EXPERIMENTAL GLOMERULONEPHRITIS IN MICE AS A MODEL FOR IMMUNOPHARMACOLOGICAL STUDIES

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Abstract—The experimental glomerulonephritis was caused by the intravenous injection of a subnephrotoxic dose of nephrotoxic serum in the mice which had been previously immunized with rabbit IgG and complete Freund’s adjuvant. The elevation of urinary protein excretion, serum blood urea nitrogen level and cholesterol level and the decrease of serum albumin level were demonstrated in nephritic mice. Hypercellularity in the glomerulus and hyalinosis in tubular system were observed in histopathological studies. The clinical signs in this experimental model were similar to human glomerulonephritis. In a transfer experiment, sensitized lymphocytes against rabbit IgG were necessary for the onset of disease. Clear remission of glomerulonephritis was indicated by the administration of cyclophosphamide or 6-mercaptopurine. Glucocorticoids showed moderate suppression of the development of this nephritis. These evidences suggest that this experimental model is useful for the immunopharmacological research of glomerulonephritis.

Several kinds of experimental glomerulonephritis can be produced in most laboratory animals. These experimental models are mainly employed for the research in pathology and immunology. In the field of pharmacology, there are a few kinds of experimental models. The commonly employed models are nephritis caused by immune complex or nephrotoxic serum (NTS) in rats. The clinical signs of these two models show a strict correlation to the human disease (1, 2). However, there are still some problems in these two models with respect to the reproducibility of the experiments, the participation of immunological mechanisms and testing the efficacy of drugs. In order to solve these problems, we have examined modified NTS nephritis in mice as a model for immunopharmacological research of glomerulonephritis.

MATERIALS AND METHODS

Animals: DDY and C57BL/6 male mice weighing 18–20 g were used.

Drugs: Cyclophosphamide (CY: Shionogi, Osaka), 6-mercaptopurine (6MP: Takeda, Osaka), hydrocortisone (Hyd C: Nippon Merck-Banyu, Tokyo), prednisolone (Pred: Takeda, Osaka) and dexamethasone (Dexa: Banyu, Tokyo) were purchased.

NTS: NTS was obtained from rabbits which had been immunized by injection of 1.0 ml of an emulsion containing 10% mouse glomerular basement membrane (GBM) rich fractions and complete Freund’s adjuvant (CFA) intramuscularly 4 times weekly. GBM rich fractions were prepared according to the method of Spiro (3). In brief, thin kidney
slices from mice were forced through 150 mesh stainless sieves. The material which emerged through the sieves was collected in phosphate buffered saline (pH 7.4) without Ca**2+** and Mg**2+** and centrifuged at 400 g at 4°C for 10 min. The precipitate was obtained as a GBM rich sediment. Anti-serum was obtained 7 to 10 days after the last injection and absorbed with homologous erythrocytes after inactivation of complement at 56°C for 30 min.

Experimental glomerulonephritis: Experimental glomerulonephritis was caused in mice by a method similar to that used for the rats (4). Mice were immunized by an intraperitoneal (i.p.) injection of 0.5 mg rabbit IgG (RGG) emulsified with 0.25 ml of CFA. Five days later, an appropriate dose of NTS (0.1 ml/animal) was injected intravenously (i.v.). In order to evaluate the severity of the symptoms, urine and blood samples were collected at 1, 5, 10, 15, 20 and 25 days after the injection of NTS and measured for the amount of urinary protein, cholesterol, blood urea nitrogen (BUN) and serum albumin. Urinary protein was measured by using test paper containing tetrabromophenol blue (Combi sticks II, Miles Lab.). Cholesterol was measured by using acetic acid anhydride and sulfuric acid according to the method of Zurkowski (5). BUN was measured by the urease-indophenol method according to the method of Saito et al. (6). Albumin was assayed by the bromcresol green method as described by Dounas (7). Pathological changes in the kidney were assessed in a semiquantitative fashion after stainings with hematoxylin and eosin or periodinate-Shiff according to the method of Litwin et al. (8). When the effect of drugs on the nephritis was tested, each drug was administered i.p. for 14 days after the injection of NTS.

Adoptive transfer experiment: The adoptive transfer experiment was done in C57BL/6 mice. Donor mice were immunized by injection of 0.1 ml of an emulsion containing 0.1 mg RGG and CFA into four foot pads. Five days later, the popliteal and axillary lymph node cells or sera were collected from each of the animals and mixed homogeneously. One milliliter of Eagle's medium containing 2×10^7 lymphocytes or 1.0 ml of the serum was injected i.v. into recipient mice and then 24 hr later, a sub-nephrotoxic dose of NTS was injected. The same procedure was done by using the lymphocytes collected from mice which had been immunized with egg albumin. For investigating the role of Thy 1 positive cells in the development of disease, treated cells were obtained as follows: 5×10^7 sensitized lymphocytes were incubated with anti-Thy 1.1 or Thy 1.2 antigen serum at the dilution of 1:500, at 4°C for 30 min, washed once by centrifugation, resuspended in 1 ml of 1:20 diluted rabbit complement and incubated for a further 60 min at 37°C. After washing three times with Eagle's medium, the recovered viable cells were adjusted. The transfer procedure and nephritis experiment were done as described above.

Statistics: Results were statistically evaluated using the Student's *t*-test. In histopathological studies, the statistical significance was tested by Wilcoxon's U-test.

RESULTS

Effect of immunization with RGG on NTS nephritis: Mice which had received only 0.1 ml NTS without previous immunization with RGG and CFA showed slight changes of each of the parameters (Fig. 1). On the contrary, significant increases of urinary protein and serum cholesterol level and decrease of serum albumin level were observed in the preimmunized group. Significant decrease of BUN level was observed on the 1st and 5th days in both groups. In the pathological study, the character of the glomerular lesions was similar to the findings in human rapidly
progressive glomerulonephritis (Fig. 2). The histopathological scores of the RGG pre-immunized animals are significantly higher than those of only NTS injected animals ($P < 0.05$). When NTS in a dose of 0.2 ml or 0.3 ml was injected, similar acceleration of the symptoms was recognized.

Adoptive transfer experiments: As a similar acceleration was demonstrated in C57BL/6 mice, an adoptive transfer experiment was done to search for the mechanisms of the disease. Significant increases in urinary protein and serum cholesterol and decrease in serum albumin were found in mice which had received the lymphocytes sensitized against RGG prior to NTS treatment (Table 1). Such changes were not observed in any mice of other groups. Histological changes were slightly more moderate than the nephritis caused by active immunization and NTS injection. In order to study the character of the effector cells, the effect of anti-Thy 1.1 or 1.2 antigen serum on the ability to cause the nephritis was examined. When the lymphocytes treated with anti-Thy 1.2 antigen serum were transferred, the increase of urinary protein and the changes of serum parameters were not observed (Table 2). Contrary to anti-Thy 1.2 antigen serum, anti-Thy 1.1 antigen serum did not affect the ability of lymphocytes to cause the nephritis.

Effect of CY and 6MP: By i.p. administration of CY at doses of 5 and 10 mg/kg and 6MP at a dose of 20 mg/kg for 14 days after the NTS injection, both drugs inhibited the nephritic changes in some parameters by means of urinary protein and serum cholesterol and albumin (Figs. 3 and 4). 6MP at a dose of 10 mg/kg showed little inhibition of each of the parameters. Each histopatholo-
Table 1. Excretion of urinary protein and serum parameters in mice receiving the lymphocytes or serum 24 hr prior to NTS treatment

| Group | Transfer | Urinary protein (unit) | Alb (g/dl) | Cholesterol (mg/dl) | BUN (mg/dl) |
|-------|----------|------------------------|------------|---------------------|------------|
| I     | Normal   | 2.6                    | 3.8        | 82.0                | 32.5       |
|       | lymphocytes | (±0.16)            | (±0.09)    | (±1.17)             | (±1.04)    |
| II    | RGG-sens | 3.5*                   | 3.4*       | 127.6*              | 34.3       |
|       | lymphocytes | (±0.15)              | (±0.14)    | (±3.79)             | (±6.70)    |
| III   | RGG-sens | 2.55                   | 3.6        | 77.1                | 29.2       |
|       | serum    | (±0.189)              | (±0.08)    | (±2.06)             | (±0.63)    |
| IV    | EA-sens  | 2.70                   | 3.6        | 64.7*               | 31.9       |
|       | lymphocytes | (±0.170)             | (±0.04)    | (±2.23)             | (±1.95)    |

Each parameter was measured on the day 15 after NTS injection. Each group consists of 10 animals. *P<0.05, †P<0.01.

Table 2. Effect of anti-Thy 1 antigen serum on sensitized lymphocytes which are capable of causing IgG accelerated NTS nephritis in C57BL/6 mice

| Group | Urinary protein (score) | Alb (g/dl) | Cholesterol (mg/dl) | BUN (mg/dl) |
|-------|-------------------------|------------|---------------------|------------|
|       |                         | mean (±S.E.)|
| Normal | 1.3                     | 4.5        | 83.4                | 18.4       |
|        | (±0.33)                | (±0.05)   | (±5.24)             | (±4.20)    |
| Control | 2.5*                   | 4.3*       | 136.9†              | 30.4       |
|         | (±0.50)                  | (±0.02)  | (±18.65)            | (±10.72)   |
| Thy 1.1 | 2.6*                   | 4.2*       | 98.8*               | 20.4       |
|         | (±0.61)                  | (±0.01)  | (±3.89)             | (±5.48)    |
| Normal | 2.3                     | 4.0        | 81.5                | 27.7       |
|        | (±0.18)                | (±0.09)   | (±2.93)             | (±2.93)    |
| Control | 4.0*                   | 3.0*       | 278.0*              | 102.9†     |
|         | (±0.00)                  | (±0.08)  | (±23.08)            | (±14.98)   |
| Thy 1.2 | 2.8                     | 4.0        | 81.0                | 31.2       |
|         | (±0.24)                  | (±0.06)  | (±3.31)             | (±0.74)    |

Each parameter was measured on day 16 after NTS injection. Each group consists of 10 animals. *P<0.05, †P<0.01.

The surgical score of the mice given 5 mg/kg CY, 10 mg/kg CY or 20 mg/kg 6MP was significantly different from that of the control (P<0.05).

Effect of glucocorticoids: Hyd C at doses of 1 and 5 mg/kg indicated the tendency of inhibition or clear inhibition to the elevation of urinary protein, BUN and cholesterol and the decrease of albumin (Fig. 5). Pred at doses of 1 and 5 mg/kg indicated the clear inhibition or the tendency of inhibition to the elevation of urinary protein and BUN (Fig. 6). However, the effect of Pred on the decrease of albumin and the increase of cholesterol was obscure. Dexa at doses of 1 and 5 mg/kg indicated the inhibition or the tendency of inhibition to the increase in urinary protein (Fig. 7). However, the change in cholesterol was not influenced.

DISCUSSION

The present results indicate that RGG
accelerated NTS nephritis in mice is a good model for the pharmacological research of glomerulonephritis. There are two reasons to support the idea. One is that the disease in mice is caused relatively severely and reproducibly. The other is that the drugs which are often used as a remedy for nephritis in clinical cases show the remission of this RGG accelerated NTS nephritis.

Little attention has been paid to the experimental nephritis in mice (9-14). The nephritis in mice so far reported is mainly caused by NTS or soluble antigen-antibody complexes. When the nephritis in mice is compared to that in rats, there are slight differences between them. Unanue et al. have reported that NTS nephritis in mice is relatively mild and develops slowly in comparison to that of rats (9). On the contrary, Benacerraf et al. and Tada et al. have reported that immune complex nephritis in mice are more severe than that of rats (10, 11); and it is also reported that severe arteritis or endocarditis besides the glomerular lesion are usually found in the nephritic mice. The nephritis reported here may be the mixed type of disease of the above two nephritides. The development of the disease is similar to immune complex nephritis with respect to the severity and the time of onset, but the lesion is confined to the kidney as well as in NTS nephritis. The changes of the clinical signs by nephritis are nearly the same as in the case of rats. Therefore, this glomerulonephritis in mice is as good an experimental model as that in rats. In addition, if immunopharmacological study is planned, the mouse is more suitable species than the rat because...
the immunology in the mouse is as well investigated as human immunology.

In the present study, the acceleration of NTS nephritis by preimmunization of RGG was clearly recognized. Though a precise mechanism for the acceleration is uncertain, it is clear that the T cell plays an important role in the onset of the disease. By the transfer of RGG sensitized lymphocytes to the normal recipient, the acceleration of NTS nephritis was reproduced, and the ability of sensitized lymphocytes is diminished by the treatment with anti-Thy 1.2 antigen serum. These evidences suggest the participation of sensitized T cells against RGG for the acceleration of the disease. However, the character of the T cell is yet uncertain. Recently, the relationship between surface markers and function of T cells has been actively investigated. Helper T cells for antibody formation and delayed hypersensitivity T cells have the similar surface antigen. If the acceleration occurred through either of the nephritis would be caused by completely different mechanisms. If the transferred T cell population contained mainly helper T cells, the reaction must be caused by immune complex mechanisms. On the other hand, if the delayed hypersensitivity T cell is mainly involved, the nephritis must be caused by cell mediated mechanisms. Since it is impossible to distinguish the helper and delayed hypersensitivity T cell by their surface antigen, further experiments must be done to determine the onset mechanisms of the disease. While the mechanisms of the disease is yet uncer-
The participation of the T cell has been proven in the acceleration of the disease. Nowadays, nonspecific antiphlogistic drugs and occasionally immunosuppressive drugs such as CY and 6MP, single or combined, have been widely used as a remedy for glomerulonephritis. In the present study, CY and 6MP showed dramatic remission compared to the mild remission by glucocorticoids. The same tendency is reported in clinical studies (15). Actually, no specific drug treatment is yet available for nephritis. One of the reasons why there is no potent drug for nephritis is due to the absence of a good experimental model. Therefore, this RGG accelerated NTS nephritis in mice is one of the most useful models for immuno-pharmacological research on glomerulonephritis.

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