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TGFβ-1 and the development of chronic graft nephropathy: relative roles of gene, mRNA and protein

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ABSTRACT

The exact relationship between transforming growth factor beta-1 (TGFβ-1) and the development of chronic graft nephropathy remains uncertain; however, it would appear that TGFβ-1 is up-regulated at the protein and mRNA levels during the first year following cadaveric renal transplantation and the effect of 'high producer' gene polymorphisms may also be important. This up-regulation of TGFβ-1 in plasma may provide a novel. non-invasive means of identifying early fibrotic damage before it becomes clinically apparent thus allowing an opportunity for intervention for grafts that may otherwise fail.

KEYWORDS

TGFβ-1 – Chronic graft nephropathy – Renal transplantation

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The short-term results of renal transplantation have improved steadily over the past two decades, this being attributable to a fall in acute rejection episodes and infectious deaths. However, the annual attrition rate after the first year has changed very little and over 25% of grafts are still being lost between postoperative years 1–5 in the UK. Data from the UK Transplant Support Service Authority clearly indicates that chronic graft nephropathy (CGN) is the main cause of graft failure.[1]

Chronic graft nephropathy

CGN is defined as a progressive deterioration of graft function, in the absence of any other disorder and after confirmation of the diagnosis by pathological study.[2] The histopathological changes of CGN were defined as part of the Banff working classification of renal transplant pathology that aimed to standardise pathology interpretation and reporting in the transplant literature globally.[3] These features include a glomerulopathy together with interstitial fibrosis, tubular atrophy, and proliferation and thickening of the vascular intima – mainly in the cortical arteries (Fig. 1).

The aetiology of CGN remains uncertain; however, it is believed that a number of immunological and non-immunological factors may play a role in the pathogenesis of the condition (Table 1). There is currently no therapy for established CGN and efforts are directed at treating risk factors for the condition in the hope that this may prevent its development.

Relatively few studies have attempted to define accurately the prevalence of CGN. A study from Nottingham identified CGN in 13% of all grafts, found it to be the cause of 18% of all graft losses, and 40% of losses occurred after 6 months.[4] In relation to CGN as a cause of long-term graft failure, a Boston group found CGN to be the cause of 81% of graft losses occurring after 5 years.[5]

The prevalence of CGN is difficult to assess due to a lack, until recently, of a standardised histological definition for CGN, a paucity of biopsy confirmation of suspected diagnosis in most studies and a relatively short duration of follow-up on many clinical trials. In addition, these assessments may not be a realistic reflection of the true prevalence of the condition as such studies do not depend on a fixed follow-up period but rather rely on *ad-hoc* presentation in order to obtain biopsy material. The clinical prevalence is likely to be only the tip of the iceberg as studies in which protocol biopsies are utilised to determine prevalence paint a different picture.

Protocol biopsies

In a study from Finland in which protocol biopsies were performed at 2 years, the prevalence of CGN was determined to be in the order of 40% in patients with well-functioning grafts.[6] However, when follow-up of the same

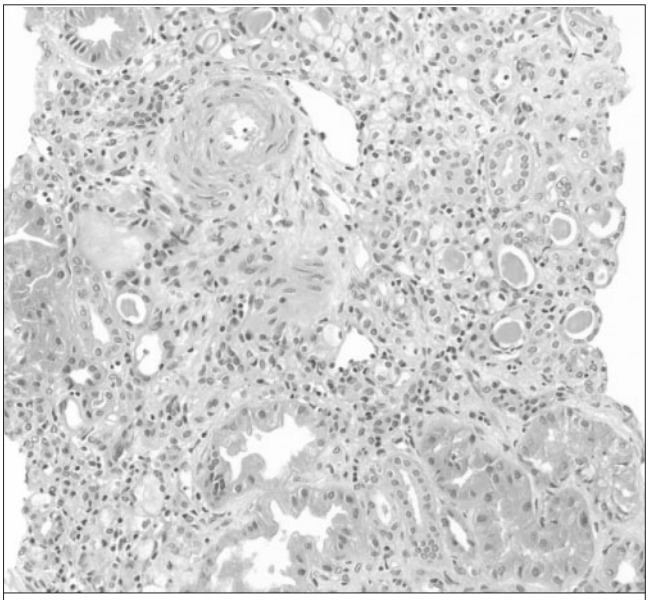

Figure 1 High-power micrograph of a kidney transplant biopsy exhibiting CGN demonstrating the typical appearances of tubular atrophy.

cohort was extended to 5 years, 30% had developed clinical CGN. Similarly, a recent report from the US tacrolimus multicentre study noted a CGN prevalence of 62% in patients receiving tacrolimus and 72% in individuals receiving cyclosporin-based immunosuppression.[7] The recent appreciation that CGN is now far more prevalent than previously believed has lead some to question whether chronic allograft nephropathy is an inevitable outcome of renal transplantation.

In addition to identifying subclinical CGN, the performance of biopsies early in the postoperative phase has also demonstrated a high prevalence of subclinical acute rejection (SCAR). The centre with the largest experience with protocol biopsies is the Winnipeg group headed by Rush.[8] Their first paper showed a 30% incidence of grade 1 acute rejection in early protocol biopsies. In a follow-up study, a group of 72 patients were randomised to protocol biopsy or no biopsy at 1, 2, and 3 months following transplantation.[9] Both groups were biopsied at 6 months and followed clinically for 2 years. Subclinical rejection was identified in 80% of patients in at least one of the three protocol biopsies. All episodes of subclinical rejection were treated with corticosteroid leading to a reduced number of subsequent acute rejection episodes and improved graft function at 2 years.

Transforming growth factor beta-1

One molecule, which has attracted attention as a potentially important factor in the pathogenesis of the fibrosing process seen in CGN, is the archetypal profibrotic cytokine transforming growth factor beta-1 (TGFβ-1).

Biology and biochemistry of TGFβ-1

The name TGFβ-1 is derived from the observation that TGFβ-1 stimulates cultured cells to grow in a manner similar to cells that have been virally transformed. Since its discovery in 1981 by two independent laboratories,[10,11] interest in this complex pleiotropic molecule has grown exponentially.

TGFβ-1 is a key member of a large family of polyfunctional molecules, which show diverse biological activities. TGFβ-1 is one of three mammalian isoforms, the others being TGFβ-2 and TGFβ-3. All 3 mammalian isoforms are homodimers in their biologically active forms[12] and show a high level of sequence conservation. TGFβ-1 is by far the most abundant and most heavily investigated of the mammalian TGF isoforms. TGFβ-1 is a 25-kDa homodimer peptide synthesised as the carboxyl-terminal 112 amino acids of a 390 amino acid precursor. The gene encoding for TGFβ-1 has been isolated to chromosome 19q13 comprises 7 exons and 9 introns and produces mRNA of 2.5 kb.[15]

Table 1 Non-immunological and immunological risk factors for the development of CGN

Non-immunological	Immunological
Donor age/sex/weight	Acute rejection
Brain death	Subclinical acute rejection
Cold ischaemic time	HLA matching
Ischaemia–reperfusion injury	Panel reactive antibodies > 50%
Delayed graft function	T-cell infiltration/activation
Hyperlipidaemia	Macrophage infiltration/activation
Hypertension	Inadequate immunosuppression
Cytomegalovirus infection	Non-compliance
Drug nephrotoxicity	

Functions of TGFβ-1

Expression of TGFβ-1 is wide-spread, the molecule having been observed in a variety of embryonic and adult tissues some of the most important sources being platelets, bone, placenta, lung and kidney.[14] The importance of TGFβ-1 to human biochemistry is indicated by the fact that is has been found to play a key role in several important processes including embryogenesis, bone development, angiogenesis, tumorogenesis, wound healing, fibrosis and immune modulation.[15,16]

It appears that this intriguing molecule has both 'good' and 'bad' properties and it is the maintenance of a delicate balance between the two that determines whether normal physiology is maintained or whether a downward spiral towards disease is followed. There is increasing evidence for the role of TGFβ-1 in the kidney, in health and disease including non-transplant and transplant disease processes. These properties are summarised in Table 2.

Relationship between TGFβ-1 and the development of CGN

It has been proposed that CGN could simply be regarded as 'over healing' of the renal tissue leading to fibrosis-mediated scar formation. In this regard, it would be logical that TGFβ-1, being a potent profibrogenic cytokine, would play an important role in the pathogenesis of CGN. There is now a considerable body of evidence relating TGFβ-1 expression to many of the known risk factors for CGN including acute rejection, ischaemia–reperfusion injury, immunosuppressive agents, drug-induced nephrotoxicity, hyperlipidaemia and hypertension as well as to CGN directly. Due to textual limitations, only the studies in which direct links have been sought between TGFβ-1 and CGN will be discussed in this review.

Experimental evidence for the role of TGFβ-1 in CGN

Most experimental studies have used murine models of CGN based upon transplantation between different rodent strains such as the Fisher 344 to Lewis combination. These studies are unanimous in showing that TGFβ-1 mRNA expression, as determined by Northern blotting, is increased in the early post-transplantation period and that the onset of CGN is associated with a further increase in TGFβ-1 mRNA expression. With the addition of immunocytochemistry, these studies have confirmed that the TGFβ-1 is predominantly distributed within the glomeruli.

Clinical evidence for the role of TGFβ-1 in CGN

TGFB-1 PROTEIN QUANTIFICATION

Coupes and colleagues from Manchester were the first to note increased expression of TGFβ-1 in renal allografts.[17] They detected active TGFβ-1 in the plasma of 23/61 (38%) cases but failed to detect any differences between patients with and without CGN. They also identified TGFβ-1 in the urine of most of the transplant patients. More recently, the same group compared active plasma TGFβ-1 levels in cyclosporin (n = 103)

Table 2 Detrimental and beneficial effects of TGFβ-1 on the kidney

Detrimental effects
Stimulatory effects of TGFβ-1
Increased transcription of extracellular matrix proteins and their receptors
Up-regulation of proteinase inhibitors
Induction of glomerular expression of integrins
Inhibitory effects of TGFβ-1
Decreased production of matrix-degrading proteinases
Beneficial effects
Stimulatory effects of TGFβ-1
Increased apoptosis within endothelial cells
Differentiation of endothelial and mesangial cells
Inhibitory effects of TGFβ-1
Reduced mitosis within mesangial, epithelial and endothelial cells
Reduced cytokine production by mesangial cells, glomeruli and by infiltrating lymphocytes and macrophages
Reduced adhesion of lymphocytes and macrophages to the endothelium
Reduced oxidant production by macrophages
Reduced nitric oxide production by macrophages

and tacrolimus (n = 26) treated patients.[18] They identified active TGFβ-1 in only 2.2% of those receiving tacrolimus but 16.7% of patients on cyclosporin therapy. At 2 years, 19 of the cyclosporin group had developed CGN though this complication was not seen in the tacrolimus group.

TGFB-1 PROTEIN LOCALISATION

Horvath and colleagues, using immunohistochemistry, identified TGFβ-1 within the glomeruli, blood vessels and inflammatory cells of patients with CGN and reduced expression in the tubules in comparison with the control population.[19] Shihab *et al.*[20] utilised immunofluorescence to investigate expression of TGFβ-1 in 10 transplant patients taking cyclosporin (5 AR, 5 CGN) and 20 controls (10 normal, 10 renal disease). They demonstrated increased immunostaining for TGFβ-1 in the tubulo-interstitium and the glomeruli in both AR and CGN whereas TGFβ-1 was rarely seen in the control patients. Pilmore and colleagues examined the distribution of TGFβ-1 in relation to macrophage and myofibroblast cell populations and noted that expression co-localised thus suggesting an origin for the TGFβ-1.[21] Iñigo *et al.*[22] determined the intensity of staining for TGFβ-1 protein in 30 transplant biopsies (16 AR and 14 CGN) and found the tubulointerstitial expression to be more intense in the CGN group. Cuhaci and colleagues[23] determined the relative level of TGFβ-1 expression in 40 patients with CGN receiving cyclosporin-based immunosuppression and found 72% had a high level of CGN expression. Furthermore, they found that the intensity of TGFβ-1 immunostaining correlated with the rate of decline in renal function.

TGFB-1 MESSAGE DETECTION

Gaciong and co-workers[24] performed reverse transcription polymerase chain reaction (RT-PCR) on kidney biopsies from patients with CGN (n = 12), long-term transplant survivors (n = 8) and controls who had undergone nephrectomy for adenocarcinoma (n = 7). TGFβ-1 mRNA levels in patients with CGN were significantly greater than for controls with stable renal function. Similar results were reported by Sharma and colleagues[25] who investigated the expression of TGFβ-1 using RT-PCR in 127 core needle biopsies taken from 107 patients with graft dysfunction. TGFβ-1 mRNA was identified in 69 (54%) of biopsies of which 43 had a histological diagnosis of AR and 26 displayed evidence of CGN. There was a strong positive correlation between TGFβ-1 and CGN (P = 0.01) and with interstitial fibrosis (P = 0.03). However, not all investigators agree with this relationship. Vuillemin and colleagues using similar methodology, found no difference in TGFβ-1 mRNA expression between the CGN and normal patient groups.[26]

Nicholson and colleagues[27] used a novel RT-PCR technique in which single glomeruli were isolated from a biopsy to determine TGFβ-1 mRNA expression and to correlate the findings with collagen expression. The group identified a positive correlation between levels of cortical collagen III staining and glomerular TGFβ-1 expression and statistically significant relationships between TGFβ-1 mRNA and mRNA for TIMP I, TIMP II, MMP-2 and tenascin. In a subsequent study, the Leicester group determined TGFβ-1 mRNA expression in 51 patients randomised to either tacrolimus- (n = 29) or cyclosporin- (n = 22) based immunosuppression but did not identify a significant difference in TGFβ-1 expression between the two treatment groups.[28]

TGFB-1 GENE POLYMORPHISM IDENTIFICATION

The recent use of PCR-SSCP (single strand conformation polymorphism) to determine the presence of polymorphisms within the TGFβ-1 gene has identified seven variations, three of which lead to a change in the amino acid encoded. Population studies have defined the prevalence of each polymorphism within the general population and genotyping studies have then allowed determination of the role of each polymorphism in the pathogenesis of CGN. Several studies have investigated the relationship between TGFβ-1 polymorphisms and CGN leading to claims of associations between various polymorphisms although the same polymorphisms are not implicated by the different groups.[29,30] However, not all studies are in agreement and indeed claim no relationship between TGFβ-1 and gene polymorphisms.[31,32]

Conclusions

The current literature would suggest that the use of protocol biopsies allows early diagnosis of subclinical rejection and provides a window of opportunity for treatment which may prevent progress to CGN. The exact relationship between TGFβ-1 and the development of CGN remains uncertain; however, it would appear that TGFβ-1 is up-regulated at the protein and mRNA levels during the first year following cadaveric renal transplantation and the effect of 'high producer' gene polymorphisms may also be important. This up-regulation of TGFβ-1 in plasma may provide a novel, non-invasive means of identifying early fibrotic damage before it becomes clinically apparent thus allowing an opportunity for intervention for grafts that may otherwise fail.

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