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Differential effects of cyclosporin A and tacrolimus on the production of TGF-β: implications for the development of obliterative bronchiolitis after lung transplantation

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Abstract The development of obliterative bronchiolitis is a common cause for failure of lung allografts. Fibrinogenesis can occur for a number of different reasons but some groups have suggested that cyclosporin A (CsA) and tacrolimus (FK506) have different effects on the cytokines which induce fibrinogenesis. We investigated the effect of tacrolimus and CsA in tissue culture and found that there was indeed a negative effect on human lung small airway epithelial cell proliferation by recombinant transforming growth factor-β (TGF-β), which was reversed by anti-TGF-β. The same effect was seen with CsA at immunosuppressive concentrations, which was also reversed by anti-TGF-β, whereas no such inhibition was seen with tacrolimus at immunosuppressive doses unless high concentrations were used. Free TGF-β was confirmed as being elevated in the supernatant of cell culture wells with standard dose CsA as opposed to low dose CsA or tacrolimus using an ELISA assay.

Key words Immunosuppression · Obliterative bronchiolitis · Transforming growth factor

Introduction

Three isoforms of transforming growth factor-β (TGF-β) are produced by mammalian cells, with TGF-β_1 being the most well studied. The biologically active form of TGF-β_1 is a 25-kDa dimer consisting of two identical 112-amino acid subunits joined by disulphide bonds. The TGF-β family is known to have many physiological functions, including wound repair and modulation of the immune system; many of these are mediated by regulation of the secretion of a range of additional cytokines. In vitro studies have shown that low concentration of TGF-β are chemotactic for fibroblasts and stimulate the deposition of extracellular matrix components such as collagen, fibronectin and proteoglycans.

A variety of studies has identified TGF-β as a powerful fibrogenic cytokine [2]. Indeed, intravenous injection of TGF-β rapidly results in systemic fibrosis, with the liver, kidney and lung being most severely affected. The correlation between elevated TGF-β levels and increased deposition of extracellular matrix is particularly clear for models of pulmonary fibrosis.

Several groups have shown that cyclosporin A (CsA) can augment the production of TGF-β by epithelial cells [3]. It is possible that this elevated production of TGF-β enhances development of the fibrotic disease obliterative bronchiolitis following lung transplantation.

This study was designed to determine whether CsA and tacrolimus have a similar effect on TGF-β production by lung tissues.

Materials and methods

Human small airway epithelial cells (SAEC) were supplied by Clonetics and expanded in serum-free medium. The cells were characterised by immunofluorescence flow cytometry and shown to be free from significant contamination by endothelial cells (CD31) or leucocytes (CD45); the cells expressed the epithelial marker cytokeratin 19. The cells were propagated in serum-free medium and they produced a doubling time of 30 h.

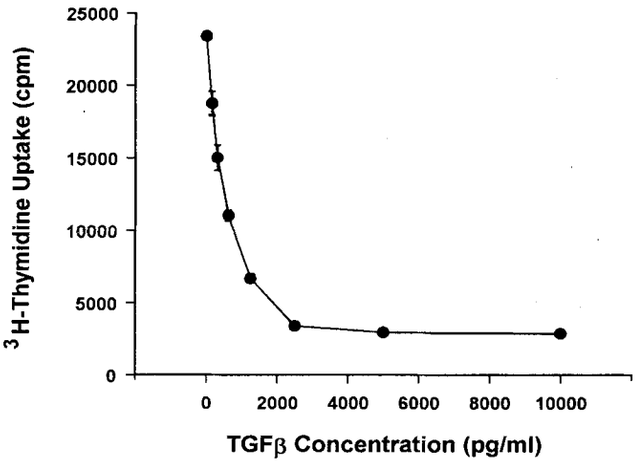

Fig. 1 Inhibition of human small airway epithelial cell (SAEC) proliferation by addition of recombinant transforming growth factor-β_1 (*TGF-β_1*). The ID_{50} for this inhibition is 600 pg/ml

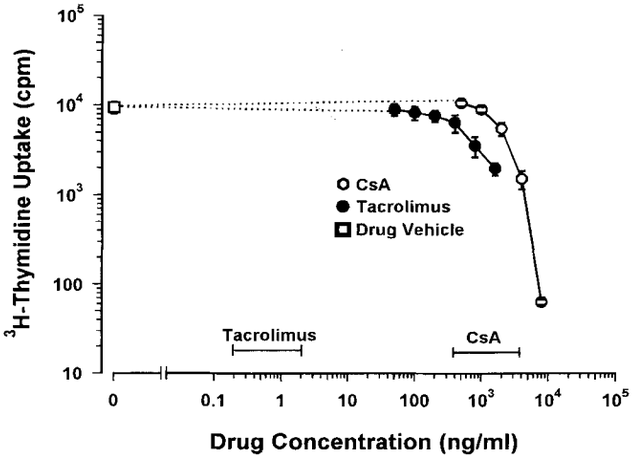

Fig. 2 Inhibition of SAEC proliferation by immunosuppressive drugs. Immunosuppressive concentrations of cyclosporin A (*CsA*) and tacrolimus in tissue culture are indicated by lines above the *x*-axis

CsA was supplied by Sandoz and tacrolimus was provided by Fujisawa; both drugs were dissolved in ethanol. The immunosuppressive doses of both CsA and tacrolimus was derived from a mixed lymphocytic reaction (MLR) Epstein-Barr virus-transformed B cells as targets. The immunosuppressive dose was defined as that producing a significant reduction of cell proliferation from the plateau of inhibition. Recombinant TGF-β_1, anti-TGF-β and ELISA kits were supplied by R&D Systems Europe.

Cell proliferation was routinely assessed by measurement of incorporated radioactivity after pulsing subconfluent cultures in flat profile 96-well plates with ^{3}H-thymidine for 18 h.

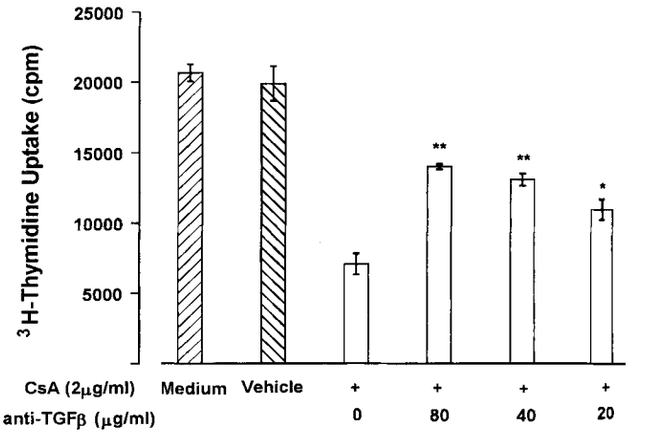

Fig. 3 Anti-TGF-β blocking antibody significantly restored proliferation of SAEC treated with an immunosuppressive concentration of CsA. An irrelevant control antibody had no effect on SAEC proliferation (* $P < 0.05$, ** $P < 0.01$ compared with SAEC proliferation in the absence of anti-TGF-β antibody)

Results

When the human SAEC were cultured with the addition of recombinant TGF-β [1] a significant inhibition of proliferation was encountered (Fig. 1). This was dose dependent with an ID_{50} inhibition of 600 pg/ml.

The same experiment was then repeated replacing recombinant TGF-β with varying doses of CsA and tacrolimus (Fig. 2). The immunosuppressive concentrations of CsA and tacrolimus in tissue culture are indicated by lines above the *x*-axis. It is clear that an immunosuppressive concentration of CsA inhibits SAEC proliferation. An immunosuppressive concentration of tacrolimus has no effect on SAEC proliferation; this drug only inhibited cell division at a concentration between 100 and 1000 times that which blocked lymphoproliferation in a MLR. The effect of CsA was non-toxic as SAEC proliferated normally when returned to drug-free medium.

A polyclonal anti-TGF-β blocking antibody significantly restored proliferation of SAEC treated with an immunosuppressive concentration of CsA (Fig. 3). An irrelevant control antibody had no effect on SAEC proliferation.

Discussion

Treatment of human SAEC with an immunosuppressive concentration of CsA upregulates the production of biologically active TGF-β. This growth factor has well-defined fibrogenic properties and may con-

tribute to the development of the fibrotic changes associated with obliterative bronchiolitis following lung transplantation. The presence of tacrolimus had no effect on the production of TGF-β by the airway epithelial cells. This would suggest that fibrosis may be a greater problem with CsA than with tacrolimus [1].

References

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