Disorganization Process in the Development of Diabetic Nodular Glomerulosclerosis

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In order to clarify the mode of development of the diabetic nodular glomerulosclerosis, 38 kidney specimens of autopsied and biopsied diabetic cases were studied light and electron microscopically including serial sections. Disorganization process occurred chiefly at the capillary region of glomeruli. Early change of this process was proliferation of intramembranous cells following histolysis in the glomerular loop and it was characterized by transformation of the glomerular loop into enmeshed or reticular structure, that is, disorganization of the glomerular architecture. In the subsequent stage intercellular matrix production and hyalinization accompanied by axial crowding of proliferated intramembranous cells and peripheral recanalization of blood spaces were clarified. And in the later stage axially crowded intramembranous cells decreased in number and the matrix was increased and hyalinized, resulting in the formation of the axial hyaline nodule. The distribution of this process was focal and segmental, lesions of various stages coexisted, and it was suggested that glomerular lesions may spread all over the kidney by recurrence of this process. This process was quite similar to those seen in disorganization process of glomerulonephritis. But another characteristic changes were the presence of foam cells, intra- and intercellular deposition of lipid droplets, and increased matrix formation.

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Diabetic nodular glomerulosclerosis, which was first described by Kimmelstiel and Wilson (1936), is thought to be the most characteristic lesion in diabetic nephropathy. In the recent decades it has been proposed that lytic changes in glomerular loops may play an important role in the morphogenesis of this lesion. Bloodworth (1978) postulated that large Kimmelstiel-Wilson nodules are formed by the organization of the glomerulocapillary microaneurysms caused by disruption of so-called “anchor points”. Saito et al. (1988) suggested the concept of “mesangiolysis” as a developmental process of nodular lesions without microaneurysm formation, in which loosening and dissociation of the mesangial matrix.
begins in the central portion of the glomerular tuft, extends to the whole tuft followed by the reticular structure, and concentric and layering re-arrangement of the mesangial matrix leads to the nodular lesion. From these two authors' opinions it seems to be suggested that disorganization and the following repair process of glomerular loops may participate in the development of the nodular lesion. However, details of this developmental process have never been clarified.

On the other hand, Fujimoto (1988) pointed out that the typical mode of development of the nodular lesion is similar in some respects to that of lobular glomerulonephritis and that lipid droplets are often found in the proliferated cells and the matrix of glomerular loops, and considered that disturbance of lipid metabolism in diabetes mellitus may play an important role in the genesis of nodular glomerulosclerosis.

In the present study, in order to clarify the mode of development of the nodular lesion, especially disorganization process, we studied diabetic kidneys in detail with light and electron microscopes including a serial section study.

**Materials and Methods**

*Patients*

Kidney specimens obtained from 38 patients were examined. Twenty-two specimens were obtained from autopsy cases with diabetes mellitus in the Osaka City University Hospital. Biopsy specimens were obtained from 16 diabetic patients by percutaneous renal biopsy at the Osaka City University Hospital and some affiliated hospitals. Diabetic patients with other renal diseases were excluded from this study.

*Light microscopic examination*

Autopsied and biopsied kidney specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at 3 μm, and stained with hematoxylin and eosin, periodic acid Schiff’s reagent (PAS), and periodic acid silver methenamine (PAM), and observed under a light microscope. For the serial section study, 11 specimens selected from the autopsy cases were cut into 70 to 150 slices serially at 4 μm.

In the 11 selected autopsy cases, the glomerular changes in the development of the nodular lesion were divided into three stages: early stage (E); intermediate stage (I); later stage (L) by the degrees of cellularity and matrix production. Glomerular changes of each stage were examined in details. And the incidences of glomerular changes of each stage were determined by counting 1000 glomeruli on each case (100 consecutive glomeruli were counted on each slide and this procedure was performed on 10 randomly selected slides of each case). But in case No. 21, 300 glomeruli were counted.

*Electron microscopic examination*

Other portions of biopsy specimens were pre-fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4), post-fixed with 2% osmium tetroxide (pH 7.4), dehydrated in an ethanol series, and embedded in Quetol 812. Ultrathin sections were stained with lead citrate and uranyl acetate, observed and photographed under an electron microscope (H-300, Hitachi; Hitachi).
RESULTS

Light microscopic findings

In the observation of 38 autopsy and biopsy cases characteristic lesions of diabetes (diffuse lesion, nodular lesion, exudative lesion, afferent and efferent arteriolosclerosis, ischemic changes, and microaneurysm) were found in varying degrees and stages (Tables 1 and 2). The incidence of mature nodular lesion in these cases was 71.0%. The degree of this lesion varied considerably from glomerulus to glomerulus and from loop to loop, since it was obvious that its distribution was focal and/or segmental. And premature forms of the nodular lesion formation in various stages were also seen in a focal and/or segmental fashion. Among 22 autopsy cases 11 with mild to severe degree of premature forms of the nodular lesion (disorganization) were selected. In these cases disorganization process was examined by serial sections.

There were various transition phases between the proliferative changes of

Table 1. Light microscopic findings of autopsy cases

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-, not found; ±, rare; +, mild; #, moderate; #, severe.
glomerular loop and the mature nodular lesion.

In the early stage (E) glomerular loops were enlarged and the normal architecture constituting the capillary and mesangium became indistinct. The cells enclosed by the glomerular basement membrane ("intramembranous" cells) increased in number and were swollen. They had a clear cytoplasm and a large nucleus with coarse chromatins and were interconnected three-dimensionally with their cytoplasmic processes. Irregular intercellular spaces were found and PAS-positive basement membrane-like structures were seen along them (Fig. 1). From these features glomerular loops appeared enmeshed or reticular. It was difficult to identify the origin of proliferated cells forming such a structure as endothelial or mesangial cells. These changes were found in 7 cases (Table 3).

In the subsequent stage [intermediate stage (I)] intramembranous cells accumulated toward the axial portion and basement membrane-like structures became thickened. Cellular arrangement became slightly concentric (Fig. 2). Foam cells were sometimes seen in the glomerular loops (Fig. 3). Intercellular spaces were spindle-shaped. These spaces were connected with each other three-dimensionally. Some of them could be identified as capillary lumina. At the peripheral portion recanalization of blood spaces was beginning (Fig. 2). Occasionally at the peripheral portions cystic dilatation of spaces in which red blood cells were filled was seen (Fig. 4). These changes were found in 10 cases (Table 3).
In the later stage (L) proliferated intramembranous cells decreased in number. PAM-positive intercellular matrix increased and became dense and hyalinized. Matrix arrangement was lamellar. Intercellular spaces appeared slit-like among the increased matrix. Red blood cells were found within some of them near the axial portion. At the peripheral portion recanalization of blood spaces (Fig. 5) or microaneurysm (Fig. 6) was seen. These changes were found in all 11 cases (Table 3).

The above mentioned process was localized at the most peripheral part of the intraglomerular vascular system, that is, the capillary region (Fig. 1). And the walls of the intraglomerular arteriolar branches were spared. Three-dimensionally in the glomerular loops in any stage of this process, as a rule, cellular components were prominent and matrix relatively scanty at the peripheral portion, and toward the axial portion intramembranous cells were reduced in number and the matrix gradually increased, dense, and hyalinized (Fig. 3). But, in some loops newly proliferated area surrounded the axial large hyaline nodule (Fig. 7).

The incidence of disorganization process ranged from 0.6 to 31.3%. But in all cases the percentage of each stage increased with advancing of stages (Table 3). In biopsy specimens this process was found in 3 cases (Table 2).

**Electron microscopic findings**

In the early stage intramembranous cells increased in number and had large cytoplasms and abundant cytoplasmic organelles, especially rough endoplasmic reticula in the enlarged loops (Fig. 8). Axially situated cells extended their cytoplasmic processes. Intercellular matrix was fibrillar. Lipid droplets some-
times were seen intra- and intercellularly. In the subsequent stage proliferated intramembranous cells still remained large. Their cytoplasmic organelles were abundant. These cells with prominent cytoplasmic processes were accumulated near the axial portion, and at the periphery, recanalization of blood spaces began (Fig. 9). Cellular arrangement was slightly concentric. Intercellular matrix became thickened. Foam cells and lipid droplets were also found (Fig. 10). Capillary lumina were sometimes seen near the axial portion (Fig. 11). In the later stage proliferated intramembranous cells decreased in number. Intercellular matrix much increased in width and became dense at the axial portion. Matrix arrangement was lamellar. Blood spaces within the nodule were sometimes seen (Fig. 12). At the peripheral portion newly proliferated area surrounding the preformed nodule was encountered (Fig. 13).

Discussion

In the present study it was revealed that early change of the development of the nodular lesion is proliferation of intramembranous cells following histolysis in the glomerular loop, which is characterized by the transformation of the glomerular loop into enmeshed or reticular structure, that is to say, disorganization of the glomerular architecture. In the subsequent stage intercellular matrix production and hyalinization accompanied by axial crowding of proliferated intramembranous cells and peripheral recanalization of blood spaces were clarified. And in the later stage axially crowded intramembranous cells decreased in number and the matrix increased and hyalinized resulting in the formation of the axial hyaline nodule. The distribution of this process was focal and segmental, lesions of various stages coexisted, and it was suggested that glomerular lesions may spread all over the kidney by recurrence of this process.

The cell proliferation in diabetic glomeruli has been pointed out in the literature (Kimmelstiel 1966; Iidaka et al. 1968). It was unclear, however, whether the cell proliferation is found or not in the early stage of nodular lesion formation. Bloodworth (1978) demonstrated transition phases between the large open microaneurysms completely filled with red blood cells and the mature Kimmelstiel-Wilson nodule, and presented that the proliferating mesangial cells push laterally into the microaneurysm which initially is quite cellular and contains fine fibers or fibrils and osminophilic granular deposits between the cells. The features of this lesion seems to correspond to those of the enmeshed or reticular structure in this study. However, in the present study it is suggested that microaneurysms don't always precede to the disorganization process and instead they seem to be sometimes accompanied by this process. Saito et al. (1988) suggested “mesangiolysis” as a leading process in the development of nodular lesions without microaneurysm formation. And they postulated that the reticular structure was formed by extension of mesangiolytic process to the whole tuft (Saito et al. 1988). But they regarded the reticular structure as glomerular
injury and didn’t refer to the cell proliferation (Saito et al. 1988). Because in our study proliferated intramembranous cells forming enmeshed or reticular structures were relatively immature according to their morphological characteristics and accompanied by the basement membrane-like structure, it is thought that they are derived from local mesenchymal cells. Bloodworth (1978) regarded proliferated cells as mesangial cells, but in the present study these proliferated cells could not be identified clearly as endothelial or mesangial cells by their morphological characteristics at least in the early stage. Therefore, we instead used the term “intramembranous cells” defined as the cells enclosed by the glomerular basement membrane. Moreover, because proliferated intramembranous cells were accompanied by irregular or round intercellular spaces and thereafter these spaces became slit-like, some of which contained red blood cells, it is thought that the cell proliferation occurs inside the original blood spaces and at least that proliferated cells are not brought about by in situ proliferation of mesangial or intercapillary cells. In addition, axially accumulated cells without facing blood stream may acquire more characters of mesangial cells, while peripherally situated cells lining recanalized blood spaces may become to have more endothelial natures.

Bloodworth (1978) regarded the cell proliferations showing the reticular structure as the organization of microaneurysm and considered that some unknown injury or stimulus causes disruption of anchor points. On the other hand, Saito et al. (1988) regarded the reticular structure as the result of mesangiolysis, probably due to glomerular ischemia caused by arteriolosclerosis. We would suggest that such a developmental process is quite similar to the disorganization process which Fujimoto (1954a, b) first suggested in rabbit Masugi nephritis and human glomerulonephritis. And Fujimoto (1964) regarded the formation of enmeshed or reticular structure as an expression of the immunologic response. Mostofi et al. (1971) postulated that mesangial cells react to most types of glomerular injury, which may be explained by the reticuloendothelial properties of these cells. Therefore, it is probable that the cell proliferation showing the reticular structure found in the development of the nodular lesion may represent some immunological response of the glomerular loop.

However, some differences between disorganization process in diabetic nodular glomerulosclerosis and that in glomerulonephritis do exist. As our study clarified, in diabetic glomerular loops showing disorganization process foam cells and lipid droplets were often found, axially accumulated cells remained active for longer duration, and intercellular matrix production was more intense. These results suggested that the disturbance of lipid metabolism in diabetes mellitus, in addition to the disorganization process, may also be important in the development of the nodular lesions. Al-Shabe et al. (1988) also demonstrated some increase of cells, increased mesangial matrix, and lipid accumulation in glomeruli in experimental hypercholesterolemic guinea pigs.

The nodular lesion has two histological characteristics: lamellar structure of
the hyaline nodule and lobulation of the glomerular loop (Heptinstall 1983). First, lamellar structure of the nodule is explained by the fact, as clarified in our study, that the concentric cellular arrangement and the following matrix production along cells with reduction in number were seen in later stages. And the presence of red blood cells within peripheral slits of the nodule was reported (Heptinstall 1983). It is thought through our study that these slits are the remnants of the original blood spaces reduced by intracapillary proliferation of cells with reticular arrangement, and that some of them, after axial crowding of proliferated cells and peripheral recanalization, have been lined by cells with endothelial characters. In addition, narrowing and obliteration of these spaces by increased matrix production may have some contribution to the formation of lamellar structure. These spaces were also described as “unusual blood spaces” in the acute phase of Masugi nephritis (Suzuki et al. 1963). Secondly, although lobulation of the glomerular loop has been reported in various conditions (Olsen 1972), it is generally considered that remarkable cell proliferation, matrix production, and deposition of abnormal substances at the centrolobular area contribute to lobulation. Fujimoto (1978) postulated that when cell proliferation and following axial fibrosis of glomerulus are pronounced, lobulation of glomerular loop is brought about, which is the lesion characteristic of lobular glomerulonephritis. Shoji (1988) demonstrated that lobular feature of glomerular loops is accentuated by the recurrence of proliferative glomerulonephritis in rabbit Masugi nephritis. Our observation of the disorganization process at the peripheral portion of glomerular loops with the axial preformed hyaline nodule indicates the recurrence of disorganization process in diabetic glomerular loops. Therefore, it is suggested that the recurrence of this process may contribute to lobulation of glomerular loops in diabetic nodular glomerulosclerosis.

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References


Fig. 1. Glomerular loops show proliferative changes. Intramembranous cells increase in number and intercellular matrix is basement membrane-like. Intercellular spaces are irregular. These loops represent enmeshed or reticular structure. (PAS, ×330).

Fig. 2. Glomerular loops show reticular structure. Intramembranous proliferated cells accumulate toward the axial portion and basement membrane-like structures become thickened. Cellular arrangement becomes slightly concentric. At the peripheral portion recanalization of blood spaces is beginning. (PAM, ×630).

Fig. 3. A glomerular loop represents reticular structure. Intramembranous cells become less prominent and intercellular matrix becomes thickened and concentric. At the axial portion foam cells are seen. (PAM, ×840).

Fig. 4. A glomerular loop shows reticular structure, in which proliferated cells are less prominent and the matrix is somewhat thickened, associated with a microaneurysm. The peripheral microaneurysm compresses the axial structure. (PAS, ×540).
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Fig. 5. A glomerular loop represents almost mature nodular lesion. Intramembranous cells decrease in number and intercellular matrix increases and becomes dense and lamellar. Intercellular spaces are slit-like. At the peripheral portion recanalization of blood spaces is seen. (PAS, ×280).

Fig. 6. A glomerular loop represents mature nodular lesion associated with microaneurysm. The nodule is markedly less cellular and lamellar. Intercellular spaces are slit-like. (PAS, ×290).

Fig. 7. A glomerular loop shows recurrence of cell proliferation, surrounding the axial large hyaline nodule. (PAS, ×350).
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Fig. 8. A glomerular loop shows cell proliferation. Intramembranous cells increase in number and have large cytoplasms and abundant cytoplasmic organelles, especially rough endoplasmic reticula. Intercellular matrix is fibrillar. (electron micrograph, ×1,150).

Fig. 9. A glomerular loop shows peripheral recanalization of blood spaces. Large intramembranous cells have cytoplasmic organelles. Axial increase of intercellular matrix is seen. Cellular arrangement is slightly concentric. (electron micrograph, ×2,240).
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Fig. 10. A glomerular loop shows appearance of foam cells and deposition of lipid droplets. Intramembranous cells increase in number and intercellular matrix increases. (electron micrograph, ×2,840).

Fig. 11. A glomerular loop represents reticular structure. Intramembranous cells accumulated toward the axial portion. Intercellular matrix increases. Capillary lumina are seen near the axial portion. (electron micrograph, ×1,830).
Fig. 12. A glomerular loop shows almost mature nodular lesion. Capillaries within the nodule are seen. Intercellular matrix much increases and becomes dense. (electron micrograph, ×1,560).

Fig. 13. A glomerular loop shows recurrence of peripheral cell proliferation which surrounds an axial preformed nodule. (electron micrograph, ×1,560).
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