Proc. R. Coll. Physicians Edinb. 1996; **26**: 352–373

MESANGIAL CELL GROWTH CONTROL IN GLOMERULAR INFLAMMATION*

H. O. Schoecklmann, H. D. Rupprecht and R. B. Sterzel,† Medizinische Klinik IV, Universität Erlangen-Nürnberg, Krankenhausstr. 12, D-91054 Erlangen, Germany

In his first edition of *The Principles and Practice of Medicine*, published in 1892, Sir William Osler described the histopathological findings in Bright's disease: 'the tufts suffer first, and there is either an acute intracapillary glomerulitis, in which the capillaries become filled with cells and thrombi, or involvement of the epithelium of the tuft and of Bowman's capsule, the cavity of which contains leucocytes and red blood-corpuscules'.[1] In chronic cases 'complete atrophy' and 'extensive hyaline degeneration' of glomeruli, as well as 'multiplication of cells between the loops' can be found. As to the prognosis of chronic disease, Osler writes: 'Chronic Bright's disease is an incurable affection, and the anatomical conditions on which it depends are quite as much beyond the reach of medicines as wrinkled skin or gray hair'.[1]

More than a century later, chronic immune-mediated inflammatory diseases of the renal glomerulus represent the major cause of end stage renal disease in the Western World, requiring chronic dialysis treatment or renal transplantation. Aside from the impact on individual patients' lives, the consequences of these diseases constitute an enormous socio-economic burden. To date, there is no specific therapy of glomerular inflammatory diseases available, with most therapeutic regimens employing systemic immunosuppressive agents. However, considerable progress has been made in the past decade leading to better understanding of the molecular mechanisms of glomerular inflammatory processes, thus providing a basis for more specific therapies in the future. Here, we will review the major causes of glomerular inflammation and will discuss some aspects and novel findings concerning the molecular pathogenesis of these diseases. In this context, special focus will be placed on the balance of mitogenic and antimitogenic responses of glomerular mesangial cells (MCs).

For long, the renal glomerulus was thought to be a passive capillary ultrafilter which merely allowed the efflux of fluid from the plasma into the urinary space. More recently, however, it has been recognized that the glomerulus is an important regulatory apparatus as well, capable of fine-tuned functional control of ultrafiltration and maintenance of filter integrity. Moreover, the glomerular cells not only respond to various neurohumoral stimuli and inflammatory mediators but are also the source of important regulatory substances.

The glomerulus is composed of a network of capillaries located between the afferent and efferent arterioles. These capillaries are formed by a specialized, flat and highly fenestrated endothelial cell layer supported by the glomerular basement membrane (GBM). Visceral and parietal epithelial cells line the urinary space of the glomerulus. The visceral epithelial cells are also termed podocytes, because they extend multiple foot processes which are based upon the lamina rara

*Based upon a lecture delivered by Professor Sterzel at the Symposium on *Renal Medicine* held in the College on 20 September 1995.

†Correspondence to Professor Sterzel.

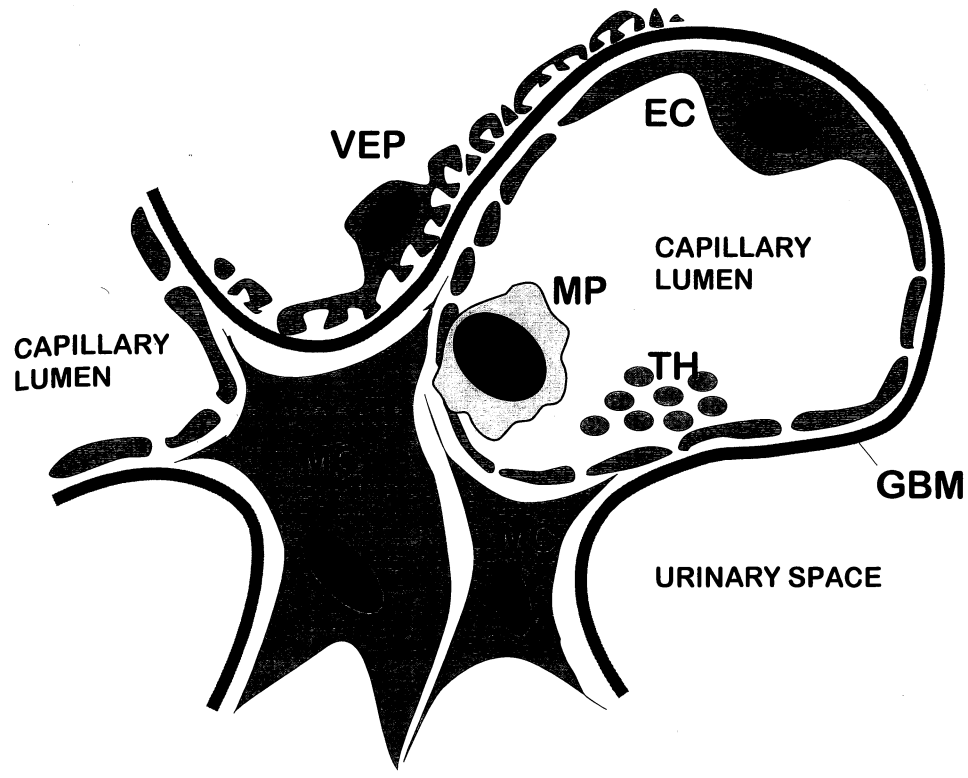

FIGURE 1

Schematic architecture of the mammalian glomerulus. MC–mesangial cell; EC–endothelial cell; VEP–visceral epithelial cell; TH–platelets; MP–macrophage; GBM–glomerular basement membrane.

externa of the GBM of one or more capillary loops. The glomerular mesangium constitutes the central region of a capillary lobule and is composed of mesangial cells (MCs) and extracellular matrix (ECM). While under normal conditions no resident macrophages are seen in the mesangium of human kidneys, bone marrow-derived inflammatory cells are frequently recruited to the mesangium in glomerular disease. MCs are located between peripheral glomerular capillaries as well as in the stalk of the tuft. They provide support to maintain the glomerular architecture and can directly influence glomerular blood flow by their contractile, smooth muscle cell-like properties. MCs are in direct contact with glomerular endothelial cells being located at the luminal side of the GBM. The absence of a continuous GBM at the endothelial-mesangial interface permits easy entry of plasma products and interaction with infiltrating bone marrow-derived inflammatory cells and their secretory products. Fig 1 shows the main structural elements of the mammalian glomerulus. It illustrates the centrolobular location of the mesangium which one can consider as the readily accessible interstitium of the glomerular capillary tuft. Also, infiltrating macrophages and platelets are depicted.

Types of injury to the glomerular mesangium

Very different types of injury can induce inflammatory responses in the renal

glomerulus. As listed in Table 1, primary causes of glomerular injury may be immune-mediated (e.g. deposition of immune complexes or autoantibodies), metabolic (e.g. hyperglycemia, oxidized LDL), infectious (e.g. bacterial breakdown products), mechanical (glomerular hypertension), toxic or of other etiologies.

TABLE 1

Types of primary injuries to mesangial cells.

1. Immunologic	• immune complexes • autoantibodies • cryoglobulins • complement factors • T cells
2. Metabolic	• hyperglycemia • AGEs • oxidized LDL • amyloid • coagulation factors
3. Infectious	• microbial proteins and bacterial breakdown products • viral components
4. Mechanical	• glomerular hypertension • turbulence/shear/stretch
5. Toxic	• poisons • enzymes
6. Others	• ischemia/hypoxia • age-related mechanisms • unknown causes

Type and duration of an immune-mediated glomerular injury determine the extent of the inflammatory response which, if successful, may lead to removal or neutralization of the injurious agent with subsequent restoration of glomerular tissue integrity. The initial inflammatory response usually includes the recruitment of bone marrow-derived inflammatory cells. Various soluble substances, released by both intrinsic glomerular cells and infiltrating inflammatory cells, act as paracrine and autocrine mediators, and regulate tissue remodeling involving changes of the proliferative and secretory MC phenotype. A common histopathological finding in most forms of immune-mediated glomerular diseases is MC hyperplasia (Fig 2). Acute glomerular inflammation, e.g. after deposition of foreign antigens during infections, may be transitory and result in complete structural and functional tissue integrity. However, if the glomerular injury is prolonged, the inflammatory process becomes chronic and the continuous release of mediators may not lead to resolution. Hence, complete tissue restoration becomes impossible and accumulation of ECM can result in replacement of the glomerular capillary tuft with scar tissue. Partial or complete glomerular sclerosis may cause additional injury due to hypertension and hyperfiltration in remaining glomerular capillaries.

The involvement of humoral immune mechanisms with deposition of immunoglobulins and complement components in the pathogenesis of mesangial pathology is well established *in vivo* and in cell culture. With few exceptions, the

specificity of the deposited antibodies is uncertain. Causative antigens have been identified as microbial breakdown products (e.g. from streptococci, Treponema, Plasmodium malariae, viral) or circulating autoantigens (e.g. DNA, histones, IgG). They are assumed to enter the mesangium either as part of circulating antigen-antibody immune complexes or separately and form immune complexes within the mesangium. The immunoglobulin class involved most frequently is IgG, but IgM and IgA are also found. IgM may be a component of cryoglobulins (large complexes of IgM reacting with other immunoglobulins) which are trapped in the mesangium. While IgA along with IgG may be part of circulating immune complexes deposited in the mesangium and elsewhere in the glomerulus, the most conspicuous and quite uniform mesangial staining for IgA is found in a renal disease originating in the mesangium. This is identified by immunofluorescence microscopy as IgA nephropathy and by light microscopy as mesangioproliferative glomerulonephritis.

Soluble antigen-antibody complexes as well as aggregates of immunoglobulins have been shown in animal experiments and in MC culture to specifically bind to Fc receptors expressed on MCs.[2] They affect the synthetic phenotype of cultured MCs, e.g. by stimulating production and secretion of pro-inflammatory cytokines and chemokines such as IL-6,[3] CSF-1, RANTES and MCP-1,[4,5] eicosanoids,[6] PAF[6] and superoxides.[7] IgG complexes also exert a contractile effect on MCs.[8]

Components of the complement system play a prominent role in immune mediated mesangial pathology. In many forms of glomerulonephritis, antibodies and complement (C3 cleavage products and the terminal complement complex C5b-9) are deposited in afflicted areas. By immunohistology, complement proteins and immunoglobulins often show similar distribution patterns, suggesting that complement has been activated by immunoglobulins. Rat and human MCs have been shown to express receptors for C1Q which may facilitate the mesangial deposition of immune complexes.[9] In addition, recent studies have indicated that complement components, such as C3, can also be synthesized by intrinsic renal cells, including MCs.[10,11]

Cellular immune mechanisms have also been implicated in mesangial injury. MCs have been shown to be activated by products of T helper cells. Also, they secrete co-stimulatory factors for T lymphocytes such as IL-1 and IL-6.[12,13] While MCs do not constitutively express MHC class II molecules, these can be induced *in vitro* by recombinant T cell lymphokines, such as INF-γ.[14] INF-γ, IL-1 and TNF-α also induce MC expression of ICAM-1.[15] MCs, therefore, can function as antigen-presenting cells and substitute for macrophages by meeting the accessory cell requirement in the interaction with T lymphocytes, as has been reported for the small population of la-positive cells found in the mesangium of healthy rats.[16]

Other prominent causes of glomerular injury include glomerular hypertension and chronic metabolic diseases. Of great clinical relevance are chronic and progressive mesangial abnormalities resulting in glomerular hyalinosis and sclerosis which are associated with long-standing diabetes mellitus. Hyperglycemia has been shown to affect the MC phenotype by multiple mechanisms, many of which induce changes of ECM production and deposition. Amyloidosis can also lead to progressive mesangial widening due to irregular deposition of amyloid fibrils throughout the mesangium. The mesangial deposition of lipids and lipoproteins can be found in states of hyperlipidemia and can contribute to MC activation and mesangial pathology. Hypercholesterolemia may aggravate glo-

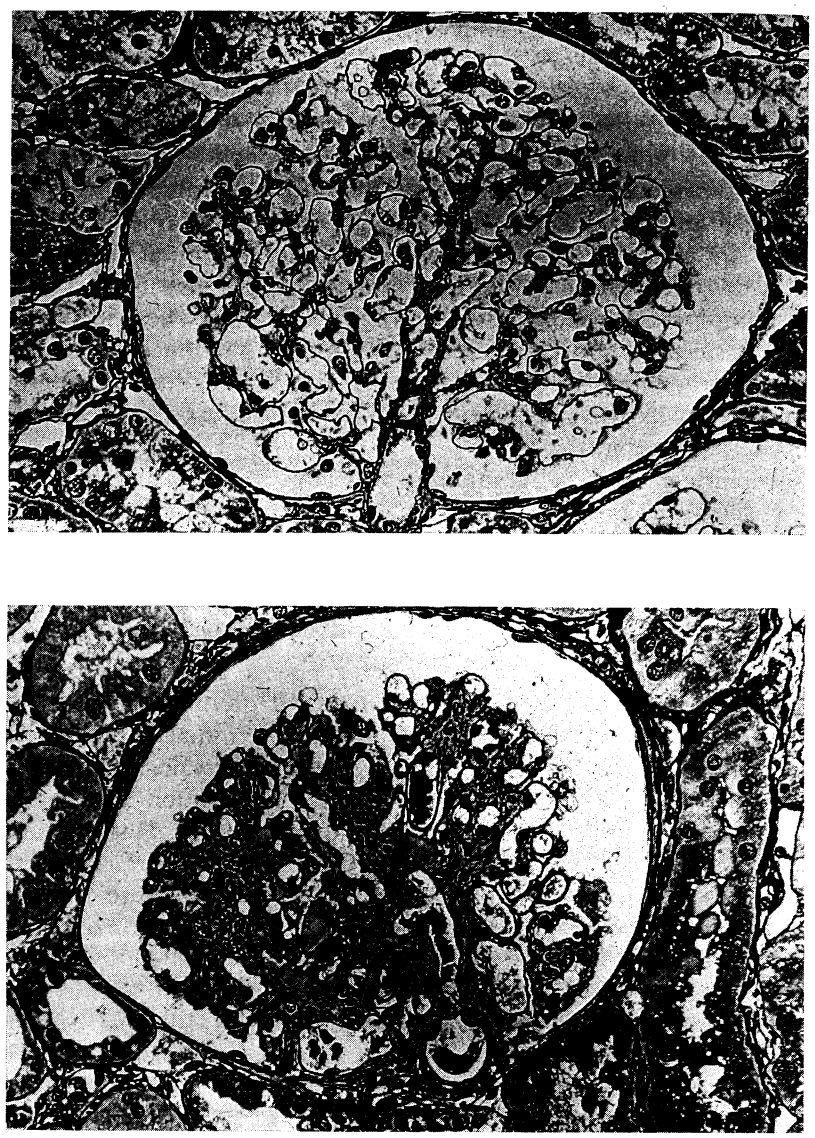

FIGURE 2

Tissue sections of human kidneys showing: (A) a normal glomerulus and (B) a glomerulus with increase of mesangial cells and widening of mesangial matrix from a patient with mesangioproliferative glomerulonephritis (Gomori, ×500).

merulosclerosis. MCs can actively participate in this process by receptor-mediated endocytosis of LDL particles. The HMG-CoA reductase inhibitor, lovastatin, has been reported to lessen renal injury in obese Zucker rats, 5/6 nephrectomy and in the aminonucleoside model of nephrotic syndrome in rats. Lovastatin inhibits proliferation of cultured MCs. Lovastatin also reduces monocyte chemotaxis effected by MC supernatants.[17] Other causes of MC injury[18–20] are not discussed here.

Intercellular crosstalk

The complex intercellular interactions which take place between intrinsic glomerular cell types and infiltrating bone marrow-derived inflammatory cells play a crucial role in the pathogenesis of glomerular inflammation. In the past, co-culture studies have tried to elucidate direct and specific interactions between different cell types in the glomerulus *in vitro*. In addition, the role of infiltrating inflammatory cells has been examined by *in vivo* depletion studies. MCs are able to secrete a variety of immune modulatory cytokines (IL-1, CSF-1, GM-CSF, TNF-α, IL-6)[21] as well as chemokines (RANTES, MCP-1, MIP-2, IL-8) which may induce the recruitment of various inflammatory cells to the glomerulus (Table 2).

TABLE 2

Chemotactic factors released by mesangial cells

	Chemotactic target cell	*Stimulator of release*	
CSF-1	monocytes, macrophages	INF-γ, IgG complexes, TNF-α	(2,4,96)
RANTES	monocytes, CD4+lymphocytes, eosinophilic granulocytes	LPS, TNF-α, IgG complexes	(5)
MCP-1	macrophages, monocytes	IL-1β, TNF-α, INF-γ, LIF, LDL, thrombin, IgG complexes	(97,98,99)
MIP-2	neutrophils	IL-1	(100)
IL-8	neutrophils, lymphocytes	IL-1, TNF-α, LPS	(101,102)

Indeed, MC expression of MCP-1 and RANTES was demonstrated to be associated with macrophage influx to the glomerulus in experimental glomerulonephritis and human proliferative glomerular diseases.[22,23] On the other hand, soluble mediators secreted by activated leukocytes, e.g. cytokines, chemokines, eicosanoids, nitric oxide (NO) and other pro- and anti-inflammatory substances can greatly affect the proliferative and synthetic phenotypes of MCs in a paracrine fashion. The amount of IL-1 released by macrophages is approximately 10–100 higher than that secreted by resident MCs. Also, glomerular IL-1 production in a rat anti-GBM nephritis model was shown to be largely dependent on infiltrating macrophages.[24] In the same type of experimental glomerulonephritis, a reduction in mesangial hypercellularity was achieved by macrophage depletion,[25] confirming the important pathogenetic rule of macrophage influx for the induction of MC proliferation.

Intraglomerular platelet accumulation and aggregation is observed in several animal methods of glomerular disease,[26,27] as well as in human disease.[28] Platelets are the source of multiple growth factors for MCs, including PDGF, EGF, IGF-1, IL-1, nucleotides, serotonin and TXA2. *In vivo*, platelet depletion studies have supported a role for platelets in mediating MC proliferation. In glomerulopathies caused by Habu snake venom or anti-Thy 1·1 IgG, experimental induction of thrombocytopenia with anti-platelet serum did not affect the initial mesangiolysis but greatly reduced the subsequent MC hyperplasia.[29,30] Similar effects were seen with the anti-platelet agent dipyridamole.[31] Thus, it appeared that platelets not only contribute to initial MC injury in these models, but their products stimulate proliferation of activated MCs.

Few data exist on the interactions of MCs with other intrinsic glomerular cell

types. Cultured endothelial cells can secrete mediators which affect the MC phenotype, e.g. PDGF, bFGF, IL-1, endothelins, NO and eicosanoids. MCs respond to co-culture with endothelial cells with enhanced synthesis of PGE2, which is dependent on the release of ET-1 by endothelial cells.[32] The local release of vasoconstrictors, such as ET-1 and vasodilators, such as NO, is likely to participate in the regulation of glomerular hemodynamics through alterations of MC tone. Co-culture also led to a 5–6 fold increase in MC cGMP content, caused by endothelial cell NO production. Crosstalk between endothelial cells and MCs is also suggested by the finding that a heparin-like molecule from culture medium conditioned by endothelial cells inhibits MC growth.[33]

Cultured glomerular epithelial cells, similar to endothelial cells, have also been reported to secrete heparin-like substances which inhibit MC proliferation.[34] However, the *in vivo* relevance of this finding is unclear, since it is unkown whether soluble epithelial cell products are able to diffuse across the GBM against a pressure gradient to reach target cells in mesangial location.

Regulation of mesangial cell mitogenesis

Pathogenetic linkage of MC proliferation with glomerular sclerosis. Cellular hyperplasia in the mesangium due to MC proliferation is a prominent histopathological finding in most types of glomerular inflammatory diseases. Under normal conditions in the adult mammalian kidney, proliferation of MCs is tightly regulated with a growth rate of less than 1 per cent.[35] Increased MC proliferation appears to play an early and potentially crucial role in the pathogenesis of progressive glomerular lesions and glomerular sclerosis. It has been observed that in different experimental models of glomerulonephritis, MC proliferation is linked to the development of mesangial ECM expansion and glomerular sclerosis.[36,37] For example, mice transgenic for the SV40 T antigen, which has growth promoting functions, develop MC proliferation followed by progressive sclerosis.[38,39] Mice transgenic for growth hormone finally develop severe progressive mesangial sclerosis. They show a 5-fold increase in 3H-thymidine labelling index of glomerular tuft cells. Interestingly, the labelling index remained increased even at late timepoints in densely sclerotic glomeruli,[36] indicating that increased MC turnover can be a significant feature associated with sclerosis, both at the onset and later stages of glomerular disease. In addition, experimental therapeutic approaches which reduce MC proliferation in glomerular disease models, such as treatment with heparin,[40] low protein diet,[41] or neutralizing antibodies against PDGF,[42] were found to prevent or reduce ECM expansion and sclerotic changes. Further evidence for the role of uncontrolled MC proliferation in the pathogenesis of sclerosis is provided from studies in rats with spontaneous, age-related glomerulosclerosis. MCs cultured from these rats demonstrated increased proliferative potential as a function of donor age, whereas in control animals with little or no glomerular sclerosis, the proliferative potential of MCs decreased with age of the donor.[43]

Regulators of MC proliferation. Over the past decade, much research has addressed the questions: which are the molecular mechanisms controlling MC growth and how is increased MC proliferation induced and maintained under disease conditions? *In vitro* and *in vivo* studies have identified a variety of factors which stimulate or inhibit MC proliferation, including various cytokines, autacoids and hormones. Many of these soluble or insoluble ligands are produced by MCs and possess autocrine growth-modulating activity (Tables 3 and 4).

TABLE 3

Regulators of mesangial cell proliferation in culture.

Mitogenic		*Anti-mitogenic*		*Variable*	
PDGF-BB	(103)	TGF-β (high	(105)	TNF-α	(103)
PDGF-AB	(104)	conc. > 250 pg/ml)		↑/↓	(106)
bFGF	(103)	INF-γ	(107)	angiotensin II	(110)
				~/↑	(111)
EGF	(103)	IL-4	(109)	nitric oxide	(116)
	(105)			~/↓	(123)
insulin	(114)	IL-10	(112)	TXA2	(119)
				↑/↓	
IGF-I	(114)	PGE2	(115)	high glucose	(126)
	(118)			↑/↓	
IL-1	(12)	PGI2 (stable	(119)		
		analogue iloprost)	(120)		
IL-6	(13)	ANP	(121)		
	(122)				
TGF-β(low conc.	(105)	cGMP	(123)		
0·01–1 ng/ml)	(124)				
AVP	(45)	LDL oxidized	(125)		
	(46)				
ET-1	(60)	Ca-channel	(127)		
		blockers			
serotonin	(45)	phosphodiesterase	(128)		
	(110)	antagonists			
ATP,	(130)	P450 MOX-	(129)		
dinucleotides	(131)	inhibitors			
PGF2α	(120)	Lovastatin	(17)		
		Simvastatin	(63)		
thrombin	(132)	heparin sulfate	(90)		
		proteoglycan			
bradykinin	(110)	heparin	(34)		
oxytocin	(110)				
PMA	(110)				
LDL native	(125)				
thrombospondin	(88)				
fibronectin	(87)				
stretch	(133)				

Post-receptor signalling pathways. In an attempt to define novel therapeutic targets in glomerulonephritis, many investigators have tried to elucidate post-receptor signalling pathways which are initiated by the interaction of growth-regulatory substances with MCs.

Many of these pathways follow a common pattern in that ligand-receptor binding initiates activation of various enzymatic steps which trigger the release of intracellular second messengers. Subsequently, multiple protein kinase systems can be activated, leading to post-translational modifications of cellular proteins. Both serine/threonine and tyrosine kinases are essential elements of the mitogenic signal transduction cascade. Preincubation of MCs with genistein, an inhibitor of protein tyrosine phosphorylation, blocks PDGF-induced MC proliferation[44] and depletion or inhibition of protein kinase C (PK C) blunts mitogenesis induced by

arginine vasopressin (AVP).[45,46] Phosphorylation or dephosphorylation processes may represent a final step in extranuclear signalling. These events initiate the modification and activation of nuclear transcription factors. This mechanism allows coupling of short term signalling events to more prolonged processes involving alterations of gene expression and protein synthesis, ultimately resulting in changes of the cellular phenotype. Communication between different signal transduction pathways, along with the release of eicosanoids, other autacoids and cytokines acting as autocrine or paracrine intercellular mediators, promotes the potential for interactive regulation of glomerular cell functions.

TABLE 4

Regulators of mesangial cell proliferation *in vivo*.

Mitogenic		*Anti-mitogenic*	
PDGF-BB		*Heparin*	
↑MC prolif. after infusion in normal rats or glomerular gene transfer. ↓proliferation after PDGF Ab in anti-Thy1·1 nephritis	(134) (51) (42)	Anti-Thy1·1 nephritis Habu snake venom model Puromycin aminonucleoside nephrosis Remnant kidney model	(40) (135) (136) (137)
bFGF		*INF-γ*	
No proliferative response after bolus or 1 wk infusion in normal rats, but 5-fold ↑ after subnephrit. dose of anti-Thy1·1	(94) (134)	Reduction of MC proliferation by 44% in anti-Thy1·1 model, but increase in glom. macrophages and TGF-β	(138)
IL-1		*Ca channel blockers*	
Constant infusion of IL-1 receptor antagonist reduced proteinuria, hypercellularity and glom. necrosis in rabbit and rat anti-GBM nephritis	(139) (140) (141)	Nifedipine reduced MC number and mesangial expansion in anti-Thy1·1 nephritis	(142)
IL-6		*ACE inhibitors*	
Mesangioproliferative glomerulonephritis in mice transgenic for human IL-6	(143)	Enalapril had similar effect as nifedipine in anti-Thy1·1 nephritis	(142)
ET-1		*Phosphodiesterase antagonists*	
ET A receptor antagonist in remnant kidney model reduced proteinuria and glomerular injury	(144)	Phosphodiesterase III or IV antagonists (Lixazinone, Rolipram) reduced proteinuria, α-actin expression and MC proliferation	(145)
SV40			
Mice transgenic for SV40 T antigen showed MC proliferation and sclerosis after age of 2 months	(146)		
Growth hormone (GH)			
Mice transgenic for GH or GH-releasing factor showed MC proliferation followed by progressive sclerosis	(36) (147)		

Here, we will discuss examples of some of the more frequently studied signalling transducing systems in MCs, including receptor tyrosine kinase-mediated signalling and signals originating from G-protein-coupled receptors.

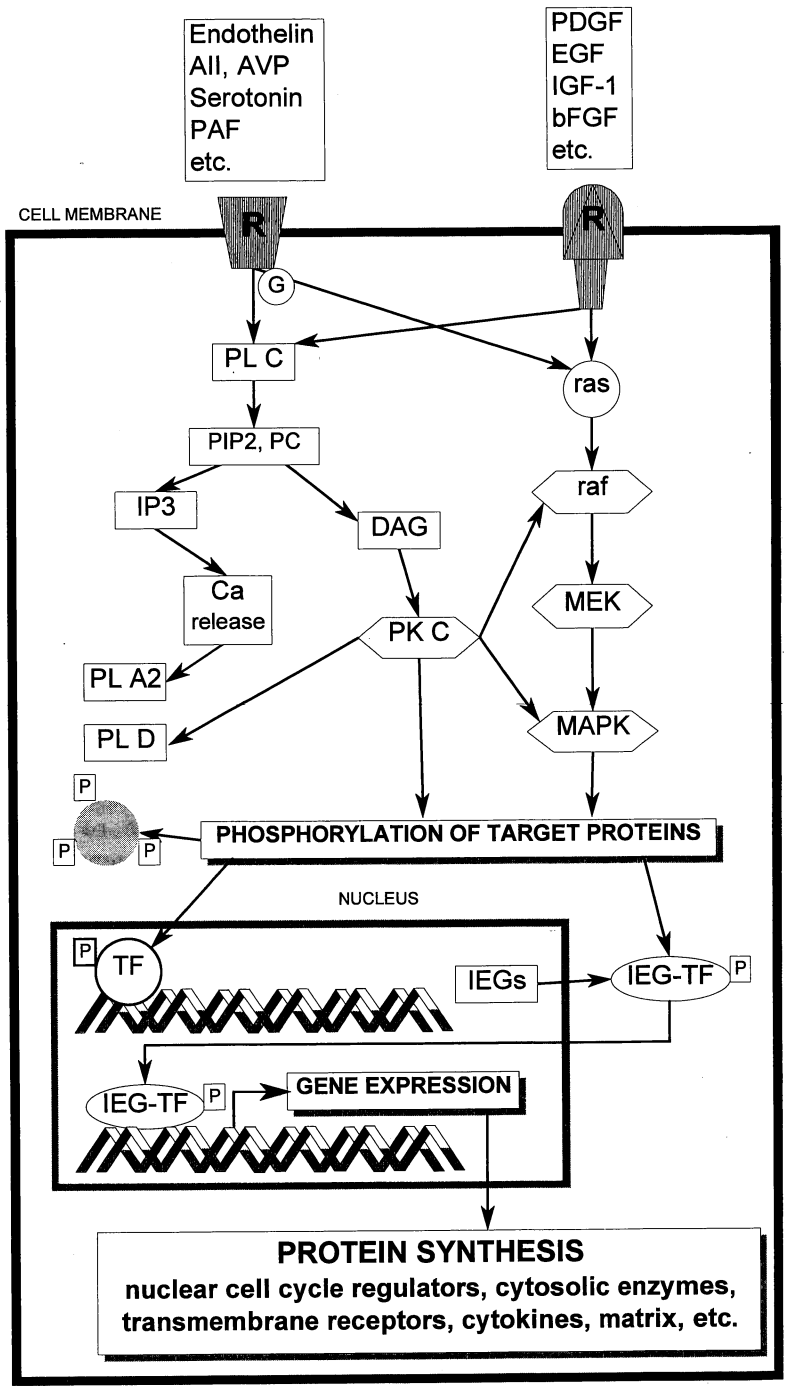

FIGURE 3

Scheme of ligand-induced post-receptor signal transduction pathways resulting in changes of the MC phenotype. R–receptor; G–G-protein; PL C–phospholipase C; PLA2–phospholipase A2; PL D–phospholipase D; PIP2–phosphatidylinositol-4,5-biphosphate; PC–phophatidylcholine; IP3–inositoltriphosphate; DAG–diacylglycerol; Ca–intracellular calcium; PK C–protein kinase C; MEK–mitogen activated protein kinase kinase; MAPK–mitogen-activated protein kinase; TF–transcription factor; IEG–immediate early gene.

Receptor tyrosine kinase-mediated signalling includes ligand-induced activation and autophosphorylation, e.g. by PDGF or bFGF, followed by generation of a multiprotein complex forming at the intracellular domain of these receptors. Intracellular signalling pathways are linked to the cascade of the mitogen-activated protein kinase (MAPK) and often also include phospholipase C (PL C) activation. One of the best-studied receptor tyrosine kinase signal transduction systems in glomerular cells is the PDGF receptor. In the mesangium of normal human kidneys, low level expression of both α- and β-subunits of the PDGF receptor has been demonstrated.[47] Remarkably, in studies of experimental and human glomerular disease, MC proliferation is associated with upregulation of the PDGF-β receptor.[47–50] In the anti-Thy 1·1 rat model of immune-mediated glomerulonephritis, administration of neutralizing PDGF antibodies reduced MC proliferation and deposition of ECM.[42] In addition, transfer of the PDGF B gene into the glomerulus by *in vivo* gene transfer using the hemagglutinating virus of Japan (HVJ)-liposome method resulted in proliferation and glomerulosclerosis.[51]

Activation of the PDGF receptor tyrosine kinase leads to autophosphorylation of the receptor and to phosphorylation of multiple cytoplasmic proteins, causing, for example, activation of PL C. This, in turn, induces generation of inositol triphosphate (IP3) which mobilizes intracellular Ca^{++} and diacylglycerol (DAG), resulting in PK C activation. These PDGF receptor-mediated intracellular events have been demonstrated to occur in cultured MCs.[52, 53]

Similarly, signals transmitted via G-protein-coupled receptors result in the activation of PL C with subsequent generation of IP3 and DAG. IP3 induces a rise in intracellular Ca^{++} concentration, which, in turn, activates various Ca^{++}-dependent processes like activation of phospholipase A2 (PL A2) and of the arachidonic acid (AA) metabolism. DAG leads to activation of PK C, a key enzyme in a variety of intracellular processes. MCs have been reported to express multiple, both Ca^{++}-dependent and Ca^{++}-independent, subtypes of PK C.[54]

Among the best studied G-protein-coupled receptor signalling systems are the endothelins and their receptors. Three structurally distinct endothelin isoforms, ET-1, ET-2 and ET-3 bind to two G-protein-coupled receptors, ETA and ETB. MCs have been shown to express both receptor subtypes.[55, 56] MCs produce ET-1, which can act via ETA and ETB receptors in an autocrine manner.[57] ET-1 induces contraction of cultured MCs[58] and reduces glomerular filtration surface area and glomerular ultrafiltration coefficient after intravenous injection.[59] In addition to these hemodynamic effects, ET-1 is a mitogen for MCs.[60] The mitogenic signal is transmitted by the ETA receptor since an ETA-receptor antagonist blocks and an ETB-receptor agonist fails to induce MC proliferation.[61] ET-1 induces elevation of phosphatidic acid by activation of phospholipase D (PL D), an enzyme linked to mitogenesis in other cell types. PL D activation was shown to be PL C-dependent and constitutes an event downstream of PK C activation.[62] In addition, it has been demonstrated, that PK C and non-receptor tyrosine kinase activity (such as c-src) contribute to mitogenic signalling of ET-1.[21]

The mitogen-activated protein kinase pathway and early gene expression. The MAPK pathway plays a critical role in the regulation of MC proliferation and represents a common final pathway leading to nuclear signal transduction for a variety of initial signalling events. The MC mitogens PDGF, EGF, AVP and ET-1[63, 64]

have all been shown to activate MAPK. On the other hand, inhibitors of MC growth, like PGE2 or other cAMP-elevating agents as well as simvastatin inhibit MAPK.[63] Activation of MAPK by ET-1 was shown to involve at least two different pathways, one of which is PK C-dependent and one involving tyrosine kinase activity[64] and which requires stimulation of ras and the c-Raf-1 kinase.[65] Activation of the ras/Raf-1/MAPK cascade is one of the major signalling pathways by which growth factors transmit mitogenic messages from their transmembraneous receptors to the nucleus.

In the nucleus, these messages are translated into early changes of gene expression, the earliest being the induction of so-called immediate early genes (IEGs). Some of the IEGs, like c-fos or Egr-1, encode nuclear transcription factors which initiate the expression of additional genes. It was shown that overexpression of activated delta Raf-1 in MCs activates the serum response element in the promoter of the IEG, c-fos. ET-1-induced activation of the c-fos serum response element which was blocked by transfection of a dominant negative c-Ha ras mutant.[65] MAPK can also activate another IEG, the early growth response gene-1 (Egr-1).[66] Rupprecht *et al.* have demonstrated a very close correlation between the induction of MC growth and the induction of Egr-1 mRNA and protein.[44, 45, 67] By using antisense oligonucleotides to Egr-1, it became evident that Egr-1 induction is a necessary step in the mitogenic signal transduction cascade in MCs. As shown in Fig 4, Egr-1 antisense oligonucleotides inhibit serum-induced Egr-1 mRNA and protein as well as MC growth. Sense or scrambled control oligonucleotides were without significant effects.[68]

Molecular control of mesangial cell cycle progression. The replication cycle of a eukaryotic cell is controlled by a series of checkpoints and transitions in which temporal order is imposed by a family of serine/threonine kinases, acting in concert with their regulatory subunits, the cyclins.

In the past few years, considerable advances have been made in this field of research, and nephrologists are applying this knowledge to elucidate the molecular control of cell cycle progression of renal cells, including MCs.

In other experimental cell systems, *in vivo* gene transfer of specific cell cycle inhibitory regulators has been successfully applied in proliferative disorders. In angioplasty animal models of vascular injury, adenovirus-mediated overexpression of $p21^{Cip1}$ or of the retinoblastoma gene product (Rb) inhibited vascular smooth muscle cell proliferation and neointima formation, thereby attenuating arterial restenosis.[69, 70] Currently, novel gene delivery systems targeting the glomerulus are being developed,[71] providing an experimental tool to counteract increased MC proliferation by transfer of negative nuclear regulators.

Cell cycle progression is controlled by cyclin-dependent kinases (CDKs), whose catalytic activity is modulated by association with different cyclins, functioning as regulatory subunits. In mammalian cells, several classes of cyclins have been identified[72] which can associate with different CDK catalytic subunits.[73] Three diffeent D-type cyclins (D1, D2, D3) and cyclin E are involved in controlling G1 phase progression and entry into S phase. D-type cyclins are growth factor-regulated genes whose products preferentially associate with CDK4 or CDK6. Cyclin D1 can bind to the tumor suppressor gene product Rb, and is able to stimulate phosphorylation of Rb by CDK4, its predominant kinase partner.[74]

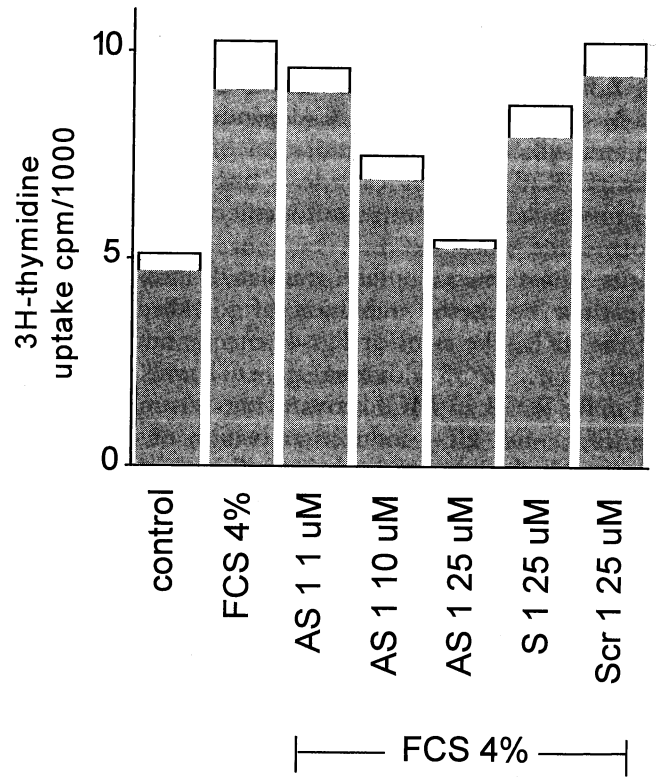

FIGURE 4

Effects of antisense oligonucleotides to Egr-1 on serum-induced growth of cultured rat MCs. Fetal calf serum (4 per cent FCS)-induced increase of 3H-thymidine incorporation is reduced by co-incubation (24 h) with antisense oligonucleotides (AS1) to Egr-1 in a dose-dependent manner. The sense (S1) and scrambled (SCR1) control oligonucleotides have no effect. Data shown as x±SEM. (From Rupprecht *et al.*, unpublished results).

The enzymatic activity of several cyclin/CDK complexes can be modulated by a group of nuclear kinase inhibitors.[74] The protein $p21^{Cip1}$ is a potent inhibitor of several cyclin/CDK complexes, with apparently limited specificity. $p27^{Kip1}$ binds tightly to cyclin D/CDK4, as well as to cyclin E/CDK2 complexes and inhibits their CDK activities in a stoichiometric fashion. $p16^{INK4a}$ and the recently cloned $p15^{INK4b}$[75] specifically inhibit the cyclin D-type CDKs, CDK4 and CDK6.

Recently, we have investigated some of the nuclear regulation events induced by an endogenous inhibitor of MC growth, transforming growth factor-β (TGF-β). TGF-β (5 ng/ml) significantly reduced mitogenesis of cultured MCs induced by PDGF, EGF, serotonin, ET-1 or bFGF. FACS analysis revealed that incubation of cultured MCs with TGF-β blocks progression of MC cycle in G1 phase of the cell cycle (Schoecklmann *et al.*, unpublished results). Our preliminary findings indicate that TGF-β-elicited signalling keeps the tumor suppressor gene product Rb in its underphosphorylated, active form and that TGF-β induces expression of the CDK4 inhibitor, $p15^{INK4b}$.

A recent report showed that overexpression of $p16^{INK4a}$ and $p21^{Cip1}$ in cultured MCs, by using an adenovirus-mediated gene transfer, inhibited entry of cells

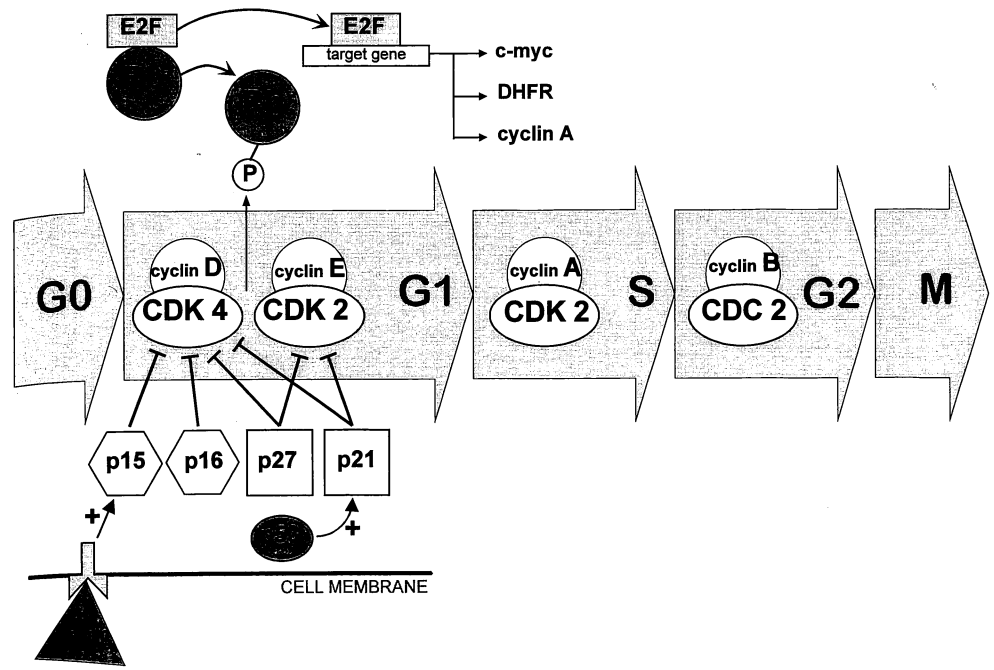

FIGURE 5

Schematic diagram of the signalling pathway that mediates cell cycle progression through G1 into S phase. Cell cycle progression is controlled by cyclin-dependent kinases (CDKs) and their regulatory subunits, the cyclins. Nuclear kinase inhibitors (p15, p16, p21, p27) modulate the enzymatic activity of cyclin/CDK complexes. External (e.g. TGF-β) or internal regulators (e.g. p53) influence the activity of these inhibitors. The transcription factor E2F is repressed by its binding to the underphosphorylated form of the retinoblastoma gene product (Rb). Phosphorylation of Rb activates E2F, which induces transcription of target genes essential for S phase.

from G1 into S phase.[76] Another study, investigating *in vivo* protein expression of cyclin kinase inhibitors in the anti-Thy1·1 model of experimental glomerulonephritis in rats, found high endogenous expression of $p27^{Kip1}$ in quiescent MCs under control conditions. After induction of nephritis, $p27^{Kip1}$ expression was reduced, inversely correlating with MC proliferation. This coincided with an increased protein expression of cyclin A and CDK2. During resolution of MC proliferation, $p21^{Cip1}$ expression was increased, whereas $p27^{Kip1}$ remained undetectable.[77]

Other work has addressed the role of the nuclear transcription factor E2F in MC growth control. E2F regulates expression of several genes involved in cell growth-control including c-myc, proliferating cell nuclear antigen (PCNA), dihydrofolate reductase (DHFR) and cyclin A.[74] E2F is a direct target for several components of the cell cycle control machinery. The fact that E2F activity is repressed by its binding to the underphosphorylated form of Rb led to the hypothesis that Rb may exert growth suppression by blocking cellular E2F target genes, and that cyclin D1 might relieve this block by inducing Rb phosphorylation.[78] Inhibition of E2F in cultured MCs by their transfection with a double-stranded oligonucleotide decoy with high affinity to E2F resulted in decreased MC proliferation.[79] Under these conditions, serum-stimulated upregulation of

PCNA and CDK2 kinase gene was inhibited. E2F decoy oligonucleotides were also used for a novel *in vivo* gene therapy approach in experimental anti-Thy 1·1 glomerulonephritis.[80] E2F decoy double-stranded oligonucleotides could be delivered efficiently *in vivo* to the glomeruli of rats using the HVJ-liposome method. Application of E2F decoy significantly inhibited glomerular expression of PCNA and CDK2 and reduced total glomerular celllularity and lesion formation in nephritic animals.

Apoptosis: a mechanism balancing mesangial cell hyperplasia. As discussed above, mesangial hypercellularity is thought to be one of the major pathogenetic factors in glomerular inflammatory disease. If recovery of the disease occurs, the question is, how is the resolution of hypercellularity achieved? Theoretically, this resolution would require reduction of the baseline regeneration or proliferation rate and/or increased removal or death of MCs.

Recent research has examined apoptosis, a mechanism which uses an intrinsic program for cell death. In the kidney, apoptotic cells were first described in renal biopsies from patients with proliferative glomerulonephritis or hemolytic uremic syndrome.[81,82] Cultured MCs are capable of undergoing apoptosis after having been deprived of serum growth factors.[83] Baker *et al.* demonstrated that MC apoptosis is an important cell clearance mechanism counterbalancing cell division in self-limited anti-Thy1·1 glomerulonephritis, thereby mediating resolution of glomerular hypercellularity in experimental MC proliferation in rats.[84] In these experiments, mitotic and apoptotic cells were frequently detected in the same glomerulus, suggesting that both processes can occur simultaneously. These results were recently confirmed by another study using the same model of rat glomerulonephritis. Apoptosis was upregulated at the beginning of the hypercellular state, with the majority of apoptotic cells being MCs.[85]

A recent report demonstrated that the expression of the apoptotic regulators Fas and Bcl-2 is upregulated in several types of human glomerulonephritis.[86] The Fas antigen-Fas ligand system mediates apoptosis in several cell types. In contrast, the protein encoded by the Bcl-2 proto-oncogene can block apoptotic cell death and prolong the lifespan of cells. The number of intraglomerular cells positive for Fas protein was shown to be high in Henoch–Schönlein purpura and lupus nephritis. Bcl-2 positive cells were increased in lupus nephritis, focal glomerular sclerosis, and IgA nephritis. By dual-labelling on serial sections it could be demonstrated that mainly MCs and occasionally infiltrating leucocytes expressed Fas antigen and Bcl-2. Electron microscopy revealed apoptotic cells in areas of MC proliferation and within the glomerular capillaries. In mesangioproliferative types of glomerulonephritis, the population of Bcl-2-positive intraglomerular cells, but not that of Fas-positive cells, was significantly correlated with the number of PCNA positive cells, the grade of mesangial cell increase and the magnitude of proteinuria. Based on these findings, one could speculate that *in vivo* Bcl-2 overexpression might contribute to maintain MC hyperplasia in proliferative glomerulonephritis by preventing apoptotic cell death. Obviously, much research is required to elucidate the pathophysiological relevance of apoptosis in glomerular inflammation.

Mesangial cell growth modulation by extracellular matrix

The composition of the mesangial ECM appears to be a major determinant of the physical, mechanical and functional properties of the glomerulus. Changes in matrix composition can directly affect MC biology and, in turn, changes in MC activation status may result in altered ECM synthesis. Under normal conditions, the matrix compounds are being synthesized and degraded in a highly regulated manner, since the integrity of glomerular matrices is strictly maintained throughout most of adult life. However, loss of this coordinated regulation with increase in matrix deposition occurs in the development of glomerulosclerosis, a common feature in most forms of chronic inflammatory glomerular diseases.

As discussed above, proliferation of MCs is controlled by many soluble regulator molecules, including various cytokines, autacoids and hormones. In addition, studies in the last decade have revealed that ECM can also regulate MC growth. *In vitro*, freshly seeded MCs do not only plate with much higher efficiency on collagen types, I, II and IV and fibronectin compared to plastic but also show greater replication activity.[87] Thrombospondin, an anti-adhesive matrix glycoprotein which promotes cell rounding and division in a variety of cell types, also increases MC proliferation. This effect might be mediated in part by upregulation of EGF and PDGF secretion, which, in turn, can increase MC proliferation in an autocrine manner.[88] In recent cell culture studies, we observed that the rapid attachment of MCs to fibronectin is significantly inhibited by the heparan sulfate proteoglycan, perlecan.[89] Several investigators reported a dose-dependent inhibition of MC proliferation by heparan sulfate.[90,91]

In additon to direct receptor-mediated effects, ECM molecules can indirectly affect the MC phenotype, e.g. by binding and sequestering various cytokines. For example, the small proteoglycan, decorin, the production of which is increased in MCs by TGF-β,[92] was shown to neutralize the action of TGF-β. This effect could reflect an autoregulatory negative feed-back mechanism. Furthermore, bFGF strongly binds to heparan sulfate proteoglycans.[93] Since bFGF has been reported to influence experimental kidney diseases,[94] interactions of basement membrane proteoglycans with bFGF may have important regulatory functions.

Parallel increases of MC proliferation and MC growth have been observed in several *in vivo* models of glomerular disease. For example, in rat anti-Thy1·1 glomerulonephritis, an increase of ECM synthesis was demonstrated during proliferation of MCs.[95] On the other hand, inhibition of MC proliferation in this model by administration of neutralizing PDGF antibodies[42] or heparin[40] was shown to reduce mesangial ECM deposition. Taken together, there is ample evidence that mesangial ECM acts not only as a mechanical, inert structural support system of the glomerular capillary tuft. Rather, it has become clear, that ECM can affect and regulate MC proliferation in a specific manner, similar to and in concert with growth factors or cytokines.

ACKNOWLEDGEMENTS

Research data for this review were obtained with the support of the Deutsche Forschungsgemeinschaft, Bonn; Sonderforschungsbereich 263/B5 and Klinische Forschergruppe Ste 196/3-1.

REFERENCES

1 Osler W. The principles and practice of medicine. New York: Appleton & Co. 1892, 741–57.
2 Schlondorff D, Mori T. Contributions of mesangial cells to glomerular immune functions. *Klin Wochenschr* 1990; **68:** 1138–44.
3 Chen A, Chen WP, Sheu LF, Lin CY. Pathogenesis of IgA nephropathy: *in vitro* activation of

human mesangial cells by IgA immune complex leads to cytokine secretion. *J Pathol* 1994; **173:** 119–26.

[4] Hora K, Satriano JA, Santiago A *et al.* Receptors for IgG complexes activate synthesis of monocyte chemoattractant peptide 1 and colony-stimulating factor 1. *Proc Natl Acad Sci USA* 1992; **89:** 1745–9.

[5] Wolf G, Aberle S, Thaiss F *et al.* TNF alpha induces expression of the chemoattractant cytokine RANTES in cultured mouse mesangial cells. *Kidney Int* 1993; **44:** 795–804.

[6] Neuwirth R, Singhal P, Diamond B *et al.* Evidence for immunoglobulin Fc receptor-mediated prostaglandin2 and platelet-activating factor formation by cultured rat mesangial cells. *J Clin Invest* 1988; **82:** 936–44.

[7] Sedor JR, Carey SW, Emancipator SN. Immune complexes bind to cultured rat glomerular mesangial cells to stimulate superoxide release. Evidence for an Fc receptor. *J Immunol* 1987; **138:** 3751–7.

[8] Mené P, Dubyak GR, Emancipator SN, Dunn MJ. Stimulation of cytosolic free calcium and contraction by immune complexes in cultured rat mesangial cells. *Trans Assoc Am Physicians* 1987; **100:** 179–86.

[9] van den Dobbelsteen ME, van der Woude FJ, Schroeijers WE, Klar Mohamad N, van Es LA, Daha MR. C1Q, a subunit of the first component of complement, enhances the binding of aggregated IgG to rat renal mesangial cells. *J Immunol* 1993; **151:** 4315–24.

[10] Abe K, Miyazaki M, Furusu A *et al.* Intraglomerular C3 synthesis and its activation in IgA nephropathy. *J Am Soc Nephrol* 1995; **6:** 917 (Abstract).

[11] van den Dobbelsteen ME, Verhasselt V, Kaashoek JG *et al.* Regulation of C3 and factor H synthesis of human glomerular mesangial cells by IL-1 and interferon-gamma. *Clin Exp Immunol* 1994; **95:** 173–80.

[12] Lovett DH, Ryan JL, Sterzel RB. Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. *J Immunol* 1983; **131:** 2830–6.

[13] Horii Y, Muraguchi A, Iwano M, Matsuda T, Hirayama T, Yamada H, Fujii Y, Dohi K, Ishikawa H, Ohmoto Y *et al.* Involvement of IL-6 in mesangial proliferative glomerulonephritis. *J Immunol* 1989; **143:** 3949–55.

[14] Martin M, Schwinzer R, Schellekens H, Resch K. Glomerular mesangial cells in local inflammation. Induction of the expression of MHC class II antigens by IFN-gamma. *J Immunol* 1989; **142:** 1887–94.

[15] Brennan DC, Jevnikar AM, Takei F, Reubin Kelley VE. Mesangial cell accessory functions: mediation by intercellular adhesion molecule-1. *Kidney Int* 1990; **38:** 1039–46.

[16] Schreiner GF, Kiely J-M, Cotran RS, Unanue ER. Characterization of resident glomerular cells in the rat expressing la determinants and manifesting genetically restricted interactions with lymphocytes. *J Clin Invest* 1981; **68:** 920–31.

[17] Kasiske BK, O'Donnell MP, Kim Y *et al.* Cholesterol synthesis inhibitors inhibit more than cholesterol synthesis. *Kidney Int Suppl* 1994; **45:** S51–3.

[18] Lovett DH, Sterzel RB. Cell culture approaches to the analysis of glomerular inflammation. *Kidney Int* 1986; **30:** 246–54.

[19] Mené P, Simonson MS, Dunn MJ. Physiology of the mesangial cell. *Physiol Rev* 1989; **69:** 1347–1424.

[20] Sterzel RB, Lovett DH. Interactions of inflammatory and glomerular cells in the response to glomerular injury. In: *Immunopathology of renal disease*. Wilson C, ed. New York: Churchill Livingstone 1988, 137–73.

[21] Sterzel RB, Schulze-Lohoff E, Marx M. Cytokines and mesangial cells. *Kidney Int Suppl* 1993; **39:** S26–31.

[22] Stahl RA, Wolf G, Thaiss F. The possible role of chemotactic cytokines in renal disease. *Clin Investig* 1994; **72:** 711–2.

[23] Rovin BH, Rumancik M, Tan L, Dickerson J. Glomerular expression of monocyte chemoattractant protein-1 in experimental and human glomerulonephritis. *Lab Invest* 1994; **71:** 536–42.

[24] Tipping PG, Lowe MG, Holdsworth SR. Glomerular interleukin 1 production is dependent on macrophage infiltration in anti-GBM glomerulonephritis. *Kidney Int* 1991; **39:** 103–10.

[25] Matsumoto K, Hatano M. Effect of antimacrophage serum on the proliferation of glomerular cells in nephrotoxic serum nephritis in the rat. *J Clin Lab Immunol* 1989; **28:** 39–44.

[26] Johnson RJ, Alpers CE, Pruchno C *et al.* Mechanisms and kinetics for platelet and neutrophil localization in immune complex nephritis. *Kidney Int* 1989; **36:** 780–9.

[27] Cattell V, Bradfield JW. Focal mesangial proliferative glomerulonephritis in the rat caused by habu snake venom. A morphologic study. *Am J Pathol* 1977; **87:** 511–24.

[28] Clark WF, Lewis ML, Cameron JS, Parsons V. Intrarenal platelet consumption in the diffuse proliferative nephritis of systemic lupus erythematosus. *Clin Sci Mod Med* 1975; **49:** 247–52.

[29] Cattell V. Focal mesangial proliferative glomerulonephritis in the rat caused by Habu snake venom: the role of platelets. *Br J Exp Pathol* 1979; **60:** 201–8.

[30] Johnson RJ, Garcia RL, Pritzl P, Alpers CE. Platelets mediate glomerular cell proliferation in immune complex nephritis induced by anti-mesangial cell antibodies in the rat. *Am J Pathol* 1990; **136:** 369–74.

[31] Cattell V, Mehotra A. Focal mesangial proliferative glomerulonephritis in the rat caused by Habu venom: the effect of antiplatelet agents. *Br J Exp Pathol* 1980; **61:** 310–14.

[32] Uhida K, Ballermann BJ. Sustained activation of PGE2 synthesis in mesangial cells cocultured with glomerular endothelial cells. *Am J Physiol* 1992; **263:** C200–9.

[33] Castellot JJ, Jr, Hoover RL, Karnovsky MJ. Glomerular endothelial cells secrete a heparinlike inhibitor and a peptide stimulator of mesangial cell proliferation. *Am J Pathol* 1986; **125:** 493–500.

[34] Castellott JJ, Jr, Hoover RL, Harper PA, Karnovsky MJ. Heparin and glomerular epithelial cell-secreted heparin-like species inhibit mesangial-cell proliferation. *Am J Pathol* 1985; **120:** 427–35.

[35] Pabst R, Sterzel RB. Cell renewal of glomerular cell types in normal rats. An autoradiographic analysis. *Kidney Int* 1983; **24:** 626–31.

[36] Pesce CM, Striker LJ, Peten E *et al.* Glomerulosclerosis at both early and late stages is associated with increased cell turnover in mice transgenic for growth hormone. *Lab Invest* 1991; **65:** 601–5.

[37] Floege J, Burns MW, Alpers CE *et al.* Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Int* 1992; **41:** 297–309.

[38] MacKay K, Striker LJ, Pinkert CA. Glomerulosclerosis and renal cysts in mice transgenic for the early region of SV40. *Kidney Int* 1987; **32:** 827–37.

[39] Striker LJ, Peten EP, Elliot SJ *et al.* Mesangial cell turnover: effect of heparin and peptide growth factors. *Lab Invest* 1991; **64:** 446–56.

[40] Floege J, Eng E, Young BA *et al.* Heparin suppresses mesangial cell proliferation and matrix expansion in experimental mesangioproliferative glomerulonephritis. *Kidney Int* 1993; **43:** 369–80.

[41] Fukui M, Nakamura T, Ebihara I *et al.* Low-protein diet attenuates increased gene expression of platelet-derived growth factor and transforming growth factor-beta in experimental glomerular sclerosis. *J Lab Clin Med* 1993; **121:** 224–34.

[42] Johnson RJ, Raines EW, Floege J *et al.* Inhibition of mesangial cell proliferation and matrix expansion in glomerulonephritis in the rat by antibody to platelet-derived growth factor. *J Exp Med* 1992; **175:** 1413–6.

[43] Pugliese F, Ferrario RG, Ciavolella A. Growth abnormalities in cultured mesangial cells from rats with spontaneous glomerulosclerosis. *Kidney Int* 1995; **47:** 106–13.

[44] Rupprecht HD, Sukhatme VP, Lacy J *et al.* PDGF-induced Egr-1 expression in rat mesangial cells is mediated through upstream serum response elements. *Am J Physiol* 1993; **265:** F351–F60.

[45] Rupprecht HD, Dann P, Sukhatme VP *et al.* Effect of vasoactive agents on induction of Egr-1 in rat mesangial cells: correlation with mitogenicity. *Am J Physiol* 1992; **263:** F623–F36.

[46] Ganz MB, Pekar SK, Perfetto MC, Sterzel RB. Arginine vasopressin promotes growth of rat glomerular mesangial cells in culture. *Am J Physiol* 1988; **255:** F898–F906.

[47] Gesualdo L, Di Paolo S, Milani S *et al.* Expression of platelet-derived growth factor receptors in normal and diseased human kidney. An immunohistochemistry and in situ hybridization study. *J Clin Invest* 1994; **94:** 50–8.

[48] Iida H, Seifert R, Alpers CE *et al.* Platelet-derived growth factor (PDGF) and PDGF receptor are induced in mesangial proliferative nephritis in the rat. *Proc Natl Acad Sci USA* 1991; **88:** 6560–4.

[49] Gesualdo L, Pinzani M, Floriano JJ *et al.* Platelet-derived growth factor expression in mesangial proliferative glomerulonephritis. *Lab Invest* 1991; **65:** 160–7.

[50] Fellstrom B, Klareskog L, Heldin CH *et al.* Platelet-derived growth factor receptors in the kidney-upregulated expression in inflammation. *Kidney Int* 1989; **36:** 1099–1102.

[51] Isaka Y, Fujiwara Y, Ueda N *et al.* Glomerulosclerosis induced by *in vivo* transfection of transforming growth factor-beta or platelet-derived growth factor gene into the rat kidney. *J Clin Invest* 1993; **92:** 2597–601.

[52] Bonventre JV, Weber PC, Gronich JH. PAF and PDGF increase cytosolic [Ca2+] and phospholipase activity in mesangial cells. *Am J Physiol* 1988; **254:** F87–F94.

[53] Mené P, Abboud HE, Dubyak GR *et al.* Effects of PDGF on inositol phosphates, Ca2+, and contraction of mesangial cells. *Am J Physiol* 1987; **253:** F458–F63.
[54] Huwiler A, Schulze-Lohoff E, Fabbro D, Pfeilschifter J. Immunocharacterization of protein kinase C isoenzymes in rat kidney glomeruli, and cultured glomerular epithelial and mesangial cells, *Exp Nephrol* 1993; **1:** 19–25.
[55] Sugiura M, Snajdar RM, Schwartzberg M *et al.* Identification of two types of specific endothelin receptors in rat mesangial cell. *Biochem Biophys Res Commun* 1989; **162:** 1396–1401.
[56] Martin ER, Brenner BM, Ballermann BJ. Heterogeneity of cell surface endothelin receptors. *J Biol Chem* 1990; **265:** 14044–9.
[57] Sakamoto H, Sasaki S, Hirata Y *et al.* Production of endothelin-1 by rat cultured mesangial cells. *Biochem Biophys Res Commun* 1990; **169:** 462–8.
[58] Simonson MS, Dunn MJ. Endothelin-1 stimulates contraction of rat glomerular mesangial cells and potentiates beta-adrenergic-mediated cyclic adenosine monophosphate accumulation. *J Clin Invest* 1990; **85:** 790–7.
[59] Marsen TA, Schramek H, Dunn MJ. Renal actions of endothelin: linking cellular signaling pathways to kidney disease. *Kidney Int* 1994; **45:** 336–44.
[60] Simonson MS, Wann S, Mené P *et al.* Endothelin stimulates phospholipase C, Na+/H+ exchange, c-fos expression, and mitogenesis in rat mesangial cells. *J Clin Invest* 1989; **83:** 708–12.
[61] Simonson MS, Herman WH. Protein kinase C and protein tyrosine kinase activity contribute to mitogenic signaling by endothelin-1. Cross-talk between G protein-coupled receptors and pp60c-src. *J Biol Chem* 1993; **268:** 9347–57.
[62] Kester M, Simonson MS, McDermott RG *et al.* Endothelin stimulates phosphatidic acid formation in cultured rat mesangial cells: role of a protein kinase C-regulated phospholipase D. *J Cell Physiol* 1992; **150:** 578–85.
[63] Ishikawa S, Kawasumi M, Saito T. Simvastatin inhibits the cellular signaling and proliferative action of arginine vasopressin in cultured rat glomerular mesangial cells. *Endocrinology* 1995; **136:** 1954–61.
[64] Wang Y, Simonson MS, Pouyssegur J, Dunn MJ. Endothelin rapidly stimulates mitogen-activated protein kinase activity in rat mesangial cells. *Biochem J* 1992; **287:** 589–94.
[65] Herman WH, Simonson MS. Nuclear signaling by endothelin-1. A Ras pathway for activation of the c-fos serum response element. *J Biol Chem* 1995; **270:** 11654–61.
[66] Hipskind RA, Büscher D, Nordheim A, Baccarini M. Ras/MAP kinase-dependent and independent signaling pathways target distinct ternary complex factors. *Genes Dev* 1994; **8:** 1803–16.
[67] Rupprecht HD, Sukhatme VP, Rupprecht AP *et al.* Serum response elements mediate protein kinase C dependent transcriptional induction of early growth response gene-1 by arginine vasopressin in rat mesangial cells. *J Cell Physiol* 1994; **159:** 311–23.
[68] Rupprecht HD, Hofer G, Faller G *et al.* Expression of the transcriptional regulator Egr-1 in experimental nephritis: correlation with mesangial cell proliferation. [Abstract] *J Am Soc Nephrol* 1995; **6:** 882.
[69] Chang MW, Barr E, Lu MM *et al.* Adenovirus-mediated over-expression of the cyclin/cyclin-dependent kinase inhibitor, p21 inhibits vascular smooth muscle cell proliferation and neointima formation in the rat carotid artery model of balloon angioplasty. *J Clin Invest* 1995; **96:** 2260–8.
[70] Chang MW, Barr E, Seltzer J *et al.* Cytostatic gene therapy for vascular proliferative disorders with a constitutively active form of the retinoblastoma gene product. *Science* 1995; **267:** 518–22.
[71] Kitamura M, Taylor S, Unwin R *et al.* Gene transfer into the rat renal glomerulus via a mesangial cell vector: site-specific delivery, in situ amplification, and sustained expression of an exogenous gene *in vivo*. *J Clin Invest* 1994; **94:** 497–505.
[72] Sherr CJ. Mammalian G1 cyclins. *Cell* 1993; **73:** 1059–65.
[73] Pines J. Cyclins and cylin-dependent inases: take your partners. *Trends Biochem Sci* 1993; **18:** 195–7.
[74] Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995; **81:** 323–330.
[75] Hannon GJ, Beach D. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* 1994; **371:** 257–60.
[76] Terada Y, Yamada T, Sasaki S, Marumo F. Overexpression of cyclin D1 and cell cycle inhibitors (p16INK4 and p21CIP1) using adenovirus vectors regulates proliferation of rat mesangial cells. [Abstract] *J Am Soc Nephrol* 1995; **6:** 778.
[77] Shankland SJ, Hugo CH, Coats SR *et al.* Cyclin kinase inhibitors: potential regulators of mesangial cell proliferation *in vivo*. [Abstract] *J Am Soc Nephrol* 1995; **6:** 776.
[78] Weintraub SJ, Prater CA, Dean DC. Retinoblastoma protein switches the E2F site from a

positive to negative element. *Nature* 1992; **358:** 259–61.
[79] Tomita N, Kim J, Gibbons GH *et al.* Oligonucleotide decoy for transcriptional factor E2F inhibits rat mesengial cell proliferation *in vitro*. [Abstract] *J Am Soc Nephrol* 1995; **6:** 778.
[80] Tomita N, Kim J, Gibbons GH *et al. In vivo* gene therapy of anti-Thy 1 nephritis using E2F decoy oligonucleotide. [Abstract] *J Am Soc Nephrol* 1995; **6:** 887.
[81] Rifai A. Experimental models for IgA-associated nephritis. *Kidney Int* 1987; **31:** 1–7.
[82] Arends MJ, Harrison DJ. Novel hisopathologic findings in a surviving case of hemolytic uremic syndrome after bone marrow transplantation. *Hum Pathol* 1989; **20:** 89–91.
[83] Savill J. Apoptosis and the kidney. *J Am Soc Nephrol* 1994; **5:** 12–21.
[84] Baker AJ, Mooney A, Hughes J *et al.* Mesangial cell apoptosis: the major mechanism for resolution of glomerular hypercellularity in experimental mesangial proliferative nephritis. *J Clin Invest* 1994; **94:** 2105–16.
[85] Shimizu A, Kitamura H, Masuda Y *et al.* Apoptosis in the repair process of experimental proliferative glomerulonephritis. *Kidney Int* 1995; **47:** 114–21.
[86] Takemura T, Murakami K, Miyazato H *et al.* Expression of Fas antigen and Bcl-2 in human glomerulonephritis. *Kidney Int* 1995; **48:** 1886–92.
[87] Simonson MS, Culp LA, Dunn MJ. Rat mesangial cell-matrix interactions in culture. *Exp Cell Res* 1989; **184:** 484–98.
[88] Marinides GN, Suchard SJ, Mookerjee BK. Role of thrombospondin in mesangial cell growth: possible existence of an autocrine feedback growth circuit. *Kidney Int* 1994; **46:** 350–7.
[89] Gauer S, Schulze-Lohoff E, Schleicher E, Sterzel RB. Glomerular basement membrane derived perlecan inhibits mesangial cell adhesion to fibronectin. *Eur J Cell Biol* 1995; (in press).
[90] Groggel GC, Marinides GN, Hovingh P *et al.* Inhibition of rat mesangial cell growth by heparin sulfate. *Am J Physiol* 1990; **258:** F259–F65.
[91] Caenazzo C, Garbisa S, Ceol M *et al.* Heparin modulates proliferation and proteoglycan biosynthesis in murine mesangial cells: molecular clues for its activity in nephropathy. *Nephrol Dial Transplant* 1995; **10:** 175–84.
[92] Border WA, Okuda S, Languino LR, Ruoslahti E. Transforming growth factor-beta regulates production of proteoglycans by mesangial cells. *Kidney Int* 1990; **37:** 689–95.
[93] Saksela O, Rifkin DB. Release of basic fibroblast growth factor-heparin sulfate complexes from endothelial cells by plasminogen activator-mediated proteolytic activity. *J Cell Biol* 1990; **110:** 767–75.
[94] Floege J, Eng E, Linder V *et al.* Rat glomerular mesangial cells synthesize basic fibroblast growth factor: Release, upregulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. *J Clin Invest* 1992; **90:** 2362–9.
[95] Floege J, Johnson RJ, Gordon K *et al.* Increased synthesis of extracellular matrix in mesangial proliferative nephritis. *Kidney Int* 1991; **40:** 477–88.
[96] Mori T, Bartocci A, Satriano J *et al.* Mouse mesangial cells produce colony-stimulating factor-1 (CSF-1) and express the CSF-1 receptor. *J Immunol* 1990; **144:** 4697–702.
[97] Rovin BH, Yoshiumura T, Tan L. Cytokine-induced production of monocyte chemoattractant protein-1 by cultured human mesangial cells. *J Immunol* 1992; **148:** 2148–53.
[98] Satriano JA, Hora K, Shan Z *et al.* Regulation of monocyte chemoattractant protein-1 and macrophage colony-stimulating factor-1 by IFN-gamma, tumor necrosis factor-alpha, IgG aggregates, and cAMP in mouse mesangial cells. *J Immunol* 1993; **150:** 1971–8.
[99] Hartner A, Sterzel RB, Reindl N *et al.* Cytokine-induced expression of leukemia inhibitory factor in renal mesangial cells. *Kidney Int* 1994; **45:** 1562–71.
[100] Largen PJ, Tam FWK, Rees AJ, Cattell V. Rat mesangial cells have a selective role in macrophage recruitment and activation. *Exp Nephrol* 1995; **3:** 34–9.
[101] Brown Z, Strieter RM, Chensue SW *et al.* Cytokine-activated human mesangial cells generate the neutrophil chemoattractant, interleukin 8. *Kidney Int* 1991; **40:** 86–90.
[102] Kusner DJ, Luebbers EL, Nowinski RJ *et al.* Cytokine- and LPS-induced synthesis of interleukin-8 from human mesangial cells. *Kidney Int* 1991; **39:** 1240–8.
[103] Silver BJ, Jaffer FE, Abboud HE. Platelet-derived growth factor synthesis in mesangial cells: induction by multiple peptide mitogens. *Proc Natl Acad Sci USA* 1989; **86:** 1056–60.
[104] Abboud HE, Grandaliano G, Pinzani M *et al.* Actions of platelet-derived growth factor isoforms in mesangial cells. *J Cell Physiol* 1994; **158:** 140–50.
[105] Jaffer F, Saunders C, Schultz P *et al.* Regulation of mesangial cell growth by polypeptide mitogens. Inhibitory role of transforming growth factor beta. *Am J Pathol* 1989; **135:** 261–9.
[106] Baud L, Perez J, Friedlander G, Ardaillou R. Tumor necrosis factor stimulates prostaglandin

production and cyclic AMP levels in rat cultured mesangial cells. *FEBS Lett* 1988; **239:** 50–4.
107 Kakizaki Y, Kraft N, Atkins RC. Differential control of mesangial cell proliferation by interferon-gamma. *Clin Exp Immunol* 1991; **85:** 157–63.
108 Floege J, Eng E, Young BA, Johnson RJ. Factors involved in the regulation of mesangial cell proliferation *in vitro* and *in vivo*. *Kidney Int Suppl* 1993; **39:** S47–S54.
109 Nakazato Y, Okada H, Sato A *et al.* Interleukin 4 downregulates cell growth and prostaglandin release of human mesangial cells. *Biochem Biophys Res Commun* 1993; **197:** 486–93.
110 Ganz MB, Perfetto MC, Boron WF. Effects of mitogens and other agents on rat mesangial cell proliferation, pH, and Ca2+. *Am J Physiol* 1990; **259:** F269–F78.
111 Ray PE, Bruggeman LA, Horikoshi S *et al.* Angiotensin II stimulates human fetal mesangial cell proliferation and fibronectin biosynthesis by binding to AT1 receptors. *Kidney Int* 1994; **45:** 177–84.
112 Osawa H, Yamabe H, Inuma H *et al.* Interleukin 10 (IL-10) modulates mesangial cell proliferation and its IL-6 production. [Abstract] *J Am Soc Nephrol* 1995; **6:** 846.
113 Budde K, Coleman DL, Lacy J, Sterzel RB. Rat mesangial cells produce granulocyte-macrophage colony-stimulating factor. *Am J Physiol* 1989; **257:** F1065–F78.
114 Conti FG, Striker LJ, Lesniak MA *et al.* Studies on binding and mitogenic effect of insulin and insulin-like growth factor I in glomerular mesangial cells. *Endocrinology* 1988; **122:** 2788–95.
115 Stahl RA, Thaiss F, Haberstroh U *et al.* Cyclooxygenase inhibition enhances rat interleukin 1 beta-induced growth of rat mesangial cells in culture. *Am J Physiol* 1990; **259:** F419–F24.
116 Mohaupt M, Schoecklmann HO, Schulze-Lohoff E, Sterzel RB. Altered nitric oxide production and exogenous nitric oxide do not affect the proliferation of rat mesangial cells. *J Hypertens* 1994; **12:** 401–8.
117 Haralson MA, Jacobson HR, Hoover RL. Collagen polymorphism in cultured rat kidney mesangial cells. *Lab Invest* 1987; **57:** 513–23.
118 Doi T, Striker LJ, Elliot SJ *et al.* Insulinlike growth factor-1 is a progression factor for human mesangial cells. *Am J Pathol* 1989; **134:** 395–404.
119 Mené P, Abboud HE, Dunn MJ. Regulation of human mesangial cell growth in culture by thromboxane A2 and prostacyclin. *Kidney Int* 1990; **38:** 232–9.
120 Mené P, Dunn MJ. Prostaglandins and rat glomerular mesangial cell proliferation. *Kidney Int* 1990; **37:** 1256–62.
121 Johnson A, Lermioglu F, Garg UC *et al.* A novel biological effect of atrial natriuretic hormone: inhibition of mesangial cell mitogenesis. *Biochem Biophys Res Commun* 1988; **152:** 893–7.
122 Ruef C, Budde K, Lacy J *et al.* Interleukin 6 is an autocrine growth factor for mesangial cells. *Kidney Int* 1990; **38:** 249–57.
123 Garg UC, Hassid A. Inhibition of rat mesangial cell mitogenesis by nitric oxide-generating vasodilators. *Am J Physiol* 1989; **257:** F60–F6.
124 Haberstroh U, Zahner G, Disser M. TGF-beta stimulates rat mesangial cell proliferation in culture: role of PDGF beta-receptor expression. *Am J Physiol* 1993; **264:** F199–F205.
125 Coritsidis G, Rifici V, Gupta S *et al.* Preferential binding of oxidized LDL to rat glomeruli *in vivo* and cultured mesangial cells *in vitro*. *Kidney Int* 1991; **39:** 858–66.
126 Wolf G, Sharma K, Chen Y *et al.* High glucose-induced proliferation in mesangial cells is reversed by autocrine TGF-beta. *Kidney Int* 1992; **42:** 647–56.
127 Shultz PJ, Raij L. Inhibition of human mesangial cell proliferation by calcium channel blockers. *Hypertension* 1990; **15:** 176–80.
128 Matousovic K, Grande JP, Chini CC *et al.* Inhibitors of cyclic nucleotide phosphodiesterase isozymes type-III and type-IV suppress mitogenesis of rat mesangial cells. *J Clin Invest* 1995; **96:** 401–10.
129 Sellmayer A, Uedelhoven WM, Weber PC, Bonventre JV. Endogenous non-cyclooxygenase metabolites of arachidonic acid modulate growth and mRNA levels of immediate-early response genes in rat mesangial cells. *J Biol Chem* 1991; **266:** 3800–7.
130 Schulze-Lohoff E, Zanner S, Ogilvie A, Sterzel RB. Extracellular ATP stimulates proliferation of cultured mesangial cells via P2-purinergic receptors. *Am J Physiol* 1992; **263:** F374–F83.
131 Heidenreich S, Tepel M, Schluter H *et al.* Regulation of rat mesangial cell growth by diadenosine phosphates. *J Clin Invest* 1995; **95:** 2862–7.
132 Shultz PJ, Knauss TC, Mené P, Abboud HE. Mitogenic signals for thrombin in mesangial cells: regulation of phospholipase C and PDGF genes. *Am J Physiol* 1989; **257:** F366–F74.
133 Cortes P, Riser BL, Zhao X, Narins RG. Glomerular volume expansion and mesangial cell mechanical strain: mediators of glomerular pressure injury, *Kidney Int Suppl* 1994; **45:** S11–S6.

134 Floege J, Eng E, Young BA *et al.* Infusion of platelet-derived growth factor or basic fibroblast growth factor induces selective glomerular mesangial cell proliferation and matrix accumulation in rats. *J Clin Invest* 1993; **92:** 2952–62.
135 Coffey AK, Karnovsky MJ. Heparin inhibits mesangial cell proliferation in habu-venom-induced glomerular injury. *Am J Pathol* 1985; **120:** 248–55.
136 Diamond JR, Karnovsky MJ. Nonanticoagulant protective effect of heparin in chronic aminonucleoside nephrosis. *Renal Physiol* 1986; **9:** 366–74.
137 Purkerson ML, Tollefsen DM, Klahr S. N-desulfated/acetylated heparin ameliorates the progression of renal disease in rats with subtotal renal ablation. *J Clin Invest* 1988; **81:** 69–74.
138 Johnson RJ, Lombardi D, Eng E *et al.* Modulation of experimental mesangial proliferative nephritis by interferon-gamm. *Kidney Int* 1995; **47:** 62–9.
139 Lan HY, Nikolic Paterson DJ, Zarama M *et al.* Suppression of experimental crescentic geomerulonephritis by the interleukin-1 receptor antagonist. *Kidney Int* 1993; **43:** 479–85.
140 Nikolic Paterson DJ, Lan HY, Hill PA. Suppression of experimental glomerulonephritis by the interleukin-1 receptor antagonist: inhibition of intercellular adhesion molecule-1 expression. *J Am Soc Nephrol* 1994; **4:** 1695–1700.
141 Tang WW, Feng L, Vannice JL, Wilson CB. Interleukin-1 receptor antagonist ameliorates experimental anti-glomerular basement membrane antibody-associated glomerulonephritis. *J Clin Invest* 1994; **93:** 273–9.
142 Kiyama S, Nanishi F, Tomooka S *et al.* Inhibitory effects of antihypertensive drugs on mesangial cell proliferation after anti-thymocyte serum (ATS)-induced mesangiolysis in spontaneously hypertensive rats. *Life Sci* 54: 1891–900.
143 Suematsu S, Matsuda T, Aozasa K *et al.* IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc Natl Acad Sci USA* 1989; **86:** 7547–51.
144 Benigni A, Zoja C, Corna D *et al.* A specific endothelin subtype A receptor antagonist protects against injury in renal disease progression. *Kidney Int* 1993; **44:** 440–4.
145 Tsuboi Y, Shankland S, Grande JP *et al.* Treatment of anti-Thy1·1 mesangioproliferative glomerulonephritis with cyclic-3′,5′-nucleotide phosphodiesterase (PDE) isozyme antagonists, types PDE-III and PDE-IV. [Abstract] *J Am Soc Nephrol* 1995; **6:** 856.
146 MacKay K, Striker LJ, Pinkert CA *et al.* Glomerulosclerosis and renal cysts in mice transgenic for the early region of SV40. *Kidney Int* 1987; **32:** 827–37.
147 Doi T, Striker LJ, Quaife C *et al.* Progressive glomerulosclerosis develops in transgenic mice chronically expressing growth hormone and growth hormone releasing factor but not in those expressing insulinlike growth factor-1. *Am J Pathol* 1988; **131:** 398–403.