AGE-RELATED IMMUNOHISTOPATHOLOGICAL CHANGES OF MURINE LUPUS NEPHRITIS IN NZB/NZW F₁ MICE USING SERIAL RENAL BIOPSY

HITOSHI TANAKA, TOSHIO KANEMURA, SHUNJI MATSUDA,
FUKUMI FURUKAWA, YOJI NAKAI, KENICHI SEKITA
AND YOSHIHIRO HAMASHIMA

*Department of Pathology, Faculty of Medicine,
Kyoto University, Sakyo-ku, Kyoto 606*

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Serial renal biopsies were performed to examine the age-related morphological changes of murine lupus nephritis in NZW/NZB (NZB/W) F₁ mice. Renal biopsies were performed at 3, 5, 7 and 9 months of age in the same mouse. The earliest histological change was slight mesangial thickening. Mesangial hypercellularity was seen in one-third of all the cases at 7 months. With age, the incidence of mesangial thickening was progressively increased, and capillary wall thickening appeared simultaneously. The incidence of interstitial cell infiltration was found in 100% at 11 months. Significant correlations among the capillary wall thickening, crescent formation and proteinuria were observed. In an immunofluorescent study, a significant correlation between immunofluorescent deposition in the capillary wall and proteinuria was observed. The pattern of immunoglobulin deposition changed from mesangial type to capillary type with age. However, the pattern of gp 70 (retroviral antigen) deposition in glomeruli varied considerably, and in some mice the gp 70 deposition decreased with age. Furthermore, a significant correlation between the titer of circulating gp 70 immune complex (IC) and gp 70 deposits in glomeruli was found. These results suggest that the age-related morphological changes are due to multifarious characters of IC and due to numerous immunological disorders.

NZB × NZW F₁ (NZB/W F₁) mice spontaneously develop an autoimmune disease resembling human systemic lupus erythematosus and die of immune complex (IC) glomerulonephritis (7, 10). It has been demonstrated that the development of autoimmunity in NZB/W F₁ mice results from the interaction of genetic, immunological, viral and endocrinological factors (17, 21, 23). Among these various factors, retroviral antigen (gp70) and nuclear antigen seem to represent a major feature in development of murine lupus nephritis. In these mice, gp70-anti-gp70 IC was detected in sera, and gp70 deposits were observed in glomeruli. Furthermore, antinuclear factor was eluted from renal lesions of NZB/W F₁ mice (14). Many investigators have reported the histopathological and immunopathological findings in the kidney of NZ mice (15, 5, 3, 2). It is well known that the histological and immunopathological findings in the kidney of NZB/W F₁ mice are too

various. Also various lesions at varying stages may be seen in the same kidney or even in the same glomerulus. However, these age-related histopathological changes have not been studied in the same mouse in detail.

In this study, we tried to analyze the age-related morphological changes in each mouse by performing serial renal biopsies at 3, 5, 7 and 9 months of age, in order to pursue the morphological changes with age individually. The morphological changes were examined in light and immunofluorescent microscopy. We found that the morphological abnormalities were variable and they changed with age. Furthermore, it was found that the pattern of immunofluorescent (IF) deposition was too various and changed with age.

MATERIALS AND METHODS

Mice: NZB/W F₁ hybrid mice were obtained by mating NZB females with NZW males in our colony. Female mice were used in this study. Sera were obtained at 4, 7 and 10-11 months of age and stored at -70°C until used.

Renal biopsy technique: Mice kidneys were biopsied at 3, 5, 7 and 9 months of age. All biopsies were performed as sterile open procedures under general anesthesia induced by intraperitoneal injection of sodium pentobarbital about 0.5 mg per 10 g body weight. Mice were fully anesthetized within 5-10 min after injection. The mice were stretched in a prone position on a cork board, and each foot was secured with string. Following the shaving and disinfection, dorsal skin and fascia were incised. Connective-tissue and fatty-tissue were gently and carefully separated from the kidney. The kidney block was obtained by using the trepan and/or ophthalmic curved scissors. Hemorrhage was slight and, if present, it was prevented by compression with absorbent cotton. The skin and fascia were sutured together. At the second biopsy of the same side, a slight adhesion was observed. The number of mice biopsied at 3, 5, 7 and 9 months were 80, 75, 75 and 47 respectively. At 11 months, the surviving 40 mice were sacrificed; other mice died of renal insufficiency or of complications after biopsy.

Light microscopy: Renal fragments were divided into two pieces for light and immunofluorescent microscopy. Renal tissue for light microscopy was fixed in 10% formalin, embedded in paraffin, sectioned at $2\text{ }\mu\text{m}$ and stained with hematoxylineosin (HE) and periodic acid-Schiff (PAS). Histological abnormalities of the kidney were observed on the basis of the following 10 categories (7) capillary wall thickening, (10) wire loop lesion, (17) mesangial thickening, (21) mesangial hypercellularity, (23) endocapillary hypercellularity, (14) karyorrhexis, (9) hyaline thrombus, (22) crescent formation, (1) tubular cast, (15) interstitial cell infiltration. These categories were seen in high frequency in lupus nephritis. Appearance of over 4 cells in one mesangial area or in one capillary lumen was regarded as hypercellularity.

Immunofluorescent (IF) microscopy: Renal tissue for immunofluorescent study was embedded in optimum cutting temperature (OCT) compound Tissue-Tek II (Lab-Tek products, Division of Miles Laboratory, Napier, Ill) and was frozen in dry ice-acetone. Cryostat sections of $2\text{ }\mu\text{m}$ thickness were stained by anti-mouse IgG₁ (Nordic, Netherlands), IgG₂ (Meloy, U. S. A.), IgA (Meloy, U. S. A.), IgM (Cappel, U. S. A.), C₃ (MBL, Nagoya, Japan) and anti-goat Rauscher gp70 antisera (a kind gift of Dr. Yoshiki, Sapporo City General Hospital, Japan) using

direct or indirect method. Direct IF staining, fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG₂, IgA, FITC-labelled rabbit anti-mouse C₃ were used. Indirect IF staining was performed by using unlabelled rabbit anti-mouse IgG₁, unlabelled goat anti-mouse IgM absorbed with non-IgM fraction containing IgG and IgA obtained by separation of BALB/c and C57BL/6 pooled sera on a Sephadex G-150 column. Murine retroviral antigen, gp70, was sought by using goat anti-Rauscher virus gp70 followed by FITC-conjugated rabbit anti-goat IgG serum. FITC-labelled rabbit anti-goat immunoglobulin (Ig) and FITC-labelled goat anti-rabbit Ig were prepared in our laboratory and absorbed with BALB/c sera before use. The immunological specificity of these monospecific antisera directed against mouse IgG₁, IgG₂, IgA and IgM was confirmed by IF test using the mouse myeloma cells, MOPC 31C (γ_1 , κ), HOPC 1 (γ_2a , λ_1), MOPC 141 (γ_2b , κ), J. 606 (γ_3 , κ), MOPC 511 (a_1 , κ), TEPC 183 (μ , κ), which were a gift of Prof. Honjo, Kyoto and Osaka University, Faculty of Medicine.

At least 15 glomeruli were examined in each section.

Detection of circulating gp70 immune complex (IC): Partial purification of gp70 in NZB mice sera was performed according to the method, as previously described by Yoshiki *et al.* (22). Using this gp70 in NZB mice sera, circulating gp70 IC was examined by inhibition radioimmunoassay (RIA). Briefly, a mixture of 20 μ l of the test samples adjusted to 70 μ l in RIA medium and 20 μ l of goat anti-Rauscher gp70 antiserum diluted in RIA medium containing 5% normal goat serum (NGS) was first incubated for 2 hr at room temperature, then mixed with 20 μ l of radio-labeled NZB gp70 diluted in RIA medium containing 5% NGS and was further incubated at room temperature overnight. After 100 μ l of rabbit anti-goat IgG serum were added, the reaction mixture was incubated for 2 hr at room temperature, followed by incubation at 4°C for 1 hr. Then the immunoprecipitate was washed twice, and the radioactivity was measured in a gamma counter. Percent inhibition was calculated and the amount of gp70 IC was estimated as units/ml according to the standard curve obtained by using pooled NZB serum which was regarded to have 1,000 units of free gp70 activity.

Measurement of anti-ds-DNA antibodies: Measurement of antibodies against double stranded DNA (dsDNA) was carried out by modified Farr assay (1). Briefly, a mixture of 0.1 ml serum sample diluted to 1: 10 in borate buffer (pH 8.4; ionic strength; 0.1) previously heated at 56°C for 30 min, and 0.1 ml ¹⁴C-dsDNA solution (20 ng dsDNA) was incubated at 37°C for 1 hr, followed by further incubation at 4°C for 18 hr. After 1 ml of cold 60% saturated ammonium sulfate was added and mixed, these mixtures were incubated and the precipitates were washed once with 1 ml of 50% saturated ammonium sulfate. The precipitates were dissolved in 0.1N HCl and mixed with scintillation fluid (PCS; The Radiochemical Centre, Amersham), and the radioactivity was measured by Packard liquid scintillation counter. The results were expressed as the percentage of ¹⁴C-dsDNA precipitated.

Measurement of proteinuria: Proteinuria was checked every two weeks, as described by Knight *et al.* (9). The mice that showed proteinuria of 111 mg/100 ml or more were regarded as positive.

Statistical analysis: Statistical analysis was performed using χ^2 test with Yates' correction and Student's t-test.

RESULTS

Light microscopic findings: The renal biopsy specimen was examined by light microscopy in the ten categories as described in "Materials and Methods". A section in which at least one of the ten categories was observed was regarded as abnormal. The incidence of abnormalities was 23% at 3 months, 40% at 5 months, 74% at 7 months, 90% at 9 months, and 100% at 11 months. Subsequently, we

TABLE 1. *Incidence of morphological abnormalities in light microscopic findings*

LM findings	Age				
	3M (n=80)	5M (n=75)	7M (n=75)	9M (n=47)	11M (n=40)
(1) Capillary wall thickening	0	12.0	38.6	59.5	50.0
(2) Wire loop	0	1.3	12.0	12.7	20.0
(3) Mesangial thickening	12.5	18.6	36.0	85.1	67.5
(4) Mesangial hypercellularity	0	1.3	4.0	31.9	27.5
(5) Endocapillary hypercellularity	0	9.3	33.3	34.0	45.0
(6) Karyorrhexis	0	5.3	9.3	10.6	30.0
(7) Hyaline thrombus	1.3	12.0	16.0	31.9	42.5
(8) Crescent formation	0	0	2.6	2.0	32.5
(9) Tubular cast	1.3	4.0	14.6	8.5	47.5
(10) Interstitial cell infiltration	2.6	4.0	42.6	51.0	100

M, months; n, number of mice.

FIG. 1. Light microscopic findings (HE, $\times 400$): Endocapillary hypercellularity, wire loop lesions and hyaline thrombus are seen in glomerulus at 7 months NZB/W F₁ mice.

TABLE 2. *Relationship between LM findings in each category and proteinuria (10–11 M)*

LM findings		Proteinuria		χ^2	p values
		+	—		
(1) Capillary wall thickening	+ —	14 4	6 16	8.18	0.01 > p > 0.001
(2) Wire loop	+ —	6 12	2 18	3.26	n.s.
(3) Mesangial thickening	+ —	14 4	13 9	1.17	n.s.
(4) Mesangial hypercellularity	+ —	7 11	4 18	2.12	n.s.
(5) Endocapillary hypercellularity	+ —	7 11	11 11	0.49	n.s.
(6) Karyorrhexis	+ —	7 11	5 17	1.23	n.s.
(7) Hyaline thrombus	+ —	10 8	7 19	2.28	n.s.
(8) Crescent formation	+ —	10 8	3 19	6.13	0.01 > p
(9) Tubular cast	+ —	11 7	8 14	2.43	n.s.
(10) Interstitial cell infiltration	+ —	18 0	22 0	0	n.s.

(+) positive (—) negative n.s.: not significant

found that many mice had histological abnormalities without proteinuria. The percentage in each category is shown in detail in Table 1. At 3 months, only slight mesangial thickening was found. At 5 months, capillary wall thickening and hyaline thrombus were observed in addition to the mesangial thickening. At 7 months, various histological abnormalities were observed (Fig. 1); capillary wall thickening, endocapillary hypercellularity and mesangial thickening were observed in over 30%, and interstitial cell infiltration was increased remarkably. At 9 months, capillary wall thickening, mesangial thickening and interstitial cell infiltration were increased in over 50%; especially, mesangial thickening was seen in 85%. At 11 months, all mice were sacrificed. Capillary wall thickening and tubular cast were seen in almost 50%, and interstitial cell infiltration was observed in all mice. In some cases, diffuse severe hyalinization was seen in glomeruli. However, wire loop lesions and mesangial hypercellularity were seen less frequent at 11 months than at 7 months. The incidence of wire loop lesions was not so high as expected. At that age, cell infiltration was seen in various organs; especially in kidney, lung, liver, salivary gland and thyroid gland.

Correlation between light microscopic findings and proteinuria: The relationship between the positive rate of light microscopic findings and proteinuria was investigated. It was found that most of the mice had some histological abnormalities without proteinuria at a young age. Table 2 shows the relationship between

TABLE 3. *Incidence of immunofluorescent deposition with age*

Age	anti-sera					
	gp70	IgG ₁	IgG ₂	IgA	IgM	C ₃
3 M (80)	1.2%	53.7%	17.5%	7.1%	63.5%	67.1%
5 M (75)	20.9	74.6	53.8	9.4	92.5	89.6
7 M (75)	61.2	95.3	92.4	40.6	90.0	96.9
9 M (47)	57.8	100	100	63.5	97.8	100
11 M (40)	69.4	100	97.3	62.1	100	100

(): number of mice tested

light microscopic findings in each category and proteinuria. Capillary wall thickening and crescent formation were significantly associated with proteinuria. There was no significant correlation between the other categories and proteinuria.

Immunofluorescent (IF) microscopic findings: The incidence of IF deposition in glomeruli is given in Table 3. At 3 months, deposits of gp70 and IgA were found in less than 10% of the mice; especially deposits of gp70 were found in only 1%. On the other hand, deposits of IgG₁, IgM and C₃ were observed in 50%, but IgG₂ deposits were seen in only 17%. Even at 5 months, deposits of IgA were present in less than 10% of the mice; however, deposits of gp70 and IgG₂ were increased. Deposition of gp70 was seen in about 20%, and IgG₂ was seen in 50%. The majority of these deposits were distributed in the mesangial area. At 7 months, deposits of IgG₁, IgG₂, IgM and C₃ were observed in over 90% of the mice, whereas deposits of gp70 and IgA were seen in a maximum of 60%. At this time, IF deposition was seen in the capillary wall as in the mesangial area. At 9 months, deposits of gp70 and IgA were also present in about 60% of the mice, but the deposits of other immunoglobulin and C₃ were seen in almost 100%.

The pattern of IF deposition was classified into the following 4 types; i) mesangial type, ii) mesangial and capillary type (deposits were seen in the mesangial area and capillary wall in one glomerulus), iii) capillary type, iv) mixed type (above three types were seen in one section). The changing patterns of deposits with age are summarized in Fig. 2. At the age of 3 to 5 months, IF deposition was seen in the mesangial area in most cases (Fig. 3). However, with age, the pattern of deposition changed from mesangial type to capillary type (Fig. 4). After 7 months, 4 types of patterns were seen in one section. At 11 months, the percentage of mixed type was relatively increased. However, the deposition of IgM was usually seen in the mesangial area. The changing patterns of IgG₂ and IgA deposits with age were similar to those of IgG₁ deposits.

The pattern of gp70 deposits were pursued in each individual mouse: The results are summarized in Table 4. Age-related changes of gp70 deposition were observed in each mouse. Group 1 had no deposition at any age, and group 2 showed gp70 deposition in the mesangial area only. In group 3, the pattern of deposits changed from mesangial type to capillary type. In group 4, capillary type was first observed. Other changing patterns were shown in group 5. On the other hand, deposition of IgG₁, IgG₂, IgA, IgM and C₃ changed basically from mesangial type to capillary type.

Relationship between distribution of immunofluorescent deposits and protein-

FIG. 2. Age-related changes of pattern in IF depositions of gp 70, IgG₁, IgM and C₃ in NZB/W F₁ mice. (□) negative, (▲) mesangial type, (●) mesangial and capillary type, (○) capillary type, (*) mixed type.

uria (11M): Significant correlation between IF findings and proteinuria was shown in only gp70 deposition. Relationship between the pattern of IF deposits and proteinuria is shown in Table 5. The capillary pattern in deposition of gp70, IgG₂, and C₃ was significantly associated with proteinuria.

Circulating gp70 IC: Circulating gp70 IC was examined at 4, 7 and 10-11 months. Fig. 5 shows the titer of circulating gp70 IC in each age of NZB/W F₁

FIG. 3. Immunofluorescent microscopic (IF) findings: Granular deposits of gp 70 are seen in glomerulus at 7 months NZB/W F₁ mice. $\times 400$

FIG. 4. IF findings: The same mouse (Fig. 3) showed fine granular gp 70 deposition along the capillary wall at 11 months. $\times 400$

TABLE 4. *Age-related changes of gp70 deposition pattern in individual mice*

Group	Mouse NO.	Age (M)				
		3	5	7	9	11
(1)	5, 12, 73, 84, 85, 93, 98, 102, 106, 108, 109, 113, 139 (total 13)	(-)	(-)	(-)	(-)	(-)
(2)	8, 9, 10, 15, 17, 50, 53, 59, 66, 76, 82, 83, 86, 91, 92, 94, 97, 119, 128 (total 19)	(-)	(-)	(-)	(-)	Me
		(-)	(-)	(-)	Me	Me
		(-)	(-)	Me	Me	Me
(3)	3	(-)	(-)	(-)	Me	Cap
	16, 103, 114, 121, 69, 75	(-)	Me	Me	Me	Cap
	81, 131	(-)	Me	Cap	n.d.	n.d.
	65, 95, 123	(-)	(-)	Me	Mix	n.d.
(4)	116, 125	(-)	(-)	Cap	Cap	Cap
	6, 107	(-)	(-)	Cap	n.d.	Mix
	1, 110	(-)	(-)	Mix	Mix	n.d.
(5)	68	(-)	Me	Me+Cap	n.d.	Me
	79	(-)	(-)	Me	Mix	Cap
	104	(-)	(-)	Cap	Me+Cap	n.d.
	123	(-)	Me	Cap	Cap	Me+Cap
	4	(-)	(-)	Me	Cap	(-)
	78, 80, 96, 118	(-)	(-)	(-)	Me	(-)
	117	Me	Me	Mix	(-)	n.d.
	126	(-)	Me+Cap	Me	(-)	Cap
	129	(-)	(-)	Me	(-)	Mix
	133	(-)	(-)	Me+Cap	(-)	n.d.
(-): negative n.d.: not done Me: mesangial type Me+Cap: mesangial and capillary type Cap: capillary type Mix: above three types are observed						

TABLE 5. *Relationship between distribution of immunofluorescent deposits and proteinuria (11 M)*

anti-sera	distribution	Proteinuria		χ^2	P Values
		(+)	(-)		
gp70	Me	4	7	6.23	0.01 < p < 0.02
	Cap	9	0		
IgG ₁	Me	8	8	0	n.s.
	Cap	2	2		
IgG ₂	Me	8	10	5.73	0.01 < p < 0.02
	Cap	9	0		
C ₃	Me	5	11	8.43	0.001 < p < 0.005
	Cap	9	0		
IgA	Me	10	9	0.20	n.s.
	Cap	2	1		
IgM	Me	23	8	0	n.s.
	Cap	0	0

FIG. 5. Age-related changes of circulating gp 70 IC in NZB/W F₁ mice. Over 20 units were regarded as positive.

mice. The mean level of gp70 IC was low at 4 months, but it increased progressively with age. Since over 20 units were regarded as positive, the positive rate was 25%, 91% respectively in each age. Furthermore, gp70 IC was observed in the individual mice. The majority of the mice that were negative at 4 months became positive at 7 and 10-11 months. However, some of the mice changed from negative at 4 and 7 months to positive at 10-11 months, and a few mice were negative at any age.

Relationship between circulating gp70 IC deposition of gp70 in IF staining: Table 6 shows the relationship between circulating gp70 IC and gp70 deposits in glomeruli. There was a significant correlation between circulating gp70 IC and gp70 deposits in glomeruli. However, there was no significant correlation between the titer of circulating gp70 IC and the distribution of gp70 deposits in glomeruli.

Anti-dsDNA antibodies: Anti-dsDNA antibodies were examined at 4, 7 and

TABLE 6. Relationship between circulating gp70 IC and gp70 deposits in glomeruli

Age	gp70 IC (+)		gp70 IC (-)		χ^2	P Values
	gp70 deposits (+)	gp70 deposits (-)	gp70 deposits (+)	gp70 deposits (-)		
4 M	4	5	6	39	2.97	n.s.
7 M	9	4	1	0	0.43	n.s.
10-11 M	21	9	2	0	0.834	n.s.
Total	34	18	9	39	20.2	p<0.001

FIG. 6. Age-related changes of anti-dsDNA antibodies in NZB/W F₁ mice. Over 10% of binding was regarded as positive.

10-11 months, as shown in Fig. 6. The respective mean level (Mean $\pm$ SEM) of anti-dsDNA antibodies was 8.10 ± 1.05 , 14.23 ± 2.16 and 25.44 ± 3.26 at each age. The level of anti-dsDNA antibodies increased progressively with age. More than 10% binding was regarded as positive, the positive rate was 25% at 4 months, 54% at 7 months and 67% at 10-11 months.

Proteinuria: The proteinuria of 111 mg/100 ml or more was regarded as positive. Proteinuria was first detectable at 5 months, and the cumulative incidence was 13.8% at 7 months and 45% at 11 months. The results are consistent with the findings of previous reports (9).

DISCUSSION

Many investigators have described the morphological changes of murine lupus nephritis, but they have not observed the age-related changes in the same

mouse. In the present study, we attempted to examine the age-related changes of murine lupus nephritis in individual mice using serial renal biopsies. Renal biopsies were performed 4 times bilaterally, and the mice were sacrificed at 11 months. Therefore, we serially observed the kidney tissues 5 times in a mouse. Consequently, we confirmed that the histological and immunopathological findings changed with age and that a variety of glomerular lesions was observed in one specimen. It should be considered whether the natural course of the renal disease was influenced by a sequence of renal biopsies, e.g. compensatory changes, stimulated adrenal cortical hormone and anesthesia. However, we found that it was not influenced by these factors, because the results of proteinuria, circulating gp70 IC and anti-dsDNA antibodies were consistent with those of the previous reports (9, 15, 16).

Some investigators reported that the early glomerular changes were hypercellularity with unfolding and thickening of the capillary walls to form typical wire loop deformities (10, 13, 5). After an interval of 1 to 2 months, the glomerulonephritis develops with focal and irregular hyaline thickening and deformity of the capillary basement membrane. The glomerulonephritis becomes more florid and clearly fibrinoid necrotic over the next 2 months (7, 10, 13, 5). In this study, the earliest histological changes was a slight mesangial thickening. Mesangial hypercellularity and endocapillary hypercellularity were seen in one-third of all cases at 7 months. With age, the incidence of mesangial thickening was progressively increased, and capillary wall thickening appeared simultaneously. Furthermore, hypercellularity of the mesangial area and endocapillary lumen was observed. The incidence of interstitial cell infiltration which was frequently observed in autoimmune disease mice (NZB, NZB/W F₁, MRL/l, BXSB) (18, 19), increased progressively, and it was seen in 100% at 11 months. However, mesangial thickening, capillary wall thickening and mesangial hypercellularity were less frequent at 11 months than 9 months. These findings showed that the glomeruli became homogeneously hyalinized at the end stage and lost intrinsic glomerular structures.

We attempted to correlate light microscopic findings and proteinuria. Lambert *et al.* (10) reported that the animals died of renal failure 3-4 week after the onset of proteinuria. It has been thought that proteinuria is an index of renal disease (9). But many mice had no proteinuria with morphological abnormalities in this study. Our results indicated that proteinuria was not the index of the onset of renal disease. Table 2 showed the significant correlations among capillary wall thickening, crescent formation and proteinuria. It should be considered that the damage of glomerular basement membrane was the most crucial factor in the induction of proteinuria. We have confirmed that mesangial alterations and the other morphological abnormalities did not seem to influence the occurrence of proteinuria.

Immunofluorescent deposits were observed in the mesangial area at an early age. With age, however, they were transferred to the capillary wall. These results were in agreement with the changes of light microscopic findings. However, Hicks *et al.* (6) reported that the glomerulitis of NZB/W F₁ mice at an early stage was associated with the deposition of immunoglobulin in fine granular line in the region of the basement membrane. The deposition of IgG₁ preceded that of IgG₂, whereas C₃ deposits paralleled the deposition of IgG₁ and IgM (Table 3). These results conflicted with the biological activities of mouse immunoglobulins, since mouse IgG₂ was known to fix the complement, while mouse IgG₁ was not.

On the other hand, deposition of IgM was usually seen in the mesangial area. Therefore, the complement was consumed with not only IgG₂-associated IC, but also IgM-associated IC. It is unclear whether the deposition of IgM in the mesangium was only influenced by the size of IgM-associated IC.

It has been reported that circulating gp70 IC, using conglutinin binding assay, was detected at 2 months preceding glomerular lesions, and reached the highest level at 4 months, subsequently decreasing with age (8). In this study the mean level of circulating gp70 IC increased progressively with age. However, since over 20 units of gp70 IC were regarded as positive, the positive rate did not increase after 7 months. Nevertheless, as shown in Table 6 deposition of gp70 was much influenced by circulating gp70 IC.

The deposition of gp70 showed various patterns in individual cases. Group 5 in Table 4 showed the particular changing pattern of gp70 deposits. Taken altogether, the renal changes were supposed to be influenced by several factors, such as amounts of antigens and antibodies, level of circulating IC, and IC size. Furthermore, these changes might be influenced by immunoglobulin class and subclass of anti-gp70 antibodies. Steward *et al.* (20) reported age and sex-related changes in immunoglobulin class and subclass of anti-DNA antibodies which can be correlated with the natural history of the murine lupus syndrome. In some cases, deposits of gp70 became negative at 9 months. These mice had severely hyalinized and or sclerosed glomeruli in light microscopy. Therefore, it should be considered that retroviral antigen, gp70, might disappear from the glomeruli. Mannik *et al.* (12) reported the removal of glomerular deposits of IC in mice by administration of excess antigen. On the other hand, deposition of IgG₁, IgG₂, IgM and C₃ was found homogeneously in these glomeruli.

There are several reports concerning the pathogenesis of glomerulonephritis in autoimmune disease mice. Andrews *et al.* (4) reported that the autoimmune mice had accumulated glomerular deposition of IgG and C₃, and in some cases, DNA and gp70 retroviral antigen. They all developed immune complex glomerulonephritis, a principal cause of death. Dubois *et al.* (4), Lambert *et al.* (11) and Burnet *et al.* (3) reported that the NZB/W F₁ mice developed a high titer of anti-nuclear antibodies associated with the development of rapidly progressive and lethal glomerulonephritis. Additionally, anti-DNA antibodies could be eluted from the glomerular lesions (14). In this study, however, no significant correlation between anti-ds DNA antibodies and proteinuria was observed. It may be considered that many other factors including gp70 retroviral antigen and nuclear antigen may be involved in the pathogenesis of murine lupus nephritis.

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ABBREVIATION

NZ mice; New Zealand mice, NZB mice; New Zealand Black mice, NZW mice; New Zealand White mice, NZB/W F₁ mice; (NZB×NZW) F₁ hybrid mice, gp70; major glycoprotein constituent of endogenous retrovirus envelope, gp70IC; retroviral gp70-anti-gp70 immune complexes, dsDNA; double stranded DNA.

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