TGF-β and glomerulonephritis: anti-inflammatory versus prosclerotic actions

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Introduction

It is almost two decades since transforming growth factor-β (TGF-β) emerged from the backwater of modern biology [1,2]. During the latter years, numerous studies have been pursued to elucidate the biological properties of this molecule. It is now well recognized that TGF-β is a pleiotropic and complex cytokine involved in a wide range of cell behaviour [3]. Expression of TGF-β is observed in various embryonic tissues and adult organs [4–6], and physiological levels of TGF-β are supposed to be essential for normal development, tissue repair, and maintenance of organ functions. On the other hand, overexpression of TGF-β is closely linked to certain pathologies including fibrogenesis [7]. In the kidney, a number of reports have suggested contribution of TGF-β to renal diseases [8]. In the process of glomerulonephritis, for example, upregulated TGF-β stimulates production of extracellular matrix (ECM), leading to excessive matrix deposition [9]. Several reviews have described the ‘dark side’ of TGF-β in the kidney [7,8,10,11]. In contrast, little attention has been paid to the ‘bright side’ of this molecule in the glomerulus. The aim of this article is to address the therapeutically relevant potential of TGF-β in the process of glomerular inflammation.

The biology of TGF-β

The TGF-β superfamily consists of more than 25 molecules isolated from many species. The main subgroup includes three mammalian isoforms, TGF-β1, -β2 and -β3, that are 60–80% homologous [3]. Among these, TGF-β1 is most abundantly produced by mammalian cells. Mature TGF-βs are homodimeric polypeptides with a molecular weight of 25 kDa [3]. These are synthesized and secreted as inactive pro-peptides consisting of a 5′ signalling sequence, a pro-domain called ‘latency associated peptide’ (LAP) and a mature protein domain. The latent TGF-β may exist as complexes with latent TGF-β binding proteins (LTBP) of varying size [12]. After secretion, the latent molecules are proteolytically cleaved to the mature forms. The in vivo process of this activation has not been fully elucidated, but evidence suggests the contribution of plasmin, a serine protease, to the cleavage of latent TGF-β [13].

TGF-β is produced by various cell types, and the major sources are platelets, bone, kidney, lung, and placenta [3]. Expression of TGF-β receptors is ubiquitous as well, and almost all cells respond to TGF-β in a cell-type-specific manner. Biological actions of TGF-β are mediated by TGF-β receptors type I (RI) and II (RII) [14]. TGF-β initially binds to RII that has a constitutively active kinase. RI then binds to the TGF-β and is phosphorylated by RII. RI and RII form a heteromeric complex and generate signals via serine threonine kinases [14]. Although currently the signal transduction molecules responsible for propagating TGF-β’s effects are not fully elucidated, recent investigations have indicated the involvement of tumour suppressor proteins, mitogen-activated protein kinases, and Mad family of molecules [14–16]. TGF-β possesses a diverse range of biological actions on mammalian cells, i.e. control of cell proliferation, adhesion, migration and phenotypic change, and thereby either positively or negatively regulates organogenesis/embryogenesis, tissue repair, inflammation, fibrosis, and oncogenesis [3]. Although TGF-β family of molecules are multifunctional, current dogma is that TGF-β is generally an inhibitor of cell growth and a stimulator of matrix formation. It is supposed that the biological properties of TGF-β1, -β2 and -β3 are nearly identical, but in certain pathophysiological situations, each isoform may have distinct biological effects [17,18].

Expression of TGF-β in the normal and nephritic glomerulus

It has been reported that normal glomeruli express or contain detectable levels of TGF-β [9,19–21].
physiological meaning of this observation is currently unknown. Based on the fact that TGF-β generally functions as a differentiation factor [3], the basal levels of TGF-β could contribute to the maintenance of normal glomerular structure and function.

In various pathological conditions, TGF-β is upregulated in the glomerulus. The induction of TGF-β has been reported in experimental models including anti-Thy 1 glomerulonephritis, antiglomerular basement membrane (GBM) nephritis, Habu-venom glomerulonephritis, puromycin-induced nephrosis, diabetic glomerulopathy, and nephropathy associated with ureteral obstruction, as well as in human diseases such as IgA nephropathy, focal/segmental glomerulosclerosis, crescentic glomerulonephritis, lupus nephritis, diabetic nephropathy, and human immunodeficiency virus nephropathy [7,21]. In these pathological circumstances, TGF-β1, -β2 and -β3 may be similarly upregulated [21].

The cell type responsible for TGF-β production in the glomerulus is not fully elucidated, but mesangial cells may be one of major sources [9,22]. In vitro, mesangial cells have the ability to secrete a substantial amount of latent TGF-β1 and to convert it to the mature form [23,24]. Glomeruli as well as cultured mesangial cells synthesize plasminogen activator [25,26] that participates in the in vivo activation of TGF-β [13]. These findings suggest that mesangial cells are involved in both production and activation of TGF-β1 in the affected glomeruli. Platelets and infiltrating cells, especially macrophages, are other potential sources of TGF-β [27–29]. Currently it remains unclear whether or not resident endothelial or epithelial cells contribute to the production of glomerular TGF-β in pathological conditions.

**Profibrotic action of TGF-β and its potential role in glomerulosclerosis**

Excessive deposition of ECM is the characteristic feature of tissue fibrosis. Numerous reports have suggested the role of TGF-β in fibrogenesis of various organs including kidney, liver, lung, brain, joint, and skin [7].

In glomerular disease, TGF-β has been regarded as a ‘blackguard’ that contributes to glomerulosclerosis [7,8,10,11]. Generally, TGF-β induces deposition of ECM via stimulating production of matrix proteins, decreasing synthesis of ECM-degrading proteinases and upregulating synthesis of proteinase inhibitors [30]. In vitro, glomerular mesangial and epithelial cells produce collagens, fibronectin and proteoglycans in response to TGF-β1 [31]. TGF-β upregulates production of plasminogen activator inhibitor in isolated glomeruli and thereby downregulates the activity of plasminogen activator that contributes to activation of matrix-degrading metalloproteinases [32]. TGF-β also induces glomerular expression of integrins that enhances cell-matrix interaction [33] and stimulates ECM assembly [34]. These data suggest the role of TGF-β in the generation of glomerulosclerosis (Figure 1).

In both acute and chronic glomerular diseases, upregulation of local TGF-β and accumulation of ECM are concomitantly observed [7,8,10,11]. In addition to abundant descriptive data based on immunohistological analyses, the crucial role of TGF-β1 in the accumulation of ECM in vivo has been demonstrated by several investigators. Isaka et al. reported that introduction of a TGF-β1 cDNA into the glomerulus induced ECM deposition [35]. Using an anti-TGF-β antiserum or a natural inhibitor of TGF-β, decorin, Border and co-workers demonstrated that systemic inhibition of TGF-β activity attenuated accumulation of ECM in an acute model of anti-Thy 1 glomerulonephritis [36–39]. Similarly, Akagi et al. showed that inhibition of local TGF-β1 expression via introduction of antisense oligodeoxynucleotides repressed the glomerular ECM deposition in the same experimental disease [40]. These data provided evidence that overexpression of TGF-β1 contributes to the accumulation of ECM in certain acute and chronic glomerulonephritides.

In contrast to the accumulative data on the pathological role of TGF-β1 in acute, reversible injury, information is limited regarding whether long-term expression of TGF-β1 induces irreversible glomerulosclerosis. One report has provided evidence on this issue. Sanderson et al. generated transgenic mice that express TGF-β1 under the control of an albumin promoter [41]. These mice abundantly produced biologically active TGF-β1 in the liver, exhibited high levels of circulating TGF-β1 and developed progressive glomerulosclerosis. This finding suggests the potential role of overexpressed TGF-β1 in the generation of irreversible glomerulosclerosis.

**Protective action of TGF-β against tissue injury**

There is a body of literature that has described the ‘beneficial’ aspects of TGF-β in diseases. For example,
it is well known that TGF-β enhances wound healing process and bone formation [42,43]. This molecule acts as an autocrine tumour suppressor [44,45] and may function as a repressor of atherosclerosis [46,47]. TGF-β1 protects tissue cells from reperfusion injury via inhibiting adhesion of leukocytes to the endothelium [48] and/or opposing actions of certain cytokines and superoxide radicals [49]. This molecule is also useful for the treatment of septic shock by inhibiting production of nitric oxide [50].

TGF-β is known to be a potent regulator of immune systems and inflammatory processes, generally functioning as an endogenous immunosuppressant. This molecule represses B cell proliferation and immunoglobulin secretion [51,52], mitogenesis and cytokine production by thymocytes/T lymphocytes [53,54] and the function of natural killer cells [55]. TGF-β inhibits neutrophil and T cell adhesion to the endothelium [56] and deactivates macrophages [57]. This molecule also antagonizes immunomodulatory effects of inflammatory cytokines IL-1, IL-2, IL-3, colony stimulating factors, and interferons α and γ [58].

In several inflammatory diseases, especially in autoimmune disorders, TGF-β exerts anti-inflammatory functions. Systemic administration of TGF-β attenuates the activity of experimental autoimmune diseases including arthritis, encephalomyelitis, insulinitis, Sjögren syndrome and systemic lupus erythematous without obvious unfavourable effects [59–63]. TGF-β also prolongs survival of cardiac allografts in mice [64].

Constitutively expressed endogenous TGF-β1 is essential for maintenance of normal immune and organ functions since targeted disruption of TGF-β1 via homologous recombination induced multiorgan inflammatory diseases and early death in mice [65,66]. Organs isolated from the TGF-β1 null-mutant mice exhibited substantial expression of proinflammatory cytokines including interferon-γ, tumour necrosis factor-α (TNF-α) and macrophage inflammatory protein-1α (MIP-1α) [65], suggesting the critical role of TGF-β1 as an endogenous, housekeeping immunosuppressant.

**TGF-β as a potential ‘defender’ against glomerular injury**

Typical pathological features of glomerulonephritis are proliferation of resident cells and infiltration of leukocytes, especially macrophages. Based on the growth-inhibitory and immunosuppressive actions of TGF-β, it is not surprising that TGF-β has anti-inflammatory effects during the process of glomerular injury. The ‘bright side’ of TGF-β in glomerulonephritis is addressed below (Figure 2).

**TGF-β and glomerular cell proliferation**

In general, TGF-β is an inhibitor of mitogenesis in glomerular cells. Several investigators have reported that, in vitro, externally added TGF-β inhibits mitogenic responses of cultured mesangial, epithelial and endothelial cells [67–69]. Similarly, the proliferative response of isolated normal glomeruli is also inhibited by TGF-β1 [70]. A possible mechanism of the antiproliferative action of TGF-β on glomerular cells is via modulation of the cell-cycle machinery [15,71,72].

In glomerulonephritis, mitogenic mediators elaborated by infiltrating cells play crucial roles. In vitro, macrophage-derived IL-1 stimulates mesangial cells to mitogenesis [73]. Our group recently reported that TGF-β1 is a predominant suppressor of macrophage cytokine synthesis produced by mesangial cells [23]. TGF-β may repress proliferation of glomerular cells, in part, via inhibiting release of cytokines by activated local macrophages.

In in vivo situations, however, the effect of TGF-β on glomerular cell proliferation is still controversial. Isaka et al. have reported that introduction of a TGF-β1 gene into the normal rat glomeruli induced modest mitogenesis of resident cells [35]. Using antisense oligonucleotides, the same group recently showed that suppression of TGF-β1 mRNA in anti-Thy 1 glomerulonephritis did not affect the hypercellularity [40]. Using the similar experimental model and an ex vivo gene transfer approach, we have demonstrated that mitogenesis of glomerular cells could be repressed by introduction of a mutated gene coding for the active form of TGF-β1 [70]. Further studies will be needed to determine the in vivo effect of TGF-β on the glomerular cell proliferation.

**TGF-β and glomerular cell apoptosis**

Apoptosis is an innate programme of cell suicide that is required for removal of unnecessary or damaged cells from bodily structures. In the glomerulus, apoptotic cell death is observed in several types of glomerular disease [74–76] and may play a role in the recovery from proliferative nephritis [77,78]. Currently, how-
ever, information is limited regarding the regulation of apoptosis in the glomerulus. It has been reported that certain growth factors such as insulin-like growth factor-I and II and basic fibroblast growth factor function as survival factors of cultured mesangial cells [79]. In contrast, proinflammatory cytokines IL-1α, IL-1β and TNF-α may act as apoptosis inducers during an early phase of glomerulonephritis [80,81].

TGF-β1 is known as a trigger of apoptosis in several cell types including epithelial cells, hepatocytes, fibroblasts, endothelial cells, osteoclasts and leukocytes [82–86]. We recently found that TGF-β1 induces apoptosis in isolated glomeruli (Y. Ishikawa and M. Kitamura, unpublished data). Cultured mesangial cells produce the active form of TGF-β1 [23,24], and mesangial cells expressing a dominant negative mutant of TGF-β RII are resistant to oxidant-initiated apoptotic death (M. Kitamura, unpublished data). These data suggest that TGF-β1 is an autocrine stimulator of mesangial cell apoptosis. In the acute, reversible model of anti-Thy 1 glomerulonephritis, Baker et al. reported that apoptosis of mesangial cells is the major mechanism for resolution of glomerular hypercellularity [77]. In this experimental model, expression of TGF-β1 is sustained over the recovery phase [9]. This molecule might contribute to the resolution of inflamed glomeruli from hypercellular states via inducing apoptosis.

**TGF-β and homeostasis of ECM**

ECM is known to have profound effects on the regulation of cell behaviour [87]. In the glomerulus, mesangial cells are surrounded by mesangial matrix consisting of basement-membrane-type collagens, glycoproteins and proteoglycans [88]. Using artificial gel matrices reconstituted in vitro, we and others previously reported that three-dimensional ECM inhibited migration, proliferation and expression of α-smooth muscle actin in cultured mesangial cells [89–92]. A gene transfection study explored that mesangial cells with aberrant matrix-degrading activity exhibited accelerated mitogenesis and migration in ECM [93]. This line of evidence implies that the mesangial matrix controls the behaviour of mesangial cells and maintains their differentiated phenotype in the glomerulus. This hypothesis has been further supported by our recent findings [92,94]. Prolonged culture of mesangial cells forms nodular structures composed of cells and surrounding extracellular matrix, that mimics the situation in the glomerular mesangium. Incorporation of mesangial cells into this 'natural' matrix structure allows for transition of the cellular phenotype toward deactivated and differentiation [94]. Interestingly, the differentiated phenotype of mesangial cells in nodules was correlated with upregulation of TGF-β1 mRNA [94]. Basal levels of glomerular TGF-β1, an important regulator of ECM homeostasis [95], may participate in the maintenance of ECM structures and thereby could contribute to the maintenance of differentiated phenotypes of glomerular cells.

Generally, TGF-β upregulates production of ECM and proteinase inhibitors and downregulates synthesis of proteinases [30]. However, there are some exceptions in this story. Expression of matrix-degrading metalloproteinase-2 (MMP-2; gelatinase A; 72 kDa type IV collagenase) in cultured mesangial cells is enhanced by externally added TGF-β1 or transfection with a TGF-β1 cDNA [70,96]. A similar effect was observed in isolated glomeruli treated with TGF-β1 protein or transferred with a TGF-β1 gene [70]. This upregulation is due to transcriptional activation since TGF-β1 transfectants expressed higher levels of MMP-2 mRNA compared to mock transfectants [70]. Consistent with these *in vitro* and *ex vivo* data, MMP-2 is induced in nephritic glomeruli where TGF-β1 is upregulated [97]. Although the pathophysiological relevance of this observation remains to be determined, the negative contribution of TGF-β1 to degradation of glomerular ECM may not be as simple as has been proposed.

**TGF-β and cytokine responses of the glomerulus**

Prototypic proinflammatory cytokines IL-1 and TNF-α play a central role in various inflammatory processes [98]. In the kidney, IL-1 and TNF-α are involved in several glomerular diseases [99,100]. These cytokines stimulate mesangial cells to mitogenesis and production of inflammatory mediators including cytokines, chemokines, neutral proteinases, bioactive lipids and reactive oxygen/nitrogen species [99,101,102]. These mediators subsequently induce cell proliferation, leukocyte influx and destruction of glomerular structure, leading to progression of injury. TGF-β1 has several actions that counteract against these effects [98,103–105]. We and others previously reported that mesangial cells and/or glomeruli treated with TGF-β1 exhibited depressed responses to IL-1β and TNF-α [70,106–108]. In some particular pathological situations, TGF-β1 could function as a ‘defender’ of the glomerulus by opposing the actions of these proinflammatory cytokines. Preliminary evidence showed that administration of decorin, a natural inhibitor of TGF-β1, into rats subjected to anti-GBM nephritis induced deterioration of the disease; i.e. enhanced glomerular cellularity, accelerated crescent formation, and increased proteinuria [109]. In this experimental model, IL-1 plays a crucial role in the generation of glomerular injury [110,111]. If TGF-β1 has an opposing effect on IL-1 action, the anti-inflammatory potential of TGF-β1 in this nephritis model is raised.

**TGF-β and macrophage function**

One of the most common pathological features of glomerular disease is infiltration of mononuclear cells. These cells are mainly monocytes/macrophages, with T lymphocytes present in smaller numbers [112]. Several investigators have disclosed a link between macrophage infiltration and histological damage in the glomerulus, e.g. mesangial/endothelial proliferation,
matrix accumulation, and crescent formation [113]. These data suggest that glomerular macrophages play a crucial part in the generation of glomerular injury. The pathological importance of macrophages has been investigated more extensively in several experimental glomerulonephritis [113]. Using these models in combination with manipulations for macrophage depletion, a close correlation between macrophage accumulation and onset of glomerular injury has been established.

TGF-β is a prominent 'macrophage deactivator' [114]. This molecule suppresses functions of macrophages at picomolar concentrations [57]. From this viewpoint, TGF-β may act as a potential 'defender' against macrophage-mediated glomerular injury.

Adhesion. Adhesiveness is an important factor that determines retention of macrophages at inflammatory sites. Adhesion also serves as a priming stimulus for functional alteration in monocytes/macrophages; e.g. adhesion promotes differentiation of monocytes to tissue macrophages and induces migration, phagocytosis, respiratory oxidative burst, and expression of certain cytokines and proto-oncogenes [115–119]. These findings indicate that adhesiveness of macrophages within the glomerulus controls macrophage function.

Macrophages use scavenger receptors and complement receptor 3 (CR3; β2 integrin) to adhere to local tissues [120,121]. It has been reported that TGF-β1 downregulates expression of these adhesion receptors in macrophages [122,123]. Consistent with these data, we recently reported that mesangial cell-derived TGF-β1 impairs adhesiveness of macrophages in vitro [24]. Furthermore, compared to adherent cells, detached macrophages showed blunted expression of cytokines in response to lipopolysaccharide (LPS).

In the acute, reversible model of anti-Thy 1 glomerulonephritis in rats, a transient accumulation of monocytes/macrophages is observed within 24 h [124]. The increased number of local macrophages is sustained for up to 7 days and declines thereafter. At day 14, the majority of the inflammatory macrophages disappear from the glomerulus [124], probably by trafficking to draining lymph nodes [125]. In this nephritis model, upregulation of glomerular TGF-β1 is detected from day 4 and continues at least until day 14 [9]. Interestingly, the anti-Thy 1 antibody re-injected at day 14 does not lead to macrophage accumulation [124]. The upregulation of TGF-β1 in this nephritis model is thus correlated not with accumulation but with reduced macrophage retention in the glomerulus.

Cytokine synthesis. Macrophage-derived proinflammatory cytokines play crucial roles in glomerular injury. For example, IL-1, IL-6 and TNF-α are detectable in several types of glomerular disease where macrophages are implicated [100]. These macrophage-derived factors induce cell proliferation, overproduction of ECM, and secretion of inflammatory mediators by glomerular cells and contribute to glomerular injury [73,126]. In this context, inhibition of macrophage cytokine synthesis would be a potential strategy for therapeutic intervention in glomerular disease.

Previous reports have provided evidence that TGF-β inhibits production of IL-1α, TNF-α, lymphotoxin, and interferon-γ by peripheral blood mononuclear cells and peritoneal macrophages [127,128]. We recently found that cultured mesangial cells secrete a factor that strongly inhibits production of IL-1β, IL-6, TNF-α and monocyte chemoattractant protein-1 by activated macrophages [23,129]. Using a specific neutralizing antibody, we have identified that this active entity is TGF-β1.

To further investigate the suppressive action of mesangial cell-derived TGF-β1 on macrophages in vivo, reporter macrophages prestimulated with LPS were transferred into normal rat glomeruli or glomeruli in the regeneration phase of acute glomerulonephritis where mesangial TGF-β1 is upregulated [130,131]. In the normal glomeruli, cytokine-inducible metalloproteinases were markedly induced in resident glomerular cells following the transfer of activated macrophages. In contrast, this induction was repressed in the TGF-β1-expressing, nephritic glomeruli. This result points to the novel potential of glomerular TGF-β1 to suppress macrophage-mediated activation of glomerular cells.

Generation of reactive oxygen metabolites. A body of evidence has suggested pathological roles of reactive oxygen species in a wide range of glomerular injury [132]. The oxygen radical metabolites generated by infiltrating cells and/or resident glomerular cells induce degradation of GBM and inhibit de novo synthesis of heparan sulphate proteoglycans, leading to proteinuria [133]. Oxidants also initiate glomerular expression of chemoattractants of monocytes, reduce the activity of ADPase that inhibits thrombus formation, and thereby contribute to the generation of proliferative glomerulonephritis [133].

In glomerular inflammation, major sources of reactive oxygen species are neutrophils and monocytes/macrophages [133]. These cells exhibit a respiratory burst with marked increase in the generation of reactive oxygen metabolites in response to various stimuli. Prevention of the oxidant production by infiltrating cells would be a potential therapeutic approach to glomerulonephritis.

Enhanced generation of reactive oxygen metabolites has been reported in macrophages isolated from glomeruli subjected to anti-GBM nephritis and anti-Thy 1 glomerulonephritis [134,135]. It is known that TGF-β1 and TGF-β2 strongly inhibit the respiratory burst of macrophages at picomolar concentrations [57]. In both experimental diseases, TGF-β is upregulated in the glomerulus [7]. TGF-β possibly functions as a defender molecule in these pathological circumstances via deactivation of macrophages.

Production of nitric oxide (NO). The l-arginine-NO pathway is involved in the physiological function of many mammalian organs, including the kidney. In the glomerulus, constitutively expressed NO synthase (NOS) is observed. The basal generation of NO parti-
icipates in the regulation of glomerular haemodynamics [136]. In contrast, during glomerulonephritis, the generation of NO increases to picomolar levels via the inducible NO synthase (iNOS) principally expressed in infiltrating macrophages [137]. Increased glomerular production of nitrite has been reported in anti-GBM nephritis, Heymann nephritis, anti-Thy 1 nephritis, and in situ immune complex glomerulonephritis [137].

The pathophysiological role of NO during glomerular injury is controversial. Its potentially beneficial actions include inhibition of thrombosis, scavenging of superoxide, repression of cytokine expression and inhibition of mesangial cell proliferation [138–141]. On the other hand, a high concentration of NO is cytotoxic, and macrophage-derived NO possibly contributes to glomerular injury [137]. Indeed, several reports demonstrated that administration with NO inhibitors attenuates macrophage-mediated glomerular injury including autoimmune glomerulonephritis and anti-Thy 1 nephritis [142,143].

Several endogenous mediators function as inhibitors of iNOS expression in macrophages. Those include IL-4, IL-10 and TGF-β family of molecules [144]. It is well known that TGF-β1, -β2 and -β3 inhibit generation of NO by activated macrophages [145]. TGF-β1 inhibits iNOS expression at multiple levels; i.e. it decreases stability of iNOS mRNA, reduces translation of the mRNA, and increases degradation of iNOS protein [146]. In certain pathological conditions, locally produced TGF-β may act against the NO-mediated glomerular injury.

Unanswered questions

Accumulative data have proposed a role for TGF-β in glomerular injury, especially in the generation of glomerulosclerosis. However, this is largely dependent on histology-based studies to detect TGF-β expression in affected glomeruli. Since expression of certain molecule in disease sites does not directly mean its pathological significance, more definitive evidence is required to determine the role of TGF-β. For this purpose, gene transfer strategies would provide useful tools [147,148]. However, current in vivo gene transfer methods only allow for transient expression of transgenes [149,150], and long-term action of TGF-β has not been tested in vivo. It is necessary to clarify whether or not sustained, local expression of TGF-β1 is sufficient for the generation of irreversible glomerulosclerosis and scarring.

A transgenic approach has given one answer to this question. Sanderson et al. generated transgenic mice that express TGF-β1 in the liver. These mice developed progressive glomerulosclerosis, leading to chronic renal failure [41]. However, this is not an appropriate model to examine the local, direct action of TGF-β1 in the glomerulus because; (i) the levels of circulating mature TGF-β were extremely high, and (ii) massive deposition of immunoglobulins was observed in the glomerulus [151]. The glomerulosclerosis observed in this model may be an outcome of immune-complex-mediated glomerular injury.

By combining an experimental model of glomerulonephritis with manipulations to reduce TGF-β activity in vivo, several studies have suggested the pathogenic contribution of TGF-β [36–40]. However, it should be pointed out that all these studies utilized the acute model of anti-Thy 1 glomerulonephritis in the rat. This experimental model mimics mesangial proliferative glomerulonephritis in human, but the pathological changes are transient and reversible. After several weeks, the lesions spontaneously disappear without irreversible sclerosis or scarring [152,153]. This is obviously different from the human counterpart. To determine whether inactivation of TGF-β provides a general therapeutic strategy for progressive glomerulosclerosis, two-shot models of anti-Thy 1 nephritis [22,124] or other chronic experimental diseases must be tested.

In the acute, reversible model of anti-Thy 1 glomerulonephritis, expression of TGF-β1 is maintained during the reconstruction of the normal glomerulus [9]. Since, generally, TGF-β plays an important role in the repair of injured tissues [42], this molecule could contribute to the repair of affected glomeruli in this model. Indeed, in vivo inactivation of TGF-β1 attenuates ECM accumulation in the glomerulus, whereas it disturbs repair of mesangiolysis caused by anti-Thy 1 antibodies (Dr Seiya Okuda, personal communication). The role of TGF-β1 in the repairing process of the glomerulus should be investigated further in a novel context.

As noted above, TGF-β is a prominent macrophage deactivator that inhibits production of potentially injurious mediators including cytokines and reactive oxygen/nitrogen species. Therapeutic utility of TGF-β should therefore be tested in certain types of glomerular injury, especially in macrophage-mediated glomerular diseases. Autoimmune-associated glomerular disease is another target of therapeutic intervention via TGF-β. It has been reported that intramuscular injection of a TGF-β1 cDNA suppressed production of autoantibodies in lupus-prone mice [63]. Using the same gene transfer technique, Raz et al. reported that long-term treatment of murine systemic lupus erythematosus with a TGF-β1 gene prolonged survival, improved renal function, and suppressed glomerular inflammation [154].

In this review, we did not emphasize the effect of TGF-β on functions of infiltrating cells other than macrophages. However, this molecule may affect local accumulation and activity of other leukocytes, including neutrophils, another important player in the acute phase of glomerular inflammation [155]. It has been reported that TGF-β inhibits endothelial production of IL-8, a neutrophil chemoattractive/activating factor [156]. Furthermore TGF-β suppresses both adhesiveness of neutrophils to the endothelium and their transmigration into the extravascular space via inhibiting the endothelial expression of E-selectin and/or IL-8 [156,157]. Since infiltration of neutrophils is an essential event for certain glomerular inflammation, TGF-β...
may exert its anti-inflammatory action, in part, via inhibiting accumulation of neutrophils.

The data from TGF-β1 knockout mice have elucidated the physiological importance of TGF-β [65,66]. The mice in which TGF-β1 gene was abrogated via the homologous recombination technique showed multiorgan inflammation, including the kidney. Since expression of TGF-β1 is observed in the normal glomerulus and renal tubules [4,9,19–21,158], its physiological function in the kidney needs to be addressed further.

Currently, the majority of the studies focus on the role of TGF-β1 but not on other isoforms. It is generally believed that the biological actions of TGF-β1, -β2 and -β3 are almost identical, but in some situations, each isoform may have different biological actions [17,18,159]. For example, excessive TGF-β1 and -β2 enhance scarring of wounds whereas TGF-β3 reduces scar formation in the skin [17]. The pathological role of TGF-β2 and β3 in the glomerulus is another important issue to be investigated in the future. In addition, roles of other members of the TGF-β superfamily of molecules, such as bone morphogenetic proteins (BMPs), in the glomerular pathophysiology should be evaluated further. Recent studies have elucidated the crucial role of BMP-7 in glomerulogenesis [160] and in the maintenance of glomerular function during the course of renal disease [161].

Summary

TGF-β has been considered as the key regulator in the generation of glomerulosclerosis. Despite abundant descriptive data, it still remains undetermined whether sustained, local expression of TGF-β leads to irreversible glomerulosclerosis. There is no doubt that TGF-β stimulates ECM production in the glomerulus, but this molecule has several anti-inflammatory properties as well. Towards a better understanding of the pathogenesis of glomerulonephritis and for the development of novel and efficient therapeutic interventions, extensive efforts should be made to clarify the 'bright side' of TGF-β as well as its 'dark side' in individual experimental and human diseases, focusing especially on the concentration and the time-point at which its anti-inflammatory properties spill over into its proscelerotic actions.

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