked incompatibilities were stronger than the Y-linked ones in relation to otherwise identical male recipients. The existence of multiple *H-X* alleles in mice has been confirmed and extended by D. W. Bailey (*11*), although Barnes (*12*) has not found expected H-X associated skin graft rejection in certain F_3 male → F_1 male hybrid experiments. We noted (*7*), in a report not cited by Silvers *et al.* (*1*), that reactions to these weak sex-associated antigens can be greatly influenced by such variables as prior immunization, graft dosage, and the particular allelic combination. Indeed, "X-Y barriers in these male to male combinations are so weak that many large grafts remained viable for nearly the entire life span of the recipients (*7*, p. 27).

The failure to confirm H-Y allelism, reported by Wachtel *et al.* without supporting data [reference 7 in (*2*)], is hardly acceptable, especially when extensive experiments with numerous controls and subtleties are involved. It is easy to get long-term acceptance of skin grafts across the weak H-Y (or

H-X) barrier, especially in F_1 hybrid male recipients, simply by using thoracic skin grafts of moderate size (8 to 12 mm in diameter). To obtain effective immunization and test graft rejection requires far more attention to details.

We are not dealing merely with the trivial issue of how weak a histocompatibility difference can be before its detection becomes difficult. The issue of the existence and extent of H-Y allelism is important because it bears on the question of evolutionary conservatism of Y-determined characters. If the *H-Y* locus (or loci) were functionally immutable (which our evidence says is not so), it would be the only known histocompatibility locus having this property. By contrast, the highly variable recipient responsiveness to sex-associated antigens, male-specific or otherwise, has been conclusively demonstrated by numerous investigators.

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In the report of Silvers and Yang (*1*) it was demonstrated that male lymphoid cells from some but not all strains of rats can sensitize C57BL/6 (B6) female mice against B6 male skin grafts. For example, the median survival time (MST) of B6 male skin grafted onto B6 females sensitized with Lewis rat female cells was 22.2 days (negative control), and the MST following sensitization with CBA mouse male cells was 14.6 days (positive control). When recipients of the same strain were injected with Lewis male cells, the MST was 14.5 days. This can hardly be considered a modest degree of sensitization.

Table 1. Results of grafting skin from parental strain males to various male F_1 hybrid recipients.

Experiment		Recipient	Donor	Number of grafts surviving after 175 days/ number grafted
1	A	♂ (♀B6 × ♂A/J)F_1	♂A/J	9/22
	B	♂ (♀B6 × ♂A/J)F_1	♂B6	23/23
	C	♂ (♀A/J × ♂B6)F_1	♂A/J	7/7
	D	♂ (♀A/J × ♂B6)F_1	♂B6	6/6
2	A	♂ (♀B6 × ♂A)F_1	♂B6	16/16*
	B	♂ (♀B6 × ♂C3H)F_1	♂B6	3/3
	C	♂ (♀B6 × ♂T6)F_1	♂B6	4/4*
	D	♂ (♀B6 × ♂GR)F_1	♂B6	4/4
	E	♂ (♀B6 × ♂AKR)F_1	♂B6	4/4*

* These mice were observed until their death. In no case did a rejection occur, and in 19 cases the grafts survived for more than 375 days.

It was pointed out by Silvers and Yang that five of the nine rat strains were ineffective in sensitizing against the male antigen, and that this cannot be explained by differences in the Y antigen. For example, BN male cells were ineffective while Fischer cells were highly effective. If Hildemann's thesis were correct, the cells of male (♀ BN × ♂ Fischer)F_1 hybrids should be effective in sensitization (whether the male specific antigen is determined by the Y chromosome or an autosome), when in fact they were completely ineffective. Silvers and Yang therefore concluded that those cells which were ineffective in sensitization may not have survived long enough in the B6 recipients to induce sensitization because their Ag-B antigens elicited stronger rejections than the Ag-B^1 antigens of those strains that were effective.

The report of Wachtel *et al.* (*2*) is not related to earlier reports of an *H-2*–linked *Ir* gene which influences male skin rejection (*3*). All of the recipients in the experiment to which Hildemann refers were genetically identical females of the (B10 × B10.BR)F_1 generation. The observed differences in skin graft rejection therefore cannot be attributed to *Ir* gene differences among the recipients or to "the disparate hormonal environment of males and females."

In the experiments we reported (*2*), we made no attempt to identify the site or sites within the *H-2* complex which might be responsible for the observed effect. We are now exploring this problem by the use of recombinant *H-2* haplotypes, but as yet we have no definitive data. A recent report by Micková and Iványi, however, suggests that two sites within the *H-2* complex (one in the *K* end and the other in the *Ss-Slp* region) influence the immunogenicity of the Θ-C3H antigen (*4*).

We alluded to experiments in which we failed to confirm the report of Hildemann and Cooper (*5*) on allogeneic differences in the Y antigen [reference 7 in (*2*)]. The data from these experiments are shown in Table 1. Experiment 1 was conducted by D. L. Gasser at the University of Pennsylvania and experiment 2 was conducted by S. S. Wachtel at the Memorial Sloan-Kettering Cancer Center. In experiment 1, all grafts were 3 by 3 mm pieces of ear skin, whereas in experiment 2 tail skin grafts 2 by 2 mm to 2 by 4 mm were used.

In experiment 1A, we confirmed Bailey's report (*6*) of an X-linked histocompatibility locus at which strains B6 and A/J differ. On the basis of Hildemann and Cooper's report (*5*), however, we would have expected rejections to occur in experiments 1B and 2A. If any of the strains C3H, T6, GR, or AKR possesses a Y-linked allele that differs from that of B6, we would have expected rejections in the appropriate experiment from 2B through 2E. On the basis of these data, we stated that we attempted to confirm the findings of Hildemann and Cooper but were unable to do so.

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