Clin. exp. Immunol. (1989) **75**, 129–135

Autoimmune abnormalities in a murine model of accelerated senescence

H. YOSHIOKA, H. YOSHIDA, T. DOI, E. MUSO*, G. OHSHIO, K. HIGUCHI†, M. INADA‡, T. MIYAKE‡, T. KITA‡, Y. HAMASHIMA, & T. TAKEDA† *Department of Pathology, *The Third Division Department of Internal Medicine, †Department of Senescence Biology, Chest Disease Research Institute, and ‡Department of Geriatric Medicine, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan*

(Accepted for publication 16 August 1988)

SUMMARY

Immunopathological abnormalities in senescence-accelerated mice (SAM) were studied by comparison of senescence-prone (SAM-P/1) and senescence-resistant (SAM-R/1) mice. Sera from SAM-P/1 mice contained a number of autoantibodies, including natural thymocytotoxic autoantibody (NTA), anti-nuclear antibodies (ANA) and IgG anti-single-stranded and anti-double-stranded (ss and ds) DNA antibodies. Furthermore, an earlier increase in serum IgG2 levels and an earlier appearance of IgG circulating immune-complexes (CIC) associated with low C3 levels, were observed in SAM-P/1 mice. These serological findings were distinctive features in SAM-P/1 mice, which could almost discriminate these mice from SAM-R/1 mice. In addition, age-associated glomerular mesangial and capillary lesions with granular IgG and C3 deposition were frequently observed in SAM-P/1 mice, whereas SAM-R/1 mice even at 10 months of age showed only mild mesangial lesions. These findings suggest that autoimmune abnormalities may contribute to the accelerated senescence in these mice.

Keywords autoantibodies circulating immune complexes glomerulonephritis senescence-accelerated mice

INTRODUCTION

Recently a murine model for accelerated senescence, called SAM, was successfully established from AKR/J mice (Takeda *et al.*, 1981; Hosokawa *et al.*, 1984). In the selection and maintenance of this senescence-prone series of animals, senescence-resistant mice (SAM-R/1, 2 and 3) were also produced. The former series, including SAM-P/1, 2, 3, and 4 strains, show irreversible advancement of senescence after 6 months of age, manifested by clinical signs and gross lesions, such as alterations in general behaviour, skin and hair abnormalities, cataracts and increased lordokyphosis of the spine. In these mice, spontaneous age-associated systemic amyloidosis has been described as one of the notable pathological findings (Shimizu *et al.*, 1981; Takeda *et al.*, 1981). The mean life spans of SAM-P/1 and SAM-R/1 animals were reported to be 9·7 and 13·3 months respectively, and in the SAM-P/1 mice, abscess formation and interstitial pneumonia were, besides amyloidosis, the major autopsy findings (Takeda *et al.*, 1981).

Accumulating evidence has suggested that immunopathological abnormalities normally occur in aged mice and in older humans (Price & Makinodan, 1972; Hallgren *et al.*, 1973; Edmond *et al.*, 1976; Kishimoto *et al.*, 1978; Gillis *et al.*, 1981). In SAM-P mice abnormal immune responses such as low *in vitro* anti-SRBC antibody response and the defect in helper T cell function have been described (Hosokawa *et al.*, 1987a, b). However, it still has not been clarified whether or not the SAM-P/1 mice bear immunopathological abnormalities, in addition to amyloidosis, which could be related to their terminal infectious states and/or to the accelerated ageing process itself. In the present study we studied immunopathological abnormalities in the sera and kidneys of SAM-P/1 mice in comparison with the control SAM-R/1 mice.

Correspondence: Dr Haruyoshi Yoshida, Department of Pathology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

MATERIALS AND METHODS

Mice

SAM-P/1 and SAM-R/1 mice were maintained in our laboratories. BALB/c mice and autoimmune female MRL/MpJ-1pr/lpr(MRLl/l) and New Zealand black (NZB) mice were used as controls. Mice were bled and killed at different ages up to 10 months old.

Proteinuria

Female mice were tested for proteinuria using paper test strips (Hemacombistix, Ames, IA). Protein concentrations in the urine of 1 mg/ml or more were regarded as positive proteinuria.

Measurement of serum immunoglobulin and C3 levels
Serum levels of IgM, IgG1, IgG2a, IgG2b, IgA and C3 were measured by a single radial immunodiffusion method. The C3 levels were expressed as a percentage of the amount found in pooled sera from 2 month-old BALB/c mice.

Detection of anti-nuclear antibodies (ANA)
ANA were detected by indirect immunofluorescence (Lambert & Dixon, 1968), using FITC-labelled goat anti-mouse IgG antibodies (Cappel Laboratories, Cochranville, PA).

Measurement of IgG anti-ssDNA and anti-dsDNA levels
Heat-denatured calf thymus DNA (Type V, Sigma Chemical Co., St Louis, MO) was used as ssDNA. Double-stranded DNA was prepared by digestion with S1 nuclease and subsequent fractionation by benzoyl-naphthoyl-DEAE cellulose column chromatography (Yoshida *et al.*, 1985). Enzyme-linked immunosorbent assays (ELISA) for anti-ssDNA and anti-dsDNA antibodies were carried out according to a modification of the method described by Kavai *et al.* (1982), using anti-mouse IgG antibodies conjugated with alkaline phosphatase (Zymed Laboratories, Inc., San Francisco, CA).

Measurement of IgG circulating immune complex (CIC) levels
An ELISA for CIC was performed according to a modification of the previous method using ELISA plates coated with IgG anti-mouse C3 antibodies (Pereira, Theofilopoulos & Dixon, 1980). After serum samples diluted to 1:2000 were reacted to the wells, alkaline phosphatase-labelled goat anti-mouse IgG antibodies (Zymed Laboratories, Inc.) were added to each well. The results were expressed as absorbance at 405 nm after incubation with the substrate.

Test for natural thymocytotoxic autoantibody (NTA)
Measurement of NTA was performed by a cytotoxicity test according to the method of Shirai & Mellors (1971). Briefly, a mixture of 50 μl of serum sample and 25 μl of BALB/c thymocyte suspension (1×10^7 cells/ml) was incubated at 4°C for 60 min. After washing, 50 μl of rabbit serum diluted to 1:6 was added as complement, and the mixture was incubated at 37°C for 30 minutes. The percentage of cells killed was calculated by the trypan blue dye exclusion method.

Glomerular examination
Renal sections were stained with haematoxylin and eosin, periodic acid Schiff (PAS) and Congo red for light microscopy. Increase in mesangial matrix, mesangial cell proliferation, endocapillary cell infiltration, and capillary wall thickening were scored separately on a scale of 0–2, the scores being based on the increasing intensity and extent of the abnormalities. A general severity index for glomerular histopathology was then calculated for each mouse by summing the individual scores. Frozen sections from each kidney were examined for deposition of IgM, IgG, IgA, and C3 by direct staining with the respective FITC-conjugated antiserum (Cappel Laboratories). According to the intensity and distribution of the fluorescence, observations were graded from 0–3.

Deposition of amyloid materials was also studied by the peroxidase/anti-peroxidase method using specific antiserum against amyloid protein (AS_{SAM}) of these mice (Higuchi *et al.*, 1983).

Statistical analysis
Statistical analysis was performed using the Wilcoxon rank sum test for non-paired data.

RESULTS

Proteinuria
In SAM-P/1 mice the incidence of proteinuria in groups of 10 mice at 2, 6, and 10 months of age were 40%, 70%, and 80% respectively. In contrast, SAM-R/1 mice showed a much lower incidence of 0%, 30% and 50% at 2, 6 and 10 months of age respectively.

Serum immunoglobulins
In both series of mice the levels of all of the IgG subclasses and IgA antibodies began to increase at as early as 2 months of age, whereas the levels of IgM antibodies did not increase with aging (Table 1). Interestingly, the mean levels of IgG2a and IgG2b in

Table 1. Age-related changes of serum levels of immunoglobulins in SAM-P/1 and SAM-R/1 mice

Mice	Immunoglobulins	Age (months) 1	2	4	6	8	10
SAM-P/1	IgM	32±7	31±9	29±5	26±3	30±7	33±8
	IgG1	141±18	366±115	376±105	341±121	463±86	591±145
	IgG2a	262±37	407±52	625±195	519±229	479±91	604±166
	IgG2b	146±76	392±140	528±120	536±197	510±98	563±137
	IgA	58±12	100±34	141±69	157±92	135±16	225±129
SAM-R/1	IgM	40±11	45±13	38±12	38±8	46±3	55±21
	IgG1	129±47	259±151	218±34	276±66	304±28	343±29
	IgG2a	194±22	338±126	384±52	510±101	421±51	460±93
	IgG2b	117±35	245±46	367±22	542±173	548±68	538±85
	IgA	38±7	72±4	98±32	158±28	106±17	154±48

Results are expressed as mean concentrations of immunoglobulins (mg/dl) ± 1 s.d.

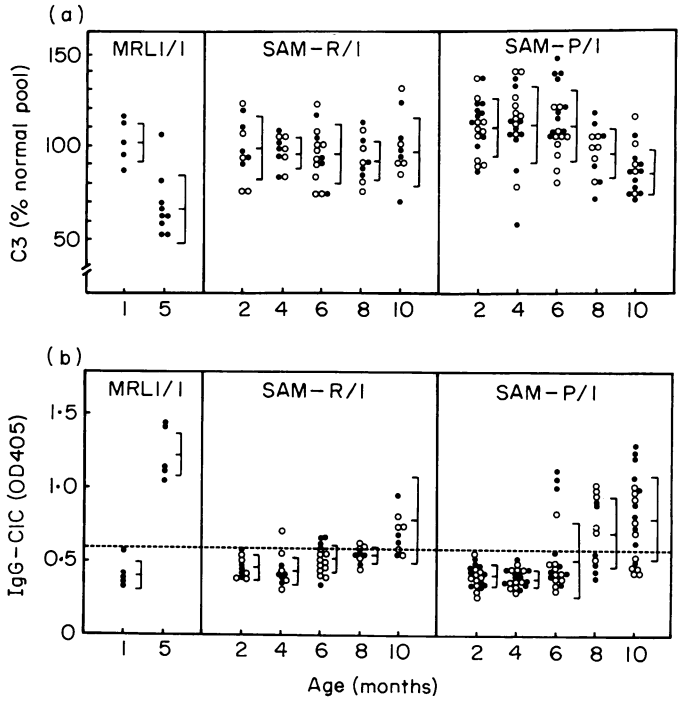

Fig. 1. Age-related changes in serum C3 levels (a) and IgG-CIC levels (b) in SAM-R/1 and SAM-P/1 mice. For comparison, data of MRL1/1 mice are shown on the left. The bars indicate the means ± 1 s.d. at each age. The dotted line indicates the upper limit of the normal range (the mean + 2 s.d. value) shown by BALB/c mice. ○, male mice; ●, female mice. There was no significant difference between males and females.

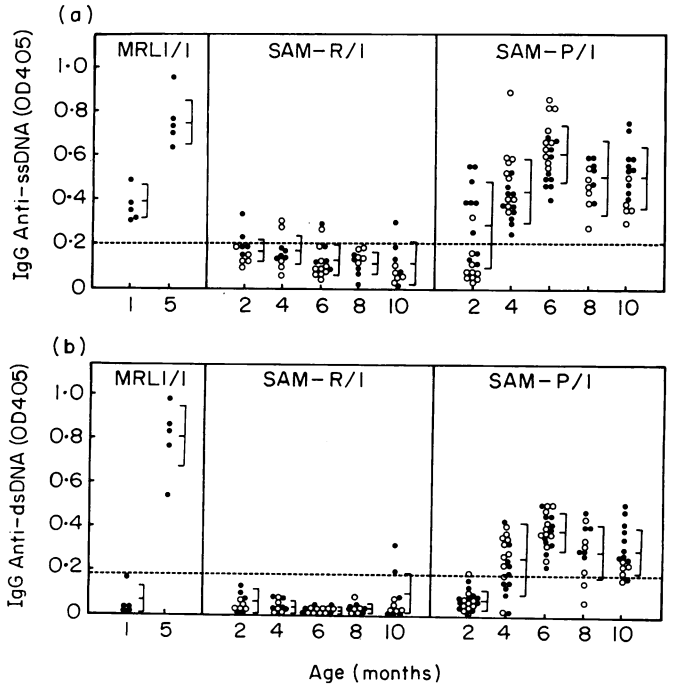

Fig. 2. Age-related changes in IgG anti-ssDNA (a) and IgG anti-dsDNA (b) antibodies in SAM-R/1 and SAM-P/1 mice. On the left, data from MRL1/1 mice are shown. The bars, the dotted line, and (○), (●) are the same as indicated in the legend for Fig. 1. There was no significant difference between males and females.

SAM-P/1 mice showed sharp increases, almost reaching their maximum levels by the time the animals were 4 months of age. On the other hand, in SAM-R/1 mice at this age, the mean levels of these antibodies were significantly low and their maximum means were reached after 6 months of age. Apart from proteinuria all of the data in the present report showed no significant differences between male and female mice.

Serum C3 and IgG-CIC levels

SAM-P/1 mice began to show significant decreases in the mean C3 level after 8 months of age, values being 108% in 2-month-old animals, 95% at 8 months old ($P < 0.005$), and 84% at 10 months old ($P < 0.005$), (Fig. 1a). SAM-R/1 mice up to 10 months of age showed no significant decrease in C3 levels.

As shown in Fig. 1b, comparison of the mean levels of IgG-CIC showed that they increased in SAM-P/1 mice earlier than in SAM-R/1 mice (0·70 and 0·80 in SAM-P/1 mice vs. 0·55 and 0·75 in SAM-R/1 mice at 8 and 10 months old respectively). It should be noted that these abnormalities were not so severe as those observed in MRL1/1 mice (Fig. 1).

ANA

Sera from groups of 10 SAM-R/1 mice at 2, 6, and 10 months of age were negative at 1 : 10 dilution. In contrast, sera at the same dilution from SAM-P/1 mice at 2, 6, and 10 months of age showed increasing levels of ANA; 5% (1/20), 45% (9/20), and 93% (14/15) respectively.

Serum anti-ss and ds DNA antibody levels

In SAM-R/1 mice the mean level of both anti-DNA antibodies was low at 2 months of age and did not increase with aging (Fig. 2). On the other hand, in SAM-P/1 mice anti-ssDNA antibodies were detected in 40% (8/20) of animals at as early as 2 months of age, with the mean value being 0·30. Thereafter these antibodies were detected in all of the mice tested, with the mean level increasing to a maximum (0·62) at 6 months of age. The mean levels of anti-dsDNA antibodies in SAM-P/1 mice began to increase at 4 months of age and reached the maximum at 6 months old. In comparison with MRL1/1 mice, which show the highest anti-DNA levels among autoimmune mice (Yoshida *et al.*, 1985), both anti-DNA antibody levels in SAM-P/1 mice were relatively low.

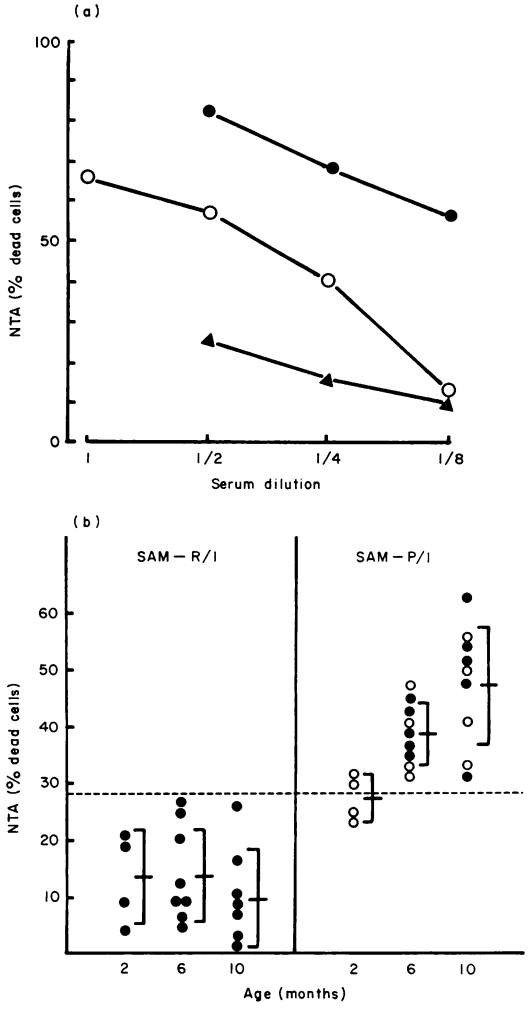

Fig. 3. (a) NTA titres at different dilutions of serum from a 10 month-old SAM-P/1 mouse (○). For comparison, data of a 10 month-old NZB (●) and a 2 month-old BALB/c (▲) are shown. (b) Comparison of NTA titres between SAM-R/1 and SAM-P/1 mice with aging. The bars, the dotted line and (○), (●) are the same as indicated in the legend for Fig. 1. There was no significant difference between male and female in SAM-P/1 mice.

NTA

Figure 3a shows NTA titres at different dilutions of a representative serum from a 10 month-old SAM-P/1 mouse. Significant levels of NTA were observed in the aged SAM-P/1 mouse, although not so high as those in the NZB mouse. As shown in Fig. 3b, no sera from SAM-R/1 mice, aged from 2–10 months old, showed significant levels of NTA. On the other hand, SAM-P/1 mice showed increasing levels of NTA as they aged. Mean NTA killing rates in these mice were 28%, 39% and 48% at 2, 6 and 10 months of age respectively. Notably, all sera from SAM-P/1 mice more than 6 months old were positive (Fig. 3b).

Glomerular histopathology

In SAM-P/1 mice increasing numbers of mesangial and endocapillary cells and thickening of the mesangial matrix became apparent at 6 months of age (Fig. 4A). At 10 months of age, in addition to these findings, these mice showed a marked thickening of the peripheral capillary walls (Fig. 4b). A comparison of severity indexes for these glomerular abnormalities between SAM-R/1 and SAM-P/1 mice at different ages is shown in Fig. 5. Late in life SAM-R/1 mice showed a slight increase in this index from 1·9 at 2 months to 6·2 at 10 months of age. On the other hand, SAM-P/1 mice began to show a prominent increase in the index from 6 months of age, with it reaching 4·0, 9·6 and 14·2 at 2, 6, and 10 months of age respectively. Furthermore, global glomerular sclerosis, capillary fibrinoid necrosis and crescent formation or adhesions were observed, with high incidence rates of 50%, 36%, and 71% respectively, in 10 month-old SAM-P/1 mice. No such lesions were found in 10 month-old SAM-R/1 mice.

The positivities of amyloid deposits with anti-AS_{SAM} antiserum in groups of 12 mice at 2–4, 6, 8 and 10 months of age were 0, 40, 50 and 83% respectively. With Congo red staining no deposits were detected in 2 to 6 month-old mice, and an incidence of 58% was noted at both 8 and 10 months of age. By both methods glomerular amyloid deposits were found only in

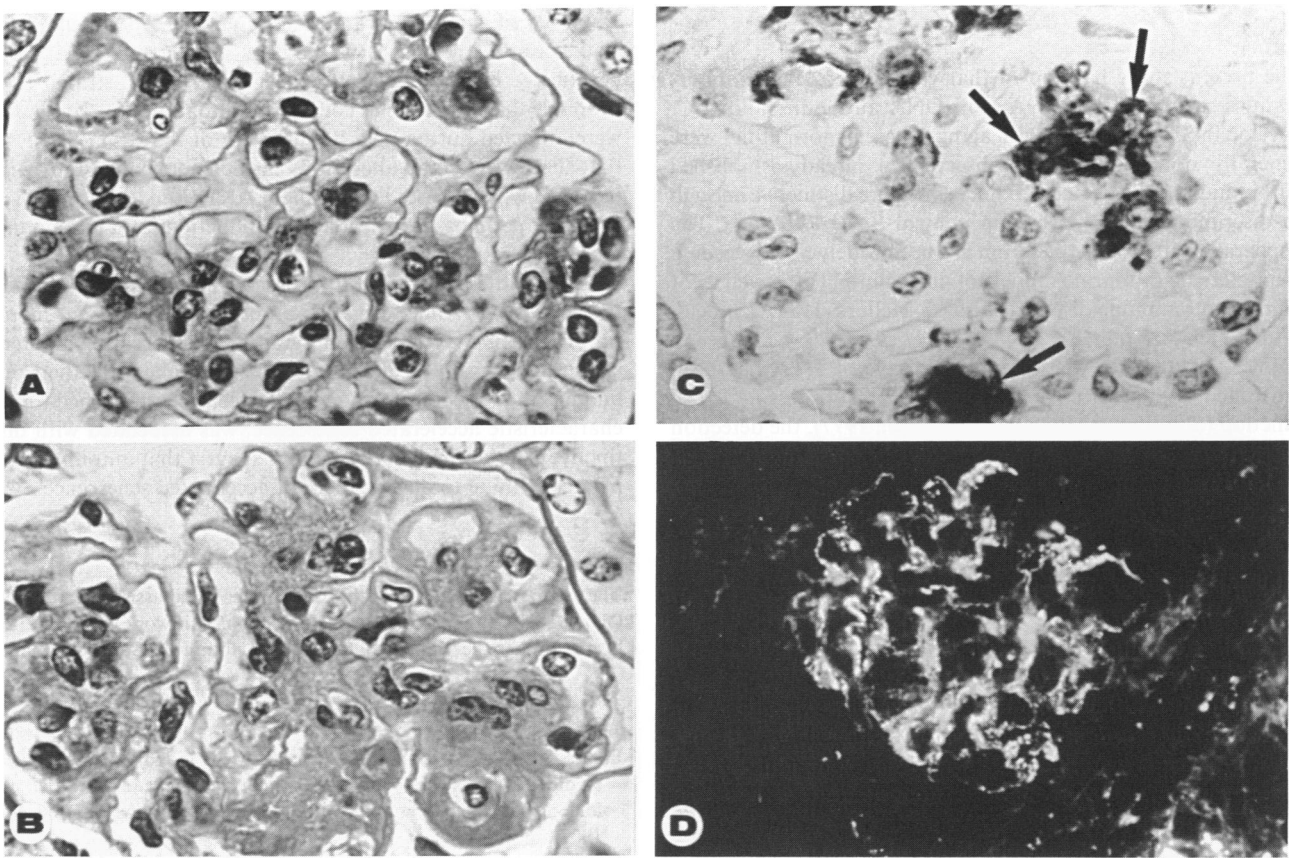

Fig. 4. Representative light microscopic observations in SAM-P/1 mice. (A) Mesangial proliferation with endocapillary cell infiltration in a glomerulus from a 6 month-old mouse (PAS staining, × 1150). (B) Markedly-increased mesangial matrix with thickened peripheral capillary walls in a glomerulus from a 10 month-old mouse (PAS staining, × 1150). (C) Amyloid deposits detected by immunoperoxidase staining with antiserum to AS_{SAM} (× 1150). AS_{SAM} deposits could be detected in the mesangial area (arrows). (D) Characteristic IgG staining in glomerular mesangium and capillary walls in a 10 month-old SAM-P/1 mouse. Original magnification for each section × 1150.

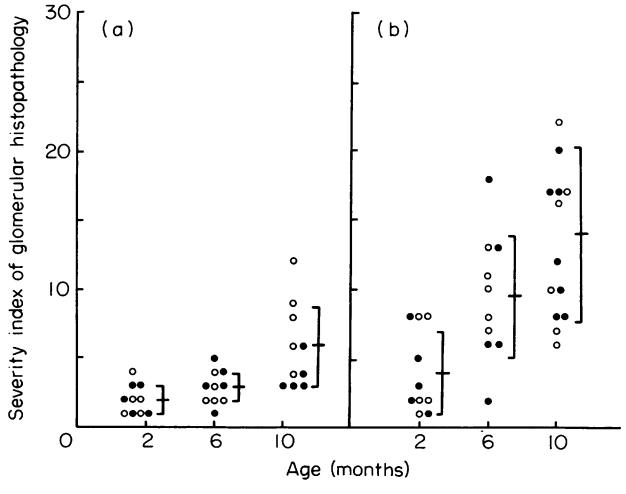

Fig. 5. Comparison of the severity index for glomerular histopathology in SAM-R/1 (a) and SAM-P/1 (b) mice at 2, 6 and 10 months-old. The bars and (○), (●) are the same as indicated in the legend for Fig. 1. There was no significant difference between males and females.

the mesangial area, none being detected along the peripheral capillary walls (Fig. 4C). In SAM-R/1 mice, as reported previously, amyloid deposits were less frequently observed.

Glomerular immunofluorescence

Glomerular immunofluorescence findings in SAM-P/1 mice were compared between different ages (2, 6 and 10 months old), in groups of 20 mice. At 6 months of age the mesangial deposits of all immunoglobulin classes and of C3 were increased above those observed at 2 months of age. At 10 months of age, the mean scores of mesangial IgG and IgM deposits were greatly increased (2·00 and 2·10 respectively) above the levels detected at 6 months of age (0·95 and 1·00 respectively). Significantly, peripheral capillary wall deposits of IgG and C3 became evident at 10 months of age, their mean values being 1·19 and 0·75 respectively, in contrast to those of IgM (0·38) and IgA (0·29). In 50% of the 10 month-old SAM-P/1 mice IgG deposits were found in both the mesangial area and peripheral capillary walls. However, at no time during the study did SAM-R/1 mice show such considerable deposition of either immunoglobulins or of C3 in the peripheral capillaries. Figure 4D shows characteristic granular deposits of IgG in the mesangial area and capillaries of a 10 month-old SAM-P/1 mouse.

DISCUSSION

The present study has shown that various serological abnormalities such as NTA, ANA, anti-DNA antibodies and CIC, develop in SAM-P/1 mice with aging. These abnormalities were generally not so severe as observed in already-established autoimmune MRLl/l or NZB mice, but were distinctive enough to discriminate SAM-P/1 from control SAM-R/1 mice. The appearance of NTA in the serum has been widely noted as being characteristic of murine models of autoimmunity, as well as of human autoimmune disease (Shirai & Mellors, 1971; Winfield, Winchester & Kunkel, 1975; Koike *et al.*, 1979; Schocket & Kohler, 1979). Since the pathological role of NTA has been attributed to its selective impairment of suppressor T cell function (Shirai *et al.*, 1978; Klassen *et al.*, 1977), the detection of NTA in the SAM-P/1 mice suggests that these animals suffer from T cell abnormalities that contribute to the production of autoantibodies as well as of earlier increases of serum immunoglobulin levels. In this regard, patients with Werner's syndrome, characterized by a range of symptoms for accelerated senescence, have been shown to have a high incidence of NTA-like autoantibody in the serum, associated with changes in the T cell subpopulations, although in normal aged individuals this antibody could not be detected (Goto *et al.*, 1979). Thus it is likely that the early appearance of NTA in the sera of SAM-P/1 mice may be a fundamental immunological abnormality related to the pathogenesis of their accelerated senescence. In addition the earlier appearance of IgG-CIC associated with decreasing C3 levels and the production of IgG anti-ss and ds DNA antibodies suggests the presence in these mice of organ involvements such as lupus glomerulonephritis. In fact the development of the glomerulonephritis, frequently associated with capillary fibrinoid necrosis, was observed with aging in SAM-P/1 mice. These glomerular lesions seem to be independent from the amyloid deposition, and rather are related to the IgG and C3 deposition, because the amyloid materials were confined to the mesangial area and mesangial proliferative lesions were frequently observed in mice without apparent renal amyloid deposition.

As to the mechanism of autoantibody production in SAM-P/1 mice, the appearance of NTA suggests that these animals may suffer from certain viral infections. Indeed, production of NTA has been observed in a variety of disease states associated with polyclonal B cell activation (Izui *et al.*, 1979). However, infections of SAM with mouse hepatitis virus or haemagglutinating virus of Japan have been excluded (Takeda *et al.*, 1981). Instead, genetic factors or endogenous retroviral infections as described in NZB and (NZB × NZW) F1 mice should be considered (Yoshiki *et al.*, 1974; Izui *et al.*, 1981; Maruyama *et al.*, 1983). In this context AKR/J mice, from which the SAM were derived, have been shown to be in an immunodeficient state associated with the onset of leukaemia (Metcalf & Moulds, 1967; Roman & Golub, 1976), and AKR/J mice homozygous for the lpr gene produce relatively few autoantibodies in comparison with MRLl/l, C3H/HeJ-lpr/lpr and C57BL/6J-lpr/lpr mice (Izui *et al.*, 1984). However, in contrast to these findings, SAM-P/1 mice produced substantial amounts of various autoantibodies, associated with high IgG2 levels. Furthermore, it has been shown that SAM exhibit a low incidence of lymphoma and a high incidence of amyloid deposition, whereas AKR/J mice are known to have a high incidence of lymphoma and a low incidence of amyloidosis (Takeda *et al.*, 1981). These findings suggest that SAM have genetically deviated from the original AKR/J strain. It is probable that either genes coding for autoantibody production were inducted during the establishment of the strain SAM, or that there were some alterations in the endogenous retroviral infection which causes leukaemia in AKR/J mice, but which in SAM is perhaps responsible for induction of autoimmune abnormalities.

It should be noted that the observed serological abnormalities in SAM-P/1 mice began to appear during normal maturation before the accelerated aging process became apparent after 6 months old (Takeda *et al.*, 1981). Taken together, our findings that this murine model, selected and established for its characteristic accelerated senescence, was associated with distinctive autoimmune abnormalities suggest that autoimmunity may contribute to development of accelerated senescence.

ACKNOWLEDGMENTS

The authors are grateful for the excellent technical assistance of Mr T. Obata, Miss M. Asano and Miss H. Minami. This work was supported by grants from the Ministry of Education, Culture and Science of Japan.

REFERENCES

EDMOND, A., INNES, J.B. & WEKSLER, M.E. (1976) Immunological studies of aging. II. Loss of IgG and high avidity plaque-forming cells and increased suppressor cell activity in aging mice. *J. exp. Med.* **144,** 1037.

GILLIS, S., KOZAK, R., DURANTE, M. & WEKSLER, M.E. (1981) Immunological studies of aging; Decreased production of and response to T cell growth factor by lymphocytes from aged humans. *J. clin. Invest.* **67,** 937.

GOTO, M., HORIUCHI, Y., OKUMURA, K. & TADA, T. (1979) Immunological abnormalities of aging; An analysis of T lymphocyte subpopulations of Werner's syndrome. *J. clin. Invest.* **64,** 695.

HALLGREN, H.M., BUCKLEY, C.E., GILBERTSEN, V.A. & YUNIS, E.J. (1973) Lymphocyte phytohemagglutinin responsiveness, immunoglobulins and autoantibodies in aging humans. *J. Immunol.* **111,** 1101.

HIGUCHI, K., MATSUMURA, A., HONMA, A., TAKESHITA, S., HASHIMOTO, K., HOSOKAWA, M., YASUHIRA, K. & TAKEDA, T. (1983) Systemic senile amyloid in senescence-accelerated mice; A unique fibril protein demonstrated in tissues from various organs by the unlabelled immunoperoxidase methods. *Lab. Invest.* **48,** 231.

HOSOKAWA, M., KASAI, R., HIGUCHI, K., TAKESHITA, S., SHIMIZU, K., HAMAMOTO, H., HONMA, A., IRINO, M., TODA, K., MATSUMURA, A., MATSUSHITA, M. & TAKEDA, T. (1984) Grading score system: A method for evaluation of the degree of senescence in senescence accelerated mouse (SAM). *Mech. Ageing Dev.* **26,** 91.

HOSOKAWA, T., HOSONO, M., HIGUCHI, K., AOIKE, A., KAWAI, K. & TAKEDA, T. (1987a) Immune responses in newly developed short-lived SAM mice. I. Age-associated early decline in immune activities of cultured spleen cells. *Immunology* **62,** 419.

HOSOKAWA, T., HOSONO, M., HANADA, K., AOIKE, A., KAWAI, K. & TAKEDA, T. (1987b) Immune responses in newly developed short-lived SAM mice. II. Selectively impaired T-helper cell activity in *in vitro* antibody response. *Immunology* **62,** 425.

IZUI, S., KOBAYAKAWA, T., LOUIS, J. & LAMBERT, P.H. (1979) Induction of thymocytotoxic autoantibodies after injection of bacterial lipopolysaccharides in mice. *Eur. J. Immunol.* **9,** 338.

IZUI, S., MCCONAHEY, P.J., CLARK, J.P., HANG, L.M., HARA, I. & DIXON, F.J. (1981) Retroviral gp70 immune complexes in NZB × NZW F2 mice with murine lupus nephritis. *J. exp. Med.* **154,** 517.

IZUI, S., KELLEY, V.E., MASUDA, K., YOSHIDA, H., ROTHS, J.B. & MURPHY, E.D. (1984) Induction of various autoantibodies by mutant gene lpr in several strains of mice. *J. Immunol.* **133,** 227.

KAVAI, M., BANYAI, A., ZSINDELY, A., SONKOLY, I. & SZEGEDI, G. (1982) Enzyme-linked immunosorbent assay for antibodies to native DNA in sera of patients with SLE. *J. immunol. Methods* **48,** 169.

KISHIMOTO, S., TOMINO, S., INOMATA, K., KOTEGAWA, S., SAITO, T., KUROKI, M., MITSUYA, H. & HISAMITSU, S. (1978) Age-related changes in the subsets and functions of human T lymphocytes. *J. Immunol.* **121,** 1773.

KLASSEN, L.W., KRAKAUER, R.S. & STEINBERG, A.D. (1977) Selective loss of suppressor cell function in New Zealand mice induced by NTA. *J. Immunol.* **199,** 830.

KOIKE, T., KOBAYASHI, S., YOSHIKI, T., ITOH, T. & SHIRAI, T. (1979) Erythrocyte rosette inhibition as an assay for naturally occurring T lymphocytotoxic antibody in systemic lupus erythematosus. *Arthritis Rheum.* **22,** 1064.

LAMBERT, P.H. & DIXON, F.J. (1968) Pathogenesis of the glomerulonephritis of NZB/W mice. *J. exp. Med.* **127,** 507.

MARUYAMA, N., FURUKAWA, F., NAKAI, Y., SASAKI, Y., OHTA, K., OZAKI, S., HIROSE, S. & SHIRAI, T. (1983) Genetic studies of autoimmunity in New Zealand mice; IV. Contribution of NZB and NZW genes to the spontaneous occurrence of retroviral gp70 immune complexes in (NZB × NZW)F1 hybrid and the correlation to renal disease. *J. Immunol.* **130,** 740.

METCALF, D. & MOULDS, R. (1967) Immune responses in preleukemic and leukemic AKR mice. *Int. J. Cancer* **2,** 53.

PEREIRA, A.B., THEOFILOPOULOS, A.N. & DIXON, F.J. (1980) Detection and partial characterization of circulating immune complexes with solid-phase anti-C3. *J. Immunol.* **125,** 763.

PRICE, G.B. & MAKINODAN, T. (1972) Immunologic deficiencies in senescence. I. Characterization of intrinsic deficiencies. *J. Immunol.* **108,** 403.

ROMAN, J.M. & GOLUB, E.S. (1976) Leukemia in AKR mice. I. Effects of leukemic cells on antibody-forming potential of syngeneic and allogeneic normal cells. *J. exp. Med.* **143,** 482.

SHIMIZU, K., KASAI, R., YAMAMURO, T., HOSOKAWA, M., TAKESHITA, S. & TAKEDA, T. (1981) Amyloid deposition in the articular structures of AKR senescent mice. *Arthritis Rheum.* **24,** 1540.

SHIRAI, T., HAYAKAWA, K., OKUMURA, K. & TADA, T. (1978) Differential cytotoxic effect of natural thymocytotoxic autoantibody of NZB mice on functional subsets of T cells. *J. Immunol.* **120,** 1924.

SHIRAI, T. & MELLORS, R.C. (1971) Natural thymocytotoxic autoantibody and reactive antigen in New Zealand Black and other mice. *Proc. natl. Acad. Sci. USA* **68,** 1412.

SCHOCKET, A.L. & KOHLER, P.F. (1979) Lymphocytotoxic antibodies in systemic lupus erythematosus and clinically related diseases. *Arthritis Rheum.* **22,** 1060.

TAKEDA, T., HOSOKAWA, M., TAKESHITA, S., IRINO, M., HIGUCHI, K., MATSUSHITA, T., TOMITA, Y., YASUHIRA, K., HAMAMOTO, H., SHIMIZU, K., ISHII, M. & YAMAMURO, T. (1981) A new murine model of accelerated senescence. *Mech. Ageing Dev.* **17,** 183.

WINFIELD, J.B., WINCHESTER, R.J. & KUNKEL, H.G. (1975) Association of cold-reactive antilymphocyte antibodies with lymphopenia in systemic lupus erythematosus. *Arthritis Rheum.* **18,** 587.

YOSHIDA, H., YOSHIDA, M., IZUI, S. & LAMBERT, P.H. (1985) Distinctive clonotypes of anti-DNA antibodies in mice with lupus nephritis. *J. clin. Invest.* **76,** 685.

YOSHIKI, T., MELLORS, R.C., STRAND, M. & AUGUST, J.T. (1974) The viral envelope glycoprotein of murine leukemia virus and the pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J. exp. Med.* **154,** 517.