Evolution of Glomerular Lesions in Rats with Spontaneous Diabetes

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YAGIHASHI, S., KASEDA, N., KAKIZAKI, M. and GOTO, Y. Evolution of Glomerular Lesions in Rats with Spontaneous Diabetes. Tohoku J. exp. Med., 1979, 127 (4), 359-367 — The development of glomerular lesions associated with ageing was investigated electron microscopically in rats with spontaneous diabetes. In young diabetic rats at eight weeks of age, there was no particular difference in ultrastructure from age-matched control rats showing an even contour of glomerular basement membrane, whereas in the diabetic rats at 12 weeks of age thickening of basement membrane with irregular protrusion of the epithelial side of lamina densa and accumulation of basement membrane-like materials in the mesangial regions could be observed. After 16 to 24 weeks of age in the diabetic rats, hemispherical thickening in addition to diffuse thickening of glomerular basement membrane was noted. The thickening of basement membrane was due to widening of lamina densa consisting of accumulation of basement membrane materials on the epithelial side of the lamina densa, all the way along the peripheral capillary loops. These features of glomerular lesions in the diabetic rats were progressively accentuated accompanying ageing. The early glomerular ultrastructural alterations in the diabetic rats were very compatible with those seen in the elder control rats. The results indicated that the development of diabetic glomerulopathy might be destined very early in life of the spontaneously diabetic rats supposedly by their diabetic genes. — diabetic glomerulopathy; diabetic microangiopathy; spontaneously diabetic animal; ultrastructure; ageing

Death in diabetic patients is very commonly ascribed to diabetic nephropathy, leading to renal failure (Goto et al. 1974). By electron microscopy, early renal lesions in human diabetics are represented by diffuse thickening of glomerular basement membrane and an increase in mesangial matrix (Bloodworth 1963; Østerby Hansen and Lundbaek 1970). In animals with spontaneous diabetes or experimental diabetes, thickening of glomerular basement membrane and other morphological abnormalities have been reported by various workers (Østerby Hansen et al. 1967; Shirai et al. 1967; Orci et al. 1970; Like et al. 1972). Little is known, however, about the nature and progression of diabetic glomerulopathy.

In our previous report (Yagihashi et al. 1978), we have shown definite thicken-
ing of glomerular basement membrane in rats with spontaneous diabetes that were bred in our own laboratory. These diabetic rats, new animal model for human diabetics, were produced by repetition of selective breeding of normal Wistar strain rats with glucose intolerance (Goto et al. 1975, 1976), and it was demonstrated that they have characteristic diabetic features: glucose intolerance, low insulin secretion and other biochemical abnormalities (Goto et al. 1977).

In this report, we present the results of ultrastructural study on the glomeruli in spontaneously onset diabetic rats at various ages.

**MATERIALS AND METHODS**

Twenty-two spontaneously diabetic rats of male Wistar strain were divided into six groups: three eight-week-old, three 12-week-old, two 16-week-old, six 24-week-old, four 25- to 28-week old and four diabetic rats 29 to 32 weeks of age. Thirty-six normal rats of male Wistar strain were used for age-matched control rats and divided into nine groups by four weeks intervals until 36 weeks of age. All the rats were fed with water and standard rat chow (Oriental MF) ad libitum until sacrifice.

Tissue specimens for electron microscopic observations were prepared by the following procedures: After an overnight fast, rats were killed by decapitation. The abdominal cavity was opened and the left renal cortex was cut into small pieces. The small specimens of renal tissue were immersed in 4% ice-cold glutaraldehyde in 0.15 M phosphate buffer, pH 7.4, for 1 1/2 hr and rinsed with the same buffer overnight. After postfixation in 1% osmium tetroxide, specimens were dehydrated through an ascending series of ethanol. They were then embedded in Maraglas 655 and polymerized at 60°C for 48 hr (Spurlock et al. 1963). Ultrathin sections were obtained by Porter-Blum MT 2B type ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds 1963). A Hitachi-HU 12AS electron microscope was used for the observation.

All the experimental rats were given glucose tolerance test (2 g/kg, orally), using a glucose oxidase method for blood glucose determination. The results on laboratory data and quantitative estimations of glomerular basement membrane thickness in these experimental rats have been already published elsewhere (Yagihashi and Kaseda 1978; Yagihashi 1978; Yagihashi et al. 1978).

**OBSERVATIONS**

The glomerular ultrastructures of the young diabetic rats at eight weeks of age were not different from those seen in age-matched control rats. In the diabetic rats at eight weeks of age, uniform glomerular basement membrane was observed (Fig. 1). Mesangial regions of these diabetic rats were narrow as compared with those of older rats. Mesangial cells had large indented nuclei and abundant cytoplasmic organelles. Epithelial cells of the young diabetic rats were also rich in cytoplasmic organelles; prominent areas of Golgi apparatus, well developed granular endoplasmic reticula and many free ribosomes (Fig. 2).

On the other hand, in the diabetic rats at 12 weeks of age, diffuse thickening of glomerular basement membrane appeared with an uneven contour showing more or less irregular protrusions of the epithelial side of the lamina densa in planes (Fig. 3). The unevenly thickened basement membrane could be recognized in normal control rats at 20 to 24 weeks of age. The thickening of glomerular basement membrane was due to irregular widening of lamina densa consisting of
Fig. 1. Glomerular ultrastructure of a diabetic rat at eight weeks of age, showing an even contour of basement membrane (BM). Mesangial cell (Mes) is rich in cytoplasmic organelles. BS, space of Bowman; Cap, capillary lumen; En, endothelial cell; Ep, epithelial cell; mm, mesangial matrix. ×12,400.

Fig. 2. Epithelial cell (Ep) of a young diabetic rat at eight weeks of age, showing prominent areas of Golgi apparata, many mitochondria and granular endoplasmic reticula. Basement membrane (BM) also shows even thickness. ×12,000.
Fig. 3. Diffuse thickening of glomerular basement membrane with irregular protrusions of the epithelial side of lamina densa seen in a diabetic rat at 12 weeks of age (arrows). Cap, capillary lumen; En, endothelial cell; Mes, mesangial cell. × 7,200.

accumulation of basement membrane materials, all the way along the peripheral capillary loops, neither preferentially near nor far from mesangial parts. In the mesangial regions, an increase in mesangial matrix was noted in the diabetic rats after 12 weeks of age. The cytoplasm of mesangial cells was fragmented into small pieces in mesangial matrix where there increased filamentous network (Fig. 4).

With increasing age, thickening of basement membrane became more prominent. In the diabetic rats at 16 to 24 weeks of age, hemispherical thickening of glomerular basement membrane with diffuse thickening could be seen (Figs. 5 and 6). The thickening of basement membrane in hemispherical form was not apparent in control rats until at 28 weeks of age. Near the thickened areas of basement membrane were observed pinocytotic vesicles and pores in the epithelial cytoplasm and foot processes. Epithelial cells had widened cisterns of granular endoplasmic reticulum and convoluted nucleus.

After 24 weeks of age, some diabetic rats showed large masses of basement membrane-like materials in the folded parts of peripheral capillary loops (Fig. 7). In the cytoplasm of the epithelial cells, there appeared many autophagosomes characterized by myelinated structures (Fig. 8). Mesangial regions of the old diabetic rats had large mesangial matrix which were continuous with the peripheral capillary basement membrane (Fig. 9). The thickened basement membrane in diffuse and hemispherical form was most remarkable in the diabetic rats at 32 weeks of age. However, old control rats also showed both diffuse and hemispherical thickening of basement membrane. The alterations of glomerular ultrastructure in the diabetic rats were very compatible with those seen in the elder control rats.
Fig. 4. An increase in mesangial matrix of the mesangial region observed in a diabetic rat at 12 weeks of age. The cytoplasm of the mesangial cell is fragmented into small pieces in the mesangial matrix (arrows). Cap, capillary lumen. ×11,000.

Fig. 5. Hemispherical thickening of glomerular basement membrane (*) in addition to diffuse thickening seen in a diabetic rat at 16 weeks of age. Widened cisterns of granular endoplasmic reticulum are noted in the epithelial cell (Ep). ×10,800.

Fig. 6. Magnified view of hemispherical thickening of basement membrane. In the epithelial cytoplasm, vesicles and pores are noted (arrow). ×30,000.
Fig. 7. The accumulation of basement membrane-like materials in the folded parts of peripheral capillary loops (*), seen in a diabetic rat at 24 weeks of age. There also appears diffuse thickening of basement membrane. Cap, capillary lumen; Ep, epithelial cell. × 7,500.

Fig. 8. Autophagosomes (AP) figured by myelinated structures in the epithelial cell cytoplasm (Epi) seen in a diabetic rat at 24 weeks of age. The nucleus of the epithelial cell is convoluted. × 11,000.

DISCUSSION

In this study, young rats with spontaneous diabetes have shown the diffuse thickening of glomerular basement membrane with irregular protrusions of the epithelial side of the lamina densa and accumulation of mesangial matrix in the mesangial regions. These ultrastructural alterations developed, progressively along
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Fig. 9. Diffuse thickening of basement membrane continuous with increased mesangial matrix of the mesangial region (Mes) seen in a diabetic rat at 24 weeks of age. BS, space of Bowman; En, endothelial cell. × 6,000.

with ageing. However, the figures seen in the diabetic rats were also recognized in the elder control rats.

It has been noticed that experimental diabetic animals induced by alloxan showed diffuse thickening of glomerular basement membrane with more or less irregularity, which appeared after a certain period of diabetic state; about 8 months or more (Osterby Hansen et al. 1967). Therefore, it is likely to be accepted that diabetic state provokes such renal lesions. However, the genesis of diabetic nephropathy will never be elucidated by a study using experimental diabetic animals such as drug-induced diabetic animals or pancreatectomized diabetic animals, when we aim to clarify the participation of the genetic factors responsible for the development of diabetic glomerulopathy.

In human diabetics, although it has been established that there needs the "time" to develop microangiopathy (Osterby Hansen 1965; Osterby Hansen and Lundbaek 1970), attempts to link the severity of diabetes with the appearance and progression of angiopathy have failed. It is not infrequently impressed that diabetic patients with less dysmetabolism have an early onset and manifest rapidly progressive vasculopathy (Ellenberg 1962; Sabour et al. 1962; Takazakura et al. 1975).

Evolution of glomerular lesions in the animals with spontaneous diabetes such as diabetic chinese hamsters (Shirai et al. 1967), diabetic spiny mice (Orci et al. 1970) or diabetic mutant mice (Like et al. 1972), was reported and the characteristic figures of glomeruli were represented by thickening of glomerular basement membrane in both diffuse and hemispherical form and an increase in mesangial matrix just as observed in our study. In those series, there was no evidence that littermates of diabetic animals exhibited such glomerular lesions in the absence of
overt diabetes. Those lesions, however, were related to the relatively short duration of diabetic state and developed progressively with ageing.

On the other hand, Opperman et al. (1975) found an early occurrence of definite glomerular lesions in spontaneously diabetic mice with diabetic genes (diabetic KK mice) at only two months of age. From their study, they concluded that diabetic glomerulopathy was regulated by diabetic genes and that hyperglycemia was not an absolute prerequisite acting as an accelerator of thickening of glomerular basement membrane.

Our previous studies with quantitative estimation well demonstrated constant age-related increase in basement membrane thickness both in normal and in spontaneously diabetic rats (Yagihashi 1978; Yagihashi et al. 1978). Moreover, we could detect an early significant thickening of glomerular basement membrane in the diabetic rats at 12 weeks of age. Two out of three diabetic rats at eight weeks of age showed larger thickness of basement membrane when compared with those of age-matched control rats, although it was not statistically significant. In addition to these results, the present study indicated that the glomerular lesions in the spontaneously diabetic rats seemed to be an accentuation of age-induced glomerular changes in normal rats. Hence, we may assume that progression of glomerular lesions in the spontaneously diabetic rats should be destined very early in life, probably concomitantly with the onset of carbohydrate intolerance by their diabetic genes.

Our morphological study also indicates that the first demonstrable changes of the glomeruli in the diabetic rats are neither preferentially near nor far from the mesangial regions, but start evenly all the way along the loops of capillaries in concert with an increase in mesangial matrix of mesangial regions.

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References

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