Transpl Int (2000) 13 [Suppl 1]: S295–S298

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Active TGF-β_1 Expression in kidney transplantation: a comparative study of Cyclosporin-A (CyA) and tacrolimus (FK506)

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Abstract Chronic rejection is a major cause of graft dysfunction following kidney transplantation. This fibroproliferative disease may be promoted by overproduction of transforming growth factor beta (TGF-β). Previous studies have suggested that cyclosporin-A (CyA) might increase production of this growth factor. The current study was designed to measure the expression of TGF-β in renal transplant biopsies from patients immunosuppressed with either CyA or tacrolimus. Paraffin-embedded renal biopsies were sectioned, dewaxed and incubated with primary antibody against active TGF-β_1 antibody. After washing, the sections were treated with secondary antibody conjugated with fluorescein isothiocyanate (FITC). In each case the sections were assessed by semi-quantitative scanning laser confocal microscopy. Biopsies from patients receiving CyA expressed significantly more active TGF-β_1 than biopsies from patients receiving tacrolimus ($P < 0.0001$, Mann-Whitney test). The increased level of active TGF-β_1 expression in renal biopsies of patients receiving CyA may indicate a mechanism of chronic rejection.

Key words TGFβ · Cyclosporin-A (CyA) · tacrolimus (FK506) · Chronic rejection · Kidney transplantation

Introduction

Fibrosis is a pathological process that results from some form of injury and can occur in any organ. When fibrosis develops it typically causes tissue dysfunction and ultimately organ failure. The biological cause of fibrosis is the accumulation of excess amounts of extracellular matrix within a tissue. Fibrosis within glomeruli and between tubules (tubulointerstitial fibrosis) causes the progressive loss of renal function. It is believed that this pathological fibrosis represents an excess of the normal repair process that follows tissue injury, and accordingly fibrosis has been termed the 'dark side' of tissue repair [4]. The idea that normal tissue repair and pathological fibrosis are closely related is based on the evidence that both involve similar biological processes that are regulated by the same groups of molecules [3].

TGF-β is a cytokine or signalling molecule, whose potent fibrogenic properties are derived from its multiple actions in tissue repair [17]. It is a prototypical, multifactorial cytokine, being synthesised as a 391 amino acid precursor molecule which is cleaved to yield a peptide fragment, and a 112 amino acid subunit [18]. Active TGF-β_1 is a 25-kDa dimer protein composed of two subunits linked by a disulphide bond. It binds to at least three membrane receptors (type I, II and III) that exist on virtually all cells. Sustained or excessive production of TGF-β is a key molecular mediator of tissue fibrosis including fibrosis of the kidney [9]. TGF-β is unique in its ability to stimulate strongly the deposition of extracellular matrix by increasing the synthesis of most matrix molecules including fibronectin, collagen, and proteoglycans. At the same time it blocks the degradation of matrix by inhibiting the secretion of proteases by protease inhibitors, and it also modulates the expression of

integrin matrix receptors on cells to facilitate cell matrix adhesion and matrix deposition. Finally TGF-β autoinduces its own production, which greatly amplifies its biological actions [2].

Cyclosporin A (CyA), a potent immunosuppressant, has improved graft survival in clinical organ transplantation. Cyclophilin is a cellular receptor for CyA [8–10], which when bound to CyA, inactivates calcineurin [5–7], and thereby inhibits IL2 production. This is currently considered to be the primary mechanism for the immunosuppressive efficacy of CyA [16–19]. Tacrolimus, a much newer immunosuppressant, has been used in primary treatment against allograft rejection and also for immunological rescue of rejection not responding to CyA and steroid therapy [13–22]. It is said to be 100 times more potent than CyA in vitro [21]. Tacrolimus also inhibits calcium-dependent signal transduction pathways in lymphocytes through interaction with a specific cytoplasmic immunophilin [11–15]. The observation that CyA enhances the production of functionally active TGF-β protein suggests a possibility of cell growth inhibition and tissue fibrogenesis by a TGF-β dependent mechanism [1]. Such an association between CyA and fibrosis, if it exists, would have a significant negative effect on long-term graft outcome. If differences were found between the varying immunosuppressive agents with respect to induction of fibrosis, this could have a profound effect on long-term transplant management.

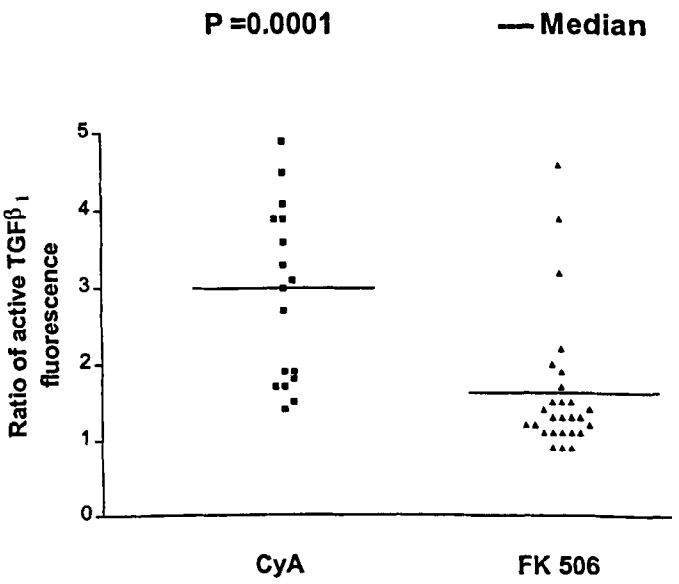

Fig. 1 Ratio of mean fluorescence of active TGF-β_1 of biopsies from patients receiving CyA or tacrolimus

Materials and methods

Tissue samples

Diagnostic transplant renal biopsies were divided into two groups on the basis of primary immunosuppression with CyA or tacrolimus. The two groups were selected to be close to each other with regard to A, B, RD mismatching, donor age, and cold ischaemic time.

Immunofluorescence for active TGF-β_1

Biopsies from patients immunosuppressed with CyA (n = 17) and tacrolimus (n = 25) were selected. The immunofluorescence procedure and subsequent confocal analysis was based on that of Robertson et al. [14]. Sections were dewaxed and rehydrated through absolute and 95% alcohol, washed in water and Tris-buffered saline (TBS), pH 7.6. The sections were then incubated with blocking serum composed of normal human serum 1/15, and normal rabbit serum 1/5, in TBS, for 2 h at 4 °C. The primary antibody, chicken anti-human TGF-β_1 (R&D Systems) at 1/100 in blocking serum, was added to sections which were incubated overnight at 4 °C. Subsequently, sections were washed in TBS for 10 min and incubated with secondary antibody in the form of rabbit anti-chicken IgG, preadsorbed with human serum proteins and conjugated to fluorescein isothiocyanate (FITC) at 1/400 in blocking serum for 2 h at 37 °C. Following a further 10-min wash in TBS, sections were mounted in fluorescence mounting medium (DAKO).

Matched negative control sections for active TGF-β_1 were prepared, in which primary antibody was replaced with secondary antibody alone or primary antibody alone. Sections were analysed by semi-quantitative scanning laser confocal microscopy. Data were expressed as the ratio of mean fluorescence over the selected area of experimentally stained tissue (excluding the tubular lumen) to the corresponding value in control sections.

Results

Biopsies from patients receiving CyA showed a significantly greater mean fluorescence ratio of active TGF-β_1 to the control (median 3, and range 1.4–4.9) when compared with biopsies from patients receiving tacrolimus (median 1.3, range 0.9–4.6; $P < 0.0001$, Mann-Whitney test) (Fig. 1). Figure 2 shows representative sections of transplant renal biopsies stained for active TGF-β.

Discussion

The latent form of TGF-β is a high molecular weight complex, in which TGF-β is non-covalently bound to another dimer peptide, the latency-associated peptide (LAP). This is cleaved to produce the active TGF-β. TGF-β is a cytokine or signalling molecule, whose potent fibrogenic properties are derived from its multiple actions in tissue repair. The fibrogenic potential of TGF-β is revealed if repeated injections of high doses of recombinant TGF-β are administered into a rat model. Serious systemic effects, including marked fibrosis in kidney, liver, lung, and injection sites are produced in consequence [20]. The role of TGF-β in pulmonary fi-

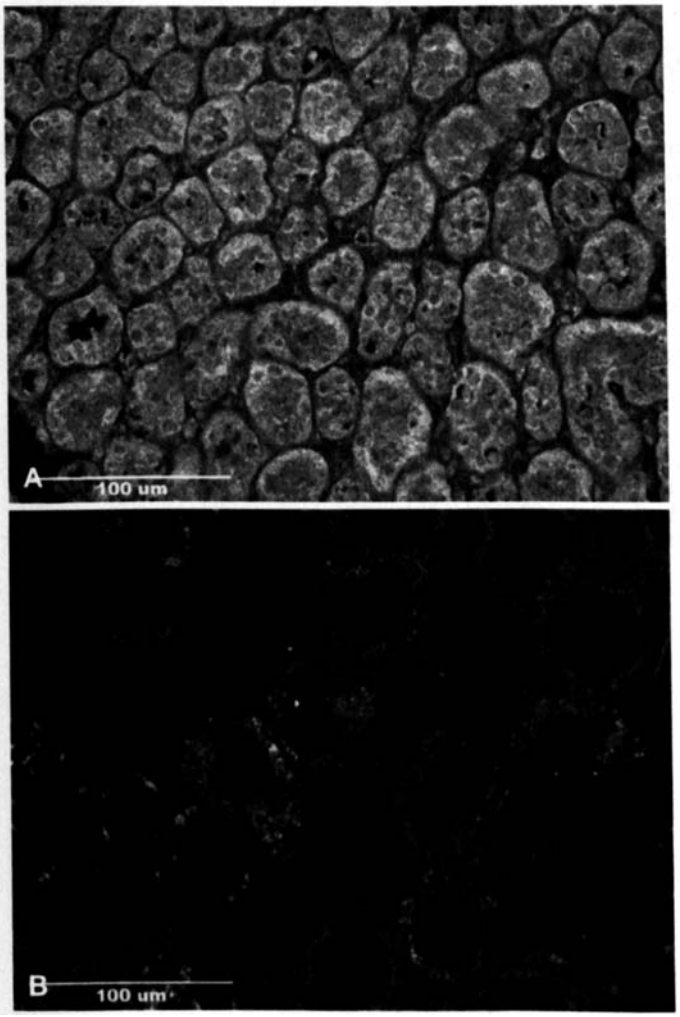

Fig. 2 A, B Immunofluorescence detection of active TGF-β1 on transplant renal biopsy sections by scanning laser confocal microscopy. **A** Section from a patient treated with CyA. **B** Section from a patient treated with tacrolimus

brosis is clear in that it is increased in alveolar walls at the sites at which extracellular matrix has accumulated [12]. Broncheoalveolar cells obtained by lavage from patients with autoimmune disease and lung fibrosis contained ten times more TGF-β_1 mRNA than similar cells obtained from normal subjects [6]. This would suggest that even though the role of TGF-β in post-transplant obliterative broncheolitis is unproven, it is probably a detrimental one. Immunosuppressive drugs that induce TGF-β expression in high levels may be responsible for graft fibrosis and chronic rejection in the long term.

The association of increased expression of active TGF-β_1 in transplant renal biopsies in patients receiving CyA as opposed to tacrolimus is potentially an important finding. CyA may directly increase the expression of TGF-β_1 and be involved in its activation; however, the renal transplant biopsies were diagnostic biopsies and were taken when there were events that led to the need for the biopsy. Therefore, these differences in active TGF-β_1 could reflect the events occurring in the graft at that time rather than a primary association with the immunosuppressive drugs.

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