MECHANISMS AND FACTORS REGULATING THE GROWTH OF GLOMERULAR CELLS

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Summary

The hallmark of end-stage renal disease is progressive sclerosis. The composition of the sclerotic material and its cellular source are under study and only partly elucidated. Sclerosis, in part, is composed of extracellular matrix components normal to the area, the sole exception thus far recognised is crescentic glomerulonephritis associated with Bowman's basement membrane disruption in which the sclerotic tissue contains interstitial connective tissue.

The source of the extracellular matrix is the local glomerular cells. The complete composition of the extracellular matrix synthesised by individual glomerular cells is under current study, but it appears that all glomerular cells are capable of synthesising many of the various basement membrane components. The respective role of each cell type in sclerosing diseases and the initiating and propagating factors await further investigation.

Introduction

End-stage renal disease is one of the urgent unsolved problems in modern nephrology. Even though it was first described in the 19th century by Sir Richard Bright [1], there has been surprisingly little new information on the link between the initial renal inflammatory process and the development of end-stage renal disease. The lack of an experimental animal model of progressive sclerosis following an initial acute inflammatory response has considerably hindered progress in this field.

The pathogenesis of progressive sclerosis thus remains one of the important unsolved problems in modern nephrology. It is known that progressive glomerular diseases are characterised by an increasing accumulation of eosinophilic extracellular material which distorts and eventually effaces the overall glomerular architecture. This process and the accumulating material has been called sclerosis. The exact chemical composition of the sclerotic material is unknown
but it has been thought to be composed principally of extracellular matrix. Similarly the site of origin of the sclerotic tissue is unknown, the postulate being that its source is mainly glomerular cells. Finally, the stimuli which lead to the deposition of the sclerotic tissue have not been elucidated. Thus, there is a broad range of opportunities for research into the pathogenesis of sclerosis at both the fundamental and applied levels.

The purpose of this communication is to consider two diseases as models of progressive sclerosing diseases, focal sclerosing glomerulonephritis and crescentic glomerulonephritis. In addition, the composition of the normal extracellular matrix and the contribution of individual glomerular cells, as studied in vitro, will be presented.

Composition of the glomerular extracellular matrix

*Normal distribution*

The extracellular matrix in glomeruli consists of the basement membranes of Bowman's capsule, the peripheral vascular loops and the mesangial region. While there is some evidence that there is a difference in the character of the basement membrane of the peripheral vascular loop and the mesangial matrix [2], more recent evidence using immunohistochemical probes suggest that they are composed of similar components [3]. Generally speaking the extracellular matrix consists of collagenous proteins, proteoglycans, and glycoproteins. The principal collagens are of the basement membrane and cell associated type (see [4] for a review). The proteoglycans have been extensively studied by several investigators in both experimental animals and man. The principal proteoglycan in the peripheral vascular wall contains heparan sulphate glycosaminoglycan. A number of glycoproteins have been identified in the glomeruli including fibronectin, laminin, and entactin. All of these components have been found throughout the extracellular matrix, i.e. in Bowman’s capsule, the peripheral vascular basement membranes and the mesangial matrix. There is some controversy about the exact localisation of fibronectin and the most recent evidence would suggest that its distribution is limited to the mesangial zone. The distribution of collagens, proteoglycans and glycoproteins within the peripheral vascular basement membrane are not known with certainty but it is believed that the proteoglycans are principally restricted to the lamina rara interna and externa.

*Distribution in focal sclerosing glomerulonephritis*

The distribution of type IV collagen, laminin and heparan sulphate containing proteoglycan was studied in patients with focal sclerosing glomerulonephritis at different stages [5]. The composition of the sclerotic areas was similar to the normal mesangial matrix and peripheral basement membrane. No interstitial matrix components were noted within the glomerular sclerotic areas or in the synechiae. There was, however, an increased concentration of interstitial collagen in the periglomerular interstitium adjacent to the synechiae. Thus, it
appeared that intraglomerular sclerosis contained components normal to the glomerular basement membranes.

**Distribution of extracellular matrix components in crescentic glomerulonephritis**

Crescentic glomerulonephritis in infectious diseases and systemic vasculitis were examined. Of the former, the extracellular matrix in the crescents consisted entirely of basement membrane components. Rarely, a small amount of interstitial (type III) collagen was present, and this appeared to be associated only with extensive crescent formation. In contrast, the crescents of patients with vasculitis contained large amounts of interstitial collagen. It was significant that in these patients there was early disruption of Bowman’s capsular basement membrane and an increased number of interstitial and inflammatory cells in the periglomerular region.

**Summary**

The components of the extracellular matrix in the glomerulus appear to be found in all basement membrane structures and in the mesangium. The techniques thus far employed do not allow an accurate determination of the relative or absolute amounts of these materials in the various glomerular locations. In those diseases where Bowman’s capsule remains intact, the sclerosis appears to consist, in large part, of normal glomerular basement membrane components. Diseases, such as systemic vasculitis, which are associated with disruption of Bowman’s capsule result in the early appearance of interstitial collagen within Bowman’s space. Thus, it would appear that when Bowman’s capsule is breached, the disrupted architecture heals by the formation of interstitial collagenous scars, whereas progressive sclerosis in the presence of intact basement membranes is characterised by the deposition of basement membrane components normal to the area.

**Source of the extracellular matrix**

**General**

Save for deposits of amyloid, immunoglobulins and possibly glycosylated proteins in diabetes mellitus, the increased extracellular matrix associated with the process of sclerosis consists primarily of locally synthesised materials. The following is a description of the current status of the knowledge in the origin of extracellular matrix in the glomerulus, using cell culture techniques.

**Glomerular epithelial cells**

It was initially assumed that glomerular epithelial cells synthesised the normal peripheral vascular basement membrane based on studies by Kurtz and Feldman [6]. Doubt was cast on the validity of the model in more recent studies [7]. However, using developing human embryos and mouse metanephratic cultures, the
contribution of glomerular epithelial cells to the peripheral glomerular vascular basement membrane has been established [8–10].

Glomerular epithelial cell cultures have been obtained in several animal species and man. Visceral epithelial cells are the first glomerular cells to appear in an outgrowth from isolated, single glomeruli [10]. They are first seen at from 7–14 days in humans [11] and if isolated from the rest of the glomerulus prior to the time that mesangial cells appear, they form a monolayer of cytokeratin-positive, large, flattened cells which have C3bi receptors on their surface [12]. These cells synthesize basement membrane collagen (type IV), heparan sulphate-containing proteoglycans, and fibronectin [13].

**Glomerular mesangial cells**

The glomerular mesangium contains two cell types. After its original description in light and electron microscopy, it was recognised that cells within the mesangium were capable of ingesting particles injected into the blood stream [14]. From this observation, the concept evolved that the mesangial cells were a part of the mononuclear phagocyte system. Electron microscopic studies raised the question that there was more than one cell type within the mesangium [15]. The validity of this concept was established by bone marrow transplant experiments using Chediak-Higashi mice [16]. In such mice, cells contain abnormally large lysosomes rendering them recognisable in radiation chimeras. When bone marrow from Chediak-Higashi mice was injected into syngeneic, normal recipients, their mesangial regions became populated with a small number of cells containing large lysosomes. Following injection of preformed immune complexes which deposited in the mesangial region, it was found that the cells which ingested the complexes also contained large lysosomes. These data were strong evidence that the cells in the mesangium responsible for the uptake of immune complexes were derived from the bone marrow and were distinct from the resident cells which have a contractile function. Thus, the identity of at least two separate populations of cells within the mesangium was established, one phagocytic and one contractile.

In cell culture, mesangial cells are noted to emerge from isolated, single glomeruli at a time later than that required for epithelial cells. They are spindle-shaped and rapidly form multilayers, resembling smooth muscle cells [11–13]. Their intermediate cytoskeletal filaments have not been identified. They do not have surface receptors for C3bi or Fc and do not phagocytose particles in vitro. It should be noted that there is some controversy about this point in cultures from rats. One group of investigators finds that in freshly isolated rat glomerular cells, approximately 2–8 per cent of the population contain Fc and C3 receptors and are positive for the common lymphocyte antigen [17]. A second group finds that nearly 50 per cent of cells in glomerular outgrowths are phagocytic [18] and demonstrate an oxidative burst, suggesting they are actively phagocytic. These discrepancies need to be clarified in future studies.
Glomerular endothelial cells

Glomerular endothelial cells have recently been isolated from humans and mice [19]. They are cuboidal, pavementous cells which grow in a monolayer and contain factor VIII antigen in cytoplasmic droplets. Their extracellular matrix synthesis has not been studied in detail but they appear to synthesise basement membrane collagens (types IV and VIII), glycoproteins, fibrinectin and laminin and a heparan sulphate-containing proteoglycan. Their successful isolation and propagation is dependent on the presence of purified platelet-derived growth factor.

Influence of inflammatory mediators on isolated glomerular donor cells in vitro

Glomerular epithelial cells were either isolated from glomeruli which had been treated by brief digestion with purified collagenase or from the initial outgrowth from adherent untreated glomeruli. Mesangial cells were isolated from the glomerular fragments resulting from the collagenase digestion or from the late appearing cells in adherent single glomeruli. Endothelial cells were isolated from single glomeruli exposed to purified platelet-derived growth factor. The individual cell types were each subjected to dilute plating, isolation of small groups of cells with a cloning ring and subsequent propagation.

Proliferation and extracellular matrix synthesis was studied as previously described in the presence of materials from either platelets or inflammatory cells [13]. That from platelets consisted of a supernatant obtained by multiple freezing and thawing of isolated platelets or from purified platelet-derived growth factor. That material from inflammatory cells was obtained from either the supernatant from Zymosan-treated peritoneal macrophages or purified interleukin-1.

Neither purified plate-derived growth factor or platelet releases influenced the proliferative capacity of mesangial cells or epithelial cells [20]. However, purified platelet-derived growth factor was required for the isolation, subsequent cloning, and proliferative capacity of endothelial cells. Crude supernatant from peritoneal macrophages was assayed on epithelial cells and mesangial cells. There was a 2.5-fold increase in cell number and tritiated thymidine incorporation in mesangial cells but no effect on epithelial cells. Interleukin-1 did not alter the proliferative response of mesangial cells in log-phase growth but when added to cultures which had come to confluence there was an increase in tritiated thymidine incorporation. These data are consistent with those recently published, demonstrating that mesangial cells in log-phase produce an autocrine which resembles interleukin-1 and has been called MC-TAF [21]. There is no effect of interleukin-1 on the proliferative capacity of glomerular visceral epithelial cells.

The effect of these inflammatory mediators on extracellular matrix synthesis has only been examined in mesangial cells. Monocyte supernatants were noted to have no effect on either total protein or collagen synthesis in cultures in either sparse or dense cultures. However, interleukin-1 stimulated both total
protein and collagen synthesis in dense cultures, consistent with the effect of this material on cell turnover.

In summary, there is little known about the influence of inflammatory mediators on cell proliferation and protein synthesis in isolated glomerular cells, the current data suggest that further investigations would be productive.

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Open Discussion

ANDREUCCI (Naples) What do you think about the suggestion that Dr Giordano* has made regarding progression of the lesion to chronic renal failure?

STRIKER Well, I think it is very difficult to speculate about the interactions once the disease becomes well established. At that point the mesangial cells are already embedded in connective tissue. The endothelial cells may not release PGI₂ and present a hypercoaguable surface or alter in other ways. I think that later there are quite different questions. The 7/8 nephrectomy model is not a model of chronic renal disease it is a model of 7/8 nephrectomy. Renal disease does not begin by chopping out 7/8 of the kidney, it begins in the individual nephron, maybe not all and maybe not all simultaneously but certainly it does not leave 1/8 free and 7/8 totally affected. I think one needs to look at all of these models with a little bit of a jaundiced eye but certainly the 7/8 nephrectomy model is the first real window into progression in the animal.

*Giordano C. Proc EDTA-ERA 1984; 21: this volume

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PECCHINI (Cremona) If platelet derived growth factor modulates generation of membrane products there is a theoretical basis for treatment by fibrinolytic drugs.

STRIKER Oh, I think that becomes the question of the hour. What is it that modulates endothelial cell production of anticoagulant and procoagulant factors? Certainly it suggests that the endothelial cells in the microvasculature are very different from those in the aorta, for instance: that is the only other endothelial cell that has been isolated. Endothelial cells from the glomeruli may be quite different.

RITZ (Heidelberg) I have one question relating to the product and one relating to the signal for mesangial cell stimulation. There have been reports from Yale that stimulation of mesangial cells causes Ia expansion, making these capable of participating in local immune reactions. Do you have information on this? With respect to the second point, do epidermal growth factor and oxygen radicals stimulate mesangial collagen synthesis or synthesis of other matrix components?

STRIKER Those are absolutely very current questions. It is very rare that you can cause the modulation of surface receptors with gamma interferon, even on fibroblasts. I would suspect that might also be true of mesangial cells, but we have not looked at that. The question about what modulates collagen synthesis is what brought us to start looking in this direction. I think epidermal growth factor, for instance, will drive collagen synthesis in a large number of cell types, but it also drives proliferation. We have not been able to dissect increase in synthesis or increase in cell number at this point. The problem is that mesangial cells grow everywhere, so it is hard to get them at basal conditions. As for oxygen radicals, we have looked at the question of oxygen radicals-induced injury and the difficulty is that oxygen radicals produce early cytosol changes. All we know is that you get a rush of activity but in such a short time that we cannot do pulse chase experiments.

HAWKINS (Birmingham) What causes the rupture of the basement membrane of Bowman's capsule?

STRIKER I have some ideas but I do not know if any of them are true. It is clear that macrophages are part of that very early influx of cells into the Bowman's space. Macrophages synthesise and release large quantities of proteases that are capable of digesting all types of collagen. It is quite possible that they could be an initiating factor. However, remember that Bowman's cells are sitting on their own pavement and in order to keep there they must have their own proteases so all bets are off until you ask the question of a visceral or epithelial cell. What happens when you have oxygen radical-induced injury is that they may release proteases as well. I think the question is open: we really do not know the answer.
BONE (Liverpool) Ischaemia can result in glomerulosclerosis as effectively as immunologically mediated disease. Do you have any observations on the sensitivity to oxygen tension of the mesangial cells that may help to explain this?

STRIKER Again another question that I would have to have several more pairs of hands in the laboratory to answer. I can tell you, however, that these cells are relatively insensitive to the amount of oxygen that is in the medium; for instance, I can package them up and put them in the mail and ship them to a colleague across the country. We have looked at large obsolescent glomeruli in diabetes mellitus and membranoproliferative glomerulonephritis and they have all basement membrane components. Ischaemic glomeruli, however, and I do not know where the connective tissue comes from, have interstitial collagen in Bowman’s space. We did look, by the way, at the crescents of patients with post-infective glomerulonephritis. Basement membrane generally stayed intact. Vernier, a long time ago, recognised that in vasculitis the Bowman’s capsule basement membrane was ruptured and there were ruptures of the vascular basement membrane. We believe they are interstitial cells, but I would not take that as gospel truth as mesangial cells also make interstitial collagen.

I guess I should conclude by saying that if you want to blame the rain on someone, you can probably blame it on me for it always rains in Seattle.