Accelerated Glomerulosclerosis in Alloxan-Induced Diabetic Rabbits with Anti-Glomerular Basement Membrane Nephritis

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WANIBUCHI, H., FUJIMOTO, T. and UEDA, M. Accelerated Glomerulosclerosis in Alloxan-Induced Diabetic Rabbits with Anti-Glomerular Basement Membrane Nephritis. Tohoku J. Exp. Med., 1991, 163 (3), 157-165 — To find how diabetes affects the process of proliferative glomerulitis, we induced anti-glomerular basement membrane (GBM) nephritis by injection of anti-GBM antiserum in rabbits with alloxan diabetes (the DM-GN group) and in rabbits without the diabetes (the GN group), and compared the glomerular lesions between the two groups. Rabbits with alloxan diabetes only (the DM group) were also studied as control. Morphological examination showed that in the acute phase, the DM-GN and GN groups underwent histolysis of the glomerular loops, which gave rise to proliferative glomerulitis. In the later stages of glomerulitis, proliferating cells were crowded toward the axial portion of glomerular loops with an increase of intercellular matrix, and glomerular capillaries in the periphery of the glomerular loops recanalized. The amount of intercellular matrix of the axial portion increased more in the DM-GN group than in the GN group. Some of the glomerular lesions in the DM-GN group showed a formation of large nodules. The results suggested that diabetes could accelerate the formation of the intercellular matrix of glomerular loops in proliferative glomerulitis in rabbits, resulting in accelerated glomerulosclerosis. ——— accelerated glomerulosclerosis; histolysis of glomerular loops; proliferative glomerulitis; diabetes; foam cells

Primary glomerulonephritis often develops in patients with diabetes mellitus. Various kinds of glomerulonephritis associated with diabetes mellitus have been reported (Yum et al. 1984). However, the morphological differences of glomerulonephritis in diabetic and nondiabetic patients have not yet been clarified.

In experiments with rats, Okuda et al. (1984) and Abrass and Cohen (1987) have found that the renal lesions produced by immunological insult of the glomeruli in diabetic rats were more severe than those produced in nondiabetic rats. They induced Heymann nephritis as a model of idiopathic membranous
glomerulonephritis in streptozotocin-induced diabetic rats.

Here, we induced anti-glomerular basement membrane (GBM) nephritis as a model of diffuse proliferative glomerulonephritis in rabbits with alloxan diabetes, and investigated the influence of the diabetic condition on the glomerular lesions of glomerulonephritis by use of repeated biopsies.

**MATERIALS AND METHODS**

*Animals*

Adult albino rabbits weighing 2.3 to 3.3 kg were used.

*Production of anti-GBM antiserum (AGBMA)*

GBMs of rabbits were collected by the method of Spiro (1967). In brief, glomeruli were collected from rabbit renal cortex with sieves of 115, 80 and 170 mesh; sieves were used both for the disruption of the tissue and for the separation of glomeruli from other components. GBMs obtained from four kidneys of two rabbits were suspended in 10 ml of saline, mixed at a 1:2 ratio with Freund's incomplete adjuvant, and 2 ml of them were injected into hens intramuscularly once a week for 12 weeks. Two weeks after the last injection, the hens were bled to death, the blood was collected and the serum was used as AGBMA.

*Experimental design*

Rabbits were divided into four groups: (1) rabbits with alloxan diabetes and anti-GBM nephritis (the DM-GN group, n = 13); (2) rabbits with anti-GBM nephritis only (the GN group, n = 5); (3) rabbits with alloxan diabetes only (the DM group, n = 5); and (4) untreated control rabbits (the C group, n = 5).

Diabetes was induced in the DM-GN and DM groups by an intravenous injection of 5% alloxan monohydrate in saline at the dose of 150 mg/kg of body weight, and in the GN and C groups only saline was injected. A fasting blood glucose level of 200 mg/100 ml blood or more 7 days after the injection of alloxan was considered to indicate diabetes. The diabetic animals were not treated with insulin. All rabbits had free access to ordinary rabbit chow and water, and were kept in metabolic cages.

Seven days after the injection of alloxan or saline, the DM-GN and GN groups received a single intravenous injection of AGBMA (3 ml/kg of body weight). In the DM and C groups, only saline (3 ml/kg) was injected intravenously.

In the DM-GN and GN groups, renal biopsies were done to observe the acute phase (10 days after the injection of AGBMA) and recovery phase (18 and 28 days after the injection of AGBMA) of glomerular inflammation. In the DM and C groups, renal biopsies were done 10 and 28 days after the injection of saline. Autopsies were carried out 2 months after the injection of AGBMA or saline.

*Biochemistry of urine and blood samples*

The urine was collected for 24 hr in the metabolic cages. Urinary protein and glucose were measured semiquantitatively with test sticks (Hemacombistix; Miles Laboratories, Inc., Elkhart, IN, USA) daily until proteinuria appeared, and at intervals of several days after the appearance of proteinuria. Blood samples were collected from all rabbits after they had been starved for 12 hr on day 7 after the injection of alloxan or saline. Blood glucose was measured with a Beckman glucose analyzer (Dextrometer; Miles Sankyo, Tokyo), based on the glucose oxidase method.
Morphologic studies

Kidney specimens were obtained by wedge-shaped resection of cortices, and were assessed for morphologic and immunopathologic changes by light, electron, and fluorescence microscopy.

Kidney specimens were fixed in phosphate-buffered 10% formaldehyde (pH 7.2), embedded in paraffin. Sections were cut at 3 μm thick and stained with hematoxylin and eosin, and periodic acid Schiff (PAS) stains, and observed under a light microscope. Additionally, to detect lipid deposits within glomeruli and tubules, frozen sections of the kidney specimens were stained with sudan III.

For immunofluorescence microscopy, kidney specimens were frozen at −70°C in a mixture of dry ice and acetone, and cryostat sections 4 μm thick were analyzed for renal deposits of rabbit IgG and chicken IgG by the direct immunofluorescence method with fluorescein-conjugated antisera to rabbit IgG and chicken IgG (Cappel Laboratories, West Chester, PA, USA).

For the electron microscopic study, kidney specimens were diced into 1-mm³ cubes, fixed in phosphate-buffered 2% glutaraldehyde, postfixed in 1% osmium tetroxide, and

Fig. 1. Immunofluorescence micrograph of a glomerulus showing diffuse linear deposits of rabbit IgG along the glomerular capillary walls. A DM-GN rabbit 10 days after the injection of AGBMA. (×190)

Fig. 2. A reticular appearance (arrow) of the glomerular loops composed of proliferating cells and basement membrane-like materials. A DM-GM rabbit 10 days after the injection of AGBMA. (PAS, ×400)

Fig. 3. Foam cells showing numerous fat granules in their cytoplasms. A DM-GN rabbit 10 days after the injection of AGBMA. (Sudan III, ×325)

Fig. 4. A fibrin-cap lesion of a glomerular loop. DM-GN rabbit 10 days after the injection of AGBMA. (PAS, ×320)
embedded in Epon 812. Semi-thin sections 1 μm thick were stained with toluidine blue for light microscopy. Ultrathin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and observed and photographed under a Hitachi H-300 electron microscope.

Results

General

Proteinuria was observed in the DM-GN and GN groups about 7 days after the injection of AGBMA. In the GN group, proteinuria ameliorated on day 18 and thereafter, and sometimes disappeared by 28 days after the injection. In the DM-GN group, however, proteinuria persisted as long as 2 months after the injection of AGBMA. The DM and C groups did not develop proteinuria. Urinary protein excretion increased more in the DM-GN group than in the GN group.

Histological findings

Immunofluorescence. Diffuse linear deposits of rabbit IgG and chicken IgG were detected along the GBM of the GN and DM-GN groups 10 days after the injection of AGBMA (Fig. 1).

Light microscopy. The DM groups did not show distinct glomerular changes within 2 months after the development of diabetes.

About 10 days after the injection of AGBMA, the GN and DM-GN groups showed proliferative glomerulitis following histolysis of glomerular loops. The severity of change was different from glomerulus to glomerulus, but typically, the glomerular loop was transformed into a reticular structure composed of proliferating cells and basement membrane-like material around the cells (Fig. 2). In some of the DM-GN group, foam cells were observed among the proliferating cells of the glomerular loops. The foam cells contained granules stained with Sudan III in their cytoplasm (Fig. 3). Exudative glomerular lesions resembling “fibrin cap” were also formed in some of the DM-GN group (Fig. 4).

Around 18 days after the injection of AGBMA, all rabbits in the PM-UN and UN groups had accumulations of cells that had proliferated in the axial portions of the glomerular loops and recanalization in the peripheral portions of the loops.

In the GN group, within 28 days after injection of AGBMA, the axial portion of the glomerular loops became slightly expanded because of the accumulation of the cells that had proliferated with intercellular matrix around them (axial fibrosis), but this portion did not have a nodular appearance. In contrast, the DM-GN group had accentuated axial fibrosis of the glomerular loops in the stage following cell proliferation. In some of the glomeruli of the DM-GN group at 60 days after the injection of AGBMA, there was much expansion of the axial portion of the glomerular loop, because of the increase in the amount of intercellular matrix, resulting in formation of a large nodular lesion (Fig. 5). These nodular lesions somewhat resembled diabetic nodular lesions in humans (Fig. 6).
Electron microscopy. In the acute phase of glomerulitis (10 days after the injection of AGBMA) in the GN and DM-GN groups, the glomerular loop expanded because of the proliferation of cells, and its original structure became obscure (Fig. 7); some glomeruli showed that the intercellular space was widened and severely edematous, and basement membrane-like materials were seen around the

![Image](image_url)

**Fig. 5.** A nodular change in a glomerulus. The axial portion is expanded with a large amount of intercellular matrix. A DM-GM rabbit 60 days after the injection of AGBMA. (PAS, x400)

**Fig. 6.** Nodular changes in a glomerulus resembling human diabetic nodular lesions. A DM-GM rabbit 60 days after the injection of AGBMA. (PAS, x400)

**Fig. 7.** Electron micrograph of a glomerulus in acute phase of glomerulitis. The original structure of the glomerulus has become obscure because of cell proliferation. A DM-GM rabbit 10 days after the injection of AGBMA. (electron micrograph, x1,410)
proliferating cells (Fig. 8); the proliferating cells had a large cytoplasm with numerous cytoplasmic organelles. The subendothelial electron dense deposits were more abundant in the DM-GN group than in the GN group, and subepithelial deposits were seen in some of the glomeruli in the DM-GN group.

In the recovery phase of glomerulitis (18 or 28 days after the injection of AGBMA) in the GN group, the cells that had accumulated in the axial portion of the glomerular loops showed fewer cytoplasmic organelles and a smaller amount of nuclear euchromatin to compare with the proliferating cells observed in the acute phase of glomerulitis. On the other hand, in the stage after the development of glomerulitis in the DM-GN group, the cells around the axial portion of glomerular loops were rich in cytoplasmic organelles and extended their cytoplasmic processes in a tree-like pattern with a large amount of intercellular matrix (Fig. 9). The intercellular matrix was composed of the collagen fibrils and the basement membrane-like materials around the cells (Fig. 10). In this stage, like the acute phase, subendothelial electron dense deposits were more abundant in the DM-GN group than in the GN group, and subepithelial, intramembranous, and mesangial dense deposits were also observed in the DM-GN group.

**DISCUSSION**

In this study, both the DM-GN group and the GN group developed proliferating cells (Fig. 8); the proliferating cells had a large cytoplasm with numerous cytoplasmic organelles. The subendothelial electron dense deposits were more abundant in the DM-GN group than in the GN group, and subepithelial deposits were seen in some of the glomeruli in the DM-GN group.

In the recovery phase of glomerulitis (18 or 28 days after the injection of AGBMA) in the GN group, the cells that had accumulated in the axial portion of the glomerular loops showed fewer cytoplasmic organelles and a smaller amount of nuclear euchromatin to compare with the proliferating cells observed in the acute phase of glomerulitis. On the other hand, in the stage after the development of glomerulitis in the DM-GN group, the cells around the axial portion of glomerular loops were rich in cytoplasmic organelles and extended their cytoplasmic processes in a tree-like pattern with a large amount of intercellular matrix (Fig. 9). The intercellular matrix was composed of the collagen fibrils and the basement membrane-like materials around the cells (Fig. 10). In this stage, like the acute phase, subendothelial electron dense deposits were more abundant in the DM-GN group than in the GN group, and subepithelial, intramembranous, and mesangial dense deposits were also observed in the DM-GN group.
ative glomerulitis following histolysis of the glomerular loops. However, the mode of development of the glomerular changes was different between two groups.

First, electron microscopy showed that the amount of the subendothelial electron-dense deposits was greater in the DM-GN group than in the GN group. Okuda et al. (1984) and Abrass and Cohen (1987) reported that diabetic rats with Heymann nephritis developed more intensely stained immune deposits along the capillary wall and mesangium than control rats with Heymann nephritis. Okuda et al. explained this phenomenon by the contributions of three suggested mechanisms. First, the ability of the cells to remove immune complexes from the glomeruli may be impaired in diabetic rats. Second, the elevated vascular permeability in diabetes may facilitate the deposit of the immune complex in the GBM. Third, the production of autoantibody to the brush border antigen of the renal tubule may be increased in diabetic rats. Abrass and Cohen suggested that alterations in Fc-receptor-mediated phagocytosis of circulating immune complexes contribute to increased glomerular deposits in diabetic animals with an immune

Fig. 9. Electron micrograph of an axial portion of the glomerular loop. The intercellular matrix has developed around the cells that had accumulated toward the axial portion of a glomerular loop. Subendothelial, intramembranous, subepithelial, and mesangial electron dense deposits are seen. Accumulated cells have tree-like projection of cytoplasmic processes and are abundant r-ER in their cytoplasms. A DM-GN rabbit 60 days after the injection of AGBMA. (×5,900)
complex disease. Furthermore, they considered that the structural changes in the GBM, especially the reduction of net negative charges, may contribute to the shift in the site of immune deposits in the diabetic animals with Heymann nephritis. The immunological status of alloxan-induced diabetic rabbits with anti-GBM nephritis is not known, but elevated vascular permeability and impaired ability to remove the immune complex from the glomeruli may bring about the glomerular accumulation of immune complexes.

Another point of difference between the DM-GN and GN groups was that in the recovery phase of glomerulitis, the production of the intercellular matrix in the axial portion was greater in the DM-GN group than in the GN group. Abrass and Cohen (1987) reported that the increase in the amount of mesangial deposits in diabetic rats with Heymann nephritis is associated with mesangial cell proliferation and an increased amount of mesangial matrix. However, they did not describe the morphology of the proliferating mesangial cells. We observed that in the DM-GN group the cells during and after proliferation that crowded towards the axial portion of the glomerular loop extended their tree-like projections of cytoplasmic processes and their cytoplasms were rich in the rough surfaced endoplasmic reticulum (r-ER), suggesting accelerated protein synthesis. These observations suggest that cellular activity was higher in the DM-GN group than in
the GN group in the stage after the development of glomerulitis, which resulted in an increase in the amount of intercellular matrix in the axial portion of the glomerular loop. The abnormal metabolic environment in diabetes may influence the cells that have proliferated to continue the synthesis of intercellular matrix for a longer time than usual.

The third point of difference was that foam cells appeared among the proliferating cells of the glomerular loops, and exudative glomerular lesions resembling fibrin-cap lesions were observed in some of the DM-GN group. These findings suggest that the abnormal fat metabolism in diabetes may influence the glomerular changes induced in rabbits by the injection of AGBMA. There are reports that dietary-induced hypercholesterolemia worsened both the glomerular lesions and the mesangial sclerosis (Kelley and Izui 1983; Al-Shebeb et al. 1988).

The results discussed above may help the understanding of the morphogenesis of human diabetic glomerulosclerosis. Shioi and Fujimoto (1989) studied the mode of the development of human diabetic nodular glomerulosclerosis, and suggested that the disturbance of lipid metabolism in the diabetes mellitus might be important in the development of the nodular lesions. Our study also suggests that abnormal fat metabolism in the DM-GN group may in some way be concerned the glomerular changes with an increase of the mesangial matrix, resulting in a formation of nodular lesions. These findings allow us to speculate that abnormal fat metabolism may be responsible for the formation of nodular lesions in human diabetic glomerulosclerosis.

In conclusion, this study suggests that the diabetic condition can accelerate the formation of the intercellular matrix in proliferative glomerulitis in rabbits, resulting in accelerated glomerulosclerosis.

References