

T Cell-mediated Induction of Anti-GBM Glomerulonephritis in WKY Rats

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Summary. Glomerulonephritis induced in Wistar Kyoto (WKY) rats by the intravenous administration of rabbit anti-glomerular basement membrane antibody is characterized by a glomerular accumulation of T lymphocytes and monocytes/macrophages, crescent formation, and massive proteinuria. Although this model was initiated by the antibody injection, the involvement of cell-mediated immunity was also suggested since a crucial role of CD8-positive T lymphocytes was demonstrated in the induction of this model.

An implication of humoral and cell-mediated immunity in this model was examined in the present study. The glomerular lesions and urinary protein excretion were deteriorated in rats preimmunized with rabbit IgG or inoculated with rabbit IgG-sensitized rat lymphocytes before the glomerular basement membrane (anti-GBM) antibody administration. In contrast, the anti-GBM glomerulonephritis was ameliorated by the passive transfer of a rat anti-rabbit IgG antibody. These observations suggested that cell-mediated immunity advanced the disease and humoral immunity suppressed it.

We then examined the involvement of T cell receptor $\alpha\beta$ on T lymphocytes by administering a monoclonal antibody against the receptor (R73) in rats before or after the anti-GBM antibody injection. Pretreatment with the antibody markedly suppressed the glomerular lesion and proteinuria. However, the suppressive effect was minimal in rats given the antibody after the anti-GBM antibody injection. These results showed that the anti-GBM nephritis in WKY rats was mediated by T cell receptor $\alpha\beta$ to initiate cell-mediated immunity.

Key words—T cell receptor $\alpha\beta$, anti-GBM glomerulonephritis, CD8⁺ T cells, WKY rat.

INTRODUCTION

We previously demonstrated that glomerulonephritis induced in Wistar Kyoto (WKY) rats by an administration of heterologous anti-glomerular basement membrane (anti-GBM) antibody was characterized by a glomerular accumulation of CD8-positive T lymphocytes (CD8⁺ T) and monocytes/macrophages (Mo/M ϕ), crescent formation, and massive proteinuria¹. These features were different from the conventional anti-GBM nephritis models, which were characterized by a glomerular accumulation of polymorphonuclear leukocytes and the distinct involvement of a humoral immune response and complement activation². Since the anti-GBM glomerulonephritis in WKY rats was markedly suppressed in animals by the depletion of CD8⁺ T, crucial roles of the CD8⁺ T were identified in this model^{1,3}. Several key molecules such as cell adhesion molecules and chemokines were also shown to mediate the induction and progression of this model^{4,5,6}.

As CD8⁺ T are generally regarded as cytotoxic T cells in cell-mediated immune tissue injury, the cytotoxic activity of CD8⁺ T is suggested to be involved in the CD8⁺ T-dependent anti-GBM glomerulonephritis model. On the other hand, several studies have demonstrated that CD4-positive T lymphocytes

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Abbreviations—CD4⁺ T, CD4-positive T lymphocytes; CFA, complete Freund's adjuvant; GBM, glomerular basement membrane; MHC, major histocompatibility complex; Mo/M ϕ , monocytes/macrophages; PAS, periodic acid-Schiff; WKY, Wistar Kyoto.

(CD4⁺ T) were also infiltrated in the glomeruli of the anti-GBM glomerulonephritis, suggesting a role of the CD4⁺ T in the induction⁷⁻¹⁰.

Thus, it is still unclear whether cell-mediated immunity is involved or which types of T cells are major players in this model although it is evident that the rabbit anti-GBM antibody and CD8⁺ T mediated the anti-GBM glomerulonephritis model in WKY rats. We therefore aimed to clarify the involvement of the immunity for rabbit IgG and T cell receptor $\alpha\beta$ in the present study. Effects of T cell receptor $\alpha\beta$ blocking on the development of this model were examined by administering a monoclonal antibody against a constant determinant of T cell receptor $\alpha\beta$ (R73) expressed on both CD4⁺ and CD8⁺ T^{11,12}.

MATERIALS AND METHODS

Animals

Male WKY rats at 12 weeks of age (body weight: 200-250g) were used (Charles River Japan Inc., Atsugi, Kanagawa, Japan).

Rabbit anti-rat GBM antiserum

The glomeruli were isolated from kidneys of WKY rats by the sieving method, and the glomerular basement membrane (GBM) fraction was separated as reported previously¹³. The GBM fraction was solubilized by trypsin (2.5 mg for 500 mg of the GBM fraction, type III, Sigma Chemical Co., St. Louis, MO). The trypsin-solubilized GBM preparation was emulsified in complete Freund's adjuvant (CFA, Dibco Laboratories, Detroit, MI) and used for the immunization of rabbits (1 mg each of the trypsin-solubilized GBM) three times subcutaneously. The antisera collected were heat-decomplemented and absorbed with 1/10 volume of packed rat erythrocytes twice overnight at 4°C. The antisera showing selective reactivity to GBM and tubular BM in rat kidney sections by immunofluorescence microscopy were pooled as an anti-GBM antiserum.

Rat anti-rabbit IgG antibody and rabbit IgG-sensitized lymph node cells

Twenty WKY rats were immunized twice with purified rabbit IgG (1 mg) emulsified at an interval of seven days and their sera and lymph node cells were collected one week after the second boost. The immunoglobulin fraction was partially purified from the pooled sera by 40%-ammonium sulfate precipitation and adjusted to a concentration to 30 mg/ml in PBS.

The inguinal and maxillary lymph nodes were removed to isolate lymphocytes by tweezing with forceps and passing through cotton meshes. They were suspended in RPMI 1640 medium containing 10% fetal calf serum and antibiotics, and placed at 37°C for one hour in plastic dishes to remove adherent cells. Then non-adherent cells were gently recovered from the dishes and incubated with nylon wool packed in a 20 ml-syringe column at room temperature for 30 min to separate T lymphocytes¹⁴. The cells recovered from the column as non-adherent cells were examined by membrane immunofluorescence for rat CD5 (Serotec, Oxford, England), indicating that most of the recovered cells were CD5⁺ T lymphocytes (86%).

Similarly, normal rat immunoglobulin fraction and normal T lymphocytes were prepared from twenty control WKY rats.

Experimental schedule

To examine the involvement of immunity to rabbit IgG in the induction of anti-GBM nephritis in WKY rats, animals were actively immunized subcutaneously either with 1 mg of rabbit IgG emulsified in CFA (n=10) or with complete Freund's adjuvant (CFA) alone (n=10) in their backs three days before intravenous injection with the anti-GBM antiserum or normal rabbit serum (5 rats each, 0.25 ml/kg body weight).

The effect of the passive transfer of rat anti-rabbit IgG immunoglobulins on the induction of this anti-GBM nephritis was investigated: ten rats were given anti-rabbit IgG immunoglobulins (n=5, 30 mg/rat) intraperitoneally 12 hours after injection of the anti-GBM serum injection (0.25 ml/kg body weight). The time point and administration route were determined for the anti-rabbit IgG antibody to react primarily to the anti-GBM antibody fixed to GBM and to minimize the formation of anti-rabbit IgG-rat antibody complexes in the circulation.

As a control, a group of 5 rats were given rat anti-rabbit IgG immunoglobulins (30 mg/rat) 12 h after normal rabbit serum (0.25 ml/kg body weight) injection.

The effect of the passive transfer of rabbit-IgG-sensitized lymph node cells on the anti-GBM nephritis was also examined by intravenous injection with the lymph node cells 7×10^7 cells/rat separated from rats sensitized with rabbit IgG (n=5) or untreated animals (n=5) 3 h after the anti-GBM antibody serum administration (0.25 ml/kg body weight).

A monoclonal anti-rat T cell receptor $\alpha\beta$ antibody (R73, ECACC, England, 1 mg/kg body weight) was

administered twice to five WKY rats intraperitoneally 1 and 2 days before the anti-GBM antiserum injection. As a control, irrelevant mouse IgG was administered in 5 rats by the same protocol. Another group of 5 rats was injected with the anti-GBM antibody and then given a monoclonal anti-rat T cell receptor $\alpha\beta$ antibody (R73) three times at days 2, 3, and 4.

All animals were sacrificed at day 5 to examine their kidneys by histology and immunohistochemistry.

Urine samples were collected by housing the animals for 24 hours in metabolic cages before and 1, 3, and 5 days after the anti-GBM antiserum injection. The amount of urinary protein was measured by using a protein assay kit (Nippon Bio-Rad Laboratories, Tokyo, Japan)

Histological examination

Each kidney specimen was divided into two parts: one was fixed in methyl-Carnoy's fixative and embedded in paraffin for histology and immunohistochemistry, and the other was quick frozen in n-hexan at -70°C for immunofluorescence and the detection of CD4^{+} T by immunohistochemistry.

The paraffin-embedded sections were stained with periodic acid-Schiff (PAS) for light microscopy. For the detection of CD8^{+} T and Mo/M ϕ , the paraffin embedded sections ($4\ \mu\text{m}$ -thickness) were deparaffined and incubated sequentially with 1:20 diluted normal goat serum, anti-CD8 antibody (OX8, PHLS Centre for Applied Microbiology & Research, Wiltshire, UK, 1:200 dilution), or anti-monocyte/macrophage antibody (ED1, Dainippon Seiyaku Co., Tokyo, Japan, 1:500 dilution) for 1 hr, and peroxidase-goat anti-mouse immunoglobulin antibody-dextran polymer (EnVision DAKO Japan, Kyoto, Japan) for 30 min. The reactivity was visualized with 3' tetrahydroxyl diaminobenzidine and hydrogen peroxide¹⁵.

The frozen samples were sectioned in a cryostat at $4\ \mu\text{m}$ -thickness and the sections were fixed in cold acetone for 5 min. By a computer-based immunofluorescence microscopy (FW 400, Leica Microsystems, Cambridge, UK), immunofluorescence intensity and the localization of rabbit IgG, rat IgG and rat C3 in the kidney were investigated by using FITC-conjugated goat anti-rabbit IgG and anti-rat C3 (Organon Teknika Corporation, West Chester, PA) and goat anti-rat IgG (Seikagaku Kogyo Co., Tokyo, Japan). The sections for immunohistochemistry were incubated sequentially with 1:20 diluted normal goat serum for 30 min, an anti-CD4 antibody (OX35, BD Biosciences, Franklin Lakes, NJ, 1:100 dilution) for

1 hr, peroxidase-goat anti-mouse immunoglobulin antibody-dextran polymer (EnVision DAKO Japan, Kyoto, Japan) for 30 min, and then with 3' tetrahydroxyl diaminobenzidine and hydrogen peroxide.

The numbers of CD8^{+} T, ED1^{+} Mo/M ϕ and CD4^{+} T were counted in more than 50 glomeruli of each kidney sample as reported previously⁶.

Statistical analysis

Data are expressed as means \pm SD. Statistical significance was evaluated by non-parametric Mann-Whitney analysis.

RESULTS

The glomerular hypercellular lesion was more extensive in rats immunized actively with rabbit IgG before injection of the anti-GBM antiserum than in control animals injected with the anti-GBM antiserum alone (Fig. 1) or those immunized with CFA and then given the antibody. The glomeruli were enlarged, with an increase in the number of infiltrating cells mostly observed in the capillary lumen, and were associated with crescent formation.

Interestingly, these glomerular lesions subsided with the passive administration of rat anti-rabbit IgG immunoglobulins, compared with those in control rats (Fig. 1). The glomerular hypercellularity was focal and segmental. The administration of a rat anti-rabbit IgG antibody did not interfere with the binding of the rabbit anti-GBM antibody to GBM (Fig. 2). Fixation of rat IgG and C3 along GBM was faint or negligible in rats 5 days after injection with the anti-GBM antibody alone or combined with normal rat immunoglobulins. However, a significant increase in rat IgG deposition was observed in rats given a rabbit anti-GBM antibody and an additional rat anti-rabbit IgG antibody, although the enhancement of complement deposition was still vague (Fig. 2).

The glomerular lesions were more intense than those in the control rats and the glomerular structure was severely impaired in rats receiving rabbit IgG-sensitized T lymphocytes after anti-GBM antibody injection (Fig. 1). In these glomeruli, giant cells containing several nuclei were frequently observed, and most of the glomerular capillary lumen was occluded by mononucleated cells and a PAS-positive proteinaceous substance. No effects on the glomerular lesions were observed in animals receiving unsensitized T lymphocytes (data not shown).

By immunohistochemistry, an accumulation of CD4^{+} T was detected in each glomerular cross-

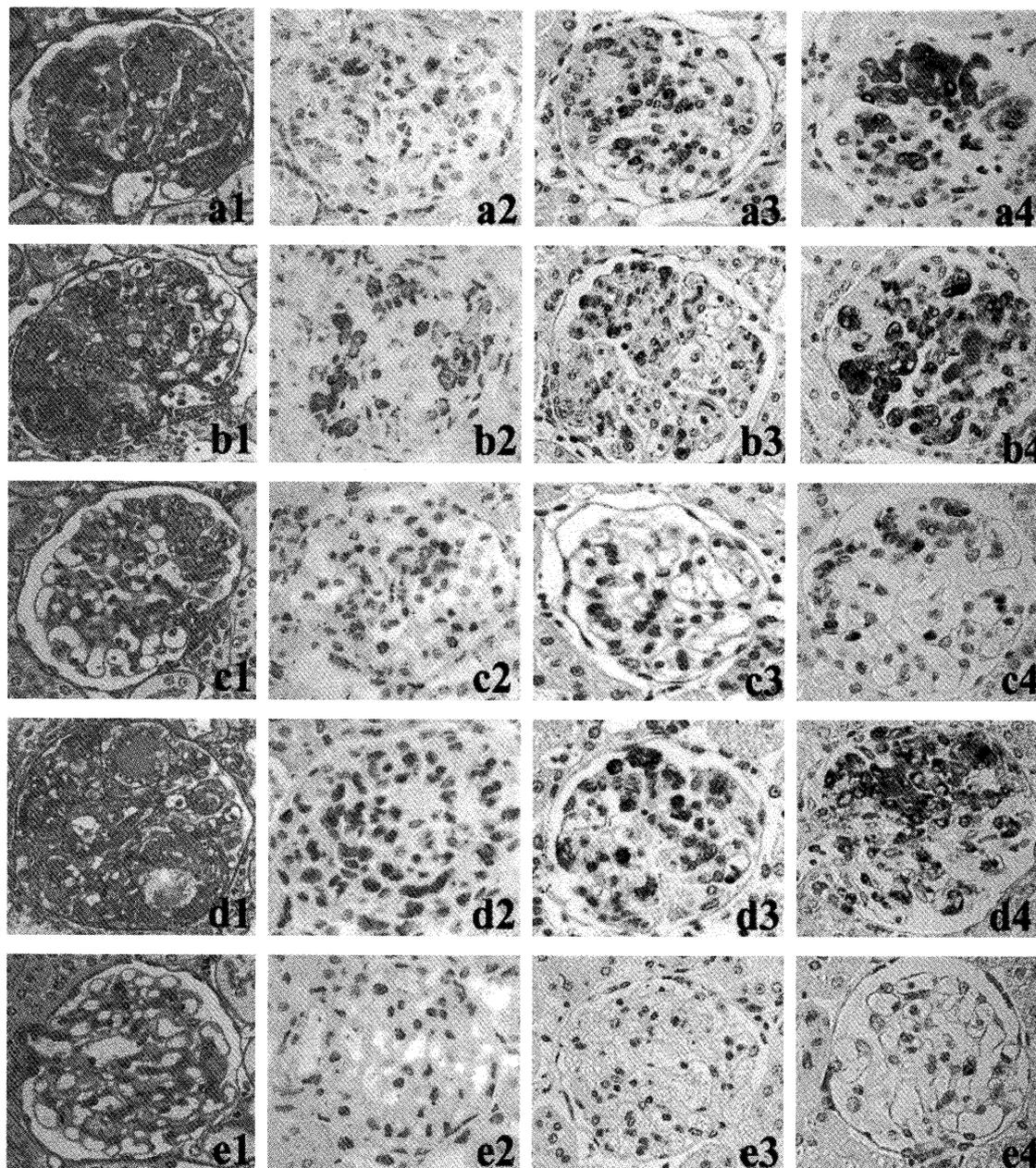


Fig. 1. Glomerular histology and CD4-, CD8- and ED1-immunostaining

A glomerular hypercellular lesion with mononuclear cell infiltrates is observed at day 5 after anti-GBM antibody administration (a1-e1). The hypercellularity (a1) and accumulation of CD4⁺ T (a2), CD8⁺ T (a3) and Mo/M ϕ (a4) observed in a rat given the anti-GBM antibody alone is enhanced by preimmunization with rabbit IgG (b1-4) but is diminished by rat anti-rabbit IgG antibody administration (c1-4). The passive transfer of T cell-rich lymph node cells obtained from rats sensitized with rabbit IgG also accelerates the glomerular lesion (d1-4). No significant glomerular lesions or leukocyte accumulation are observed in a control rat given the rat anti-rabbit IgG antibody and normal rabbit serum (e1-4). 1: histology, 2: CD4, 3: CD8, 4: ED1 immunostaining, Original magnification, $\times 400$.

section of the kidneys obtained from rats given the anti-GBM antibody alone (Fig. 1). The number of CD4⁺ T in the glomerulus increased significantly in

the rats by pretreatment with rabbit IgG immunization or by passive transfer of rabbit IgG-sensitized T cells (Table 1). In contrast, the glomerular accumula-

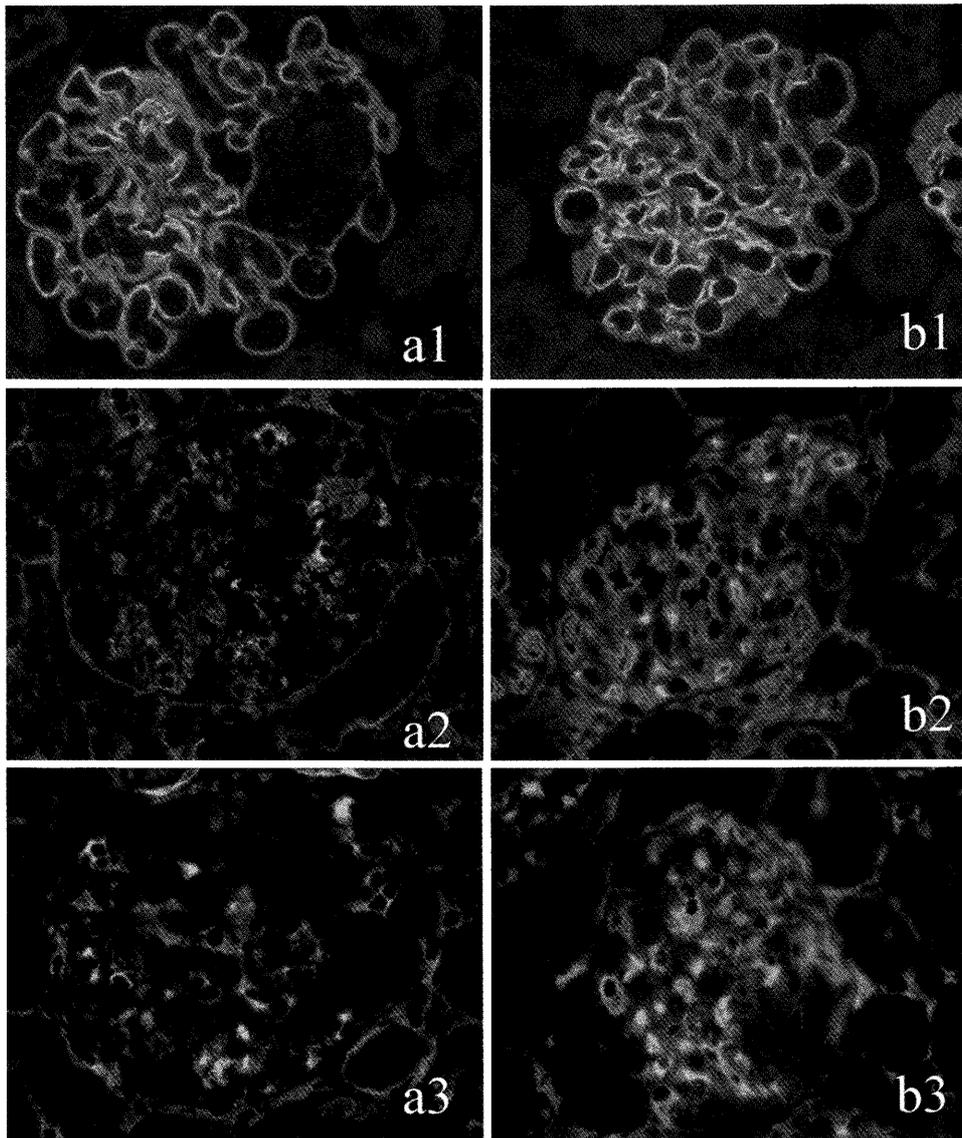


Fig. 2. Immunofluorescence photomicrographs for rabbit IgG, rat IgG and rat C3 in the glomeruli.

Deposition of rabbit IgG (a1, b1), rat IgG (a2, b2) and rat C3 (c1, c2) examined by computer-based immunofluorescence microscopy in rats given anti GBM antiserum alone (a1-3) and anti-GBM antiserum plus anti-rabbit IgG antibody (b1-3). Rabbit IgG is localized in a linear pattern along the GBM in both groups with the same intensity. Immunostaining for rat IgG and rat C3 is negligible or faint in a control rat given the anti-GBM antibody alone. On the other hand, the deposition of rat IgG and rat C3 is weak but significant in a rat given the anti-rabbit IgG antibody. Original magnification, $\times 400$.

tion of CD4⁺ T was significantly decreased by the passive transfer of the anti-rabbit IgG antibody (Fig. 1).

Substantial numbers of cells were immunostained with the anti-CD8⁺ antibody in the glomeruli five days after anti-GBM antibody injection (Fig. 1). The num-

ber of CD8⁺ T was almost comparable to that of CD4⁺ T accumulated in the glomerulus (Table 1). The glomerular accumulation of CD8⁺ T was also increased by active immunization with rabbit IgG and the passive transfer of rabbit IgG-sensitized T cells. In contrast, the numbers of CD8⁺ T and CD4⁺ T were

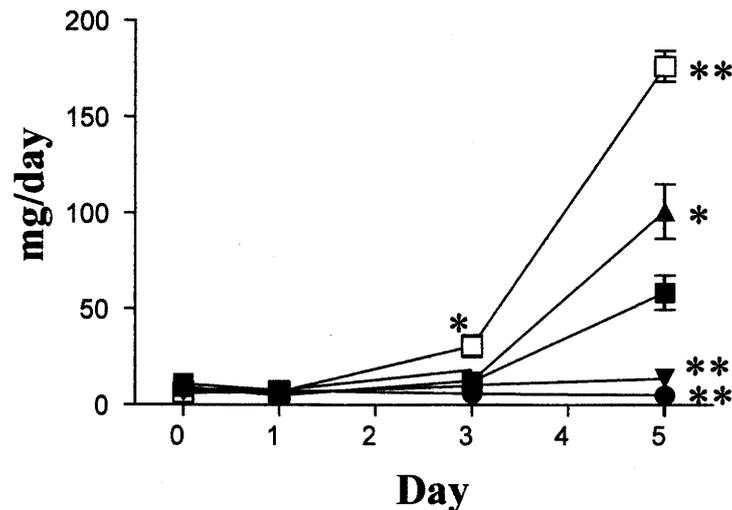


Fig. 3. Urinary protein excretion.

The excretion of protein in the urine is obvious at day 5 in rats given the anti-GBM antibody alone (■). The urinary protein excretion is enhanced by the active immunization of rats with rabbit IgG emulsified in an adjuvant (▲) or by the passive administration of T cell-rich lymph node cells obtained from rats sensitized with rabbit IgG (□). On the other hand, the amount of urinary protein is reduced by the administration of a rat anti-rabbit IgG antibody (▼). No significant proteinuria is observed in control rats given both a normal rabbit serum and rat anti-rabbit IgG antibody (●). * $P < 0.05$, ** $P < 0.01$; statistical significance vs anti-GBM antiserum group.

Table 1. Numbers of CD4⁺, CD8⁺ lymphocytes and ED1⁺ monocytes/macrophages in the glomerular cross-section

	CD4 ⁺ T	CD8 ⁺ T	ED1 ⁺ Mo/M ϕ	Proteinuria (mg/day) at Day 5
Anti-GBM alone	7.6 \pm 1.8	8.0 \pm 1.4	31.5 \pm 6.4	56.9 \pm 22.7
Rabbit IgG preimmunization + anti-GBM	11.2 \pm 3.0**	10.0 \pm 2.6*	38.2 \pm 7.4*	101.0 \pm 32.1*
Anti-GBM+anti-rabbit IgG antibody	4.0 \pm 1.7**	4.5 \pm 1.9**	17.8 \pm 3.9**	13.7 \pm 5.0**
Anti-GBM+rabbit IgG-sensitized LN	10.8 \pm 2.9**	12.5 \pm 1.9**	37.6 \pm 6.9*	176.6 \pm 17.6**
Normal rabbit serum+anti-rabbit IgG	0.0 \pm 0.0	0.2 \pm 0.4	0.2 \pm 0.5	5.1 \pm 1.3

LN, lymph node cells; Statistical significance vs anti-GBM antiserum group; * $p < 0.05$, ** $P < 0.01$.

significantly decreased in the glomerulus by the passive transfer of the anti-rabbit IgG antibody (Table 1 and Fig. 1).

The accumulation of ED1⁺ Mo/M ϕ in the glomerulus was more than that of CD4⁺ or CD8⁺ T (Fig. 2). The numbers of ED1⁺ Mo/M ϕ per glomerular cross-section were significantly higher in rats immunized with rabbit IgG and in animals transferred with rabbit IgG-sensitized T cells than control rats (Table 1.) Again, the numbers of ED1⁺ Mo/M ϕ were

significantly reduced by the passive transfer of rat anti-rabbit IgG immunoglobulins.

The glomerular injury was also evaluated by the amount of protein excreted in the urine. The amounts were significantly elevated by the preimmunization with rabbit IgG and by the passive transfer of rabbit IgG-sensitized T cells, while the urinary excretion of protein was significantly suppressed by the passive transfer of anti-rabbit IgG rat immunoglobulins (Table 1 and Fig. 3).

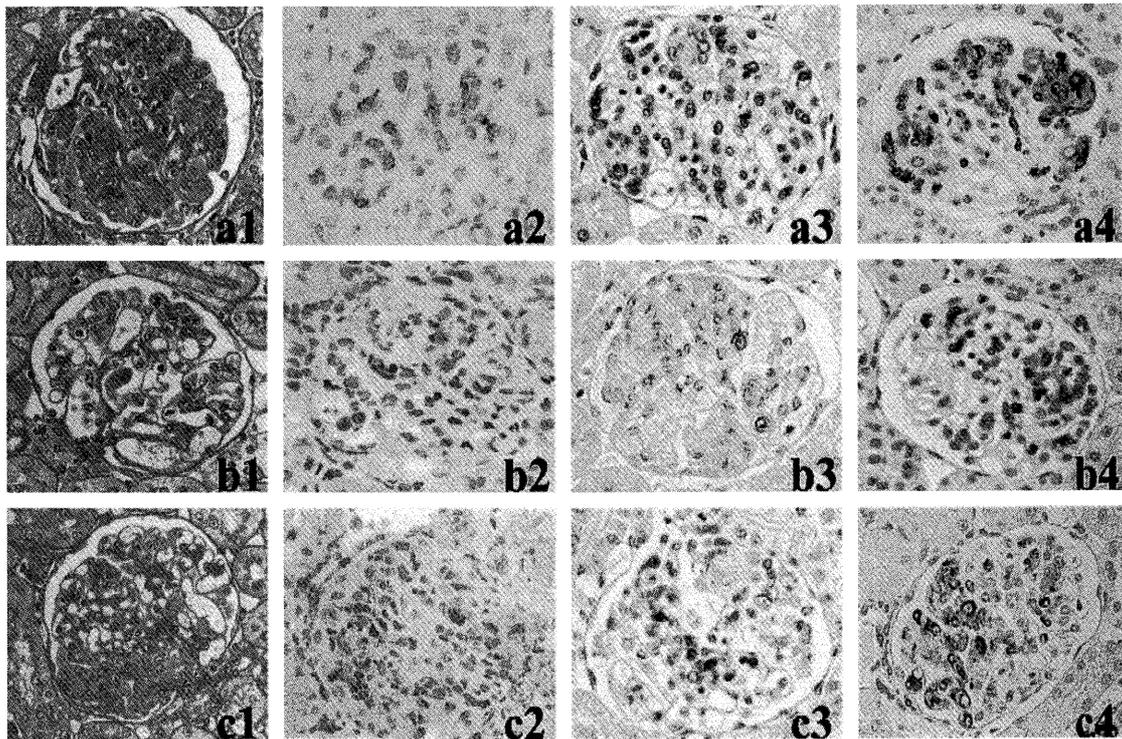


Fig. 4. Effect of anti-T cell receptor $\alpha\beta$ antibody administration on glomerular histology and leukocyte accumulation.

Light micrographs show that glomerular lesions induced by the administration of anti-GBM antibody alone (a1) are markedly (b1) and partially (c1) suppressed by the administration of an anti-T cell receptor $\alpha\beta$ antibody to animals before and after the anti-GBM antibody injection, respectively. The glomerular accumulation of CD4⁺ T (a2), CD8⁺ T (a3) and ED1⁺ Mo/M ϕ (a4) in rats injected with the anti-GBM antibody alone is apparently reduced in animals given the anti-rat T cell receptor $\alpha\beta$ antibody before (b2-4) and after (c2-4) the anti-GBM antibody administration. 1: histology, 2: CD4, 3: CD8, 4: ED1 immunostaining; Original magnification, $\times 400$.

To know whether T lymphocytes accumulating in the glomerulus mediate the induction of the glomerulonephritis model through the recognition of antigens by their T cell receptor $\alpha\beta$, effects of the receptor blocking were examined by the administration of an anti-rat T cell receptor $\alpha\beta$ antibody on induction of the model. Administration of the anti-rat T cell receptor $\alpha\beta$ antibody prior to anti-GBM antibody injection markedly suppressed the glomerular hypercellularity and crescentic lesions induced by the anti-GBM antibody administration (Fig. 4). The numbers of CD4⁺, CD8⁺ T, and ED1⁺ Mo/M ϕ were significantly reduced in the glomerulus by pretreatment of animals with the T cell receptor $\alpha\beta$ antibody (Table 2 and Fig. 4). Proteinuria was also suppressed markedly in these animals (Table 2 and Fig. 5).

On the other hand, suppressive effects were not remarkable in animals given the anti-rat T cell rece-

ptor $\alpha\beta$ antibody after injection of the anti-GBM antibody. The glomerular lesions and accumulation of leukocytes were partially suppressed in these animals but the amounts of protein excreted in the urine were almost at the same levels as in animals given the anti-GBM antibody alone (Table 2 and Fig. 5).

DISCUSSION

Our previous study showed that CD8⁺ T plays a crucial role in the WKY rat anti-GBM glomerulonephritis since the induction of this model was almost completely suppressed by the depletion of CD8⁺ T in the circulation¹. However, how the CD8⁺ T mediates development of the glomerular lesions and injury remained unknown although the observations suggested an involvement of cell-mediated immunity in

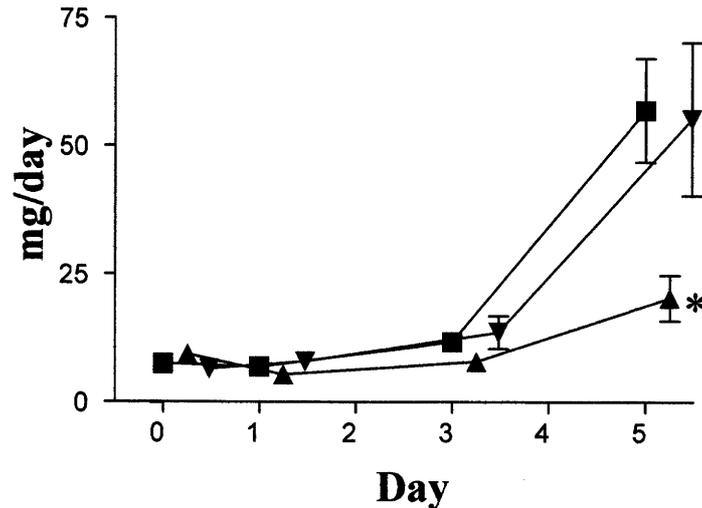


Fig. 5. Effect of anti-T cell receptor $\alpha\beta$ antibody administration on urinary protein excretion.

Urinary protein excretion induced by the anti-GBM antibody injection (■) is significantly reduced by the pretreatment of rats with an anti-rat T cell receptor $\alpha\beta$ antibody (▲). The suppressive effect is not demonstrated in rats given the anti-rat T cell receptor $\alpha\beta$ antibody after the anti-GBM antibody injection (▼). ** $P < 0.01$; statistical significance vs anti-GBM antibody alone.

Table 2. Numbers of CD4⁺, CD8⁺ lymphocytes and ED1⁺ monocytes/macrophages in the glomerular cross-section

	CD4 ⁺ T	CD8 ⁺ T	ED1 ⁺ Mo/M ϕ	Proteinuria (mg/day) at Day 5
Anti-GBM alone	7.9 \pm 1.9	8.3 \pm 1.8	33.5 \pm 5.1	58.4 \pm 20.0
Anti-TCR $\alpha\beta$ +anti-GBM	5.1 \pm 1.7*	4.2 \pm 1.5*	23.3 \pm 5.8*	20.3 \pm 9.9*
Anti-GBM+anti-TCR $\alpha\beta$	7.0 \pm 1.3	7.3 \pm 1.5	29.5 \pm 6.6	55.4 \pm 33.4

TCR, T cell receptor; Statistical significance vs anti-GBM antiserum group; * $P < 0.01$.

the model in which CD8⁺ T may act as cytotoxic T cells. On the other hand, an implication of CD4⁺ T in this model has also been noted since the administration of the anti-CD4 antibody partially suppressed the glomerular lesions and proteinuria⁹. The current study confirmed the glomerular accumulation of CD4⁺ T in substantial numbers comparable to that of CD8⁺ T. The concomitant accumulation of CD4⁺ T, CD8⁺ T and ED1⁺ Mo/M ϕ at an inflammatory site reminds us that the CD4⁺ T are helper T cells of the Th1 subset, which stimulate CD8⁺ T and ED1⁺ Mo/M ϕ by secreting various cytokines such as interferon- γ and TNF- α and induce a cell-mediated immune response or delayed-type hypersensitivity

reaction¹⁶). This may be true because we previously demonstrated the expression of these cytokines in the glomerulus of this model⁶.

In the current study, the deterioration of glomerular lesions and injury to this model was demonstrated in animals actively immunized with rabbit IgG and in those passively transplanted with IgG-sensitized T cells. These results indicated that this model was mediated by an immune response to rabbit IgG and suggested that macrophages and CD8⁺ T were accumulated in the glomerulus and activated by the Th1 type of CD4⁺ T which releases interferon- γ , IL-2 and tumor necrosis factor in the glomerulus to induce delayed-type hypersensitivity reactions¹⁷). On

the other hand, Th2 cells are known to suppress the Th1-mediated inflammatory reactions by releasing cytokines such as IL-4, IL-5 and IL-13, which promote a humoral immune response to produce an antibody. The suppression of this model by the passive transfer of the anti-rabbit IgG antibody may be interpreted by the promotion of a shift from the Th1 reaction to Th2 reaction or by the direct blocking of rabbit IgG epitopes presented to the Th1 type of CD4⁺ T with the anti-rabbit IgG antibody injected.

The anti-GBM glomerulonephritis model was also suppressed by anti-T cell receptor $\alpha\beta$ antibody administration. This observation suggests an involvement of T cell receptor $\alpha\beta$ in sensitization in the induction. The suppression was remarkable in rats pretreated with the anti-T cell receptor $\alpha\beta$ antibody before anti-GBM antibody injection and was not so obvious in animals given the anti-T cell receptor $\alpha\beta$ antibody after induction of the disease. T cell receptor $\alpha\beta$ expressed in CD4⁺ or CD8⁺ T is utilized to recognize antigens incorporated in major histocompatibility complex (MHC) molecules of antigen-presenting cells. Mo/M ϕ may present antigens by incorporating in MHC class II molecules to CD4⁺ T at the initiation of this model. The antigen presentation may occur in the glomerulus since Mo/M ϕ had been demonstrated in the glomerulus of this model as the first comer among leukocytes immediately after the binding of the anti-GBM antibody to GBM¹⁾. Other glomerular cells may also present antigens to CD8⁺ T by incorporating them in MHC class I molecules. Although antigens presented to the CD4⁺ or CD8⁺ T are unidentified, rabbit IgG-related antigen may be one of these because active immunization with rabbit IgG or the passive transfer of rabbit IgG-sensitized T cells enhanced the glomerular lesions and injury. Thus, the presentation of rabbit IgG-related antigen was presumed either from Mo/M ϕ accumulating in the glomeruli to CD4⁺ or from glomerular cells to CD8⁺ T at the initiation of this model. The CD4⁺ or CD8⁺ T may then secrete cytokines to recruit numerous numbers of Mo/M ϕ to the glomeruli and to activate them later. The Mo/M ϕ seem to injure the glomerular structure, resulting in the excretion of massive urinary protein and development of crescentic lesions since the glomerular injury and lesions were suppressed by the inhibition of Mo/M ϕ recruitment to the glomeruli⁵⁾.

This is the first study showing significant roles of T cells sensitized with rabbit IgG and of T cell receptor $\alpha\beta$ in the induction of anti-GBM glomerulonephritis in WKY rats. These results suggest the involvement of a cell-mediated delayed-type hypersensitivity in this model.

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