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EDITORIAL REVIEW

Glomerular cells in culture

The purpose of this review is to explore the ways by which cell culture has broadened our knowledge of the biology and pathobiology of the renal glomerulus. With cell culture techniques, one can study homogeneous populations of cells under controlled conditions, without the influence of other glomerular cells and extraglomerular factors. It must be noted at the outset that there are certain inherent problems in this approach. First, cell types in culture may show phenotypic modulation, that is, the loss or gain of certain phenotypic properties. For example, smooth muscle cells rapidly lose their myosin after being cultured [1]. Second, there is a paucity of both enzymatic and morphologic markers for glomerular cells in culture. Despite these limitations, this approach to the study of the glomerulus has drawn the interest of a number of investigators [2–15, 22–29] including ourselves [16–21]. Glomerular cell types from human [2–15], rat [16–21, 24–27], dog [14, 22, 23, 28], and guinea pig [29] glomeruli have been studied to date by various investigators. We will discuss the various approaches these investigators have taken in isolating, identifying, and studying glomerular cell types as well as new information obtained from these studies. Additionally, we will discuss further areas of research that have developed as a direct result of data already obtained.

The renal glomerulus is composed of at least four cell types: *(1)* endothelial cells, *(2)* glomerular epithelial cells, *(3)* mesangial cells, and *(4)* parietal epithelial cells. The endothelial cells which line the glomerular capillary wall are characterized by the presence of fenestrae approximately 1000 Å in diameter. This layer of endothelium may participate in the restriction of macromolecules from passing across the capillary wall [30]. In certain disease states the fenestrae are replaced by a continuous layer of endothelial cytoplasm [31]. Glomerular mesangial cells are attributed with several functions: *(1)* the clearing of debris from mesangial regions by phagocytosis [32]; *(2)* the control of glomerular size and blood flow by contraction [17, 33–35]; *(3)* as a possible source of renin, since under certain physiological conditions mesangial cells have the ability to develop cytoplasmic granules similar to those found in juxtaglomerular cells [36]. Glomerular epithelial cells are also attributed with several functions: *(1)* They participate in the synthesis of the glomerular basement membrane [37–38]; *(2)* they participate in the filtration process through pinocytosis of filtered proteins that may have leaked through the glomerular basement membrane [39]; and *(3)* they participate in the filtration process by exerting an influence on water flux during ultrafiltration [40]. Additionally, intrinsic negatively charged components in the filtration barrier are important in restricting the passage of anionic molecules across the glomerular basement membrane [41–44]. Since the glomerular epithelial cell contains a cell coat rich in negatively charged sialoglycoproteins, it may participate in filtration in this manner. In many forms of renal disease associated with proteinuria, the epithelial cell foot processes are replaced by a continuous band of epithelial cytoplasm adjacent to the lamina rara externa [40, 45–47]. Loss or neutralization of negative charge may be responsible for the loss of foot processes, since infusion of polycationic molecules such as protamine sulfate causes similar structural alterations to glomerular epithelial cells [48, 49]. Finally, parietal epithelial cells together with basement membrane material form the outer wall of Bowman's capsule which surrounds the glomerular tuft. In certain disease conditions, such as rapidly progressive glomerulonephritis, it is this cell that proliferates to form crescents [50].

Techniques for isolating glomerular cells

Glomeruli are most commonly isolated from cortical renal tissue using a modification [23] of a technique first described by Krakower and Greenspon [22]. For rat glomeruli, renal cortical tissue is pressed and rinsed with Hanks' salt solution through stainless steel screens of 60 (pore size 250 μ) and 150 mesh (pore size 150 μ) and collected on a 200 mesh screen (pore size 75 μ). Glomeruli from other species can be collected by using different combinations of screens [14]. With this technique, it has been reported for the rat that 86 ± 6% of the glomeruli are free of capsules, 3.0 ± 2.1% contain vascular poles, and there is very little tubular contamination [16]. Glomerular cells can be grown as follows: *(1)* by directly plating the whole glomeruli into culture for outgrowth of cells (termed explant growth), and *(2)* by enzyme dissociation of the glomeruli and plating of the dissociated cells into culture. In either case, one has at least three cell types present in the culture, and purification procedures are necessary to ensure homogeneity. Few of the isolated glomeruli contain capsules and since capsulated glomeruli neither attach to the substratum nor do cells grow out from them, parietal epithelial cells are not present in glomerular cultures [3, 16–17, 25]. The influences of anoxic delay on cellular proliferation were assessed by killing rats immediately prior to, or 2, 4, 6, and 12 hr before isolating and culturing the glomeruli [25]. After a 12-hr anoxic delay, only glomerular mesangial cells were observed in the explants while the glomerular epithelial cells did not grow out from the glomeruli [25]. Therefore, glomeruli obtained from autopsies performed 12 or more hr postmortem may not be adequate for tissue culture. In addition, glomeruli from young rats (that is, 1 to 2 months old) grew significantly better than those from older rats (8 to 9 months old) [25]. In this regard, well established human glomerular cell lines have been obtained from infant kidneys [5, 10]. Human glomeruli for tissue culture are most often obtained from

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autopsy material (less than 12 hr postmortem) [5–15] or surgical biopsy material [4, 12].

Several methods have been used to obtain cultures of different glomerular cell types. One such technique is cloning [6, 10, 16–20] which allows one to obtain pure cultures of glomerular cell types. Cells are diluted with a sufficient volume of tissue culture medium to allow the addition of single cells to the culture [51]. Colonies of cells, derived from a single cell are individually subcultured and transferred to other dishes. Cloning has been used to obtain homogeneous cultures of rat glomerular epithelial, mesangial, and renin-producing cells [16–19]. Three cell types from explanted human glomeruli have been obtained by cloning [10]. Two of these cell types have been identified as glomerular epithelial and mesangial cells, respectively [6, 10].

Some investigators have isolated cultures of glomerular epithelial and mesangial cells from explants of whole glomeruli according to the differential growth capacities of these cells [24–27]. This procedure has been referred to as the "mosaic theory"; it states that any explanted tissue contains cells with low and high growth potential [52]. According to its proponents, glomerular epithelial cells represent a vast majority of the emigrating population from explanted glomeruli for the first 6 days of growth and mesangial cells represent 100% of the culture on day 30. Therefore, if one passaged and subcultured the cells at day 6, one should obtain essentially glomerular epithelial cells while a subculture at day 30 would represent primarily mesangial cells.

Quadracci and Striker [3], Striker et al [5], Killen and Striker [6], and Striker, Killen, and Farin [7] isolated glomerular epithelial cells from human glomeruli using collagenase digestion. After 30 min of digestion, primarily glomerular epithelial cells were obtained (90% pure). Plating of the residual dissociated glomeruli resulted in the outgrowth of mesangial cells [5]. In addition, colonies of epithelial and mesangial cells were isolated by cloning [6–7]. In another study, exhaustive digestions of glomeruli with a trypsin-collagenase-DNAase mixture followed by an EDTA step resulted in a mixed population of glomerular cells [21]. One of the cell types retrieved was a fenestrated endothelial cell, the other was a phagocytic cell that had receptors for Fc and complement (C3b).

Tissue culture medium used to culture glomerular cells

Cloned homogeneous cultures of rat glomerular epithelial, mesangial, and renin-producing cells have been isolated and maintained in culture using the following tissue culture medium: Medium RPMI 1640 (Roswell Park Memorial Institute, Buffalo, New York) with 20% fetal calf serum (FCS) and 200 mU/ml insulin, diluted in half with conditioned medium obtained from Swiss 3T3 cells in log phase growth. Swiss 3T3 cells were maintained in Dulbecco's modified eagle medium (DMEM) with 10% FCS [16–20]. Conditioned medium seems to be essential for cloning of rat cells. In such sparse cultures conditioned medium supplies the necessary "chemical messengers" for growth that can otherwise be obtained from cultures with heavier growth [53]. Insulin has been found to stimulate the growth of rat glomerular cells in vitro [16] as well as other types of cultured cells [54, 55]. In addition, it has been reported that under the above conditions nearly 100% of the cultured rat glomeruli attach to the culture flask [16]. One easy and reliable way to check the cultures for fibroblast contamination is by growing cells in medium containing D-valine substituted for L-valine, a condition in which fibroblasts cannot grow [56]. Fibroblasts do not contain the enzyme necessary to convert the D-amino acid to its essential L form (that is, D amino acid oxidase) [56].

In another report, it was noted that rat glomerular epithelial cells grew better in RPMI 1640 supplemented with 10% decomplemented FCS while the optimal growth conditions for mesangial cells was RPMI 1640 with 15% decomplemented FCS [25].

The tissue culture media most commonly used for growth of human glomerular cells has been either DMEM with 20% FCS [8], medium 199 with 20% FCS, [4, 14] or Waymouth's tissue culture medium [3, 5–11, 15] supplemented with either 15% [5], 20% FCS [4, 6–11, 14, 15], or pooled human serum [6]. Interestingly, it was found that coating of the culture dishes with human cold insoluble globulin increased the plating efficiency for human glomerular epithelial and mesangial cells tenfold [7].

Recently, studies by Oberley et al [29] with explants from guinea pig glomeruli claimed that prostaglandins, transferrin, and media fibronectin were necessary for growth and maintenance of glomerular cells. Cell types growing out from the glomeruli were not identified, however, and it is not clear whether or not the growth conditions were selected for a particular glomerular cell type or whether or not these conditions were beneficial for all glomerular cells.

Properties of glomerular cells in culture

Although endothelial-like cells can be seen emigrating from cultured glomeruli, glomerular endothelial cells, positively identified by the presence of factor VIII antigen, have not been isolated and maintained in homogeneous culture [4, 10, 16–20]. This is probably due to the particular conditions these highly differentiated cells require for growth, which are unavailable in the media presently in use.

Glomerular epithelial cells. One glomerular cell type that has been isolated in pure culture is the glomerular epithelial cell [6, 7, 16–20, 25]. It seems quite certain that this cell type is not a parietal epithelial cell since very few isolated glomeruli contain Bowman's capsule [10, 16, 25], and glomeruli with capsules attached do not adhere to the substrate and therefore cells cannot grow from them [2, 16–20]. Although the glomerular epithelial cell soon loses its podocytes in culture, it is identified by the following criteria: *(1)* It has an epithelial morphology in culture, namely, a polyhedral shape, as viewed by phase contrast microscopy, and a cobblestone-like appearance of the culture when confluency is reached [6, 15, 16, 18, 20, 25]; *(2)* the presence of cilia on its surface [16] and junctional complexes [16, 25]; and *(3)* a cytotoxic response to the aminonucleoside of puromycin [16] in accord with preferential injury of glomerular epithelial cells and not other glomerular cells in vivo [40, 45, 59, 61]. In addition, this cell type does not contain factor VIII antigen, a marker for endothelium [10, 16].

It has been demonstrated that only human glomerular epithelial cells bear receptors for C3b in situ and thus should bind circulating antigen-antibody-complement complexes [57, 58]. This putative binding of immune complexes in glomeruli might be a significant immunopathogenetic mechanism in human immune complex glomerular injury. The presence of receptors

for C3b on cultured glomerular epithelial cells is controversial [6, 11, 12, 16, 18, 19, 25]. On the one hand, it has been reported that both human and rat glomerular epithelial cells retain receptors for C3b in culture [6, 12, 16, 18, 19]. On the other hand, this binding has been reported by others to be lost from cultured glomeruli or simply not to exist on cultured glomerular cells [4, 15, 25]. Furthermore, it is not clear whether rat glomeruli possess C3b receptors in vivo. Their presence on cultured rat glomerular epithelial cells and not on frozen sections of rat kidney may be a response to the culture conditions [16] or an unmasking of specific receptor sites. Because of the inconsistent results in demonstrating C3b receptors on cultured glomerular cells, this does not as yet serve as a useful marker for glomerular epithelial cells. If it proves to be true that C3b receptors are present on glomerular epithelial cells, a potential relationship to the pathogenesis of immune complex deposition in the glomerulus could be important since a feature of immune complex glomerular nephritis is the deposition of complement containing complexes in glomeruli leading to complement mediated immune injury [12].

Glomerular epithelial cell injury has been observed in many human as well as experimental glomerular diseases [40, 45, 59–61]. One such alteration is the replacement of podocytes with flattened expanses of epithelial cell cytoplasm (that is, "fusion of foot processes"), which is often associated with altered filtration and permeability in both human and experimental nephrosis [40, 45–47, 61–63]. Infusion of polycations (for example, protamine sulfate, and poly-L-lysine) resulted in similar glomerular epithelial cell alterations as that observed with the aminonucleoside of puromycin [48, 49]. Since fixed anionic sites are important in restricting filtration of polyanionic macromolecules [41, 42], a decrease in glomerular polyanions may be accompanied by increased permeability to anionic plasma proteins, with concomitant occurrence of proteinuria and glomerular epithelial cell changes [46–48, 62, 63]. The aminonucleoside of puromycin as well as nephrotoxic serum in low doses has been shown to injure cultured glomerular epithelial cells [16, 18, 19]. Since glomerular epithelial cells contain a cell coat rich in sialic acid, metabolic alterations in these fixed negatively charged moieties by agents such as the aminonucleoside of puromycin and nephrotoxic serum could be responsible for the demise of the cell. A cell culture system affords a useful way to study the mechanisms of cell injury.

The cell type responsible for the synthesis of the glomerular basement membrane (GBM) has been the focus of numerous investigations. The GBM is composed of collagenous as well as noncollagenous components [64]. Immunofluorescent studies in the rat using antiserum against types IV and V collagens have shown localization of these collagens to the mesangial matrix region of the glomerulus as well as to the peripheral capillary wall [65–68]. In the human glomerulus, an antiserum to pepsin-digested bovine lens capsule reacted with the full thickness of the GBM as well as the tubular basement membrane and Bowman's capsule [69]. The collagenous component of the GBM is secreted in the form of procollagen and is deposited in the extracellular matrix without further reduction in molecular size [64]. The interstitial collagens, types I and III, have not been localized to the normal renal glomerulus [66, 67].

The noncollagenous components of the GBM include the sulfated glycosaminoglycans (GAGS) [43, 44], fibronectin [8, 65, 68, 69–72], laminin [65, 69, 72, 73], and entactin [74]. Among the GAGS found in the glomerulus, the highly negatively charged sulfated GAGS (primarily heparan sulfate) have been shown to be important in restricting the passage of negatively charged macromolecules across the GBM [43, 44]. It has been determined that the major sulfated GAG synthesized by cultured human and rat glomerular epithelial cells is heparan sulfate [7, 75]. Human mesangial cells synthesized predominantly chondroitin-6-sulfate [7], while rat mesangial cells have been reported to synthesize non-sulfated GAGS in the culture medium [75]. Fibronectin, a fibrillar sialoglycoprotein located on the cell surface [76] or in the extracellular matrix [77–79], is involved in cell-to-cell and cell-to-substrate attachment [80]. The exact localization of this glycoprotein to the rat and human glomerulus has been somewhat controversial [8, 65, 68–72, 81, 82]. It is agreed that fibronectin is most abundant in the mesangial matrix, especially at the interface between mesangial and endothelial cells [65, 68, 70, 72] where it may be involved with mesangial cell-to-matrix attachment. The disagreement concerns whether or not fibronectin is present in the peripheral capillary wall [65, 68, 70, 72]. Cultured glomerular epithelial and mesangial cells from humans and rats, however, have been shown to be capable of producing fibronectin [8, 10, 71, 83]. Laminin, a sialoglycoprotein [84] implicated in the attachment of epithelial cells to their basement membrane [85, 86] has been localized to the lamina rarae of the peripheral capillary wall in the rat and human glomerulus [65, 67, 69, 72, 73]; thus, laminin may be the structural protein that is involved with attachment of glomerular epithelial and endothelial cells to the basement membrane. Finally, entactin, a sulfated glycoprotein, has been localized to the lamina rarae of the rat GBM as well as the mesangium and may also be involved in cell adhesion [74].

Although the importance of heparan sulfate in glomerular filtration has been elucidated recently [44], the relative importance of each of the different sialoglycoproteins in normal glomerular function is yet to be defined. Since alterations in glomerular negative charge have been associated with glomerular disease in which altered permeability has been demonstrated [62, 63], purified cultures of glomerular cell types should help to elucidate the biochemical mechanisms that lead to alterations of these highly negatively charged substances.

In vivo studies using silver nitrate administration attempted to demonstrate GBM synthesis by glomerular epithelial cells, however, it has since been shown that silver nitrate induced changes in the synthesis or turnover of normal GBM in vivo [87]. Immunofluorescent studies with purified antibodies against the different types of collagen have been performed on cultures of human and rat glomerular epithelial cells [7, 24]. Cultured glomerular epithelial cells from both rats [24] and humans [7] were observed to have a matrix of basal lamina type IV collagen that surrounded the cells, while antibodies to types I and III collagens failed to localize these antigens to these cells [7].

Biochemical analyses of the collagenous proteins produced by cultured human glomerular epithelial cells confirmed the immunofluorescent studies [6, 7, 11]. That is, glomerular epithelial cells synthesized predominantly type IV collagen. Additionally, rat glomerular epithelial cells have been demonstrated to synthesize type IV procollagen in culture [83].

Mesangial cells. Cultured mesangial cells display numerous

bundles of microfilaments oriented parallel to the plasma membrane [16], similar to mesangial cells in situ [88]. Mesangial cells have been demonstrated to contain actin and myosin by immunofluorescent microscopy [9, 10, 24]. As mentioned above, several functions have been attributed to mesangial cells. One such function is phagocytosis [32]. However, cultured rat mesangial cells do not phagocytose particles such as polystyrene, ferritin, zymosan, or carbon [16]. An intriguing explanation for this lack of phagocytosing ability by mesangial cells may be the presence of more than one type of mesangial cell in glomeruli, that is, one phagocytic and one nonphagocytic. In a previous report, a procedure was used to isolate phagocytic cells from rat glomeruli [21]. Glomeruli were subjected to long enzymatic digestions, and cells that adhered to glass overnight were examined morphologically. These cells were phagocytic and had receptors for C3b and Fc. They had large indented nuclei, large nuclear to cytoplasm ratios and, by scanning electron microscopy, showed extensive foldings of the cell surface. It was hypothesized that this cell type was a blood-born monocyte that travels into and out from the mesangium to clear debris [18, 19].

In a more recent study, Schreiner et al [89] and Schreiner and Cotran [90], with repeated enzymatic digestions of rat glomeruli conclusively demonstrated the presence of Ia-positive cells (that is, cells that bear I-region associated antigens that permit specific interactions between phagocytes and lymphocytes) in rat glomeruli. These cells resembled mononuclear phagocytes. They were a functionally heterogenous population of cells with the capacity for Fc receptor display as well as phagocytic ability. In addition, these cells could take up antigen and stimulate immune lymphocytes in an I-region-restricted reaction. Furthermore, it appeared they comprised approximately 2% of the total glomerular cell population and were derived from bone marrow [90]. These studies implied that an inherent population of glomerular cells with the potential to process antigen and initiate cellular immune responses in situ exists [89, 90]. The significance of these cells in the pathogenesis of glomerulonephritis remains to be elucidated. In addition, if one examines explants from rat glomeruli 4 days after plating, there is a population of cells that represents approximately 5% of the glomerular outgrowth that is highly phagocytic and demonstrates histochemically demonstrable nonspecific esterase activity, (an enzymatic marker for monocytes) (Kreisberg and Karnovsky, unpublished observations). These particular cells do not survive long in culture, similar to monocytes or macrophages in culture. These cells probably represent all, or some, of the Ia-positive cells described by Schreiner et al [89].

Time-lapse cinemicroscopic studies demonstrated the presence of monocytes in glomeruli [14] which increased in number with proliferative glomerular diseases [4, 91]. Cultures of glomeruli from humans, monkeys, dogs, sheep, rabbits, and rats have been observed using time-lapse cinemicroscopy [14]. The pattern of cell outgrowth from the glomeruli appears to be similar for all of the species studied. Three cell populations have been identified with the features of glomerular epithelial cells, mesangial cells, and macrophages. The latter were only rarely observed in outgrowths from normal glomeruli; these cells were actively motile, phagocytic, and exhibited other features of macrophages, such as receptors for C3b and Fc. Glomeruli isolated from patients or laboratory animals with proliferative glomerulopathies had larger numbers (60 times normal) of macrophages migrating from the cultured glomeruli [4, 91]. However, in those glomerular diseases not accompanied by hypercellularity, such as minimal change and the membranous nephropathies, the outgrowth of cells from the isolated glomeruli resembled controls.

From these in vitro studies the existence of macrophages in normal and altered glomeruli was established [4, 14, 21, 89–91]. It appears that blood-born monocytes travel into and out from the glomerulus to assume a major phagocytic role in the glomerular mesangium.

If two populations of glomerular mesangial cells exist, what is the function of the nonphagocytic one? Again, studies on cultured cells have helped to elucidate the biology of this mesangial cell. Twelve years ago, Mary Bernik observed persistent, rhythmic, and synchronous contractions of entire human glomeruli in vitro using cinema microscopy [2]. Additionally, it appeared that cells derived from the peri- or endocapillary positions (that is, mesangial cells) exhibited contractile activity.

In vivo physiological studies using angiotensin II [92], arginine vasopressin (AVP) [93], parathyroid hormone (PTH) [94], prostaglandin E_1 (PGE_1) [95], prostacyclin (PGI_2) [96], and dibutyryl cyclic AMP (cAMP) [93] have demonstrated that these substances can decrease the ultrafiltration coefficient, Kf, one determinant of GFR. A decreased capillary surface area could possibly be accomplished via contracting mesangial cells [35]. Isolated glomeruli from rats specifically bind [97] and localize AII to the mesangium [98]. In addition, glomeruli from rats [97] and rabbits [99] contract after exposure to AII. Cultured rat glomerular mesangial cells specifically bind and undergo a contractile response after exposure to angiotensin II [17, 26, 27] and AVP [17]. This contractile response is Ca++ dependent and independent of cyclic nucleotide generation [17]. Also, the contraction to angiotensin II is inhibited by prior incubation with Sar^1-ala 8 angiotensin II [26]. PTH, PGI_2, PGE, and cAMP have been shown recently to exert their effect on Kf through renin synthesis (via increased cAMP) and local angiotensin II production [96]. Therefore, it has been reasoned that one role of mesangial cells is to control glomerular size and blood flow by contraction [17, 35].

Besides being a contractile cell, cultured mesangial cells also exhibit other properties of smooth muscle cells in vitro. Namely, these cells grow in a swirl-like fashion and pile in culture (Kreisberg and Karnovsky, unpublished observations), forming the so called "hills and valleys" that have been described in smooth muscle cells [100]. Cultured mesangial cells are growth-stimulated by platelet-derived growth factor [7] as are smooth muscle cells in vitro [101]. Additionally, activated macrophages produce factors that stimulate mesangial cell growth in vitro [7]. Also, human and rat glomerular mesangial cells excrete, in addition to type IV collagen, types I and III collagens [6, 7, 11, 83]. It is interesting that mesangial cells can synthesize interstitial collagens in vitro. Normal glomeruli have not been reported to contain types I or III collagens; however, it has been suggested that in diseased states such as diabetes mellitus, these types of collagens can accumulate in the mesangium [82]. This alteration in cultured cells points to one weakness in relating the biology of cells in vitro to what may be going on in vivo.

As can be appreciated from the above discussion, glomerular function can be altered by hormonal agents, some of which are known and others of which are postulated to act via cAMP,

cyclic GMP (cGMP) or both [102–105]. Since cAMP and cGMP may play a role in modulating cellular components (for example, platelet aggregation) which participate in tissue injury (for example, inflammation) [105, 106], their importance in the pathogenesis of glomerular diseases must be elucidated. To study alterations in cyclic nucleotide production to hormones in diseased states, one must first characterize which glomerular cell types respond to which hormones. Much of the work in this field has been performed on whole isolated glomeruli [107–110]. For example, histamine and serotonin increased cAMP production in isolated rat glomeruli [105–108]. Studies performed on cloned homogeneous cultures of rat glomerular mesangial cells have shown that both AVP (at supramaximal doses, 200 nM) and PGE_2 (1 μg/ml) significantly increased cellular cAMP levels, and AVP increased measurable cAMP concentrations in the medium as well [17]. PTH had no effect on cAMP production by glomerular mesangial cells. Glomerular epithelial and renin-producing cells did not respond to AVP [17].

Considerable evidence now exists for a close association between the renin-angiotensin system and the prostaglandin system [111–117]. An intrinsic mechanism may, therefore, exist within the glomerulus for modulating the contractile activity of mesangial cells, and therefore, glomerular function. Many studies show that isolated glomeruli release products of arachidonic acid metabolism into the culture medium [111, 115–117]; therefore, an intrinsic mechanism within the glomerulus for modulating the contractile activity of mesangial cells exists. However, there have not been many studies on prostaglandin synthesis by various glomerular cell types. Sraer et al [116], using subcultures of glomerular epithelial and mesangial cells, found that cultured mesangial cells synthesized more prostaglandins than glomerular epithelial cells. Analyses were performed using a radioimmunoassay technique. Mesangial cells synthesized prostaglandin $E_2 > PGF_{2\alpha} > PGI_2$ (measured as the stable metabolite 6-keto-$PGF_{1\alpha}$). Glomerular epithelial cells synthesized much less prostaglandins. In addition, angiotensin II stimulated PGE_2 production by both mesangial and glomerular epithelial cells. In another study, prostaglandin production by 9-day explants of rat glomeruli was performed [118]. The authors concluded from morphological examination that the predominant cell type in the culture was the glomerular epithelial cell; whole glomeruli were present in the culture during the assays. It is safe to conclude that these cultures represented a multitude of glomerular cell types. Regardless of the type of cells present, however, the cultures produced the following prostaglandins in order of amount synthesized: PGE_2 > thromboxane > $PGF_{2\alpha}$. Angiotensin II and III stimulated prostaglandin production by these cultures.

Studies in our laboratory, performed in collaboration with Dr. Lawrence Levine of Brandeis University, showed a similar prostaglandin profile for mesangial cells as reported by Sraer et al [116]; however, our results with cloned populations of glomerular epithelial cells revealed that the predominant prostaglandin produced by this cell was PGI_2 [20]. Differences in prostaglandin production by cells identified as glomerular epithelial cells could lie in the degree of purity of the cultures as well as the conditions of growth used.

Determining precisely the prostaglandins produced by the different glomerular cell types is most important due to the role prostaglandins play in the inflammatory response [119, 120] as well as vasoactivity [96, 112, 113]. In this regard, it has been reported recently by Band et al [121] that isolated rat glomeruli are stimulated to produce more prostaglandins after treatment with components of inflammatory cells (that is, superoxide and hydrogen peroxide).

Renin-producing cells. Another cell type that has been isolated by cloning and cultured from rat glomeruli is a granulated cell that contains renin-like activity (that is, cells that are able to convert angiotensinogen to angiotensin I) [18, 19]. These cells are thought to be isolated from the glomerulus and not the vascular pole region for the following reasons: *(1)* Isolated glomeruli have been shown to be able to produce renin [115]; *(2)* immunofluorescence studies by Nairn, Fraser, and Chadwick [122] using an antibody to pig renin demonstrated renin activity not only in juxtaglomerular cells but also in the glomerular tuft; *(3)* under certain physiological conditions, for example, bilateral adrenalectomy [36], agranulated mesangial cells developed cytoplasmic granules similar to those of juxtaglomerular cells; *(4)* examination of serial sections of the juxtaglomerular apparatus found that granulated as well as agranulated cells entered the glomerulus and became continuous with the glomerular mesangial cells [123]; and *(5)* only 3% of the isolated glomeruli had vascular poles attached [16], making it unlikely that these granulated renin-containing cells came from the juxtaglomerular apparatus. With renin-producing cells populating the glomerulus, the glomerulus contains the means to regulate its own hemodynamics. Furthermore, regulation of glomerular hemodynamics does not have to take place on a whole glomerular level, that is, single capillary loop flow can be regulated. Therefore, it is possible that the glomerular mesangial cell may contain the capacity to produce and respond to components of the prostaglandin-renin-angiotensin system. Thus, mesangial cells may be able to regulate their own vasoactivity.

Information gained from in vitro studies on glomerular cells

It has been demonstrated that cultures of glomerular epithelial cells from human and rat glomeruli participate in the synthesis of basal lamina collagen. Glomerular epithelial cells also can synthesize heparan sulfate, a sulfated GAG present in the GBM that forms part of the filtration barrier. Although still somewhat controversial, it appears that human glomerular epithelial cells contain receptors for complement (C3b) which are retained for long periods in culture. In this regard, glomerular epithelial cells could play an important role in the course of immune complex glomerulonephritis by binding complement-containing complexes in situ. Both glomerular epithelial and mesangial cells, produce significant quantities of vasoactive prostaglandins (PGE_2 and PGI_2) which could modulate mesangial cell contraction and consequently glomerular blood flow. In addition, prostaglandin production by glomerular cells could modify cellular activity during inflammation.

In vitro studies have demonstrated conclusively the presence of at least two populations of cells that reside in the mesangium. One cell type is the contractile mesangial cell (Ia-negative); the other is an Ia-positive cell derived from the bone marrow. The presence of antigen-processing cells in the glomerular mesangium casts new light on the pathogenesis of glomerulonephritis, as well as the mechanisms that underlie kidney transplant rejection. Additionally, this monocytic cell could provide mitogens for glomerular mesangial as well as endothelial cell proliferation. Cultured mesangial cells predominantly synthesize interstitial collagen. The possibility of a third type of mesangial

cell that produces renin exists. Alternatively, perhaps the resident contractile mesangial cell has the capability of producing renin under different physiological conditions.

Future avenues of research

It is our opinion that the search for specific markers (for example, antigenic or enzymatic) for the different glomerular cell types should continue. Markers to specifically identify glomerular cells should lay to rest any doubt as to the identity of the cell type being studied. Along with this research effort, a defined serum-free medium should be established for each glomerular cell type. This is a vast undertaking, but without such an effort, the study of hormonal regulation of glomerular cell function in normal and diseased states would be incomplete. These studies should be directed along similar avenues as that taken by Barnes and Sato [124] for such established cell lines as M2R, MDCK, HeLa and B104. Oberley et al [29] have already embarked on such a journey with guinea pig glomeruli (see *Tissue culture medium used to culture glomerular cells* section).

The next obvious area of research is the possible alterations in GBM metabolism by cultured glomerular epithelial cells in diseased states. Such studies can be accomplished by either isolating cells from diseased humans or animals or by mimicking certain diseased conditions in vitro (for example, lipoid nephrosis using the aminonucleoside of puromycin and diabetes mellitus by culturing under hyperglycemic conditions). Mesangial matrices are commonly widened in diabetic glomerulosclerosis; interstitial collagen metabolism by cultured mesangial cells under hyperglycemic conditions would be important to study.

Since many hormones exert their cellular effect via changes in cellular cyclic nucleotide production, the cyclic nucleotide responsiveness to hormones by each glomerular cell type is an important area of research. In addition, once the cyclic nucleotide responsiveness to particular hormones by glomerular cells is well established, modulation of the hormonal effect under altered culture conditions (for example, hyperglycemia and insulin deprivation) would be important to study. Further, hormonal modulation of a function of a particular glomerular cell type (for example, contraction of mesangial cells) should be studied. In this regard, it has been reported recently that insulin is required for the contraction of cultured mesangial cells by angiotensin II [125].

Finally, prostaglandin production by glomerular cells along with its modulation by various hormones should be exhaustively studied. Interaction between the renin-angiotensin system and the prostaglandin system in the glomerular mileu undoubtedly determines the state of glomerular hemodynamics.

In conclusion, we have only attempted to present a few of the important issues that should be addressed in the field of glomerular cell metabolism. With purified cultures of glomerular cell types, one should be able to characterize specific biochemical alterations in diseased states that have only been characterized up to the present time by morphological criteria alone.

JEFFREY I. KREISBERG
MORRIS J. KARNOVSKY
San Antonio, Texas, and Boston, Massachusetts

Reprint requests to Dr. J. I. Kreisberg, Department of Pathology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284

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