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EDITORIAL REVIEW

The glomerular mesangium

The one million or so glomeruli present in each of our kidneys are remarkably constructed. It is here that the ultrafiltrate of plasma is formed to be economically reabsorbed by tubules, leaving behind substances of no further use to the body. It is also here that the movement of macromolecules across the peripheral glomerular capillary is impeded by the basement membrane and by fixed polyanions in the capillary wall. Also within the glomerulus is an anatomically distinct region—the mesangium. The traffic of macromolecules through this zone appears to be significantly influenced by changes in the peripheral glomerular capillary as well as by other experimental manipulations. In human glomerulonephritis, immune deposits are found here, often exclusively. Morphologic abnormalities have ranged from the mesangial matrix expansion and the characteristic intercapillary nodule formation of diabetic nephropathy to the prominent proliferation of membranoproliferative glomerulonephritis and the segmental changes seen in a host of different diseases.

Anatomic relationships

Although the term mesangium was first used over 40 years ago to denote connective tissue cells arising in the stalk and extending to the inner aspect of the glomerular capillary [1], a number of years elapsed before there was general agreement that such a region within the glomerulus did in fact exist [2–6]. The precise relationship of the mesangium (mesangial cells and matrix) to other parts of the glomerulus was defined after the application of electron microscopic techniques to the study of the kidney [7–9]. The mesangial cell has been described as being centrolobular and deep to denote its unique location, which is distinct from regions occupied by endothelial and epithelial cells. In this locus the mesangium is bounded by the endothelium, which separates it from the capillary lumen, and the mesangial reflection of the glomerular basement membrane (GBM) and hence forms part of the glomerular capillary wall (Fig. 1). In the strictest sense, therefore, the mesangium has an intracapillary and not intercapillary location. The cells have branching cytoplasmic processes and contain a number of organelles, including course microfilaments, which resemble myosin filaments, suggesting that these cells may be modified smooth muscle cells [11, 12]. Although myosin has been demonstrated in the mesangium with immunofluorescent microscopy, its localization in filaments with ultrastructural techniques has not been documented [13–15] (Fig. 2). An intercellular substance, the mesangial matrix, is present between the cells, and although morphologically similar to the GBM, its complete biochemical and antigenic structure is unknown. Of great significance is the relationship of the mesangium to the juxtaglomerular (JG) apparatus. In the stalk region, the mesangium lies in close proximity to the lacis cells, which are contiguous with the epithelioid granulated cells of the afferent arteriole and the macula densa [10, 16–20]. Gap junctions also have been reported in mesangial and lacis cells of the rat, suggesting that these cells are coupled and may act as a functional syncitium [21]. This intimate structural relationship of the mesangium to the JG zone is important for at least two reasons: (*1*) the mesangium may play a role in the regulation of glomerular blood flow, although there is no direct evidence at the moment to support this concept; and (*2*) as described later, this region may provide a route for the egress of macromolecules from the peripheral mesangium.

The intracapillary position of the mesangium has made it relatively inaccessible to study either in the intact animal or in vitro. Although attempts have been made to isolate mesangial cells directly from glomeruli, these studies have been unsuccessful because of the cloistered centrolobular location of these cells in the glomerulus. Replicating cells have been isolated from in vitro cultures of glomeruli, but direct comparison with isolated native mesangial cells has not been possible [22–28]. Although the mesangial matrix has a morphologic appearance similar to that of GBM, and in disease states has been

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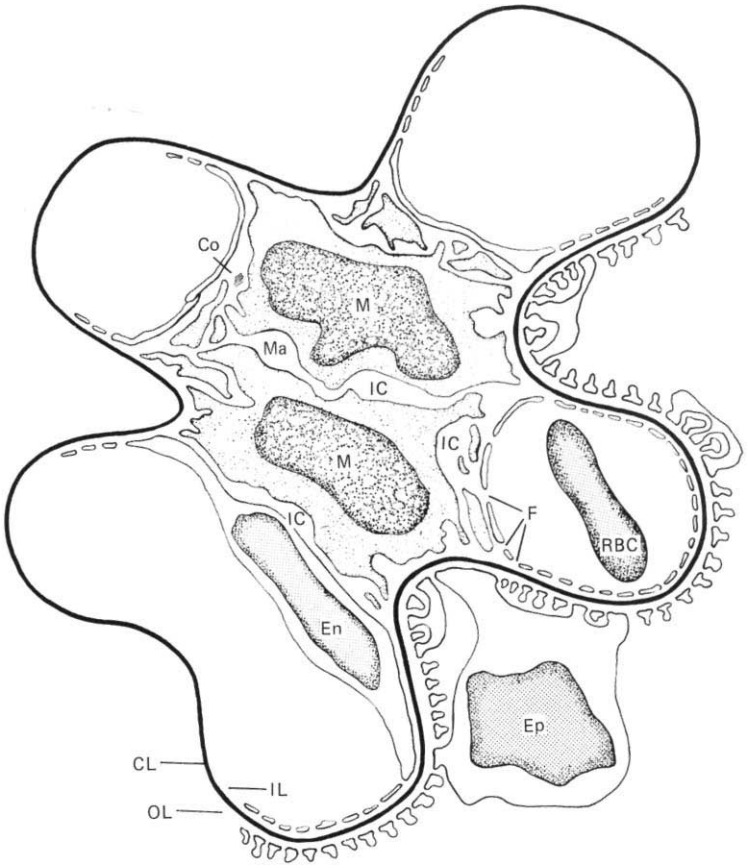

Fig. 1. *Diagram of a rat mesangial region surrounded by capillaries.* The central layer (CL) of the basement membrane invests the capillaries like a sheet and also passes over the mesangial region. The capillary boundary of the mesangium is formed by the axial portions of endothelial cells (En). Mesangial cells (M) are partially surrounded by mesangial matrix (Ma), in which bundles of collagen (Co) can sometimes be found. Fenestrations (F) of the endothelium allow passage of particles and plasma into intercapillary and intercellular channels (IC). The thicknesses of the outer layer (OL) and inner layer (IL) (lamina rara interna and externa) of the basement membrane have been drawn thicker than they are to show their relationships to the adjacent cells. The central layer (lamina densa) is thin in the rabbit, mouse, and rat (as shown here) but much thicker in the monkey and human beings. Epithelial cell bodies (Ep) extend primary processes, which branch to form the foot processes. The capillary diameter is usually somewhat greater than the diameter of a red blood cell (RBC). (Reprinted with permission of *J Ultrastr Res* [8] and American Physiological Society [10])

referred to as "basement membrane-like material," there is no evidence that these two structures are the same. Indeed, antibody eluted from the GBM of patients with anti-GBM nephritis reacts exclusively with the endothelial aspect of the GBM and not with the mesangial matrix [29]. Electron microscopic studies with tissue immersed in 5 M quanidine prior to fixation have demonstrated, however, two classes of GBM: an electron-opaque basal lamina that does not encircle the capillary (epithelial basal lamina), and an electron-lucent basal lamina that extends from the mesangium peripherally and surrounds the capillary lumen as an attentuated structure (mesangial and endothelial basal lamina) [30]. This continuity between the endothelial and mesangial basement membranes may provide a pathway for the movement of certain materials from the peripheral capillary loop to the mesangium. Immunohistochemical studies have demonstrated that certain collagen and glycoprotein antigens are present both in the mesangium and in the subendothelial region of the GBM (see below, *Mesangial antigens*).

Morphologic probes

The mesangial interstitial space is unique in that entry of a substance does not require passage through a capillary basement membrane. It has been possible to demonstrate sequestration of a number of different macromolecules within the mesangium of experimental animals, including (*1*) inorganic suspensions—thorium dioxide suspension (thorotrast), colloidal gold, colloidal carbon; (*2*) polysaccharides—dextran, iron dextran, polyvinyl alcohol polymers; (*3*) proteins—peroxidase, catalase, and ferritin; and (*4*) aggregated proteins and immune complexes (Table 1).

Following their administration to rats, thorium dioxide and colloidal gold are found within mesan-

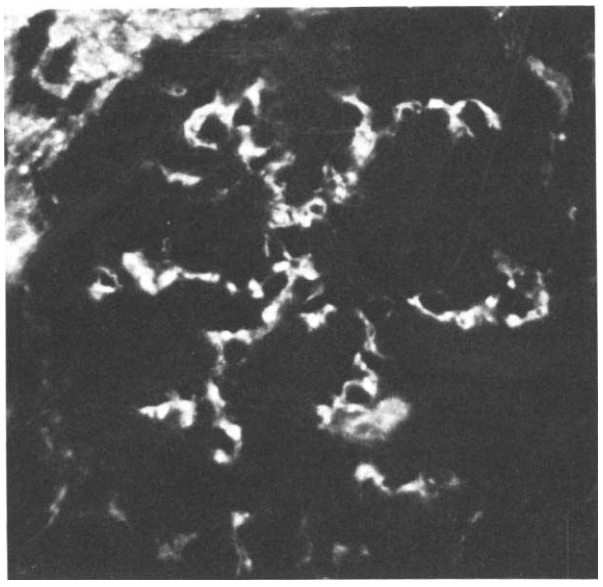

Fig. 2. *A normal glomerulus demonstrating actomyosin within the mesangium.* (Magnification, ×683) (Modified from Ref. 15)

gial channels and intercellular spaces between the endothelium and the mesangial cell within minutes of injection [8, 9]. In addition, the tracer is present within membrane-limited vesicles and vacuoles in the cytoplasm of mesangial cells. These particles are thought to enter the mesangium through the endothelial fenestra at the edge of the mesangial zone, although entry into the lamina rara interna at the periphery with migration into the mesangium cannot be excluded. In Farquhar's [9, 45] classical studies with ferritin, an iron-containing protein, penetration through the endothelial fenestra and intercellular spaces between the endothelium and mesangial cells could be demonstrated within minutes following injection. Active phagocytosis became evident within several minutes, reaching a peak within several hours, but within a period of 2 to 10 days, the mesangial zone was "cleared" of the ferritin. In nephrotic animals, the ferritin deposits at any one time period were more massive than they were in controls, a finding of importance when viewed in the light of subsequent kinetic studies with aggregated proteins.

Table 1. Substances taken up by the mesangium following their administration to animals

Probe	Mol wt (radius or s)[a] *daltons (Å or s)*
Inorganic particles	
Colloidal carbon [31-34]	(200 to 300 Å)
Thorium dioxide [8, 9]	(50 to 200 Å)
Colloidal gold [9]	(20 to 100 Å)
Polysaccharides	
Iron-dextran [35]	200,000 (37 × 220 Å)
Dextran [36, 37]	125,000 (78 Å); 250,000 (100 Å)
Polyvinyl alcohol polymer [38-40]	35,000 to 240,000
Proteins	
Horseradish peroxidase [41, 42]	40,000 (30 Å)
Myeloperoxidase [41]	160,000 (44 Å)
Catalase [43, 44]	240,000 (52 Å)
Ferritin [9, 45, 46]	480,000 (61 Å)
Aggregated protein and complexes	
Aggregated globin [47]	(250 to 1000 Å)
Aggregated human IgG [48-50]	(>7s)
Aggregated human albumin [48, 51]	—
Antigen-antibody complexes [52-54]	473,000 (IIs); >473,000 (>IIs)

[a] Adapted in part from Refs. 55 and 56.

When polysaccharide and protein tracers of varying molecular weights and sizes are administered to rats, a similar distribution of the molecules is observed between and within mesangial cells. Ultrastructural studies in the rat demonstrate that carbon particles enter the mesangial channels and are engulfed by cells within the mesangium in a manner similar to that observed for thorotrast [32]. At variance with studies carried out with other macromolecular tracers are the observations that the amount of carbon in the mesangium was maximal at 32 hours, with continued presence within the stalk region for weeks. A similar persistence has been observed following the administration of ferritin-protein conjugates to rats [46].

Because of the presence of immune deposits in the mesangium in human and experimental renal diseases, a number of studies have been carried out with aggregated proteins and preformed immune complexes [47-54] (reviewed in [56]). Observations derived from studies carried out in mice and rats following administration of aggregated human IgG (AH IgG) or albumin include the following: (*1*) These macromolecules were demonstrated almost exclusively within the mesangial region within 4 hours following administration to the animal, with a gradual disappearance over the subsequent 24 to 72 hours. Avid uptake by cells of the systemic mononuclear phagocytic system was also observed. (*2*) The accumulation in the mesangium depended on the amount administered to the animal, suggesting that the blood level was an important determinant [48, 50]. (*3*) Although AH IgG has been shown to have biologic properties, decomplementing the animal with cobra venom factor had no effect on mesangial accumulation [50]. In addition, similar results were obtained with a nonimmunoprotein, aggregated albumin [48, 51]. (*4*) The size and type of the macromolecule played an important role in view of the striking mesangial localization of AH IgG (> 7s) compared with the negligible localization of monomeric IgG (7s) [48, 49], or the rapid clearance from the mesangium of AH IgG compared with the persistence of carbon in this site [32]. (*5*) By elec-

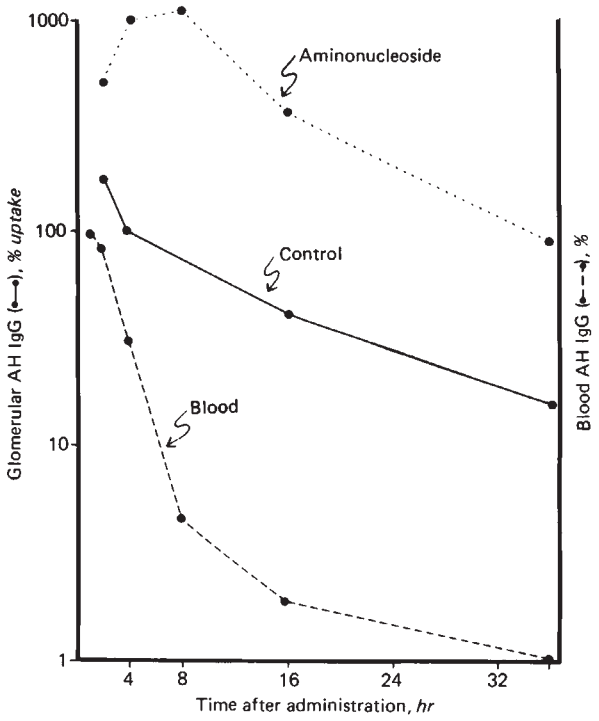

Fig. 3. *Relationship between the concentration of AH IgG in glomeruli of normal and aminonucleoside nephrotic rats and the time interval after administration of AH IgG.* The blood levels were similar in both groups of animals. Each point on the ordinate represents the concentration in glomeruli expressed as a percentage of the control value at 4 hours, the time at which aggregates were exclusively in the mesangium as demonstrated by immunohistochemical techniques. Note the striking increase in accumulation of AH IgG in aminonucleoside animals and the similar negative slopes from 4 to 36 hours. (Modified from Ref. 49)

tron microscopy, aggregated proteins were observed initially in the endothelial fenestra and the interface between mesangial cells and endothelial cells and in the channels present between mesangial cells; minimal uptake of aggregated albumin by mesangial cells was demonstrated [48, 51]. A similar intercellular distribution of antigen-antibody complexes has been demonstrated by Haakenstad and Mannik [53] and Striker, Mannik, and Tung [57] with no detectable phagocytosis by mesangial cells.

Kinetic studies of the mesangium

Most attempts to evaluate the mesangium have involved immunofluorescent and ultrastructural techniques. It has been possible, however, to study the kinetics of mesangial uptake and disposal of macromolecules by more quantitative techniques [49, 50]. Following the administration of labeled (^{125}I) AH IgG, glomeruli are isolated from groups of rats at varying time intervals thereafter, and the concentration of AH IgG (microgram per milligram of glomeruli) determined. These studies are predicated on morphologic observations showing almost exclusive localization within the mesangial zone of the glomerulus within 4 hours. In a typical experiment, a linear decrease in the concentration of glomerular aggregates is seen with time (Fig. 3). There is an associated but much more precipitous drop in the blood level, so that by 16 hours less than 2% of the initial value is present, whereas the amount remaining in the glomerulus has decreased to approximately 40% of initial values. After an initial period of rapid uptake, which in the normal animal is maximal within 4 hours occurring at a time when the blood level is relatively high, there is loss of aggregates from the mesangium as the blood level progressively decreases. Similar kinetics have been demonstrated for ferritin by Takamiya et al [46]. This movement into and out of the mesangium may be separated arbitrarily into afferent and efferent limbs. Because efferent mechanisms probably come into play shortly after uptake has occurred, a clearly defined time line does not separate these two processes. The initial 4-hour period likely represents, however, the peak of the afferent limb, whereas subsequent time periods reflect efferent mechanisms. Any attempt to separate those two phases into clear compartments is artificial because the uptake and loss of material from the mesangium represents a continuum.

Afferent mesangial limb

Substances enter the mesangial zone by way of the endothelium-mesangium interface and thence migrate by means of mesangial channels and in some instances are phagocytosed by mesangial and monocytic cells. The factors modulating uptake are probably numerous; several, however, have been identified (Table 2):

(1) Blood level and characteristics of the macromolecule. *(a)* In studies of immune complex trapping by the kidney, the blood level would appear to be a more important determinant than are other variables such as delivery rate, hydrostatic pressure, and ultrafiltration rate [58]. Similarly, the blood level of AH IgG or immune complexes is an important determinant of the amount taken up by the mesangium. This is reflected by the relationship between the amount of AH IgG or ferritin administered to rats and mesangial accumulation [46, 48, 50] (Fig. 4). When very low doses are administered, many organs trap aggregates more avidly than the renal cortex does, and no glomerular uptake is observed

Table 2. Modulation of mesangial uptake and disposal of macromolecules

Afferent limb
1. Circulating macromolecules
a. Blood level of macromolecule—related to systemic mononuclear phagocytic system
b. Characteristic of the macromolecule (size, type, digestibility, charge)
2. Characteristics of the glomerular capillary
3. Hemodynamic factors[a]
Efferent limb
1. Phagocytosis by mesangial cells or infiltrating phagocytes
2. Transport via stalk to juxtaglomerular zone
3. Capillary regurgitation[b]
4. Other modulating influences
a. Hemodynamic factors[a]
b. Ureteral obstruction
c. Capillary injury

[a] Relationship to blood flow, capillary pressure, and glomerular filtration is largely unknown.
[b] Not demonstrated

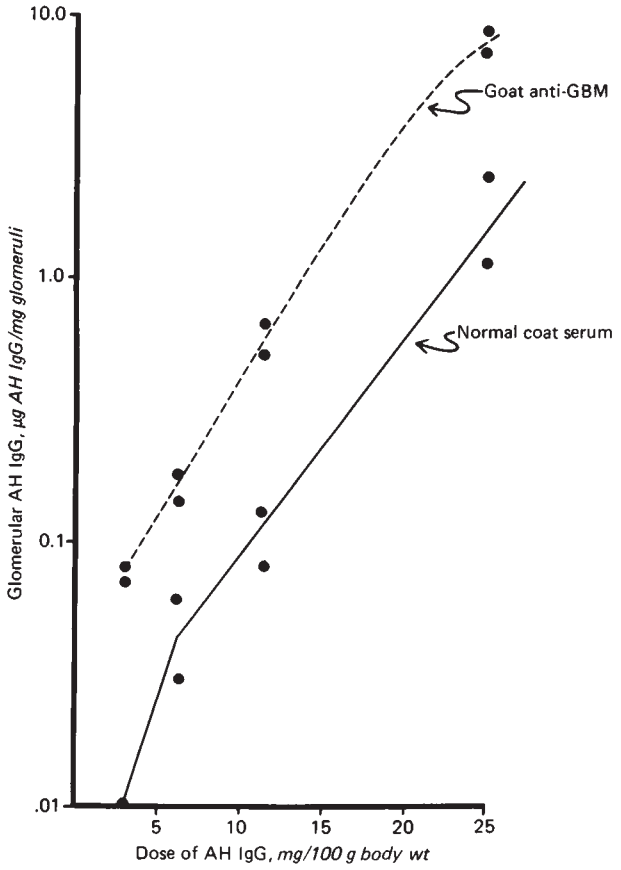

Fig. 4. *Relationship between dose and glomerular concentrations of AH IgG 8 hours following injection into nephritic and control rats.* The nephritic rats had received goat antirat GBM; and the control rats, normal goat serum 48 hours prior to administration of AH IgG. Note the dose-response in each group of animals and the significant increase in glomerular uptake in nephritic rats. Each point represents pooled glomeruli from five rats. (Data from Ref. 50)

[59]. Because many of the tracers used to evaluate mesangial function—ferritin, aggregated proteins, antigen-antibody complexes—are also taken up by the systemic mononuclear phagocytic system, it is not surprising that the latter plays an important role in modulating mesangial uptake. Indeed, in the early studies of Benacerraf, McCluskey, and Patres [31], glomerular uptake of carbon was observed only after relatively high doses that saturated the reticuloendothelial system. In experimental serum sickness nephritis, the amount of antigen-antibody complexes that become entrapped within the glomerulus represented a very small proportion of that taken up by the mononuclear phagocytic system [60]. Therefore, for any macromolecule, avid systemic phagocytic uptake would lead to relatively low blood levels and a decrease in the amount of material found in the mesangium. Conversely, reduced phagocytic uptake would favor persistence of relatively higher levels for longer periods of time and consequently increased deposition within the mesangial zone. Strong support for this viewpoint was derived from studies by Haakenstad et al [52–54, 61], who demonstrated a relationship between the glomerular localization of immune complexes and the blood level in mice. Large (> 11s) latticed complexes prepared with reduced and alkylated antibody were removed less efficiently by the hepatic mononuclear phagocytic system than were complexes made with intact antibody, leading to persistently higher blood levels of the former and increased mesangial sequestration. In mice treated with corticosteroids, the administration of preformed antigen-antibody complexes or AH IgG resulted in increased deposition within the mesangium—in part related to higher blood levels of the macromolecule secondary to a generalized decrease in vascular permeability [52], as well as changes in nonhepatic (splenic) phagocytic uptake [62]. Whether changes in glomerular hemodynamics induced by corticosteroids play a role has not been determined. The change in the distribution of immune deposits from a peripheral capillary loop to a mesangial pattern observed by Germuth and Rodriquez [63] after administration of cortisone to rabbits with serum sickness may be related to the variables discussed above as well as changes in the immunologic response of the animal. Our recent studies in endotoxin-treated mice demonstrate a discordance between mesangial or cortical uptake and the circulating level of administered AH IgG (Shvil et al, in press). Although endotoxin induces a significant in-

crease in uptake of aggregates by the mononuclear phagocytic system and as a consequence causes a reduction in the blood level, a concomitant decrease in mesangial uptake was not observed, and, in fact, at early time periods increased uptake was demonstrated. Thus, endotoxin influences mesangial uptake by a mechanism(s) that is independent of its effect on the blood level.

The concept that the mesangial uptake of macromolecules is inversely related to systemic mononuclear phagocytic activity is in keeping with studies demonstrating increased mesangial deposition of aggregated albumin in certain strains of mice with relatively decreased clearances of carbon from the blood [64], and by studies that propose but do not prove that the viremia of lymphocytic choreomeningitis leads to depressed phagocytic function, which contributes to defective immune complex clearance, and increased localization within glomeruli of nephritic animals [65].

(b) The morphologic studies cited above suggest that the mesangial transport of macromolecules of widely differing size and type is not an all or none phenomenon and that plasma percolates through the mesangium, likely under the control of hemodynamic influences. That size is an important determinant is suggested by studies demonstrating enhanced accumulation of aggregated proteins compared with that of the nonaggregated monomeric species [48, 49]. That differences other than size may be responsible for these observations has not been excluded. As discussed more completely below, increased accumulation of different macromolecules—carbon [33, 34], ferritin [45] and AH IgG [49, 50]—is observed in experimental nephrotic syndrome, a circumstance not seen with other probes such as monomeric 7s IgG [49]. It is possible that the movement of very large macromolecules through the mesangial zone is impeded compared with that of smaller molecules. The relative digestibility of a macromolecule also may play an important role in its clearance from the mesangium. For example, the prolonged persistence of carbon [32, 34] and polyvinyl alcohol polymers [38–40] is probably a consequence of overloading of phagocytes and the mesangium with indigestible residues.

There is evidence based on experimental studies that the type of antigen-antibody complex may influence the site of localization vessels and glomeruli [63, 66–68]. Poorly soluble complexes of intermediate size localize within the mesangium, resulting in a relatively innocuous lesion, whereas smaller more soluble complexes present in low concentrations are deposited in the peripheral capillary loop. Other studies have shown that immune complexes either formed in vivo or administered passively, which contain antibody of high avidity, affinity, and valence, are taken up by the mesangium in contrast to complexes containing antibody of relatively low affinity and valence, which tend to localize within the peripheral loop [69, 70]. Very little information is available regarding this relationship in human glomerulonephritis. The occurrence of membranous nephropathy in lupus erythematosus has been associated with low titers of nonprecipitating DNA antibody and lower or undetectable levels of circulating immune complexes, whereas in proliferative disease high levels of precipitating DNA antibody and circulating immune complexes are present [71, 72].

Polyanionic radicals within the glomerular capillary wall are known to play a major role in the filtration of macromolecules. With histochemical techniques that are optimal for the staining of negatively charged sialoproteins and which readily detect the epithelial polyanion, no reactivity with the mesangium could be demonstrated [73]. Recently, however, Kanwar and Farquhar [74, 75] have demonstrated a lattice-like network of negatively charged sites within the lamina rara externa, the lamina rara interna, and the mesangial matrix, which morphologically resemble proteoglycan and which contain heparan sulfate.

(2) Characteristics of the glomerular capillary. Essentially nothing is known about how the glomerular capillary itself modulates the afferent limb. Are there selective interactions between macromolecules and components of the mesangial matrix and cells? Do receptors, protein-protein binding, and intrinsic electrostatic charge play a role in the uptake of a specific macromolecule? What is the relationship between the peripheral glomerular capillary and the mesangium?

A striking increase in the mesangial accumulation of aggregated proteins, carbon, and ferritin has been demonstrated in two different forms of glomerular capillary injury—amnionucleoside nephrosis and anti-GBM nephritis [9, 33, 34, 49, 50]. Following the administration of AH IgG to the normal rat, there is prompt glomerular uptake followed by a linear fall-off in concentration, which parallels that observed in other organs such as the lung, liver, and spleen. In both experimental models, there is an increase in mesangial uptake to values tenfold higher than that observed in controls [49, 50] (Figs. 3 and 4). The rate of disappearance between 4 and 36 hours is not different because the curves derived

from normal and nephritic animals had similar negative slopes, suggesting that efferent mechanisms were relatively unaffected. Depletion of terminal complement components by cobra venom factor in nephritic animals reduced the uptake of AH IgG, although values were still significantly higher than those of controls, findings which implicate complement dependent and independent mechanisms. This enhanced uptake did not depend on the appearance of protein in the urine in either model: similar changes were observed after the administration of an amount of anti-GBM antibody that was below the threshold to induce proteinuria or very early after administration of aminonucleoside prior to the appearance of proteinuria.

That these changes were related to alterations in the kidney itself was demonstrated by induction of unilateral renal disease by direct infusion of aminonucleoside or anti-GBM antibody into one renal artery [33, 34]. Increased mesangial uptake of macromolecules (carbon or AH IgG) was demonstrated only in the perfused kidney, providing strong support for the contention that the changes were not related to alterations in the systemic milieu but rather to changes in the kidney itself (Fig. 5). These studies are consistent with the early electron microscopic observations of Farquhar et al [45] demonstrating increased amounts of ferritin within mesangial matrix and cells in nephrotic animals. In autologous immune complex nephritis, a change in the pattern of immune deposits, from epimembranous to mesangial, was demonstrated after administration of the aminonucleoside of puromycin, suggesting that alteration in glomerular capillary per-

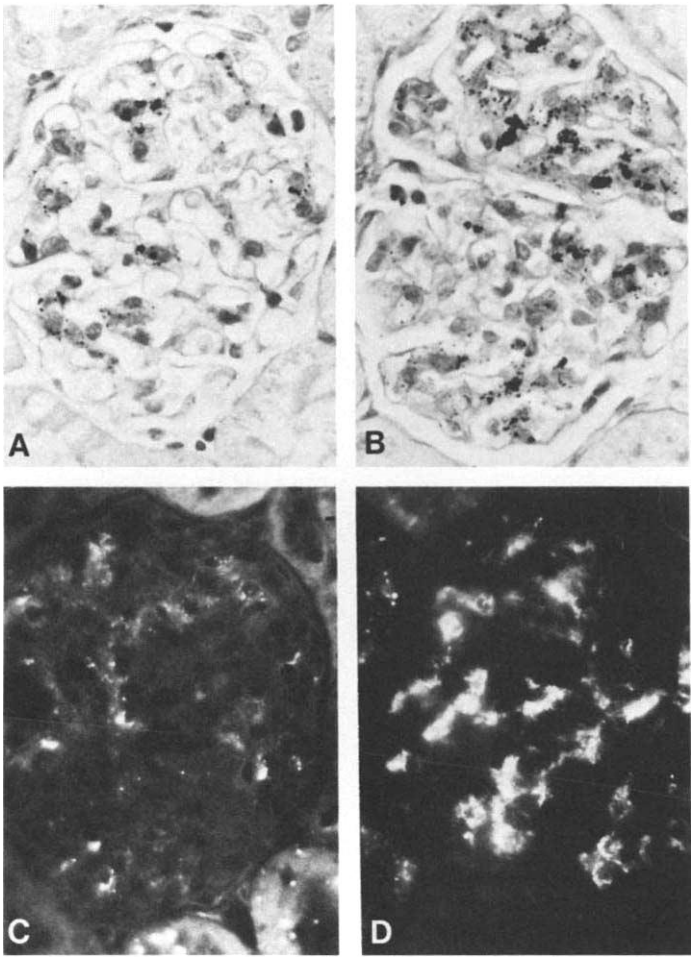

Fig. 5. A and B *Glomeruli from a rat 24 hours after administration of carbon.* The glomerulus from a normal nonperfused kidney contains small amounts of carbon (*panel A*). Note the large amount of carbon in the mesangium (*panel B*), which is from the centralateral kidney perfused with anti-GBM antibody. **C and D** *Glomeruli from a rat 12 hours after injection of AH IgG.* Trace fluorescence is noted in the mesangium of a nonperfused kidney stained for human IgG (*panel C*). Note the intense mesangial staining for AH IgG in the contralateral kidney perfused with aminonucleoside 10 days previously (*panel D*). (Magnification of A,B,C, ×450; of D, ×330) (Reprinted with permission of *J Lab Clin Med* [33] and *Lab Invest* [34])

meability may affect the locus of immune complex deposition in an ongoing autoimmune disease [76].

The cause of the change in the afferent mesangial limb induced by aminonucleoside or anti-GBM antibody is unknown. It is possible that these findings are related to the abbrogation of the negative charge in the glomerular capillary epithelium in both experimental models [73, 77]. In this regard, the observation in autologous immune complex disease of a decrease in mesangial uptake of carbon in conjunction with the failure to detect a decrease in stainable glomerular polyanion early in the disease are at variance with the findings in the other two models [78, 79]. These data suggest that the two phenomenona, electrostatic charge and mesangial traffic, may be linked in some way. Another possibility is that the alteration in the afferent limb is a consequence of hemodynamic changes.

(3) Hemodynamic factors. It is probable that the mesangium is influenced by the hemodynamic determinants that affect glomerular filtration, although very little information is currently available. The recent demonstration of normal mesangial kinetics in mercuric-chloride-induced renal failure in rats is consistent with the normal GFR and RBF observed in this model [80]. The increased macromolecular uptake in bilateral ureteral obstruction compared with the reduced uptake in unilateral obstruction is largely unexplained, however [81].

Efferent mesangial limb

What happens to macromolecules after entry into the mesangium? A number of routes of egress are possible, including degradation by phagocytosis, movement by way of the glomerular stalk to the juxtaglomerular zone, and regurgitation into the glomerular capillary (Fig. 6).

(1) Phagocytosis. That the mesangial cell has the capacity to phagocytose ferritin, thorotrast, and gold was clearly demonstrated by the studies of Farquhar and Palade [9] and Latta, Maunsbach, and Madden [8]. The hypothesis was advanced that macromolecules that become trapped along the endothelial aspect of the peripheral GBM move into the mesangium and are taken up by mesangial cells, which "police" the basement membrane and keep the filter clean [9]. That the mesangial cell acts like a component of the reticuloendothelial system was also supported by the time course for uptake and disappearance of AH IgG, which was similar to that observed for liver and spleen [49]. In contradistinction to certain tracers that were actively phagocytosed by mesangial cells, other materials, particularly antigen-antibody complexes and aggregated proteins, were seen within mesangial channels between cells and infrequently or not at all within the cell itself. In addition, studies in human and experimental forms of renal disease have generally demonstrated deposits within the mesangial matrix but not within glomerular cells. The inability, however, to detect immune complex material and aggregated proteins within mesangial cells may be methodologic, and further morphologic data are needed to settle this issue definitively. Of the macromolecules that move into and out of the mesangial zone, it has not been possible to define the relative proportion that undergoes phagocytosis. It is probable that uptake by the mesangium does not depend on the mesangial cell acting like a macrophage, actively engulfing macromolecules from the glomerular capillary lumen. Rather, it is more likely that the mesangial cell has the capacity to phagocytose certain materials that come close to it.

Monocytes have been demonstrated in diseased glomeruli by morphologic and cultural techniques [82–92]. Recent studies have emphasized the importance of these cells and cellular immune mechanisms in the pathogenesis of both anti/GBM nephritis and acute immune complex disease in animals [88–90, 92]. The relationship between mesangial cells and monocytes in the phagocytosis of immune complexes was evaluated recently by an incisive study carried out by Striker, Mannik, and Tung [57]. By exchanging marrow between Chediak-Higashi mice and syngeneic mice after radiation, it was possible to differentiate marrow-derived macrophages, which contain giant lysosomes from resident mesangial cells. Following the systemic admin-

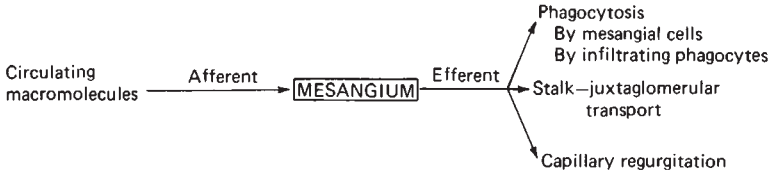

Fig. 6. *Simplified schema depicting the macromolecular traffic into and out of the mesangium.* Capillary regurgitation of macromolecules has not been definitively established.

istration of immune complexes, phagocytosis could be demonstrated only within marrow-derived monocytes present within the mesangial region; no phagocytosis could be demonstrated by mesangial cells. Additional evidence that the mesangial cell is not derived from an extrarenal cell population is shown by the frequency of the Y-chromosome in female human kidneys that had been transplanted into a male host 1 month to 8 years previously [93]. Y-body-containing cells could be demonstrated in the interstitium of the kidney and in glomerular crescents, suggesting that inflammatory cells of the male recipient populate these regions of the donated female kidney. No Y-body containing cells, however, were present within the normal or proliferating mesangium, suggesting that mesangial cells constitute a stable population and further that phagocytic cells are not present in this site in these transplanted kidneys. In analogous experimental studies, kidneys from rats injected with Habu snake venom were transplanted into recipients that had been labeled previously with tritiated thymidine; proliferating glomerular cells were not labeled, suggesting local proliferation rather than population of the mesangium by extrarenal cells [94].

Several conclusions may be derived from these studies: *(1)* monocytic cells may infiltrate the glomerulus and in certain experimental situations enter the mesangium and ingest immune complexes and macromolecules; *(2)* there is no evidence that the mesangial cell is derived from a mononuclear phagocytic bone marrow cell similar to that described for the pulmonary macrophage; *(3)* phagocytosis by mesangial cells has been observed by using certain probes such as ferritin and dextran, whereas clear evidence for uptake of aggregated proteins or immune complexes as a major pathway for disposal has not been demonstrated. It is also possible that degradation of mesangial complexes may occur extracellularly following the excretion of lysosomal enzymes and proteases.

(2) Transport via the stalk to the juxtaglomerular zone. Immunofluorescent studies of the kidney following the administration of aggregated proteins to animals often reveal significant amounts of material at the axial pole of the glomerulus [48]. In addition, iron dextran accumulates in the mesangium after administration to mice; after a period of time it is found in the region of the juxtaglomerular apparatus, the intercellular spaces of the macula densa, and the base of the distal tubular cells opposite the maxula densa beneath the tubular basement membrane [35]. In the rat, colloidal carbon gains access to the mesangial channels by way of the endothelial fenestra, and is maximally present in this site at 32 hours after administration, longer than that observed for other probes [32]. During the subsequent period of 2 months, the relative concentration of carbon in the peripheral mesangium decreases associated with a progressive increase in the stalk and lacis regions. These morphologic studies are consistent with the viewpoint that certain macromolecules move from the peripheral mesangium to the glomerular stalk and thence to the juxtaglomerular region [8, 12, 95]. Although attractive because of the anatomic relationship to the distal tubule, there is no evidence for active secretion into the urine at this site. Movement of macromolecules into this region, however, may permit entry into distal tubular cells, the interstitial space, or the lymphatics. The route of exit, however, has not been established at this time.

(3) Other mechanisms. In addition to phagocytosis and transit by way of the stalk to the juxtaglomerular zone, it is possible that regurgitation from the mesangium into the glomerular capillary might occur, although there are no morphologic studies to support this hypothesis. This would suggest that a dynamic equilibrium exists between macromolecules present within the circulation and those sequestered in the mesangium; pressure and flow relationships would permit entry into the mesangium in one region and exit in another. Thus, at high blood levels of the circulating macromolecule, entrapment within the mesangium occurs during the percolation of plasma through this site. As the plasma level decreases, a new equilibrium is established by movement of material out of the mesangial zone.

Ureteral ligation altered mesangial kinetics in a unique way, illustrating the importance of hemodynamic factors in the regulation of mesangial traffic of macromolecules [81] (Fig. 7): *(1)* A significant difference in the afferent limb was observed between bilateral and unilateral obstruction, with a higher uptake of AH IgG than normal in the former and a lower uptake in the latter; the reason for this difference is unknown and cannot be explained on the basis of differences in blood flow or GFR. *(2)* A plateau in glomerular concentration of AH IgG was observed between 4 and 16 hours in obstructed kidneys (efferent limb blockade), whereas in the control or unobstructed kidney there was a significant loss of AH IgG from the mesangium. During this 4- to 16-hour period, the concentration in the blood was relatively high. In the period of time after

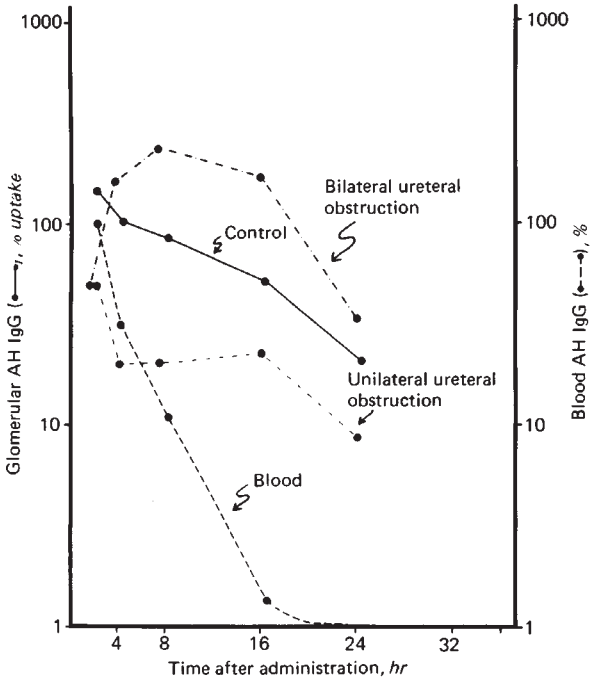

Fig. 7. *Concentration of AH IgG in glomeruli at varying time intervals after administration of AH IgG to rats with bilateral ureteral obstruction, unilateral obstruction, and controls.* The plot is as described in Fig. 3. Kinetic analysis for kidneys from control animals is the same as it is for the contralateral normal kidney from rats with unilateral obstruction and is here depicted as a single line. Blood levels in all groups of animals were similar. Note the difference in uptake between bilaterally obstructed and unilaterally obstructed kidneys. In addition, the lack of a decrease in glomerular concentration of AH IgG between 4 and 16 hours in obstructed kidneys suggests efferent mesangial blockade. (Data derived from Ref. 81)

16 hours, however, a decrease in mesangial concentration did occur, even with persistent ureteral obstruction, but associated with blood levels that were less than 5% of initial values. These findings suggest at least two mechanisms for movement of macromolecules out the mesangium—one which is impaired by ureteral obstruction and is manifested at high circulating levels of the macromolecule, and the other which is independent of ureteral obstruction and is operative at low circulating levels of macromolecules. Whether the former represents traffic through the stalk and juxtaglomerular region and the latter represents regurgitation into the glomerular capillary remains to be proven.

That injury to the glomerulus might impair macromolecular traffic through the mesangium is suggested by studies on rats with long-standing nephrotic syndrome and focal sclerosis induced by repeated injections of aminonucleoside of puromycin [96, 97]. AH IgG administered to these animals persists for prolonged periods of time within glomeruli, suggesting that mesangial clearing mechanisms are impaired as a consequence of the hyalinizing process. The presence of immunoproteins in areas of glomerular sclerosis in nonimmune forms of human renal disease may reflect a similar impedence in the traffic of endogenous complex material through the mesangium [98]. In addition, following their administration to rats, indigestible polyvinyl alcohol polymers persist for prolonged periods of time in the mesangium, inducing a remarkable ballooning deposit and altering the mesangial uptake and disappearance of AH IgG [39, 40].

Mesangial antigens

Myosin has been identified within the mesangium by immunohistochemical techniques [13–15]. The close proximity to components of the juxtaglomerular apparatus supports the hypothesis that the mesangium may have a contractile function and play an important role in regulation of glomerular blood flow. The demonstration of projections of mesangial cell cytoplasm in peripheral subendothelial portions of the glomerular capillary, especially in disease states, is consistent with the concept that the cell may be active and mobile. In addition, fibronectin or fibroblast surface antigen has also been detected within the mesangium where its distribution appears to be more extensive than that of actomyosin [15, 99]. This protein is a noncollagen glycoprotein, which is present on the surface of cultured fibroblasts and is a major component of connective tissue matrices, reticulin, and certain basement membranes [99]. On the basis of its binding to collagens, it has been proposed that this glycoprotein may mediate attachment of cells to extracellular matrix material [100]. A striking increase in fibronectin and actomyosin has been observed in the mesangium in diabetic nephropathy [14, 15]. Neither antigen can be identified in hyalinized glomeruli. The cyclic nucleotide 3′:5′-cyclic GMP has also been demonstrated in the mesangium by immunohistochemical techniques [101], and angiotensin II binds to a similar locus following its administration to rats [102]. As discussed above, the biochemical composition of mesangial matrix material is unknown, although immunohistochemical studies have demonstrated the presence of certain specific types of collagen, including type IV procollagen and type V collagen, as well as the noncollagen glycoproteins, fibronectin, and laminin; these antigens are also present along the subendothelial region of the GBM [103]. Antibody to enzyme-digested lung has been shown to react with the mesangium in vivo and in vitro, but the

nature of the antigen(s) involved is unknown [104].

From the foregoing discussion, it is possible that foreign antigens (for example, those of viral origin) could move through the mesangium in vivo and become trapped in this site, in a manner similar to that described above. The demonstration in the mouse that cytomegalovirus moves through mesangial channels to the juxtaglomerular zone supports this concept [105]. That a foreign antigen that has become planted in the mesangium may set the stage for the development of an in situ immune complex disease has been demonstrated experimentally [46, 106]. For example, after AH IgG is administered to a rabbit, the kidney containing mesangial aggregates is transplanted to a normal animal, and anti-IgG antibody is administered passively. The antibody, which reacts with the planted AH IgG, induces an acute complement-dependent mesangitis with polymorphonuclear infiltration. This study demonstrates that foreign or possibly host antigens that localize within the mesangium are not sequestered but are able to react with circulating antibody and induce an intense mesangial inflammatory disease. This type of process, however, has not been identified in human glomerulonephritis.

Conclusion

It is surprising how little we know about this unique region of the glomerulus. It contains cells with fibrils and myosin and an intercellular collagenous matrix material, situated adjacent to elements of the juxtaglomerular apparatus with which an integrated functional relationship is likely, but unproven. Studies of the mesangium in human disease have been limited to morphologic and immunohistologic studies. Abnormalities in this site have been relatively specific in some diseases: the intercapillary nodule observed in diabetic nephropathy; the mesangial ring of C3 outlining dense deposit material in type II membranoproliferative glomerulonephritis [107]; and the presence of IgA, C3, and properdin without earlier complement components in IgA nephropathy. Other mesangial abnormalities—Ig and complement component deposition, cellular proliferation, matrix expansion, sclerosis—are not characteristic of any specific disease process.

Studies in animals have begun to untangle some of the complexities involved in the movement of macromolecules into and out of the mesangium. Ultrastructural studies that use a variety of probes support the concept of plasmic flow through this region. The afferent pathway is influenced by a number of variables, including the blood level and, characteristic of the macromolecule, the glomerular blood flow, and likely other hemodynamic determinants of glomerular filtration and unknown factors that encourage movement from the capillary lumen into the mesangium. The efferent pathway includes transit by way of the glomerular stalk to the juxtaglomerular zone, but thereafter the route is obscure; phagocytosis by infiltrating cells or resident mesangial cells; and possibly regurgitation into the glomerular capillary at low blood levels of the circulating macromolecule. Increased uptake has been demonstrated in rats given aminonucleoside of puromycin or anti-GBM antibody without alteration in the efferent limb. In contrast, ureteral obstruction induces efferent blockade.

The information accumulated in the recent past by studies of the structure and function of the glomerular mesangium in laboratory animals demonstrates a number of potential mechanisms to explain some aspects of human glomerular disease. It seems likely that additional understanding of these concepts will lead to improved therapeutic approaches, as well as control and prevention of several important forms of renal disease in man.

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