CRESCENTIC TYPE NEPHRITIS INDUCED BY ANTI-GLOMERULAR BASEMENT MEMBRANE (GBM) SERUM IN RATS

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Abstract—An experimental model of crescentic type nephritis was established by immunizing rats that had been given at i.v. nephritogenic dose (0.4 ml/animal) of rabbit anti-rat glomerular basement membrane (GBM) serum [anti-GBM serum] with 5 mg of rabbit \( \gamma \)-globulin in Freund's complete adjuvant, and the process of nephritis was investigated by means of biochemical, histopathological and immunopathological analyses. Rats treated with anti-GBM serum and then with rabbit \( \gamma \)-globulin (group II) showed significantly high levels or a tendency for high levels of urinary protein content, N-acetyl-\( \beta \)-glucosaminidase activity and plasmin-like activity from the 20th day to the 40th day observations after the induction of nephritis, when compared to rats given anti-GBM serum alone (group I). On the 40th day, plasma urea nitrogen, cholesterol and fibrinogen levels were significantly higher in group II than in group I. Glomerular histopathological examination on the 40th day revealed that the incidence and the degree of severity of crescent formation, adhesion of capillary walls to Bowman's capsule and fibrinoid degeneration were remarkably greater in group II than in group I. However, no significant difference was seen between both groups on the thickening of capillary walls and mesangial proliferation. Linear deposits of rabbit IgG and rat IgG along the capillary walls as well as fibrinogen-reactive material deposits in Bowman's capsular spaces were observed by the immunofluorescence technique in both groups. The deposition of fibrinogen-reactive materials was considerably greater in group II than in group I. Moreover, the deposition of rat IgG was slightly greater in group II. These results suggest that the nephritis of group II closely resembles rapidly progressive glomerulonephritis in humans and thus seems to be an adequate experimental model for screening beneficial drugs on this type of nephritis.

Currently, glucocorticoids, nonsteroidal anti-inflammatory agents, immunosuppressants, anticoagulants and fibrinolytic agents are widely used for therapy of glomerulonephritis. Human glomerulonephritis is classified into various types on the basis of clinical and histopathological findings. Masugi nephritis or its modification induced in rats or rabbits by an injection of anti-kidney serum (1, 2) or anti-glomerular basement membrane (anti-GBM) serum (3) has been chiefly employed as an experimental model for evaluating the antinephritic effect of drugs. It has been generally believed that the immunological mode of development of the anti-GBM nephritis in rats consists of the following two phases: Immediately after the injection of rabbit anti-rat GBM serum, the primary phase (heterologous phase) is caused by a reaction between anti-rat GBM antibody and host's GBM. Consecutively, 7 to 10 days later, the secondary phase (autologous phase) is induced by a reaction between rat antibody against rabbit \( \gamma \)-
globulin and rabbit anti-rat-GBM antibody already fixed to glomeruli. It has been recognized that the levels of complements in serum are decreased during the primary and secondary phases (4, 5). Hammer and Dixon (6) presented that the glomerular injury in the primary phase appeared to be dependent to a great extent upon the participation of serum complements, while that in the secondary phase appeared to depend largely upon the host’s antibody response to the heterologous γ-globulin fixed in the glomeruli. It has been shown that severe nephritis is induced by enhancing the immune response in the secondary phase (7–9). We previously reported that the nephrotic type nephritis which was characterized by massive proteinuria and prominent hyperlipemia was induced in rats by two consecutive i.v. injections of rabbit anti-rat GBM serum at a 10 day interval; and dipyridamole, an antiplatelet coagulant, or mizoribine, an immunosuppressant, showed a beneficial effect in this model (10–12). The present study was carried out to establish the crescentic type nephritis resembling rapidly progressive glomerulonephritis (RPGN) in humans by immunizing rats that had received an i.v. injection of rabbit anti-rat GBM serum with rabbit γ-globulin. Furthermore, the establishment of this nephritis was investigated in detail from biochemical, histopathological and immunopathological viewpoints.

Materials and Methods

Preparation of rat-GBM: Rat GBM was prepared according to the method previously reported (13). Renal cortical tissues obtained from rats weighing approx. 200 g were ground by a glass homogenizer. The homogenates were passed through 42 mesh and then 120 mesh metal sieves, washing with ice-cold 0.9% NaCl solution. The suspension of sieved material was then poured on the top of a 200 mesh sieve and washed with ice-cold 0.9% NaCl solution several times. The material remaining on the 200 mesh sieve was collected, suspended in 0.9% NaCl solution, and the suspension was centrifuged at 1,500 rpm at 2°C for 15 min. The glomerular pellets of the sediment were examined under a phase microscope to confirm whether it contained only glomeruli which were free of cells and tubular fragments. The GBM was obtained from the isolated glomeruli by ultrasonic disruption as follows: The glomerular pellets obtained as mentioned above were resuspended in 0.9% NaCl solution. Sonic disruption was conducted by using Tomy’s ultrasonic vibrator (model UR-200 P) at 10 kW for 20 min, until complete fragmentation of the glomeruli had occurred. The sonically treated material was then centrifuged at 3,000 rpm at 2°C for 20 min. The sediment was washed five times with ice-cold 0.9% NaCl solution and pooled.

Production of rabbit anti-rat-GBM serum: The GBM obtained from the isolated glomeruli as mentioned above was suspended in 0.9% NaCl solution and adjusted to pH 8.2 with 0.1 M sodium borate. To this suspension, crystalline trypsin (2,000 E/G, Merck) was added at the rate of 50 mg per 10 g GBM, and the mixture was incubated at 37°C for 3 hr. The pH was readjusted to 8.2 with 0.1 M sodium borate every 30 min. Thereafter, the mixture was heated at 60°C for 30 min to inactivate trypsin and centrifuged at 27,000 rpm at 2°C for 35 min (Hitachi centrifuge 55 PA type). The supernatant was then dialyzed against distilled water and lyophilized. The dried GBM antigen thus obtained was dissolved in 0.9% NaCl solution and adjusted to pH 8.2 with 0.1 M sodium borate. To this suspension, crystalline trypsin (2,000 E/G, Merck) was added at the rate of 50 mg per 10 g GBM, and the mixture was incubated at 37°C for 3 hr. The pH was readjusted to 8.2 with 0.1 M sodium borate every 30 min. Thereafter, the mixture was heated at 60°C for 30 min to inactivate trypsin and centrifuged at 27,000 rpm at 2°C for 35 min (Hitachi centrifuge 55 PA type). The supernatant was then dialyzed against distilled water and lyophilized. The dried GBM antigen thus obtained was dissolved in 0.9% NaCl solution in a concentration of 5 mg/ml. To this solution, an equal volume of Freund’s complete adjuvant (FCA) was added, and the mixture was emulsified. The emulsion was intradermally injected into the four footpads of a male albino rabbit, weighing about 2 kg, in a dose of 0.1 ml each, once a week for 3 successive weeks.
Three weeks after the first injection, blood was preliminarily taken from the ear vein, and the titer of antibody was determined to confirm a sufficient rise of titer. Furthermore, one week later, 1 mg of the GBM trypsin-digested antigen dissolved in 0.9% NaCl solution was given i.v. into the ear vein as a booster. Five weeks after the first injection, blood samples were taken, sera were separated and then inactivated by heating at 56°C for 30 min. The inactivated sera were preserved by freezing until use.

**Experimental groups:** Male Sprague-Dawley rats, weighing approx. 180 g, were used in the experiment and divided into 3 groups, each consisting of 10 animals as follows: Normal group—Untreated rats. Group I (0.4 ml of anti-GBM serum, i.v.)—The rats were injected with 0.4 ml of rabbit anti-rat-GBM serum into the tail vein. Group II (0.4 ml of anti-GBM serum i.v.+5 mg of r-globulin in FCA, s.c.)—The rats received s.c. injection of 5 mg of rabbit r-globulin in 0.25 ml of FCA into the foot pads, immediately after the injection of 0.4 ml of rabbit anti-rat-GBM serum into the tail vein.

**Urine and blood collections:** The 24 hr urine samples were obtained by keeping each animal in individual metabolic cages for 24 hr. In this case, at the beginning and 5 hr later of the urine collection, each animal received 5 ml of distilled water twice orally without feeding. The urine thus obtained was then centrifuged at 3,000 rpm for 20 min at 4°C, and the supernatant was used for determination. After the last collection of urine samples, animals were lightly anesthetized with ether, and blood was taken in centrifuge tubes in the ratio of 9 parts blood to 1 part of 3.13% trisodium citrate from the abdominal aorta. The blood was then centrifuged at 3,000 rpm for 10 min at 4°C to obtain plasma.

**Determination of biochemical parameters in urine:** The protein content in urine was determined by the method of Kingsbury et al. (14) and expressed as mg/24 hr urine. As urinary enzymes, alkaline phosphatase (ALP) activity was determined by the method of Bessey et al. (15) by using disodium p-nitrophenyl phosphate (Daiichi Kagaku Co. Ltd.) as substrate. N-acetyl-β-glucosaminidase (NA-β-G) activity was determined according to the method of Hasebe (16) by using p-nitrophenyl-N-acetyl-β-D-glucosaminidase (BDH Chemical Co. Ltd., Poole, England) as substrate. Both enzymatic activities were expressed as mg of liberated p-nitrophenol/hr/24 hr urine. Moreover, plasmin-like (PL) activity was determined by a slight modification of the fibrin plate method described by Astrup and Mullertz (17). The fibrin plate was prepared by using plasminogen-free bovine fibrinogen (Daiichi Kagaku Co. Ltd.). The urine was dialyzed against running tap water for 1.5 hr, and 0.02 ml of the dialyzed urine was then put on the fibrin plate at 37°C. The PL activity was expressed as lysis area (mm²)/20 hr × 24 hr urine volume (ml).

**Determination of biochemical parameters in plasma:** Urea nitrogen content was determined by the method of Searcy and Cox (18) and expressed as mg/dl plasma. Cholesterol content was determined according to the method of Zurkowski (19), and fibrinogen content determined using a fibrometer (BBL Co. Ltd.). In order to assess blood coagulation, recalcification time (sec) was measured by a fibrometer.

**Assessment of histological parameters:** The kidney for light microscopy study was dehydrated and fixed by immersing the tissue stepwise into low to high concentrations of alcohol. Thereafter, the tissue was embedded in paraffin and cut into 2–3 μ thick sections. The sections were stained with hematoxylin and eosin, periodic acid Schiff, periodic acid silver methenamine and Masson trichrome (MT). Fifty glomeruli per section were observed under light microscopy for
evaluating the numbers and degree of the thickening of capillary wall, mesangial proliferation, crescent formation, adhesion of capillary wall to Bowman's capsule and fibrinoid degeneration. The results were expressed as percent of the numbers and degree of these histopathological changes.

Assessment of immunofluorescent preparations: The kidney for immunofluorescent microscopy study was fixed in alcohol, embedded in paraffin and sectioned as in the case of the kidney for light microscopy. After the sections were treated with 0.01% pronase at 37°C for 7 min, they were stained with fluorescein-isothiocyanate (FITC)-labeled goat anti-rabbit IgG serum (Capel Laboratories) and FITC-labeled goat anti-rat fibrinogen serum (Capel Laboratories), respectively, by means of a direct fluorescence antibody technique. The intensity of immunofluorescence was graded as −, +, +, ++, or +++.

Statistical analyses: Statistical analyses were carried out by using Student's t-test.

Results
1. Changes in body weight and urinary biochemical parameters after the induction of nephritis in groups I and II (Fig. 1)

Body weight: The average body weight of all groups was 180±4.2 g at the beginning of the experiment. The gain in body weights of the normal group, group I (0.4 ml of anti-GBM serum, i.v.), and group II (0.4 ml of anti-GBM serum+5 mg of r-globulin in FCA, s.c.) were 130.0, 128.7 and 91.1 g, respectively, during the 39 day observation period after the injection of rabbit anti-rat GBM serum. The average body weight of group II on the 39th day was significantly lower than that of group I.

Protein content: The urinary protein content of the normal group was 5−10 mg/24 hr urine through the 40 day observation period. The urinary protein excretion of group I was already 420 mg/24 hr urine on the 1st day.
and was then maintained at the same level up to the 20th day, and after this, it decreased rapidly. On the other hand, the protein excretion of group II was rather lower than that of group I up to the 10th day. Thereafter, the level of group II rose rapidly and attained the maximum level of 620 mg/24 hr urine on the 20th day. The levels of group II were significantly higher than those of group I from the 20th day.

**NA-β-G activity**: The urinary NA-β-G activity of group I showed pronounced increases which were approx. 160% higher than the normal level on the 5th and the 10th days and then continued to reduce till the 40th day. In group II, the enzyme activity rapidly increased after the 5th day and remained higher than that of group I from the 20th to the 40th days, as in the case of the protein excretion.

**PL activity**: PL activity was not detectable in the urine from the normal group. An apparent PL activity in both groups I and II was recognized from the 1st day already. The levels of the PL activity tended to be higher in group II than in group I from the 20th day.

2. **Plasma biochemical parameters in groups I and II on the 40th day after the induction of nephritis (Fig. 2)**

- **Urea nitrogen and cholesterol levels**: The plasma urea nitrogen levels of groups I and II were 1.6 and 2.9 times higher than the normal level, respectively. On the other hand, the cholesterol levels of groups I and II were 1.6 and 2.6 times higher than the normal level, respectively. In this case, a significant difference was observed between groups I and II.

- **Fibrinogen level and blood coagulation times**: The plasma fibrinogen levels of groups I and II were 1.5 and 1.9 times greater than the normal level, respectively, and there was a significant difference between groups I and II. The recalcification time (sec) was significantly prolonged in group II as compared to those in the normal group and group I.

3. **Histopathological findings in groups I and II on the 40th day after the induction of nephritis (Fig. 3 and Photo 1)**

When histological assessment was carried out on the kidneys of the 40th day, an apparent difference was recognized between groups I and II. In glomeruli, the incidence and the degree of severity in crescent formation, adhesion of capillary walls to Bowman's capsule and fibrinoid degeneration were strikingly greater in rats of group II given anti-GBM serum plus rabbit γ-globulin.

![Fig. 2. A comparison of plasma biochemical parameters in groups I and II on the 40th day after the induction of nephritis. Each column indicates the mean±S.E. obtained from 10 rats. See Fig. 1 for the explanations of groups. * indicates a significant difference from group I at P<0.05.](image-url)
in FCA than in rats of group I given anti-GBM serum alone. However, no significant difference could be seen in the thickening of capillary walls and mesangial proliferation between these two groups. In addition, focal tubular atrophy and dilation and chronic inflammatory cell infiltration in the interstitium were also demonstrated in group II.

4. Histopathological changes during the nephritic process of group II (Table 1)

The thickening of capillary walls in glomeruli was already seen on the 1st day and persisted throughout the course of the disease. On the 20th day, the fibrinoid thrombosis in capillary lumen and necrotic changes in all regions of capillary loops were clearly detected in glomeruli, and thereafter the extent of these changes decreased gradually up to the 40th day. On the other hand, the mesangial proliferation, adhesion of capillary walls and crescent formation were observed in glomeruli from the 5th, 10th and 20th days onwards, respectively. These changes then became more prominent.
Table 1. Histopathological changes in kidneys during the nephritic process of group II

| Days | N   | Thickening of capillary walls | Leucocyte infiltration | Mesangial proliferation | Crescent formation | Adhesion | Fibrinoid degeneration | Tubular atrophy | Interstitium |
|------|-----|-------------------------------|------------------------|------------------------|-------------------|----------|------------------------|----------------|-------------|
| 1    | (10)| +                             | ±                      | -                      | -                 | -        | -                      | -              | -           |
| 5    | (10)| +                             | ±                      | ±                      | -                 | -        | -                      | -              | -           |
| 10   | (10)| ++                            | ±                      | +                      | ±                 | ±        | ±                      | ±              | ±           |
| 20   | (10)| ++                            | ±                      | ++                     | +                 | +        | ++                     | +              | +           |
| 30   | (10)| ++                            | ±                      | +++                    | +                 | +        | ++                     | ++             | ++          |
| 40   | (10)| ++                            | ±                      | +++                    | +++               | +        | +++                    | +++            | +++         |

Days: Days after the injection of rabbit anti-rat-GBM serum. N: Number of rats. The degree of histopathological changes in kidneys: -, no change; ±, minimal; +, mild; ++, moderate and +++, severe.

Table 2. Immunofluorescent findings in glomeruli of groups I and II on the 40th day after the induction of nephritis

| Groups | N   | Rabbit IgG | Rat IgG | Fibrinogen |
|--------|-----|------------|---------|------------|
| Normal | (10)| -          | -       | -          |
| I      | (10)| ++         | +       | +          |
| II     | (10)| ++         | ++      | +++        |

See Fig. 1 for the explanation of groups. N: Number of rats. The intensity of fluorescence: -, negative; +, mild; ++, moderate and ++++, severe.

Photo 2. Fluorescent micrograph of glomeruli from rats of group II on the 40th day after the induction of nephritis (all x200). a: A glomerulus stained with FITC-labeled goat anti-rabbit IgG serum. b: A glomerulus stained with FITC-labeled goat anti-rat IgG serum. c: A glomerulus stained with goat anti-rat fibrinogen serum.
progressively with time course. Likewise, tubular and interstitial damages became also more severe with time course from the 10th day onwards.

5. Immunofluorescent findings in groups I and II on the 40th day after the induction of nephritis (Table 2 and Photo 2)

   Immunofluorescent staining for rabbit IgG, rat IgG and rat fibrinogen in glomeruli was negative in the normal group. However, in groups I and II, linear deposits of rabbit IgG and rat IgG were detected along the glomerular capillary walls (Photo 2-a and Photo 2-b), whereas fibrinogen-reactive materials were seen in Bowman’s capsular spaces (Photo 2-c). The deposition of rabbit IgG in group II was moderate (++) and was to the same degree as that in group I. On the other hand, the degree (moderate, +++) of rat IgG deposition in group II was slightly greater than that (mild, +) in group I. While group I showed mild deposition (+) of fibrinogen-reactive materials, it was severe (+++) in group II.

Discussion

In the present study, we were able to establish the crescentic type nephritis which was characterized by severe glomerular lesions with extensive formation of crescents by immunizing rats that had received i.v. injection of a nephritogenic dose (0.4 ml/animal) of rabbit anti-rat-GBM serum with 5 mg of rabbit γ-globulin in FCA. The mechanisms of the crescentic type nephritis is induced by immunization of rabbit γ-globulin are not defined in detail. In the immunofluorescent study, the deposition of rat IgG along the glomerular capillary walls was slightly greater in rats of group II given anti-GBM serum plus rabbit γ-globulin in FCA than in rats of group I given anti-GBM serum alone. Furthermore, the serum antibody titer against rabbit γ-globulin in group II rapidly elevated from the 15th day and there-

after continued to elevate. However, in group I, no elevation of the antibody titer was observed throughout the 40th day measurements (unpublished data). Therefore, it is suggested from the above results that immunization with rabbit γ-globulin may serve severe glomerular lesions in this model by enhancing the immune response in the autologous phase via the persistent production of host’s antibody against rabbit γ-globulin. On the other hand, Bhan et al. (20) reported that an apparent nephritis was induced by giving lymph node cells from donors immunized with rabbit γ-globulin into rats that had received a subnephritogenic dose of rabbit anti-GBM serum. Accordingly, it cannot be denied that cell-mediated mechanisms also participate partly in the enhancement of glomerular lesions by immunizing with rabbit γ-globulin.

Immunofluorescent staining for fibrinogen in glomeruli was severe (+++) in group II, while it was mild (+) in group I. It is well-known in general that crescent formation is related to intraglomerular fibrin deposition and reflects intraglomerular coagulation. Fujimoto (21) stressed that the crescents consisted of proliferated Bowman’s capsular epithelial cells as well as a part of monocytic–epithelioid cells which migrated from the glomerular capillaries into Bowman’s capsular spaces. Although the relationship between crescent formation and fibrin deposition has not been exactly elucidated, fibrinogen-reactive materials were seen at a location corresponding to the crescents in Bowman’s capsular spaces. Therefore, a marked accumulation of fibrin in Bowman’s capsular spaces may stimulate capsular epithelial cells and cause the proliferation of these cells leading to the crescent formation. It is concluded from these results that the experimental nephritic model of the crescentic type may be produced by the persistent immune response in the autologous phase and by
Intraglomerular hypercoagulation subsequent to the immune response. Therefore, immunosuppressants, anticoagulants and fibrinolytic agents are considered to be effective on this experimental nephritis. Moreover, this model closely resembles RPGN in humans and may be suitable for screening beneficial drugs on RPGN. Work is presently being carried out to evaluate the antinephritic action of several new agents containing mizoribine, an immunosuppressant, and MD-803 ((2R, 4R)-4-methyl-1-[N2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginy]-2-piperidine-carboxylic acid monohydrate) by using this model.

References

1) Masugi, M. and Tomizuka, Y.: Über die spezifischen zytoxischen Veränderungen der Niere und der Leber durch das spezifische Antiserum (Nephrotoxin und Hepatotoxin). Zugleich ein Beitrag zur Pathogenese der Glomerulonephritis. Trans. Japan. Pathol. Soc. 21, 329–341 (1931)
2) Masugi, M.: Über die experimentelle Glomerulonephritis durch das spezifische Antinierenenserum. Ein Beitrag zur Pathogenese der diffusen Glomerulonephritis. Beitr. Pathol. Anat. 92, 429–466 (1934)
3) Thomson, N.M., Simpson, I.J. and Peters, D.K.: A quantitative evaluation of anticoagulants in experimental nephrotoxic nephritis. Clin. Exp. Immunol. 19, 301–308 (1975)
4) Hayashi, D.: Forschung über den Komplementtiter des Serums im Verlaufe der diffusen Glomerulonephritis des Menschen und der experimentellen Nephritis des Hundes. Chiba Igakkai Zasshi 18, 833–877 (1940)
5) Suzuki, Y., Nagamatsu, T. and Ito, M.: Pharmacological studies on experimental nephritic rats (7). Changes of serum complement levels in modified Masugi nephritic rats and effect of drugs on it. Pharmacometrics 18, 461–467 (1979)
6) Hammer, D.K. and Dixon, F.J.: Experimental glomerulonephritis. II. Immunologic events in the pathogenesis of nephrotoxic serum nephritis in the rats. J. Exp. Med. 117, 1019–1034 (1963)
7) Unanue, E.R. and Dixon, F.J.: Experimental glomerulonephritis Vi. The autologous phase of nephrotoxic serum nephritis. J. Exp. Med. 121, 715–725 (1965)
8) Shimotori, T.: Studies on the onset and development of nephrotoxic nephritis of rabbits. The role of kidney fixing antibodies in nephrotoxic sera and of endogenous antibodies to duck globulin. Japan. J. Nephrology 14, 289–287 (1972) (Abs. in English)
9) Shimizu, F.: Persistent Masugi nephritis induced in rats in spite of the suppression of host antibody responses against the heterologous nephrotoxic antibodies. Japan. J. Exp. Med. 44, 443–449 (1974)
10) Suzuki, Y., Ito, M., Kito, T. and Nagamatsu, T.: Pharmacological studies on experimental nephritic rats (12). Nephritogenous model induced by the repeated administration of anti-rat GBM rabbit serum. Pharmacometrics 21, 503–513 (1981) (Abs. in English)
11) Suzuki, Y. and Ito, M.: Studies on antinephritic action of diprydamole (1). The effect of diprydamole on anti-GBM induced nephritis in rats. Japan. J. Nephrology 23, 323–332 (1981) (Abs. in English)
12) Okamoto, K., Ito, M. and Suzuki, Y.: Studies on antinephritic effect of mizoribine (p-INN, Bredinin), a new immunosuppressive agent, and azathioprine (1). Effect on the nephrotoxic type of anti-GBM nephritis in rats. Japan. J. Pharmacol. 33, 541–548 (1983)
13) Ito, M., Nagamatsu, T. and Suzuki, Y.: Pharmacological studies on experimental nephritic rats (10). Changes in coagulation-fibrinolysis system in the course of anti-GBM induced nephritis. Japan. J. Nephrology 23, 297–308 (1981) (Abs. in English)
14) Kingsbury, F.B., Clark, C.P., Williams, G. and Post, A.L.: The rapid determination of albumin in urine. J. Lab. Clin. Med. 11, 981–989 (1926)
15) Bessey, O.A., Lowry, O.H. and Brock, M.J.: A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. 146, 321–329 (1946)
16) Hasebe, K.: Biochemical studies on synovial fluid. (1) Muco polysaccharase activities in synovial fluid in rheumatoid arthritis. Fukushima J. Med. Sci. 15, 35–44 (1968)
17) Astrup, T. and Müller, S.: The fibrin plate method for estimating fibrinolytic activity. Arch. Biochem. Biophys. 40, 346–351 (1952)
18) Searcy, R.L. and Cox, F.M.: A modified technique for ultramicro estimations of urea nitrogen. Clin. Chim. Acta 8, 810–812 (1963)
19) Zurkowski, P.: A rapid method for cholesterol determination with a single reagent. Clin. Chem. 10, 451–453 (1964)
20) Bhan, A.K., Schneeberger, E.E., Collins, A.B. and McCluskey, R.T.: Evidence for a pathogenic role of a cell-mediated immune mechanism in
experimental glomerulonephritis. J. Exp. Med. 148, 246–260 (1978)

21) Fujimoto, T.: Pathology of Masugi nephritis. Forty years' progress and present status. In Masugi Nephritis and Its Immunopathologic Implications, Edited by Okabayashi, A. and Kondo, Y., p. 1–35, Igaku-Shoin, Tokyo and New York (1980)