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Research Articles

A morphometric study of spermatogenesis in the testes of mice of a senescence accelerated strain

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Abstract. The morphometric parameters of spermatogenic cells in a mouse strain prone to accelerated senescence (SAM-P), a novel murine model of spontaneously promoted aging, were compared with those of a SAM resistant strain (SAM-R) after birth until 40 weeks (mean life span of SAM-P). A mixture of gonocytes and spermatogonia were present in the testis in 1-week-old mice, and no gonocytes were observed in 2-week-old mice. At 6 weeks of age, the absolute number of spermatogonia in SAM-P was 27% greater than that in SAM-R, whereas the cell number in 40-week-old SAM-P was 17% less than in SAM-R. Primary spermatocytes were first observed in 3-week-old animals, and the cell numbers in SAM-P at 3, 5 and 6 weeks were 78%, 31% and 25%, respectively, greater than in SAM-R, whereas the cell number in SAM-P at 40 weeks was 30% less than SAM-R. Round spermatids were first observed in all SAM-P at 4 weeks old, but 20% of SAM-R had no spermatids and the rest had only a few. At 5 and 6 weeks old, the absolute numbers of round spermatids in SAM-P were 38% and 41%, respectively, greater than in SAM-R, whereas the cell number in 40-week-old SAM-P was about 34% less than SAM-R. These results indicate that testicular maturation begins at an earlier age in SAM-P than SAM-R. Furthermore, at the age of 40 weeks signs of testicular deterioration are evident in SAM-P mice only.
Key words. Spermatogenesis; senescence accelerated mouse; morphometry; Sertoli cells; Leydig cells.

The senescence accelerated mouse strains (SAM) are inbred strains of AKR mice that were bred and established by Takeda et al.[1]. There are two related SAM strains: SAM prone (SAM-P) and SAM resistant (SAM-R). The SAM-P has a shortened life span and early manifestation of various signs of senescence (severe loss of activity, hair loss and lack of hair glossiness, skin coarseness, periophthalmic lesions, increased lordokyphosis of the spine). The SAM-R strain, used as the control, undergoes the normal process of development and aging. The mean life span of SAM-P (9.7 months; approximately 40 weeks) is about 27% shorter than that of SAM-R (13.3 months)[1]. Although there have been many pathological, biochemical and physiological studies on the age-related changes in SAM-P with advancing age[1-9], there are insufficient data on growing SAM-P. The male reproductive characteristics of this mouse model have yet to be described. The present investigation focuses on spermatogenesis in growing SAM-P and on the age-related deterioration of the ability to produce spermatozoa in aged SAM-P.

Materials and methods

Animals. Male SAM-P (SAMP1//Fap strain) mice and SAM-R (SAMR1/Fap strain) controls were kept with access to tap water and a standard diet (CE-2, Clea Co. Ltd., Tokyo, Japan) ad libitum in an air (22 ± 2 °C, RH $50 \pm 5\%$) and light (12L/12D) conditioned room.

Histology and morphometry. All mice were anesthetized by ether inhalation and sacrificed by decapitation. The testes were removed and weighed. The right testis was fixed by immersion in Bouin's fixative, which causes little shrinkage[10]. Following deyhdration in ethanol, the testis was embedded in paraffin. Serial sections (5 μm in thickness) were cut orthogonally to the long axis from the top to the bottom, and stained with periodic acid-Schiff strain and hematoxylin[10]. In both strains, ten male mice aged 2, 3, 4, 5, 6, 10, 20 and 40 weeks were selected randomly and used for morphometric analyses. Ten sections per testis and ten fields per section were selected at random, and then ten fields per section were photographed to count the cell numbers and to measure the sectional area of the seminiferous tubules and the Leydig cells[11]. Germ cells were classified into three categories (spermatogonia, all classes of primary spermatocytes, and round spermatids); no attempt was made to count elongated spermatids[12,13]. Sertoli cell numbers were estimated using the equation of Berndston and Thompson[14].

The numerical densities (cells per cm^3 of testis) of germ, Sertoli and Leydig cells were obtained according to the

following equations[15]: $ND = NA/(D + T\text{-}2h)$, where ND is the numerical density, NA the number of nuclei per unit area of section, D the average diameter of the cell nucleus, T the section thickness, and h the smallest recognizable cap section of the cell nucleus ($0.1 \times D$). The absolute numbers (cells per testis) of germ, Sertoli and Leydig cells were obtained by multiplying their numerical density by the weight of the testis; the specific gravity of the testis is 1.038 in the SAM (unpubl. data). Sectional areas of seminiferous tubules and Leydig cells were measured using a semiautomatic area meter (X-Plan 360-d, Ushikata Co. Ltd., Tokyo, Japan). The volume densities (volume per cm^3 of testis) of seminiferous tubules and Leydig cells were calculated, and then the absolute volumes (volume per testis) of seminiferous tubules and of Leydig cells were determined.

Statistical analysis. Analysis of variance was carried out with the StatView IV program using a Macintosh computer. Differences with a probability of $p < 0.05$ were considered significant. All data are shown as means $\pm$ SD.

Results and discussion

The body weight of both strains increased rapidly after birth until 8 weeks of age, and thereafter remained constant until 40 weeks (fig. 1a). The body weight of SAM-P mice was significantly ($p < 0.05$) smaller than that of SAM-R. As shown in figure 1b, sigmoidal growth curves for the testis were demonstrated throughout development in both strains. The SAM-P mice exhibited accelerated growth of the testis compared with the SAM-R mice, and the testis weights of SAM-P were larger than those of SAM-R ($p < 0.05$).

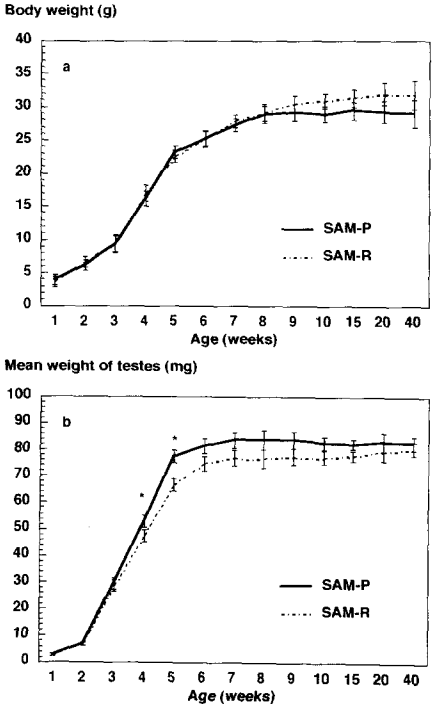

Figure 1. Age-related changes in *a* body weight and *b* testis weight in SAM-P and SAM-R. Testis weight is the average fresh weight of paired testes. Ten mice were used at each age. *$p < 0.05$ vs each SAM-R.

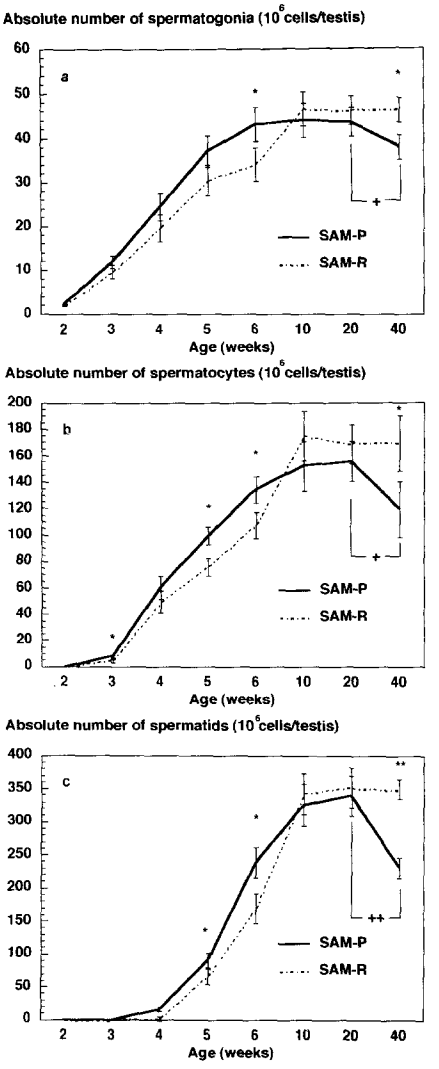

Figure 2. Age-related changes in the absolute numbers of *a* spermatogonia, *b* primary spermatocytes and *c* round spermatids in SAM-P and SAM-R mice. *$p < 0.05$ and **$p < 0.01$ vs each SAM-R. +$p < 0.05$ and ++$p < 0.01$ between 20 and 40 weeks, respectively, in SAM-P mice.

In the testes of 1-week-old mice, both gonocytes and spermatogonia were present. No gonocytes were observed in 2-week-old mice. Changes in the absolute number of spermatogonia in SAM-P and SAM-R are shown in figure 2a. At 6 weeks of age, the cell number in SAM-P was 27% greater than in SAM-R, whereas at 40 weeks the cell number SAM-P was 17% less than in SAM-R. Primary spermatocytes were absent in 2-week-old animals, and were present after 3 weeks. The cell numbers in SAM-P were greater than in SAM-R at 3, 5 and 6 weeks (78%, 31% and 25%, respectively). How-

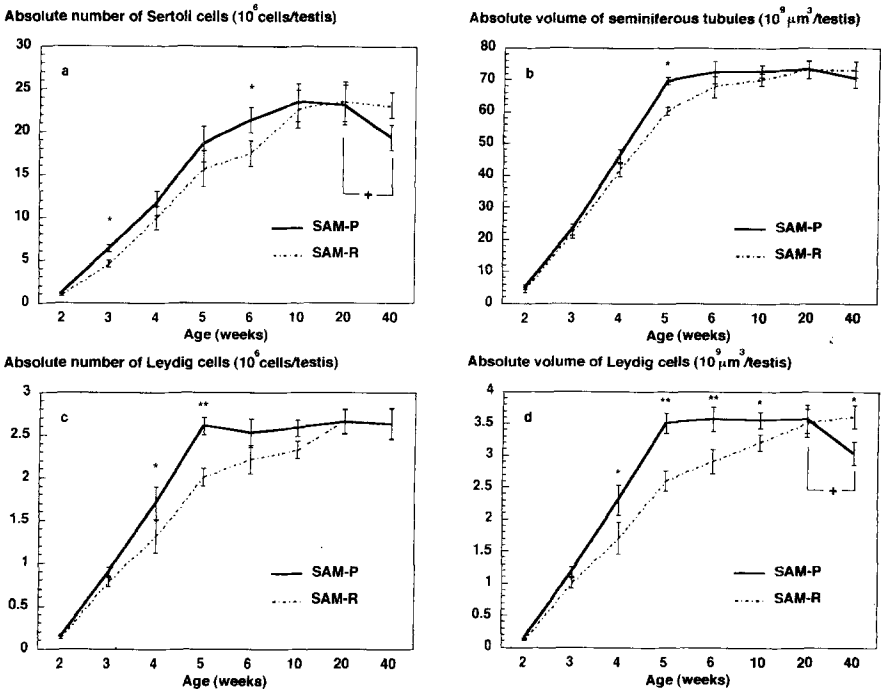

Figure 3. Age-related changes in the absolute numbers of *a* Sertoli cells and *c* Leydig cells, and of the absolute volume of *b* the seminiferous tubules and *d* Leydig cells in SAM-P and SAM-R mice. *$p < 0.05$ and **$p < 0.01$ vs each SAM-R. +$p < 0.05$ between 20 and 40 weeks in SAM-P mice.

ever, the cell number in SAM-P at 40 weeks of age was 30% less than in age-matched SAM-R (fig. 2b). Round spermatids were observed in all SAM-P at 4 weeks of age, but two of the 10 control mice had no spermatids and the remaining eight had only a few. The numbers of spermatids in SAM-P at 5 and 6 weeks of age were 38% and 41%, respectively, greater than in SAM-R, whereas the cell number in 40-week-old SAM-P was 34% less than in SAM-R (fig. 2c). The absolute numbers of spermatogonia, spermatocytes and spermatids in 40-week-old SAM-P were significantly lower when compared to those in 20-week-old SAM-P. In SAM-R, however, there was no signficant difference in any spermatogenic parameter between 20- and 40-week-old mice.

The absolute numbers of Sertoli cells in SAM-P at 3 and 6 weeks of age were 40% and 22%, respectively, greater than in SAM-R (fig. 3a). In SAM-P, a downward trend in this parameter appeared in mice from 20 to 40 weeks old. The absolute volume of seminiferous tubules in SAM-P at 5 weeks of age was 15% larger than that in SAM-R (fig. 3b). The absolute numbers of Leydig cells in SAM-P at 4 and 5 weeks old were 31% and 30%, respectively, greater than in SAM-R (fig. 3c). The absolute volumes of Leydig cells in growing SAM-P (3, 4, 5 and 6 weeks of age) were about 18%, 35%, 35% and 23%, respectively, greater than in age-matched SAM-R (fig. 3d). At 40 weeks old, however, the cell volume in SAM-P was significantly smaller than in SAM-R. In SAM-P, there was a significant difference in the absolute volume of Leydig cells between the 20- and 40-week-old groups, whereas no signficant difference was noted between 20- and 40-week-old groups in SAM-R.

Morphometric parameters of Leydig cells indicated that their development was significantly earlier in SAM-P than in SAM-R. It has been reported that the absolute number of Sertoli cells is highly correlated with daily sperm production[13], and that the increases in the absolute number of spermatogenic cells and in the absolute volume of the seminiferous tubules are caused by the increased levels of testosterone produced by Leydig cells[16]. The plasma testosterone levels in SAM-P at 4, 5 and 6 weeks old (1.6, 3.1 and 3.6 ng/ml, respectively) were significantly higher than in SAM-R (1.1, 2.3 and 2.8 ng/ml, respectively) (unpubl. data). It is considered that a greater number of Sertoli cells and a larger volume of seminiferous tubules are associated with earlier sperm production in growing SAM-P compared with age-matched SAM-R. From these morphometric parameters of spermatogenesis in growing mice, we conclude that male sexual maturation or puberty occurs earlier in SAM-P than in SAM-R.

In contrast, the absolute numbers of spermatogenic cells in SAM-P at 40 weeks of age were significantly less than in age-matched SAM-R. Only SAM-P mice demonstrated a significant downward trend in the absolute number of Sertoli cells and in the absolute volume of Leydig cells; this was noted from 20 to 40 weeks of age. The absolute volume of Leydig cells in SAM-P at 40

weeks of age was significantly smaller than in SAM-R. Nomura et al.[7] reported that the plasma concentration of testosterone/dihydrotestosterone in aged SAM-P was significantly lower than in age-matched SAM-R. We also revealed that the plasma testosterone level in SAM-P (2.9 ng/ml) at the age of 40 weeks was significantly lower than that of age-matched SAM-R (3.8 ng/ml) (unpubl. data). It is considered that the decrease in the absolute numbers of spermatogenic cell is caused by the decrease in plasma testosterone[16]. We conclude that age-related deterioration of ability to produce spermatozoa occurs with advancing age in SAM-P, but no deterioration occurs in SAM-R within 40 weeks after birth. Mice of this senescence-accelerated strain may prove to be a useful model for elucidating the mechanism of age-related deterioration of spermatogenesis in the testis.

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1 Takeda, M., Hosokawa, S., Takeshita, S., Irino, M., Higuchi, K., Matsushita, T., Tomita, Y., Yasuhira, K., Hamamoto, H., Shimizu, K., Ishii, M., and Yamamuro, T., Mech. Aging Dev. *17* (1981) 183.
2 Matsumura, A., Higuchi, K., Shimizu, K., Hosokawa, M., Hashimoto, K., Yasuhira, K., and Takeda, T., Lab. Invest. *47* (1982) 270.
3 Hosokawa, M., Kasai, R., Irino, M., Higuchi, K., Takeshita, S., Shimizu, K., Hamamoto, H., Honma, A., Irino, M., Toda, K., Matsumura, M., Matsushita, M., and Takeda, T., Mech. Aging Dev. *26* (1984) 91.
4 Higuchi, K., Matsumura, A., Hashimoto, K., Honma, A., Takeshita, S., Matsushita, M., Hosokawa, M., Yasuhira, K., and Takeda, T., J. expl Med. *158* (1983) 1600.
5 Higuchi, K., Matsumura, A., Honma, A., Toda, K., Takeshita, S., Matsushita, M., Yonezawa, T., Hosokawa, M., and Takeda, T., Mech. Aging Dev. *26* (1984) 311.
6 Miyamoto, M., Kiyota, Y., Yamazaki, N., Nagaoka, A., Matsuo, T., Nagawa, Y., and Takeda, T., Physiol. Behav. *38* (1986) 399.
7 Nomura, Y., Wang, B. X., Qi, S. B., Namba, T., and Kaneko, S., Expl Geront. *24* (1989) 49.
8 Chen, W. H., Hosokawa, M., Tsuboyama, T., Ono, T., Izuka, T., and Takeda, T., Am. J. Path. *135* (1989) 379.
9 Abe, H., and Orita, M., Mech. Aging Dev. *51* (1990) 215.
10 Culling, C. F. A., Allison, R. T., and Barr, W. T., (eds), Cellular Pathology Technique, 4th edn., p. 27. Butterworths, London 1985.
11 Manabe, N., Furuya, Y., Azuma, Y., and Miyamoto, H., Anim. Sci. Technol. *64* (1993) 1.
12 Russell, L. D., Ettlin, R. A., Hikim, A. P. S., and Clegg, E. D., (eds), Histological and Histopathological Evaluation of the Testis, p. 1. Cache River Press, Clearwater 1990.
13 Rugh, R., (ed), The Mouse; its Reproduction and Development, p. 12. Oxford University Press, Oxford 1990.
14 Bernston, W. E., and Thompson, T. L., J. Androl. *11* (1990) 429.
15 Zirkin, B. R., and Ewing, L. L., Anat. Rec. *219* (1987) 157.
16 Jong, F. H., and Sharpe, R. M., J. Endocr. *75* (1977) 197.

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