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Life Span and Renal Morphological Characterization of the SAMP1//Ka Mouse

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Abstract: *The senescence-accelerated-mouse prone 1 (SAMP1) is considered to be a model of accelerated senility and it also develops severe kidney damage. The SAMP1//Ka mouse is a specific pathogen free (SPF) subline of SAMP1. The present study examined the life span of the SAMP1//Ka mouse and morphologically investigated the kidneys of this animal at 3, 4, 5, 9, 12, 15, 18 and 24 months of age. Males survived for an average of 25 months and females for 28 months. The median lifespan was 18 months for males and 20 for females. Focal cell infiltration and thickening of the basement membrane in the glomerular capsules or tubules appeared from 4 months of age. At 12 months old, glomerular lesions with expansion of the mesangial matrix and thickening of the basement membrane as well as scar lesions in the outer cortex appeared, and amyloid was deposited in the interstitium or glomeruli from 18 months of age. Morphometrically, although the area of the kidney sections was increased at 24 months of age, the diameter of the renal corpuscles, the number of nuclei of the proximal convoluted tubules and the percentage of renal corpuscles with a cuboidal glomerular capsule did not change with age. The results of the present study indicate that the life span of the SAMP1//Ka is increased and that their age-related renal changes differ from those of the original SAMP1.*

Key words: *kidney, life span, morphology, SAMP1//Ka, SPF*

Introduction

The Institute of Laboratory Animal Sciences at the Faculty of Medicine, Kagoshima University generated specific pathogen free (SPF) mice in 1991 from the senescence-accelerated-mouse prone 1 (SAMP1) strain raised at The Council for SAM Research, Kyoto University. After confirming that no genomic alterations had arisen in the SPF SAMP1 mouse compared with the original SAMP1, the SPF SAMP1 subline was registered with The Council for SAM Research and was named SAMP1//Ka in 1995.

The SAMP1 mouse has been studied in detail as a model of accelerated senility as it has a short life span of about 9.5 months [13, 14]. Kidney damage in SAMP1 is severe and progresses early in life, starting with amyloid

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deposition and intimal elastofibrosis of the small arteries, which contract later in life [11, 12]. This renal contraction has been considered to be the main cause of SAMP1 death and is an accepted pathological feature of the SAMP1 mouse [13, 15]. However, whether or not SAMP1//Ka retains the features of a short life span and/or the renal pathology has remained unknown. The present study evaluated the life span and morphologically examined the kidneys of the SAMP1//Ka mouse.

Materials and Methods

Animals

Life span was determined using 20 male and 20 female SAMP1//Ka mice. Renal morphology was studied in male SAMP1//Ka, aged 3, 4, 5, 9, 12, 15, 18 and 24 months (n=3/age group). Animals were housed in a room with a one-way airflow system at the Institute of Laboratory Animal Sciences, Faculty of Medicine, Kagoshima University (temperature 22 ± 1 C; humidity 55 ± 10%; light period 07:00 hr–19:00 hr; ventilation 12 changes/hr) and given an autoclaved commercial diet (CE-2, Japan CLEA Co., Inc.) as well as tap water *ad libitum*. All mice were sacrificed by exsanguination under anesthesia (a mixture of ketamine and medetomidine) and the kidneys were quickly removed. All experimentation proceeded in accordance with the Guideline for Animal Experimentation of the Faculties of Medicine and Agriculture, Kagoshima University.

Tissue preparation

Central slices from the left kidneys, which included the hilum, were cut perpendicular to the long axis of the kidneys and fixed in 10% neutral buffered formalin. After routine embedding in paraffin, sections at 3 μm thick were selected every 30 μm and stained with hematoxylin-eosin (HE), periodic acid Schiff (PAS), periodic acid methenamine-silver (PAM), Azan, Weigert and thioflavin T stains. The sections stained with thioflavin T were observed using a fluorescence microscope to detect amyloid.

The right kidneys of 24-month-old mice were examined by electron microscopy. Cortical tissues were cut into pieces of approximately 1 mm^3, then fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hr. The specimens were rinsed in the same buffer, then postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hr. After dehydration through a graded ethanol series, they were embedded in Epon 812. Ultrathin sections, stained with uranyl acetate and lead citrate, were observed by transmission electron microscopy (H-7000KU, Hitachi).

Morphometric analysis

Sections stained with PAS (10 sections/each animal) were quantitatively analyzed at random as follows. 1) Light micrographs of the renal sections were taken at a primary magnification of × 2.5 and printed at a final magnification of × 12.5. As a parameter of renal size, the area of the sections, excluding the hilum, was measured on a graphic tablet (UD-1212II, WACOM) using a NIH image program (freeware). 2) The diameter of the renal corpuscles was determined. 3) The number of nuclei in the proximal convoluted tubules (PCT) was counted as a parameter of PCT size. 4) The ratio (%) of renal corpuscles with a cuboidal parietal layer (CPL) in the glomerular capsule was calculated. The latter three parameters were quantified as described previously [16].

Regrouped results were statistically analyzed using the one-way analysis of variance (ANOVA, Fisher's PLSD test) and are expressed as means ± standard error (SE) for each group. Statistical significance was defined as $p<0.01$.

Results

Lifespan

Male and female SAMP1//Ka mice survived for 25 and 28 months, with median survival times of 18 and 20 months, respectively.

Histological observation

Cell infiltration was focally observed in the interstitium around the interlobar and arcuate arteries or veins from 4 months of age (Fig. 1a). These lesions gradually widened until 18 months, and became more apparent at 24 months (Fig. 1b). Connective tissues became obvious around the arteries or veins but not within the lesions. At 15, 18 and 24 months of age, similar focal lesions also appeared around the interlobular arteries or veins. These cell infiltrates consisted mainly of lymphocytes, plasma cells and macrophages.

The basement membrane (BM) of the glomerular capsules thickened from 4 months of age and in the cortical or

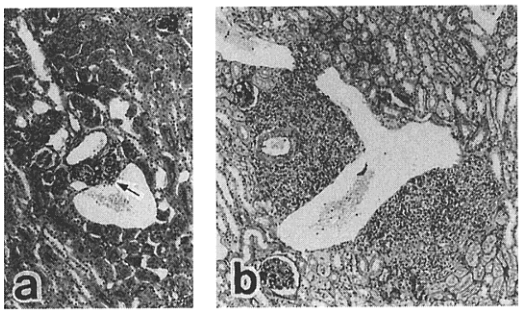

Fig. 1. Light micrographs show focal mononuclear cell infiltration around the arcuate arteries or veins. (a) Four-month-old SAMP1//Ka. (b) Twenty-four-month-old SAMP1//Ka. Arrow indicates slight cell infiltration at 4 months of age. PAS stain. × 50.

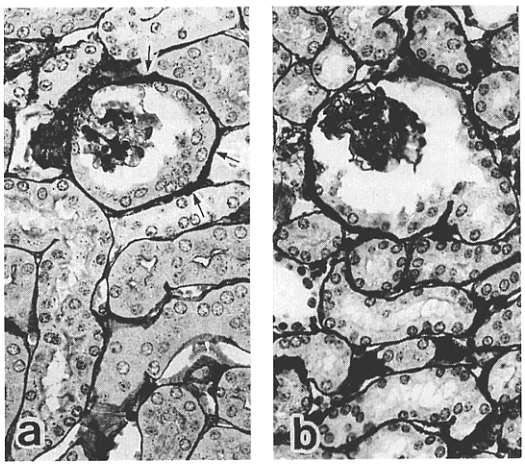

Fig. 2. Light micrographs show thickening of basement membrane. (a) Four-month-old SAMP1//Ka; basement membrane is thickened in glomerular capsule (arrows). (b) Twenty-four-month-old SAMP1//Ka; basement membrane is thickened in outer layer of glomerular capsule and cortical tubules. PAM stain. × 250.

medullar tubules at 15, 18 and 24 months of age (Fig. 2).

Wedge-shaped lesions with atrophied glomeruli and urinary tubules, cellular infiltration and an increase in connective tissues appeared in the outer cortex, and renal surfaces with these lesions were frequently irregular (Fig. 3). These lesions were observed from the age of 12 months and gradually progressed with age.

Amyloid was deposited in the interstitium of the cortex and medulla as well as in the glomeruli of 24-month-old animals (Figs. 4, 5b). In other age groups,

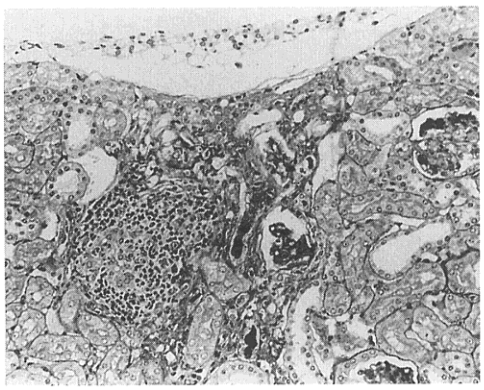

Fig. 3. Light micrograph shows scar lesion in outer cortex of 24-month-old SAMP1//Ka mouse. Atrophy of glomeruli and tubules, casts in the tubular lumen, focal cell infiltration and increase of the connective tissue are evident, and renal surface with this lesion is irregular. PAS stain. × 125.

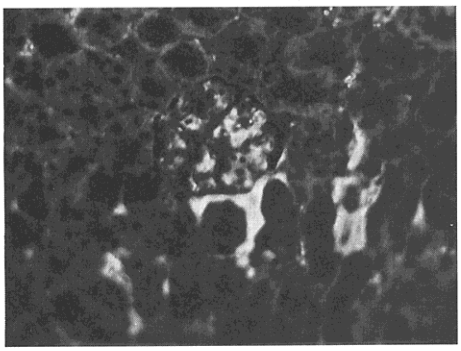

Fig. 4. Fluorescence micrograph of kidney section of 24-month-old SAMP1//Ka mouse. Fluorescent amyloid is located in the glomerulus and interstitium. Thioflavin T stain. × 250.

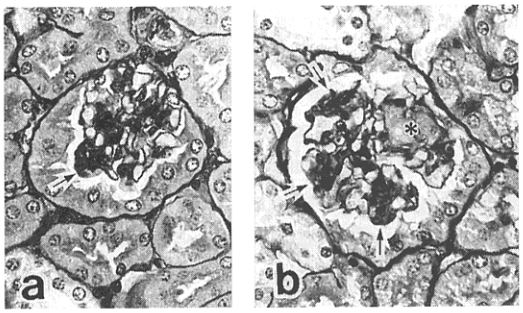

Fig. 5. Light micrographs of glomerular lesions in 12- (a) and 24-month-old (b) SAMP1//Ka mice. Arrows indicate expanded mesangial matrices. Asterisk indicates amyloid deposition. PAS stain. × 300.

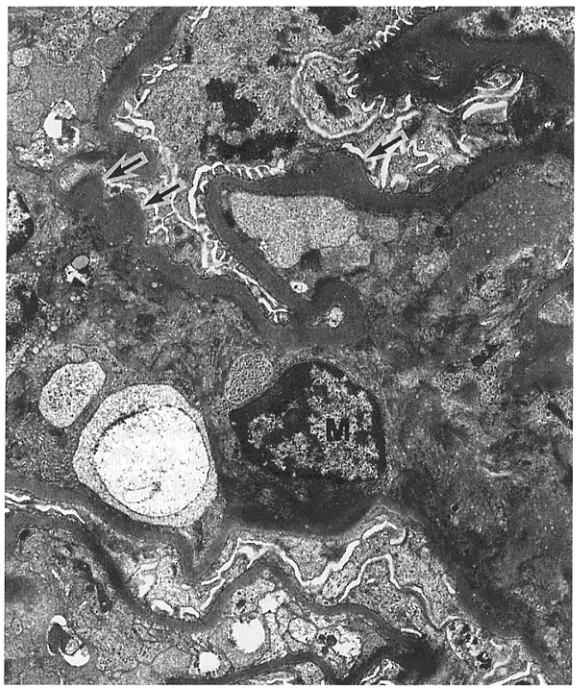

Fig. 6. Transmission electron micrograph of glomerulus of 24-month-old SAMP1//Ka mouse. (M) Mesangial cell. Expansion of mesangial matrices. Arrows indicate thickening of glomerular basement membrane. × 5,000.

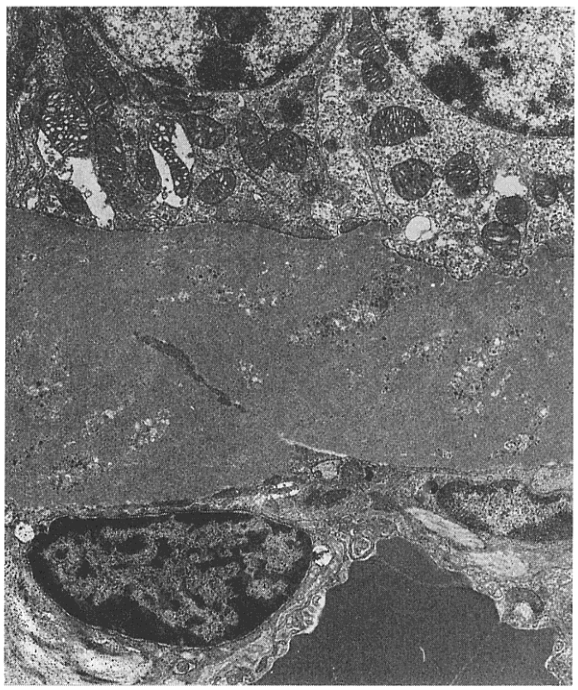

Fig. 7. Transmission electron micrograph of basement membrane of glomerular capsule. Basement membrane is very thick and crack figure is evident. × 6,000.

very few depositions were evident in the interstitium at 18 months old and none were evident from 3 to 15 months old.

Besides amyloid deposition, lesions that were heavily stained with PAS and PAM, indicative of expanded mesangial matrices, were observed in the glomeruli. These lesions were focally observed from 12 months old and became diffuse with age (Fig. 5).

Electron microscopy at 24 months of age revealed expansion of the mesangial matrices and irregular thickening of the glomerular basement membrane (GBM) in the renal corpuscles (Fig. 6). The BM of the glomerular capsules was very thick, and crack figures were frequent (Fig. 7). Ultrastructural changes in the tubules were mild. Large lysosomes and slightly thickened BM were mainly confined to the proximal tubules and changes in other organelles were not apparent (Fig. 8).

No degenerative changes were identified in the intrarenal vessels of any animals.

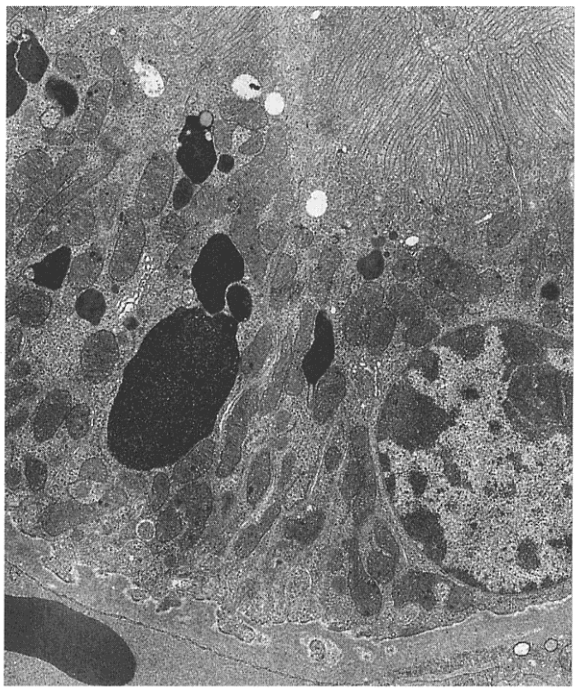

Fig. 8. Transmission electron micrograph of proximal convoluted tubule shows large size lysosomes and slight thickening of the basement membrane. × 6,000.

Table 1. Mean values (SE) of quantitative parameters in the kidney of SAMP1//Ka

Age	Area of the section (mm^2)	Diameter of renal corpuscle (μm)	Number of nuclei of the PCT (/144,000 μm^2)	Percentage of renal corpuscles with CPL (%)
3 M[1)]	15.98 (0.14)	68.43 (0.91)	776.67 (29.55)	76.50 (0.93)
4 M	15.56 (0.85)	69.10 (0.81)	791.67 (18.02)	80.97 (1.68)
5 M	16.52 (0.48)	68.40 (0.67)	823.00 (105.70)	78.60 (2.48)
9 M	17.63 (0.36)	76.53 (3.41)	760.33 (420.8)	80.93 (1.37)
12 M	17.33 (0.58)	77.60 (0.53)	833.00 (30.14)	85.77 (2.55)
15 M	17.50 (0.76)	78.17 (2.29)	782.67 (54.26)	85.93 (3.00)
18 M	16.85 (1.09)	78.27 (2.22)	825.67 (29.57)	76.70 (4.10)
24 M	19.87 (0.22)*	80.67 (3.62)	772.33 (37.53)	69.40 (2.50)

[1)]Months old. *Significantly different from before-age group ($p<0.01$).

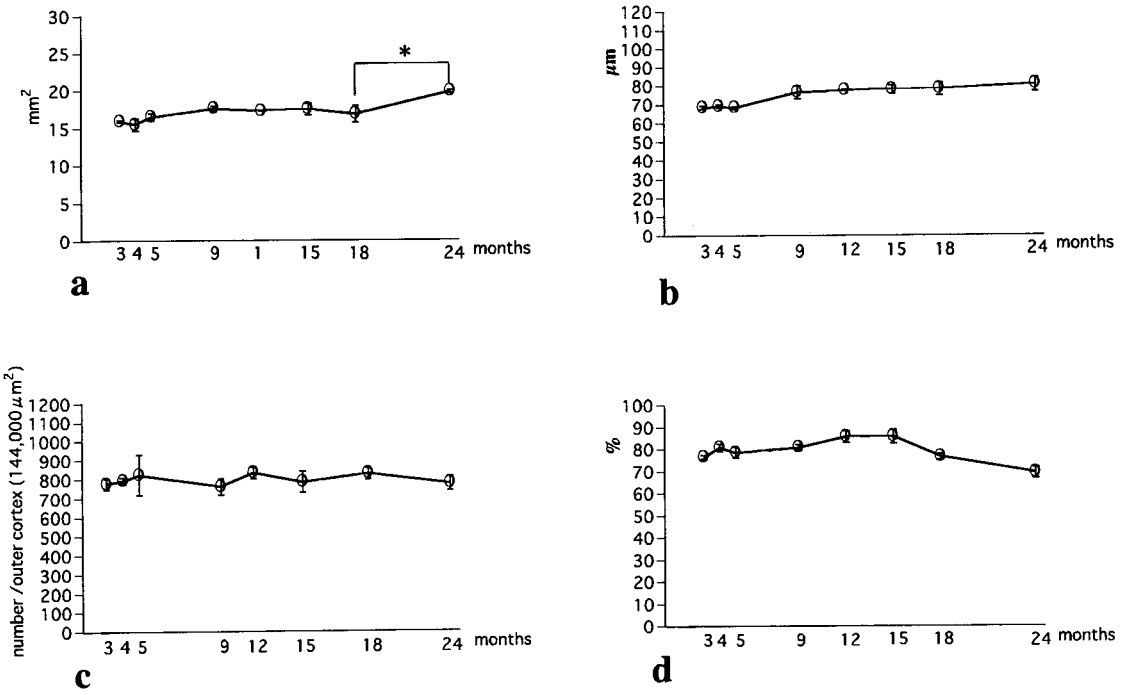

Fig. 9. Graphs of values in Table 1. a: Area of kidney sections. b: Diameter of renal corpuscles. c: Number of nuclei of the proximal convoluted tubules. d: Percentage of renal corpuscles with cuboidal parietal layer. Each value represents mean ± SE. Asterisk indicates statistically significant difference ($p<0.01$).

Morphometrical observation

Morphometrical data are shown in Table 1. The area of the kidney sections was not significantly changed from 3 to 18 months of age, but was significantly larger at 24 months of age (Fig. 9a). The diameter of the renal corpuscles (Fig. 9b), the number of nuclei of PCT (Fig. 9c) and the percentage of renal corpuscles with CPL did not significantly change (Fig. 9d).

Discussion

The life span of inbred mice is strain-specific and varies from under 1 to over 2 years [3]. The SAMP1 mouse has been regarded as a short life strain, with a reported life span of about 9.5 months [13, 14]. The SAMP1//Ka (SPF subline of the SAMP1) mouse survived for 25 months for males and 28 months for females, with a respective median survival of 18 and

20 months. Therefore, SAMP1//Ka is a long life strain. Since no genomic alteration was confirmed between SAMP1//Ka and the original SAMP1, the difference in life span may be caused by differences in microbiological status, food or the environment. However, the major causes remain unclear, since detailed information about the original SAMP1 is not currently available [11–15].

Renal contraction with an irregular surface is considered to be the main cause of death in the original SAMP1 [13, 15]. This lesion is not due to an increase in connective tissue but to renal amyloidosis that progresses rapidly from approximately 6 months of age, which is thus described as "amyloid contracted kidney" [11, 12]. In the kidney of SAMP1//Ka, although wedge-shaped scar lesions and amyloid depositions developed, they appeared later in life and the scars had already developed before amyloid deposition. In addition, connective tissue was increased within the scar lesions. These findings indicate that the renal pathological features of SAMP1//Ka differ from those of the original SAMP1. Furthermore, amyloid deposition may not be SAMP1//Ka-specific, but rather, age-associated renal change, because amyloid deposition in the kidney has been reported to be associated with age in several other mouse strains [7, 10, 17].

Besides amyloid depositions, light microscopy revealed glomerular lesions which were heavily stained with PAS and PAM from 12 months of age. At 24 months of age, electron microscopy confirmed that mesangial matrices had increased and that GBM had thickened. These glomerular changes have also been observed in aged mice of other strains [2, 17], suggesting that such glomerular lesions are age-related.

The CPL of the glomerular capsule, which has a similar function to the proximal tubular epithelium [6], is a morphological phenotype of the renal corpuscles of adult male mice of many strains [1, 9, 16]. Among ICR, BAB/cA, C57BL/6J, C3H/HeN and DBA/2Cr mice at 3 months of age, the percentage of CPL in males differed, ranging from approximately 55 to 80% [16]. This value was 76.5% in the 3-month-old SAMP1//Ka mouse, indicating that the morphological phenotype of the male renal corpuscle was the same as that of other mouse strains. The diameter of the renal corpuscles and the number of nuclei in PCT of the SAMP1//Ka mouse matched those of other strains [16].

The kidneys of SAMP1//Ka showed focal mononuclear cell infiltration and thickening of the BM from 4 months old. Thickening of the BM and cell infiltration are both histopathological features of tubulointerstitial nephritis [8]. These changes during early life are not reported in other mouse strains, but such cell infiltration has been seen in the NON (Non-Obese Non-diabetic) subline of the NOD (Non-Obese diabetic) mouse [5]. The localization and aging process of cell infiltration in the NON mouse are very similar to those in SAMP1//Ka.

Renal hypertrophy with age has been identified in the C57BL/6 and CBA/FT6 strains [4]. We found that renal size increased at 24 months of age. However, other parameters, such as the diameter of the renal corpuscles or the number of PCT nuclei, did not indicate that the size of the nephron had increased. The increased size of the kidney at 24 months of age might be related to an increase in the amount of interstitial connective tissues and amyloid.

In conclusion, SAMP1//Ka is a long life mouse strain and the age-related renal morphological changes of this strain differ from those of the original SAMP1 which is considered to be a model of accelerated senility.

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