An Aspermia Rat Mutant (*as/as*) with Spermatogenic Failure at Meiosis

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ABSTRACT. A newly identified aspermia rat mutant was investigated on testicular histology and mode of inheritance of the defect. Average testis weight of mutants was about one-third of that of phenotypically normal males. Spermatogenesis was interrupted at meiosis. Pachytene spermatocytes significantly decreased in number. Secondary spermatocytes and few round spermatids were seen, but no elongated spermatids and sperms were observed. A large basophilic inclusion-like body existed in the cytoplasm of late pachytene spermatocytes. Genetic analysis revealed an autosomal recessive transmission of the defect. Aspermia (*As*) was designated for the locus.—**KEY WORDS:** aspermia, meiosis, mutant, rat.

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Spermatogenesis is a complex process including mitosis, meiosis, and spermiogenesis sustained by hypothalamus-pituitary-testicular axis and harmonized local interactions of spermatogonia, Sertoli cells, and Leydig cells [15, 22]. Mutant models blocked at specific stages of the process can provide useful tools to elucidate genetic mechanisms regulating spermatogenesis.

We have recently identified an aspermia rat mutant. The mutant manifests the disruption of spermatogenesis at early meiosis, associating with a large inclusion-like body in the cytoplasm of late pachytene spermatocytes. Secondary spermatocytes and few round spermatids were observed, but no elongated spermatids and sperms were seen. The defect was detected exclusively in testes of the mutant, whereas sibling females were fertile. Meiotic failure with an inclusion-like body in pachytene spermatocytes has not been reported in rodents. This paper describes the testicular histology and mode of inheritance of the disorder.

MATERIALS AND METHODS

Animals: Rats with bilateral small testes were detected in 1986 in an inbred strain (TT line) having been maintained in the Institute. Since the rats were infertile, phenotypically normal male litters were mated with sibling females and successive inbreedings within litter mates of the mutant have been made. Animals were kept under a conventional condition and were fed a commercial diet (EM; Oriental Yeast, Co., Ltd., Tokyo) with tap water *ad libitum*.

Morphological observations: Groups of 5 mutants and phenotypically normal male litters at 25 weeks of age were weighed and sacrificed by decapitation. Testes, epididymides, seminal vesicles, ventral prostate, preputial glands, adrenal glands, pituitary gland, and thyroid glands were removed and weighed immediately. Testes were fixed in Bouin's solution, dehydrated in ethanol, and embedded in paraffin wax. Middle portion of testes was circularly sectioned at 6 μ m, and stained with haematoxylin and eosin for microscopic observation. Elements of germinal epithelium were quantitatively analyzed in randomly selected 4 testes in each group. Diameter of seminiferous tubules, and numbers of Sertoli cells, spermatogonia, early spermatocytes (preleptotene to zygotene) and pachytene spermatocytes were estimated in 36 tubules under a microscope. Stages of seminiferous epithelial cycle of each tubule were determined according to Perey *et al.* [19] and classified into two groups, stages XIV and I–VI, and stages VII–XIII (18 tubules respectively). Values were averaged and compared between the mutant and phenotypically normal male litters.

Genetic analysis: Since mutants were sterile, sires and dams that produced mutants, designated as carriers, were used for genetic analysis. Carriers in the line were out-crossed with rats in an unrelated line (IS line). Incidence of the mutant defined by small testes was observed in the F₁, F₂, and backcross generations. In the F₂ and backcross generations, litters produced by carrier F₁ males and females were used for statistical analysis.

RESULTS

Gross anatomy of the male reproductive tract revealed no abnormality in the mutant. The average body weight of mutants did not differ from that of phenotypically normal litters (Table 1). Weights of testes and epididymides were about one-third and two-thirds of those of phenotypically normals, respectively. The average weights of seminal vesicles, ventral prostate, preputial glands, pituitary gland, and thyroid glands did not differ from those of phenotypically normal rats.

In testes of mutants, diameter of seminiferous

tubules decreased (Fig. 1b). Spermatogenesis was interrupted at meiosis. Nearly all seminiferous tubules contained germ cells up to pachytene spermatocytes. First meiotic divisions were seen. Secondary spermatocytes, most of which stopped their division, existed in tubules at stages XIV and I-VI (Fig. 1d). Multinucleated giant cells were frequently seen in the tubules. Multinucleated giant cells were frequently seen in the tubules. Few round spermatids were recognized, but no elongated spermatids and sperms were seen. A large basophilic inclusion-like body, which was not seen in testes of normal rats, occurred in the cytoplasm of pachytene sper-

Table 1. Body and organ weights

	Phenotypically normal	Mutant
Body weight (g)	491.6± 21.4	456.3±42.5
Testes (mg)	3009.6± 89.1	971.8±38.9*
Epididymides (mg)	1603.4±102.5	1192.3±77.5*
Ventral prostate (mg)	643.0± 86.6	538.5±46.1
Preputial glands (mg)	272.4± 14.3	267.5±13.1
Seminal vesicles (mg)	1644.0±141.3	1515.3±65.9
Pituitary gland (mg)	10.4± 0.8	10.5± 1.1
Adrenal glands (mg)	63.6± 10.2	64.9± 1.3
Thyroid glands (mg)	28.9± 4.5	28.0± 2.3

Each value indicates mean±SD. *, P<0.05.

Fig. 1. Cross-sections of the testes of phenotypically normal (a) and mutant (b-d) rats. Active spermatogenesis is seen in phenotypically normal rats (a). In the mutant testes, seminiferous tubules decreased in diameter and contained multinucleated giant cells (arrowheads) and large vacuoles (asterisk). No elongated spermatids and sperms are seen (b). A large inclusion-like body (arrowheads) is seen in the cytoplasm of late pachytene spermatocytes (c). Secondary spermatocytes (asterisks) cease cell division (d). No inclusion-like body occurs in early pachytene spermatocytes (arrowheads). Magnification, $\times 150$ in a and b, $\times 600$ in c and d.

matocytes (Fig. 1c). The inclusion-like body was not observed in early pachytene spermatocytes (at stages I-VI), appeared abruptly in late pachytene spermatocytes (at stages VII-XIII), and disappeared in secondary spermatocytes. Sertoli cells showed no abnormality in their nuclear shape and location. Large vacuoles were occasionally seen in the tubules. Leydig cells in the interstitium seemed to be hyperplastic. Quantitative estimation in numbers of Sertoli cells, spermatogonia, and early spermatocytes revealed no significant difference between mutants and phenotypically normal litters (Table 2). The number of pachytene spermatocytes was, however, significantly lower than that of phenotypically normal rats.

The defect was inherited as an autosomal recessive. Incidence of mutants in the line was 43% (43 out of 100) in the sibling males of 22 litters that produced mutants. This value indicates two possibilities in mode of inheritance of the defect; X chromosome transmission and autosomal recessive transmission in which appearance of defect is limited to males. To ascertain which is the case, phenotypically normal carrier males and females in the line were out-crossed reciprocally with an unrelated line. In the F1 generation derived from carrier males and females (crosses a-1 and b-1 in Table 3), the mutant did not appear. This finding excludes the possibility of X chromosome transmission of the defect. When

the defect is controlled by an autosomal recessive locus, carrier males are heterozygous for the locus and carrier females include heterozygotes and recessive homozygotes. The F1 rats include heterozygotes and dominant homozygotes in this case. Therefore, incidences of the mutant in F2 and backcross generations were analysed in offspring from matings between proven F1 carriers and matings of proven carrier males in the line with proven carrier F1 females, respectively. The mutant appeared in the F2 generations derived from carrier males and females in the parental line (crosses a-2 and b-2) and in the backcross generation derived from parental carrier males (cross a-3). The incidence of the mutant was consistent with the expected values for an autosomal recessive transmission.

DISCUSSION

The mutant was characterized by spermatogenic failure during meiosis, associating with an inclusion-like body in the cytoplasm of late pachytene spermatocytes. Secondary spermatocytes were seen, but most of them stopped their cell division. Few round spermatids but no elongated spermatids and sperms were seen. The defect was limited to the male gonad, whereas sibling females were fertile. We designated the locus responsible for the defect

Table 2. Germinal elements of the mutant

	Phenotypically normal	Mutant
Diameter of seminiferous tubules (μm)	251 $\pm$ 22	180 $\pm$ 28*
No. of Sertoli cells	17.1 $\pm$ 4.0	18.9 $\pm$ 4.3
No. of type A spermatogonia	2.1 $\pm$ 1.1	2.3 $\pm$ 1.6
No. of intermediate to type B spermatogonia	7.0 $\pm$ 5.8	9.1 $\pm$ 8.0
No. of early spermatocytes	45.9 $\pm$ 10.9	43.1 $\pm$ 11.7
No. of early pachytene spermatocytes	52.7 $\pm$ 5.5	37.5 $\pm$ 6.7*
No. of late pachytene spermatocytes	67.6 $\pm$ 9.0	34.9 $\pm$ 9.9*

Each value indicates mean $\pm$ SD. *, $P < 0.05$.

Table 3. Segregation of aspermia males in offspring from crosses with an unrelated (IS) line

Cross		Observed	Expected for single recessive		
		Aspermia/Males(%)	Aspermia/Males(%)		χ^2
(A-1)	IS females x carrier males	0/7 (0)	0/7 (0)		0
(A-2)	Carrier F1 females x Carrier F1 males	12/52 (23.1)	13/52 (25.0)		0.103
(A-3)	Carrier F1 females x Carrier males	9/37 (24.3)	9.3/37 (25.0)		0.009
(B-1)	Carrier females x IS males	0/8 (0)	0/8 (0)		0
(B-2)	Carrier F1 females x Carrier F1 males	14/55 (25.5)	13.8/55 (25.0)		0.007

Crosses A-1-3 derived from carrier males in the line and crosses B-1 & 2 from carrier females.

as *As* representing aspermia.

The *As* locus is unique in that the effect is limited to the male gonad and the meiotic disorder is associated with an inclusion-like body in pachytene spermatocytes. Large numbers of mutant models representing spermatogenic failure have been described in mice [2, 6] and rats [8, 14–18, 24]. However, there are only two cases for meiotic disorder, *Sks* in mice and *Grc* in rats. The *Sks* locus exerts pleiotropic effects as vertebral fusion, disruption of meiosis in spermatogenesis, and sterility in females by unknown cause [8]. The *Grc* loci cause meiotic disruption in males and decreased fertility in females closely linked with depressed body growth and major histocompatibility complex [4, 5, 21].

Histochemical and electron microscopical observations on the *As* mutant testes [1] revealed that the inclusion-like body was composed of degenerating ribosomes including a large amount of ribonucleic acid. Ultrastructure of spermatogonia and early spermatocytes was normal and tight junctions between Sertoli cells were conserved. Noguchi *et al.* [17] investigated testicular histology and plasma FSH, LH, and testosterone levels of mutants from weaning to adult and revealed that the defect appears from the first cycle of spermatogenesis at weaning when hormone levels did not differ from those of phenotypically normal litters at corresponding age. Thereafter, FSH and LH levels increased significantly and testosterone level decreased slightly. Intra-testicular administration of testosterone did not repair the spermatogenesis in the mutant. These data exclude hypopituitary-hypogonadism from the cause. The defect in intratesticular elements, spermatocytes *per se* or its interactions with Sertoli cells, may be attributed to the pathogenesis. During meiosis of spermatogenesis, temporally regulated active gene expression and protein synthesis occur in both spermatocytes and Sertoli cells [3, 18, 20, 23, 25]. Further investigations such as an analysis of aggregation chimera of mutants and normal rats [16] are needed to define the primary defect.

In summary, we identified an aspermia rat mutant with meiotic failure transmitted by an autosomal recessive locus (*As*). The meiotic failure in the mutant is unique in that a large inclusion-like body occurs in the cytoplasm of late pachytene spermatocytes. The mutant will provide a useful model to elucidate the genetic mechanism controlling meiosis of spermatogenesis.

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