An Experimental Model of Nephritis Induced by Calf Serum Injection in Mice

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Accepted July 6, 1984

Abstract—Acute glomerulonephritis characterized by proteinuria, hypoalbuminemia and leukocytosis was induced in mice by repeated intraperitoneal injections of calf serum (1 ml/mouse×10). In mice treated with calf serum, hypercellularity, karyorrhexis, expansion of the mesangium and hyalinosis in the glomeruli were observed by light microscopy. Furthermore, circulating immune complexes were detected in the serum, and deposits of mouse IgG and C3 on the basement membranes of the glomeruli were demonstrated immunohistochemically. Oral administration of cyclophosphamide or 6-mercaptopurine at a dose of 20 mg/kg/day significantly suppressed the development of this nephritis. Dexamethasone (0.5 mg/kg/day) caused moderate inhibition of the nephritic changes. These results suggest that this experimental model may be useful for evaluation of anti-nephritic drugs.

Materials and Methods

Animals: Male ICR mice were obtained from Charles River Japan, Inc., and given laboratory chow and water ad libitum throughout the experiment.

Induction of nephritis: Mice (8 weeks old) were treated intraperitoneally with 1.0 ml/day of calf serum (Flow Laboratories, U.S.A.) for 10 consecutive days. The control group received the same volume of saline.

Evaluation of nephritis: Mice were placed in individual metabolic cages, and their urine was collected for 24 hr periods before and 4, 9 and 14 days after serum injection. To avoid the influence of stress, animals were kept in the cages for 1–2 days before starting to collect urine. The urine was centrifuged at 2800 r.p.m. at 4°C for 10 min, and the supernatant fluid was used for biochemical
analyses. Blood was taken by cardiac puncture under ether anesthesia, and the numbers of leukocytes and erythrocytes were counted in a TOA Microcellcounter CC-108. Biochemical parameters such as protein (5), \( \gamma \)-glutamyl transpeptidase (\( \gamma \)-GTP) (6), albumin (7), cholesterol (8) and blood urea nitrogen (BUN) (9) in the urine and serum were analyzed with an automatic analyzer (Hitachi 706D).

Serum immune complex was determined by the method of Wehler et al. (10) or by the method of Casali et al. (11). Sections of kidney were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Masson-trichrome (Masson) or periodic acid-methenamine silver (PAM) for light microscopic examination. Immunofluorescence microscopy was carried out by the method of Tsuji et al. (12). Tissues were frozen in liquid nitrogen, and 6 \( \mu \)m cryostat-sections were stained with FITC-labeled IgG of goat anti-mouse IgG (heavy and light chain specific) and FITC-labeled IgG of goat anti-mouse C3 (Cappel Laboratories, U.S.A.).

Drugs: Cyclophosphamide, 6-mercaptopurine, dexamethasone and indomethacin (Sigma, U.S.A.) were dissolved or suspended in 5% gum arabic solution. All drugs were administered orally immediately after treatment with calf serum.

Statistics: Statistical significance was evaluated by Student’s \( t \)-test.

**Results**

1. Effect of repeated injections of calf serum in mice
   1) Changes in body and organ weight: As shown in Fig. 1, repeated intraperitoneal injections of calf serum increased the body weight, associated with accumulation of ascitic fluid. It also caused dose-dependent nephromegaly and splenomegaly and decrease in thymus weight.
   2) Changes in biochemical parameters in the urine and serum: As illustrated in Fig. 2, injection of 0.5 or 1.0 ml of calf serum decreased the urinary volume and tended to increase the urinary protein level and \( \gamma \)-GTP activity on day 10. Injection of 1.5 ml of serum did not cause oliguria, but significantly increased the urinary protein level and \( \gamma \)-GTP activity on day 10 (260% and 180%, respectively), although the levels had returned almost to normal by day 15.
   3) Changes in numbers of leukocytes and erythrocytes: As shown in Table 1, injection of 1.0 or 1.5 ml of calf serum significantly increased the leukocyte count on day 10, but did not change the erythrocyte count.
   4) Serum immune complex: No circulating immune complex was detected in the control...
group on day 10 by either the Clq solid-phase binding test or the conglutinin solid-phase binding test (Table 2). However, after injection of 1.0 ml of calf serum for 10 days, it was detected in nephritic mice by both methods.

5) Light microscopic observations: Glomeruli were obtained from mice treated with calf serum (1 ml/day for 10 days) and examined histologically (Fig. 4). No abnormalities were seen in control mice (Fig. 4a). However, in mice treated intraperitoneally with calf serum, three types of alterations of the glomeruli were seen: one was glomerular hypercellularity associated with increase in the number of macrophages containing PAS-positive granules. The mesangial area was slightly expanded, but the capillary wall was not thickened (Fig. 4b, 4c). A second change was swelling of the glomerular cells and expansion of the mesangium in the glomeruli. Occasionally karyorrhexis of glomerular cells was seen. Hypercellularity was not obvious (Fig. 4d). The third type of glomerular alterations was hyalinosis in association with changes of the second type (Fig. 4e). The frequencies of these three types of glomerular changes were 50–60%, 20–30% and 10–20%, respectively.

6) Immunofluorescence microscopic observations: Mouse kidneys obtained on day

![Fig. 2. Changes in urinary volume, protein and γ-GTP activity by repeated injections of calf serum in mice. Experimental conditions and symbols are as for Fig. 1.](image)

![Fig. 3. Changes in biochemical parameters in serum by repeated injections of calf serum in mice. Experimental conditions and symbols are as for Fig. 1.](image)
10 were stained with FITC-labeled anti-mouse IgG and anti-mouse C3. Kidneys from mice treated with calf serum showed IgG staining predominantly as linear and granular patterns along the basement membranes of the glomeruli (Fig. 6a). C3 was also found to be deposited in the same location of IgG (Fig. 6b). Kidneys from mice treated with syngeneic mouse serum (Fig. 5a, b) or saline (not shown) showed only weak and homogeneous staining in the glomeruli or no fluorescence.

2. Effects of various drugs on calf serum-induced nephritis

Treatment with 1.0 ml/day of calf serum for 10 consecutive days induced appreciable nephritic changes. Therefore, this schedule was used for evaluating the effects of drugs on calf serum-induced nephritis.

Table 3 shows the effects of cyclophosphamide and 6-mercapto purine on calf serum-induced nephritis. At doses of 20 mg/kg, these immunosuppressants inhibited the calf serum-induced changes in body and kidney weight, urinary volume, urinary protein level, serum albumin level and leukocyte count. Under these experimental conditions, cyclophosphamide was more effective than 6-mercaptopurine.

As shown in Table 4, the anti-inflammatory drug dexamethasone (0.5 mg/kg) caused slight inhibition of calf serum-induced oliguria, nephromegaly, proteinuria and hypoalbuminemia. Indomethacin (1.0 mg/kg) significantly inhibited only the decrease of serum albumin level and did not affect other nephritic changes.

Table 1. Changes in number of leukocytes and erythrocytes induced by calf serum injection

| Treatment   | Serum dose (ml/day) | No. of animals | Leukocytes (x10^6/mm³) | Erythrocytes (x10^6/mm³) |
|-------------|---------------------|----------------|------------------------|-------------------------|
| Control     |                     | 11             | 101±15                 | 954±16                  |
| Calf serum  | 0.5                 | 9              | 122±22                 | 1011±23                 |
|             | 1.0                 | 10             | 156±17*                | 895±36                  |
|             | 1.5                 | 10             | 173±24*                | 935±23                  |

Values are means±S.E.M. on day 10. Significant difference from the control group (*P<0.05).

Table 2. Effect of calf serum on the level of circulating immune complex

| Treatment   | Circulating immune complex (µg/ml) |
|-------------|-------------------------------------|
|             | Clq-method | Conglutinin-method |
| Control     | N.D.       | N.D.               |
| Calf serum  | 44±19      | 24±4               |

Calf serum (1.0 ml/day) was injected intraperitoneally for 10 days. Values are means±S.E.M. for 8 mice on day 10. N.D. = not detectable.

Discussion

Recently, Kelley and Winkelstein (4) reported that injections of human plasma (1.0 ml/day, i.p., for 10 days) into mice caused glomerulonephritis. Since human plasma is not readily available, we examined whether calf serum could also induce glomerulonephritis in mice. The results reported herein show that repeated intraperitoneal injections of calf serum induced the glomerulonephritic changes with increases in the urinary protein level and γ-GTP activity, serum cholesterol and BUN level, and decrease in the serum albumin level. Furthermore, after this treatment, proliferative glomerulonephritic changes characterized by glomerular hypercellularity were seen histologically, circulating immune complex became detectable, and in immunohistochemical studies, IgG and C3 were found to be deposited along the basement membranes of the glomeruli.
Fig. 4.
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Fig. 4. (a) Glomerulus of a control mouse, showing the normal appearance. PAS, ×641. (b-e) Glomeruli of calf serum-treated mice. Calf serum (1.0 ml/day) was injected for 10 successive days. (b) Glomerular hypercellularity is seen with slight expansion of the mesangium. PAS, ×641. (c) Increase in the number of macrophages containing deposits and fibrinoid deposition are seen. Masson, ×641. (d) Swelling of the glomerular cells and expansion of the mesangium are seen. PAS, ×641. (e) Hyalinosis, swelling of the glomerular cells and expansion of the mesangium are observed. PAS, ×641.
Fig. 5. Glomeruli of mouse serum-treated mice. (a) Mouse serum (1.0 ml/day) was injected for 10 successive days, and the preparation was stained with goat anti-mouse IgG. Only weak and homogeneous staining is seen. ×400. (b) Treatment was as for (a), and the preparation was stained with goat anti-mouse C3. Only weak and homogeneous staining is seen. ×400.
Fig. 6. Glomeruli of calf serum-treated mice. (a) Calf serum (1.0 ml/day) was injected for 10 successive days, and the preparation was stained with goat anti-mouse IgG. IgG deposits are seen mainly on the basement membranes of the glomeruli. ×400. (b) Treatment was as for (a), and the preparation was stained with goat anti-mouse C3. C3 deposits are seen corresponding to those of IgG. ×400.
Table 3. Effects of cyclophosphamide and 6-mercaptopurine on calf serum-induced nephritis in mice

| Treatment           | Dose (mg/kg) | Body weight (g) | Kidney weight (mg) | Urinary volume (ml/24 hr) | Urinary protein (g/dl) | Serum albumin (g/dl) | Leukocyte count ($\times 10^2$/mm$^3$) |
|---------------------|--------------|-----------------|-------------------|--------------------------|------------------------|---------------------|----------------------------------------|
| Control             |              |                 |                   |                          |                        |                     |                                        |
| Calf serum          |              |                 |                   |                          |                        |                     |                                        |
| +cyclophosphamide   | 20           | 34.4±0.7***     | 280±10            | 3.30±0.16***             | 2.14±0.21***           | 1.9±0.0***          | 48±11***                                |
| +6-mercaptopurine   | 20           | 34.0±0.8***     | 260±10*           | 2.11±0.17*               | 2.98±0.53***           | 1.7±0.1***          | 57±3***                                 |

Values are means±S.E.M. for 10 mice on day 10. Significant difference from the group treated with calf serum only (*P<0.05, **P<0.01, ***P<0.001).

Table 4. Effects of dexamethasone and indomethacin on calf serum-induced nephritis in mice

| Treatment            | Dose (mg/kg) | Body weight (g) | Kidney weight (mg) | Urinary volume (ml/24 hr) | Urinary protein (g/dl) | Serum albumin (g/dl) | Leukocyte count ($\times 10^2$/mm$^3$) |
|----------------------|--------------|-----------------|-------------------|--------------------------|------------------------|---------------------|----------------------------------------|
| Control              |              |                 |                   |                          |                        |                     |                                        |
| Calf serum           |              |                 |                   |                          |                        |                     |                                        |
| +dexamethasone       | 0.5          | 32.3±0.7***     | 250±10**          | 2.12±0.29               | 3.82±0.94             | 1.7±0.1*            | 114±12                                 |
| +indomethacin        | 1.0          | 34.8±0.8        | 280±10            | 1.79±0.12               | 4.04±0.37             | 1.7±0.0**           | 125±19                                 |

Values are means±S.E.M. for 10 to 12 mice on day 10. Significant difference from the group treated with calf serum only (*P<0.05, **P<0.01).
In all mice treated with calf serum, antibody against calf serum was detected by double gel diffusion analysis (data not shown), whereas Kelley and Winkelstein (4) found that in mice treated with human plasma, the incidence of animals with circulating antibody against human plasma was 48%. This suggests that the features of the disease are affected by the antigen and other experimental conditions. It is likely that development of immune complex disease is influenced by various factors such as the ability to produce antibody, the affinity and class of antibody, the size and charge of the immune complex, and the rate of elimination of the immune complex (2, 13). This may explain why nephritic changes were greatest on day 10 after the first injection of calf serum and then gradually disappeared and why no clear dose-dependency on calf serum was observed for several parameters.

The changes in urinary volume, protein level, albumin level and nephromegaly observed in nephritic mice were similar to those in acute glomerulonephritis in humans.

The serum protein level decreases owing to increased glomerular permeability in human glomerulonephritis, and especially nephrosis. However, the serum protein level increased markedly in nephritic mice in this study. This increase may have been due to an inflammatory response and/or hyperglobulinemia as a result of antibody production against heterologous protein.

γ-GTP activity is high in the kidney, where it is located almost entirely in the renal tubules (14). Thus the increase in urinary γ-GTP activity in various human renal diseases is thought to be due to damage of the renal tubules (15). In calf serum-induced nephritis in mice, urinary excretion of γ-GTP was also significantly increased, and this was also probably due to damage of the tubules.

There are previous reports on induction of nephritis in mice by nephrotoxic serum (NTS) (16, 17) or soluble antigen-antibody complexes (passive serum sickness) (18-21).

However, it is difficult to prepare large amounts of NTS or soluble immune complexes for studies. There are also a few reports of experimental glomerulonephritis in mice (13, 22, 23). Nagai et al. (22) induced glomerulonephritis by injection of NTS into mice that had been immunized with rabbit IgG and complete Freund's adjuvant. This experimental nephritis was inhibited by cyclophosphamide and 6-mercaptopurine and was moderately suppressed by glucocorticoids. McLeish et al. (23) reported induction of chronic serum sickness nephritis by repeated intraperitoneal injections of apoferritin for 4 weeks. Furthermore, Isaacs and Miller (13) produced several types of nephritis by injection of dextran. However, all these methods of producing nephritis take much more time than injection of calf serum.

In the present study, we found that cyclophosphamide and 6-mercaptopurine, which are used as immunosuppressants in the treatment of human glomerulonephritis, markedly suppressed calf serum-induced nephritis. The anti-inflammatory drug dexamethasone was also moderately inhibitory.

These findings suggest that calf serum-induced nephritis in mice may be a useful model to use in a short-term screening test for the anti-nephritic effects of drugs.

Acknowledgement: The authors are grateful for the assistance in immunofluorescence studies by Dr. Takao Tsuji, Associate Professor, and Dr. Kenji Takahashi, Health Research Center, Okayama University, Okayama.

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