An Approach to the Structure and Function of the Glomerular Mesangium

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ABSTRACT

The mesangium of the glomerulus is a connective tissue tree arising at the vascular pole of the glomerulus and supporting the glomerular capillaries. It is partly covered by a basement membrane that follows the epithelial cells from the peripheral glomerular capillary wall over the supporting tissue. The capillary endothelium does not normally have a separate basement membrane. The endothelium has fenestrations that open directly into the mesangium and allow blood plasma and tracers to flow into the mesangium. The fenestrations partially restrict (or sieve) particles over 405 Å in mean length from entry. Tracers move in intercellular channels and are filtered and concentrated by the basement membrane at the sides of the mesangium or by mesangial matrix filaments in the channels between cells. The irregular distributions of flow, matrix, and concentrations of tracers may account for irregular lobular reactions in glomerular disease. Two main pathways of flow seem to be (1) through the basement membrane and between the epithelial foot processes to form part of the glomerular filtrate and (2) into the efferent capillaries through their mesangial fenestrations. Intrinsic mesangial cells can now be regarded as myofibroblasts associated with the production of the connective tissue matrix. These cells hold the basement membrane to maintain the shape of the glomerular capillaries, they swell readily, and they can constrict like smooth muscle cells with appropriate stimulation. These reactions may enable them to control the flow of blood through the capillary network in glomerular disease. Mesangial cells can take up large amounts of foreign material within 24 h. Intrinsic mesangial cells and monocytes can increase in numbers in disease. Cells in the polar cushion (Pohlskenz) react differently than do mesangial cells.

Key Words: Mesangial filtration, flow, fenestrations, basement membrane, endocytosis, mesangial cells, myofibroblast

This discussion will review the light and electron microscopy studies that describe the structure of the mesangial region, its cellular and extracellular components, its relationships to adjacent structures, some of its functions under physiologic conditions, and some of its reactions in disease. Figure 1 is a diagram of the main features of the mesangial region. Others have reviewed more recent studies of the mesangium that elucidate biosynthetic activities and reactions to various substances including enzymes, hormones, cytokines, immune complexes, and mediators of inflammation (1,2).

The mesangium was considered by Zimmermann (3,4) to be a connective tissue tree that arises from the vascular pole of the glomerulus, extends into the lobules, and supports the glomerular capillaries. He described and illustrated it in 1929 (3), but he did not use the term mesangium until 1933 (4). Zimmermann's observations were detailed and remarkable. However, they were not accepted by some investigators who used light microscopy and early electron microscopy techniques. In this period, the terms applied to this region included intercapillary, axial, and centrolobular. The development of improved electron microscopy techniques enabled confirmation of Zimmermann's observations (5). A number of studies from this laboratory have dealt with the mesangium (5-15), and we have reviewed it briefly in 1973 (11) and 1985 (15). Other studies and reviews have presented further information with electron microscopy and other techniques (1,16-21).

BASEMENT MEMBRANE

The basement membrane (basal lamina) of the glomerulus appears early in the embryologic development of the glomerulus in association with the epithelial cells that form the renal vesicle. It retains this association with epithelial cells as the glomerular capillaries and the mesangium develop. In the mature animal, the basement membrane comes to lie over
Figure 1. Diagram of the mesangial region. The mesangium is shown at the center of capillaries forming a glomerular lobule. Portions of the mesangium are covered by the lamina densa (LD) and lamina rara externa (LRE), which follow the foot processes of the epithelium (Ep) from the peripheral glomerular capillary wall over the mesangium. The capillary basement membrane (BM) (basal lamina) is composed of the LRE, the LD, and the lamina rara interna (LRI). Where it joins the mesangium, the LRI is continuous with the mesangial matrix (MM). The MM varies in density and is irregularly distributed in the mesangium. It is often more dense where it anchors processes of mesangial cells (M) to the LD of the infolded BM. The MM is usually less dense beneath central portions of endothelial cells (En) lying over the mesangium. Endothelial cells do not normally have a separate basement membrane. The central portions of endothelial cells often have fenestrations (F) that are similar in size to but fewer in number than fenestrations in the peripheral capillary wall. The fenestrations allow blood plasma and particulate tracers to directly enter into the mesangium and move in intercellular channels (IC) where there is little or no matrix. One pathway of flow out of the mesangium seems to be through the LD and between foot processes over the mesangium, contributing to the glomerular filtrate (arrow to the right). A second pathway of flow out of the mesangium seems to be through the intercellular channels and endothelial fenestrations of efferent capillaries into their lumens (arrow to the left). A third but small pathway of flow seems to be through the hilus into the polar cushion (Polkissen) of the JGA. Adjacent foot processes come from different cells, hence the number covered by epithelial cytoplasm is always odd. Modified from Latta et al. (5) with permission.

the glomerular capillary network and the mesangium like a folded sheet (11). Different stages of this process can be seen by light microscopy in developing glomeruli in the renal cortex of human fetuses under 35 wk of gestation. There is no separate basement membrane associated with glomerular endothelial
cells either in the peripheral capillary wall or over the mesangium in normal animals (5,11), but one may develop in glomerular disease as in human mesangiocapillary (membranoproliferative) glomerulonephritis (20,22). Although the term, basement membrane, seems well established in the literature on glomerular capillaries and refers to the lamina rara externa, lamina densa, and lamina rara interna, histologists now prefer the term basal lamina (23).

MESANGIAL MATRIX

The mesangial matrix was described by Zimmermann (4) as connective tissue with a fibrous network that is denser and stains more strongly with Azan than the connective tissue outside the glomerulus. Other stains indicate that it has a large polysaccharide or proteoglycan content (11,24). Electron microscopy studies showed a large component of filaments 12 to 25 Å wide (24) and a few banded collagen fibers (7). Banded collagen may become much more prominent in glomerular disease (6). Fibronectin and laminin have been described (1). Ruthenium red staining indicates a negative (polyanionic) charge on the mesangial fibrils (24), which are much more closely packed than the relatively large, negatively charged lattice-like network described by Kanwar and Farquhar (25). Deposits in the mesangium representing abnormal materials or increased amounts of normal components are recognized in diabetic glomerulosclerosis, other types of glomerulonephritis, immune complex disease, etc. (22).

Mesangial cells and fibers seem to hold the glomerular basement membrane against the blood pressure in the capillaries (11,15). This has been studied in some detail by Kriz et al. (26). Loss of this holding force would allow the basement membrane to pop out and form microaneurysms, which appear in mesangiolysis with necrosis of mesangial cells, diabetes, and other glomerular diseases (11,20).

MESANGIAL PLASMA FLOW

The concept of plasma flow in the mesangium seems to be a key to understanding the structure and functions of the mesangium.

Entry into Mesangium

The rapid entry of small tracers into the mesangium within a few minutes after i.v. injection indicates a considerable flow of blood plasma into this region (5). Entry into the mesangium seems to occur through fenestrations of the same size and shape as those in the peripheral glomerular capillary wall (15). The mean width is 376 Å in the rat. Analysis of the measurements of the fenestrations and asymmetric thorium dioxide (ThO₂) particles used as tracers indicated that particles with a mean length of 405 Å or more were partially restricted from entry. This constitutes sieving, which partially excludes larger substances. However, the presence of some large fenestrations allowed the entry of a few thorium dioxide or carbon particles over 600 Å in size. The entry of tracers into the mesangium is favored by the absence of a basement membrane under the endothelium over the mesangium. It is also favored by the lesser density of the mesangial matrix lying under the endothelium (15).

Slower entry into the mesangium of aggregates of tracers could occur in hours or days with development by the endothelium. Envelopment was suggested by clumps of ThO₂ particles in capillary lumens that appear to be incorporated into the mesangium by thin layers of endothelium moving over them (8). This would be analogous to the movement of the endothelium over the surface of a mural thrombus. This mechanism could also explain incorporation into the mesangium of masses of carbon (27), possibly fibrin, capillary thrombi, larger aggregates of protein or immune complexes, etc.

Mesangial cells can project into the capillary lumen (4,9,10,13,16). Zimmermann (4) suggested that they might do this for nourishment. No evidence has been found by electron microscopy that these processes take up tracers, and our impression is that these swollen processes are a response to ischemia or disease conditions (9,10,13).

Substances may be carried into the mesangium by cells migrating from the capillaries. After the injection of foreign organisms, which adhere to the glomerular endothelium, monocytes and neutrophils lodged in the glomeruli and began phagocytosis in 10 min (28,29). Monocytes containing ferritin began to infiltrate the mesangium in 4 h (30). Such inflammatory cells may contribute to the glomerular hypercellularity apparent by light microscopy.

Other mechanisms of incorporation have been suggested (such as by passage along the peripheral glomerular capillary wall, by transport across endothelial cytoplasm, or by mesangial cells sweeping the peripheral capillary wall), but there is little evidence for them (15,19,20).

Factors favoring mesangial localization have been discussed by Michael et al. (19), Sterzel et al. (20), and Menè et al. (1) and include high concentrations in the blood, decreased activity of the systemic phagocytic system, corticosteroids, large size, positive charge, and high avidity of immune complexes (31,32).

Pathways of Flow and Filtration in the Mesangium

If plasma flows into the mesangium rapidly, fluid must flow out rapidly (5,11,15). Pathways of flow
have been suggested by the appearance and accumulation of tracers and immune complexes. Tracers entering in a few minutes move in channels between mesangial cells where the matrix is less concentrated. With time, tracers accumulate in two places in the mesangium. One is under the dense layer of the basement membrane, which follows the epithelium over the mesangium. Tracers (hemoglobin, ferritin, dextrans, plasma proteins) and immune complexes can penetrate in this paramesangial area (see references 15, 33, and 34). This concentration suggests that these substances are being filtered out by the basement membrane and that the fluid with them flows on through the basement membrane, between foot processes, and contributes to the glomerular filtrate (34). This forms one pathway of flow through the mesangium. The behavior of these tracers suggests that the permeability of the basement membrane and foot processes over the mesangium is similar to that over the peripheral glomerular capillary wall (34).

A second pathway of flow through the mesangium was suggested by the concentration of tracers on portions of the mesangial matrix in intercellular channels (8). This pathway would conduct flow from the afferent vessels in the glomerular capillary network to the efferent capillaries. A hydrodynamic analogy is suggested by the different directions and rates of flow a river can take passing through a delta region. Another consideration favoring flow through the mesangium from afferent to efferent capillaries is that, without such flow, plasma proteins filtered out at the basement membrane over the mesangium would become unduly concentrated. They would have to be carried back into the capillary circulation for the mesangium to maintain its normal state. The accumulation of tracers on portions of the mesangial matrix represents another type of filtration in addition to filtration at the basement membrane mentioned above. Concentration on the matrix would give tracers more time to be taken up by cells in the mesangium. The irregular distribution of the mesangial matrix and variations in flow account for the different numbers of tracers found in different glomeruli and in different lobules of the same glomerulus. It may also explain the more prominent changes in some lobules in focal glomerulonephritis and other glomerular diseases. The flow of blood plasma through the mesangium would also enable the mesangial cells to react to substances carried in the plasma, such as hormones, cytokines, immune complexes, etc.

A third pathway of flow to the hilus of the glomerulus and the juxtaglomerular apparatus (JGA) has been suggested (8), but the small numbers of tracers found in the JGA in 24 h (8) and the delayed times of concentrations in the JGA (several hours for iron dextran [35] and ferritin [36] and 4 wk for carbon [27]) indicate that flow in this pathway would have to be quite small (15). Most of the hilus of a glomerulus is occupied by the two arterioles, which leaves the remaining area of contact between the mesangium and the JGA so small that it must act as a bottleneck limiting the passage of tracers, whether extracellular or intracellular. Moreover, the cells of the JGA are packed closely together, and where there is interstitial matrix between them, it is quite dense, as if to partially seal off the JGA from extraneous influences. This enables the components of the JGA to respond better to physiologic stimuli. A possible alternative means by which tracers could enter the JGA is from the adjacent interstitial tissue and vessels, but the evidence for this is less impressive than that for slow passage from the mesangium.

Other fates for tracers in the mesangium have been suggested (15, 19). These include intracellular and extracellular digestion, regurgitation into the blood, transportation by cell movement or cell-to-cell passage, passage through the basement membrane by liquefaction of its thixotropic gel structure, or dissolution of immune complexes by excess antigen. Indigestible tracers may remain for months.

CELL TYPES IN THE MESANGIUM

It is generally agreed that the normal mesangium contains at least two cell types, the intrinsic mesangial cell and the monocyte/macrophage. Each of these cell types performs multiple functions.

The Intrinsic Mesangial Cell

The Fibroblastic Nature of Intrinsic Mesangial Cells. Zimmermann (4) considered mesangial cells to be fibroblasts because they lay in a fibrous matrix that stained like connective tissue. Electron microscopy support for the connective tissue nature of the mesangium was obtained in this laboratory when banded collagen fibers were observed in experimental (6) and human (37) glomerular disease. Occasional small bundles of banded collagen fibers were also found in the normal rat mesangium (7). Increased deposition of the mesangial matrix (and sometimes collagen) can occur in diabetes and different types of glomerulonephritis and glomerulosclerosis. Amyloid deposits occur in various connective tissue sites in the body, including the mesangium (22).

The Smooth Muscle Nature of Mesangial Cells. Our early electron microscopy studies showed bundles of microfilaments and attachment bodies in mesangial cells like those in arterial smooth muscle (8). Others demonstrated actomyosin activity in mesangial cells by immunofluorescence (38, 39). Mesangial cells localize angiotensin II in situ (40) and contract in tissue culture when stimulated with an-
Mesangial cells swell easily and lift the overlying endothelium or project through it into the capillary lumen (9,10,13). Zimmermann described and illustrated this process by light microscopy in 1933 (4). Similar changes have been observed in the smooth muscle of the aortic wall (42). Contraction and swelling could control blood flow through the glomerular capillary network. Endothelial swelling may aid in this process. Considerable evidence suggests a contribution of mesangial cells to the regulation of glomerular filtration (21,43). Swelling is especially prominent after the cessation of blood flow (9) or after ischemia for 15 to 60 min (13). Such swelling could account for the loss of glomerular perfusion and filtration in shock, toxemia of pregnancy, or glomerulonephritis.

The concept of the myofibroblast developed from studies on fibroblasts in wound healing (44). Darby et al. (45) have described the development and regression of smooth muscle actin in granulation tissue fibroblasts. This is an example of the plasticity of phenotype in the expression of different features in connective tissue cells under various stimuli (46). For example, smooth muscle cells can acquire fibroblastic features (1) in the uterus (and possibly account for the development of uterine fibromyomas) or (2) when migrating from the media to the intima in atherosclerosis. It has even been suggested that the macropage can express muscle proteins (46). The fibrous histiocyte may be derived from a mesenchymal cell capable of multidirectional differentiation to express fibrous and histiocytic features (47). A contractile system would help mesangial cells hold the "infolded" basement membrane against the blood pressure in the capillaries (8,26).

Mesangial cells have been considered to be pericytes by several investigators. However, pericytes are usually described as having long branching processes that extend around capillary endothelium (23). Zimmermann (4), having earlier studied pericytes around capillaries in other tissues, was particularly interested in searching for them in the kidney. In one preparation of a cat kidney, he found five cells with small processes, apparently grasping glomerular capillaries, and concluded that they were "perhaps true pericytes." No one using electron microscopy seems to have described appropriate pericyte processes outside of the endothelium of the peripheral glomerular capillary wall in the normal animal. Therefore, it does not seem that normal mesangial cells ordinarly fulfill the histologic criteria for pericytes. Mesangial processes may appear in the capillary walls of mesangiocapillary glomerulonephritis, but these walls are greatly altered.

Endocytic Function of Mesangial Cells. In 1960, we (5) described the special features of the mesangial region and its cells with newer electron microscopy techniques. In 1961, before the concept of the mesangial region was generally accepted, Farquhar et al. (48) reported that injected ferritin accumulated on the luminal side of the glomerular basement membrane, especially in axial regions, and was taken up by the deep cells, which were considered to be endothelial. In 1962, we (8) demonstrated that large amounts of thorium dioxide particles were engulfed by centrolobular mesangial cells within 24 h. Adjacent capillary endothelial cells showed little or no uptake. Later, in 1962, Farquhar and Palade (16) wrote an article agreeing that the axial region had special characteristics and showing that the deep cells accumulated more of the tracers than the superficial endothelium. They concluded that the deep cells could be considered a distinctive "third" cell type.

A number of foreign substances have been found in cells in the mesangium (1,19,20), but the evidence needs to be reevaluated in light of two questions: were the cells that were involved intrinsic mesangial cells or macrophages, and what type of endocytosis was stimulated by each substance? The uptake of macromolecular substances into a membrane-limited organelle in a living cell is now called endocytosis and is divided into three types: (1) phagocytosis of large particles, (2) macropinocytosis, and (3) coated pit-mediated endocytosis, which accounts for the majority of uptake of fluid-phase and receptor-bound materials by most cells, with the formation of receptosomes (or endosomes) (49). These matters will be considered again below when the glomerular monocyte/macrophage is discussed.

The Multipotential Nature of Mesangial and Other Connective Tissue Cells. While the concepts of different functions for the mesangial cells have been developing, evidence has been accumulating that other connective cell types may show more than one kind of activity. Myofibroblasts occur in wound healing and in uterine myofibromas and fibrous histiocytomas have been mentioned above. Smooth muscle cells have been associated with the production of collagen and elastin in normal arteries (50). Smooth muscle cells from arteries can ingest yeast cells and latex spheres (51). They may acquire Fc and C3 receptors and the ability to ingest immunoglobulin G-coated red blood cells (52). In atherosclerosis, they migrate into the intima and produce collagen (53). Considerable evidence has led to the concept of the arterial smooth muscle cell as a multifunctional mesenchymal cell (54). Another potential is shown by connective tissue cells in muscle. When stimulated by trauma (and bone morphogenetic protein), they can produce cartilage and bone in a process called heterotopic bone formation (formerly myositis ossificans) (55). Hence, mesangial cells seem to be pluripotential and are like other connective tissue cells in being able to exhibit different functions upon appropriate stimulation.
The Monocyte/Macrophage in the Mesangium

Studies have shown that normal rat mesangia contain phagocytic cells different from intrinsic mesangial cells and derived from the bone marrow (56). Phagocytosis was considered as the uptake of latex beads, bacteria, aggregated gamma globulin, or other large particles. There were 8 to 18 mesangial phagocytes per glomerulus, and all were found to express the leukocyte common antigen. About half of these bear Ia determinants. With an average of 281 mesangial cells in a rat glomerulus (57), the phagocytes would represent between 3 and 7% (or 1 in 35 to 1 in 15) of the normal mesangial population. The kinetics of a slow traffic of monocytes from the bone marrow and the blood through the mesangium were considered (56).

According to the above measurements, 93 to 97% of the mesangial population is composed of intrinsic mesangial cells. The amount of uptake of various foreign tracers in the mesangium within a few minutes or hours of injection makes it seem likely that the intrinsic mesangial cells are involved (8,19,20); however, which type of endocytosis they use needs to be determined. The type probably depends on the tracer.

Monocytes can localize in glomeruli within minutes (29) or hours (28) of an injection of pathogenic organisms and can contribute to the hypercellularity of the developing focal glomerulonephritis. With the stimulus of foreign tracers, monocytes seem to migrate into the mesangium (30,36). After polyvinyl alcohol injections, Ia-positive mesangial monocytomacrophages showed no uptake at 24 h, whereas there was some in stellate mesangial cells (58). Considerable amounts were found in the monocytes-macrophages after 3 days. The number of monocytes-macrophages also increased between 1 and 3 days (36).

Hence, it seems that the intrinsic mesangial cells account for much of the endocytosis in the mesangium within the first 24 h, and after that, endocytosis by monocytes-macrophages becomes prominent. It may be that after encountering some substances, the intrinsic mesangial cells stimulate the recruitment of monocytes into the mesangium from the circulating blood. Macrophages can be prominent in glomerular disease and may serve as the principal effector cell in some types of glomerulonephritis (2).

OTHER FUNCTIONS AND REACTIONS IN THE MESANGIUM

The proliferation of mesangial cells has been measured in diabetic glomerulosclerosis (59), compensatory hypertrophy (57), and immunologically mediated glomerulonephritis (2). Lobular or diffuse proliferations of mesangial cells have been observed in other types of glomerular disease involving glomerulonephritis or glomerulosclerosis (22,60). Increased mesangial matrix is evident in different types of glomerulosclerosis. Deposits of amyloid, immune complexes, and other substances may occur. There is evidence for the mediation of glomerular disease by neutrophils, monocytes and macrophages, lymphocytes, and platelets (2).

In some types of glomerulonephritis, particularly membranoproliferative (or mesangiocapillary) glomerulonephritis, endothelial cells may be lifted away from the capillary basement membrane and the mesangial cells may extend out in the capillary wall in a process called mesangial interposition (22,60). A second basement membrane forms beneath the displaced endothelium, producing the double-contour effect seen by light microscopy.

THE RELATIONSHIP OF THE MESANGIUM TO THE JGA

Granular epithelioid cells had been found in the walls of afferent arterioles by Ruyter (61) and Oberg (62) when Goormaghthig (63) and Zimmermann (4) described in more detail juxtaglomerular structures containing modified smooth muscle cells. Zimmermann called a group of cells arising from the afferent arteriole outside of the vascular pole the Potkissen, which means “polar cushion” in English. The cells in it made close contact with the macula densa of the distal tubule. The cells in the polar cushion were closely packed and were separated only by a thin fibrous network, which was much less prominent than the more abundant and darker staining connective tissue fibers in the adjacent mesangium, which also contains fewer cells. The nuclei in the polar cushion may be elongated and placed parallel to each other. Striking differences at the transition from the polar cushion to the mesangium were well illustrated in several figures (4). Electron microscopy studies by us (8,11,64–68) and others (69,70) have confirmed and extended the light microscopy observations.

Although the structures of the polar cushion and the mesangium appear quite different, the cells of the polar cushion and the mesangial cells seem to be modified smooth muscle cells and functional similarities have long been postulated (8,69). However, a number of studies have shown functional differences. After adrenalectomy, rat juxtaglomerular cells had a striking increase in granularity, but no such granularity was found in mesangial cells (67). After the injection of tracers, penetration and accumulation in the mesangial region was rapid, but it was delayed and smaller in amount in the polar cushion (8,27,35,36). The delayed appearance in the polar
cushion suggested that tracers could pass into it from the mesangium. In human (71) and experimental (72) unilateral renovascular hypertension, increases in size and granularity were found in the polar cushions but not in the mesangia. With excessive NaCl intake in rats, proliferation of agranular cells was found in polar cushions but not in mesangia (73). Hence, after consideration of the anatomic and functional evidence presented above, it seems better to maintain a distinction between the mesangium and the polar cushion.

The different names that have been used in describing different parts of the JGA have been confusing. The JGA is composed of the polar cushion, the afferent and efferent arterioles, and the macula densa (11,67,69,70). It is well innervated (67,74). The polar cushion (Poltkisen, foci, juxtaglomerular cell mass, extraglomerular mesangium) contains mostly agranular cells (the pseudoeiassianerian cells described by Goormaghtigh) and a few granular cells (68). Most of the granular cells (epithelial or myo-epithelial cells) are found in the walls of the afferent and efferent arterioles (68) and are thought to contain renin (11,70).

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