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Effect of *Y* chromosome and *H-2* complex derived from Japanese wild mouse on sperm morphology

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Summary

Segregation of sperm abnormality level and *H-2* haplotypes was investigated in F_2 hybrid males obtained from reciprocal crosses involving two B10.congenic strains carrying *H-2* and the *Y* chromosome of Japanese wild mice: B10.MOL-OHM ($H\text{-}2^{wm4}$, 23·1% of sperm abnormalities) and B10.MOL-OKB ($H\text{-}2^{wm8}$, 11·1% of sperm abnormalities). In both types of crosses mean levels of abnormal spermatozoa were significantly higher for males typed as $H\text{-}2^{wm4}/H\text{-}2^{wm4}$ than for heterozygous $H\text{-}2^{wm4}/H\text{-}2^{wm8}$ or homozygous $H\text{-}2^{wm8}/H\text{-}2^{wm8}$. These results suggest that the gene for high sperm abnormality is linked to *H-2* complex of the B10.MOL-OHM strain.

1. Introduction

The spermatozoan head abnormalities in adult male mice are known to be determined by *Y*-linked factors and a small number of autosomal genes (Krzanowska, 1969, 1972, 1976). Two possible locations of these autosomal genes were chosen for investigation: the central portion of chromosome 4, and the proximity of *H-2* complex on chromosome 17. Both of these regions show homology to the sex-related sequences of *Y* chromosome (Kiel-Metzger & Erickson, 1984). In experiments involving two inbred strains: CBA/Kw (3·8% of abnormal sperm), and C57BL/Kw (21·9% of abnormal sperm) it was found that one of autosomal genes coding for sperm abnormality level is linked with *Mup-1* (major urinary protein-1) locus on the 4 chromosome (Styrna, submitted to *Genetical Research*).

The purpose of the present study was to investigate the relation between sperm abnormalities and *H-2* haplotypes.

2. Materials and methods

(i) *Mice*

B10.H-2 congenic strains were produced by introducing the *H-2* complex of Japanese wild mice, *Mus musculus molossinus*, into the C57BL/10J (B10) genetic background and have been maintained in the Department of Cell Genetics, Mishima (Moriwaki *et al.* 1982). B10.standard congenic strain was also used for crosses.

(ii) *Crosses*

Two *H-2* congenic strains, which differ considerably in the proportion of sperm head abnormalities: B10.MOL-OHM ($H\text{-}2^{wm4}$) and B10.MOL-OKB ($H\text{-}2^{wm8}$) were crossed reciprocally to produce F_2 progeny. To investigate the interaction of *H-2* haplotypes and *Y* chromosome, F_2 progeny derived from crosses between B10.MOL-OHM and B10.MOL-OKB females with B10.($H\text{-}2^{b}$) males were also used. When denoting crosses, the strain of the female is written first.

(iii) *Serological tests*

H-2 typing was carried out by the micro-cytotoxicity test, using flat-type titration plates described by Shiroishi *et al.* (1981). Appropriate antisera to *H-2* antigens were produced in the Department of Cell Genetics, Mishima, according to the immunization schedules described in the NIH catalogue (Snell, 1968).

(iv) *Sperm analysis*

After typing for *H-2* the males were killed by cervical dislocation and smears of sperm from vasa deferentia

Table 1. *Proportion of sperm head abnormalities in B10.congenic strains carrying* H-2 *haplotypes and Y chromosome of Japanese wild mice*

Genotype of males	Mean level of abnormal spermatozoa (%)	Angle ± S.D.
Standard B10.	20·2	26·68 ± 1·70
B10.MOL-TEN 1	14·0	21·97 ± 0·58*
B10.MOL-TEN 2	13·3	21·36 ± 2·13*
B10.MOL-NSB	24·5	29·65 ± 1·47
B10.MOL-OHM	23·1	28·71 ± 1·66
B10.MOL-OKB	11·1	19·37 ± 2·09*
B10.MOL-YNG	10·8	19·21 ± 0·52*
B10.MOL-MIS	14·5	22·33 ± 2·31*

* Significantly different from B10, $P < 0{\cdot}01$.

were stained with eosin Y and examined under 63 × objective. 400 spermatozoa from each male were counted and the percentage of abnormal heads was calculated. Spermatozoa were analyzed according to the scheme described previously (Krzanowska, 1976). For statistical treatment, the percentages of abnormal spermatozoa were transformed to angles (Snedecor, 1955), and compared by a Kruskall–Wallis u-test.

3. Results

(i) *Sperm abnormalities in B10.H-2 congenic strains*

The total percentage of sperm head abnormalities in B10.congenic mice carrying *H-2* haplotypes and the *Y* chromosome of Japanese wild mice is presented in Table 1. Data referring to B10.standard strain are also included for comparison. They show that the males of two congenic strains B10.MOL-NSB and B10.MOL-OHM are characterized by high percentages of abnormal spermatozoa similar to that of B10.standard strain. The other investigated strains possess lower levels of abnormal spermatozoa. All types of abnormalities present in B10.H-2 congenic males were typical to those found in C57BL (B10).

(ii) *Segregation of sperm abnormalities and H-2 haplotypes*

To investigate the genetic basis of the variation observed in sperm abnormality level among B10.H-2 congenic strains, we produced an F_2 generation from reciprocal crosses between B10.MOL-OHM and B10.MOL-OKB strains. As expected, three phenotypic classes of *H-2* appeared among progeny: *wm4/wm4*, *wm4/wm8* and *wm8/wm8*. In both types of cross hybrid males typed as homozygotes *wm4/wm4* showed a significantly higher level of sperm abnor-

Table 2. *Influence of the* H-2 *haplotypes and of the Y chromosome on the percentage of abnormal spermatozoa*

Type of cross and *H-2* haplotype of F_2 progeny	Source of *Y* chromosome	No. of males	Mean proportion of abnormal spermatozoa (%)	Angle ± S.D.
(B10.MOL-OHM × B10.MOL-OKB)				
wm4/wm4	OKB	20	22·3	28·17 ± 1·30*
wm4/wm8	OKB	20	9·3	17·65 ± 1·88
wm8/wm8	OKB	20	9·8	18·18 ± 1·40
(B10.MOL-OKB × B10.MOL-OHM)				
wm4/wm4	OHM	20	22·9	28·51 ± 1·83*
wm4/wm8	OHM	20	10·8	19·06 ± 1·93
wm8/wm8	OHM	20	11·3	19·55 ± 1·65
(B10.MOL-OHM × B10)				
wm4/wm4	B10	20	28·4	32·16 ± 3·06†
wm4/b	B10	20	21·6	27·68 ± 1·91
b/b	B10	20	23·2	28·75 ± 2·13
(B10.MOL-OKB × B10)				
wm8/wm8	B10	20	21·3	27·45 ± 1·86‡
wm8/b	B10	20	18·2	25·17 ± 2·59
b/b	B10	20	22·4	28·17 ± 2·84

* Significantly different from $H\text{-}2^{wm4}/H\text{-}2^{wm8}$ and $H\text{-}2^{wm8}/H\text{-}2^{wm8}$ brothers, $P < 0{\cdot}001$.
† Significantly different from $H\text{-}2^{wm4}/H\text{-}2^{wm4}$ males possessing the OHM or the OKB *Y* chromosome, $P < 0{\cdot}001$.
‡ Significantly different from $H\text{-}2^{wm8}/H\text{-}2^{wm8}$ males possessing the OHM or the OKB *Y* chromosome, $P < 0{\cdot}001$.

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malities than heterozygotes *wm4/wm8* and homozygotes *wm8/wm8* ($P < 0{\cdot}001$) indicating that an autosomal factor coding for a high level of sperm abnormality is linked to the $H\text{-}2^{wm4}$ complex of the B10.MOL-OHM strain and behaves as a simple unit controlled by a single recessive gene (Table 2).

(iii) *Influence of B10-derived Y chromosome on sperm abnormality*

The data collected from the crosses between B10.MOL-OHM and B10.MOL-OKB females with B10 males indicated a strong effect of the *Y* chromosome on the sperm morphology. Regardless of *H-2* haplotype, in all F_2 hybrid males high percentages of abnormal spermatozoa are recovered. The proportions of abnormalities were similar to that of B10 strain. However, the presence of a gene for high sperm abnormalities derived from B10.MOL-OHM strain additionally increased the level of abnormalities in $H\text{-}2^{wm4}/H\text{-}2^{wm4}$ homozygotes (statistically significant from males possessing $H\text{-}2^{wm4}/H\text{-}2^{wm4}$ haplotypes and *Y* chromosome of B10.MOL-OHM or B10.MOL-OKB strain, $P < 0{\cdot}001$, Table 2).

4. Discussion

Laboratory strains of mice are known to differ in the percentages of spermatozoa with abnormal head shape. The reason for sperm abnormalities is not clear. Perhaps they are the results of naturally occurring mistakes in the differentiation process (Bruce *et al.* 1974). On the other hand, there is evidence indicating strong genetical control of sperm abnormality level. The results of the F_2 and the backcrosses suggest that both autosomal and *Y*-linked factors are involved (Krzanowska, 1969, 1972, 1976). In this connexion we present data suggesting that one such gene controlling the high proportion of spermatozoa with deformed head is located close to the *H-2* complex in the B10.MOL-OHM strain.

The presence of genes affecting spermatogenesis and linked to the MHC (major histocompatibility complex) in the mouse is well known (Gill III *et al.* 1983). They are mostly related to the *T/t* locus (Bennett, 1975). The mechanism by which the *T/t* genes act is not known. At the level of the gene products, they are associated with enzymic defects in the sperm and in the male germ cells. Among the B10.H-2 congenic strains used in this study the presence of *T/t* alleles was not found (T. Shiroishi, personal communication), so specific sperm defects might be caused by a separate gene linked to the *H-2* complex of the B10.MOL-OHM strain. On the other hand, in the crosses where the *Y* chromosome was of *Mus musculus molossinus* origin a significantly lower proportion of abnormal sperm was observed than in matings with B10 males. This suggests that the B10-derived *Y* chromosome must differ from the *Y* chromosome of *Mus musculus molossinus* in gene(s) causing sperm abnormality. The results presented here confirm the earlier finding of Krzanowska (1976) indicating an important role for the *Y* chromosome in spermatogenesis.

In summary, we can conclude that production of morphologically abnormal sperm is controlled by at least three genes: one linked to the *Mup-1* locus on chromosome 4 (Styrna, submitted to *Genetical Research*), one linked to the *H-2* complex of chromosome 17 and one linked to the *Y* chromosome.

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