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Effect of FK 506 on FK-Binding Protein and Transforming Growth Factor Beta Gene Expression

P. Rao, H. Sun, J. Snyder, J.J. Fung, and T.E. Starzl

FK 506 is a recently discovered immunosuppressive macrolide[1] that has been shown to be significantly more potent than cyclosporine A (CyA) in a variety of in vitro and in vivo assays.[2–5] Like CyA, FK 506 inhibits the transcription of early T-cell activation genes[6] following binding to a cytoplasmic receptor FK-binding protein (FKBP).[7–9]

It has recently been demonstrated that transforming growth factor beta (TGF-β) has the ability to regulate immune functions in vitro,[10,11] and has therefore been used as an immunosuppressant in cardiac and islet allotransplantation.[12–14] It is interesting to speculate that one of the pathways by which immunosuppressants like FK 506 may act is by inducing endogenous TGF-β expression.

MATERIALS AND METHODS

A rat liver transplant model was used to study the effect of FK 506 administration on FKBP and TGF-β gene expression. Livers harvested from ACI donors were orthotopically transplanted in Lewis recipients.[15] Following orthotopic liver transplantation (OLTX), recipients were either treated with 1.28 mg/kg body weight of intramuscular FK 506 for 4 days (n = 15) or left untreated (control, n = 12). Three animals each were killed on postoperative days 3, 6, and 9 in the control group and on days 3, 6, 9, and 12 in the FK 506-treated group. Total cellular RNA was extracted and purified by the method of Chomczynski and Sacchi.[16] For Northern analysis, RNA samples were fractionated on 0.7% agarose, 18% formaldehyde gels and transferred to nitrocellulose paper. Hybridization was carried out at 60°C overnight in Church buffer[17] and a ^{32}P-labeled complementary DNA probe. The filters were washed twice for 20 minutes at room temperature in 1 × SSC (SSC = 150 mmol/L sodium chloride, 15 mmol/L sodium citrate, pH 7), 0.1% sodium dodecyl sulfate and 0.1 SSC, 0.1% sodium dodecyl sulfate at 65°C. The filter was exposed to Kodak X-omat film for 4 days with an intensifying screen at −70°C.

RESULTS

Fig 1 depicts the autoradiographs for FKBP expression. FKBP gene expression was observed in both untreated control, and FK 506-treated recipients on all the days studied.

Fig 2 is the autoradiographs for TGF-β expression. No evidence of TGF-β gene expression was observed in untreated control livers. On the other hand, FK 506-treated livers showed an induction of TGF-β gene expression on days 3 and 6, which tapered off on days 9 and 12 following orthotopic transplantation.

DISCUSSION

Our results demonstrated no association between treatment with FK 506 and FKBP gene expression. Both FKBP and its cyclosporine binding counterpart cyclophilin are ubiquitous immunophilins with rotamase activity.[18,19] It has recently been reported that inhibition of the rotamase activity of FKB following FK 506 binding cannot explain the biologic effects of FK 506 administration.[18] This would appear to confirm the lack of correlation between FK 506 treatment and FKBP expression observed in our study.

TGF-β gene expression was observed as early as the third postoperative day and persisted on the sixth postoperative day, tapering off on days 9 and 12. No evidence of

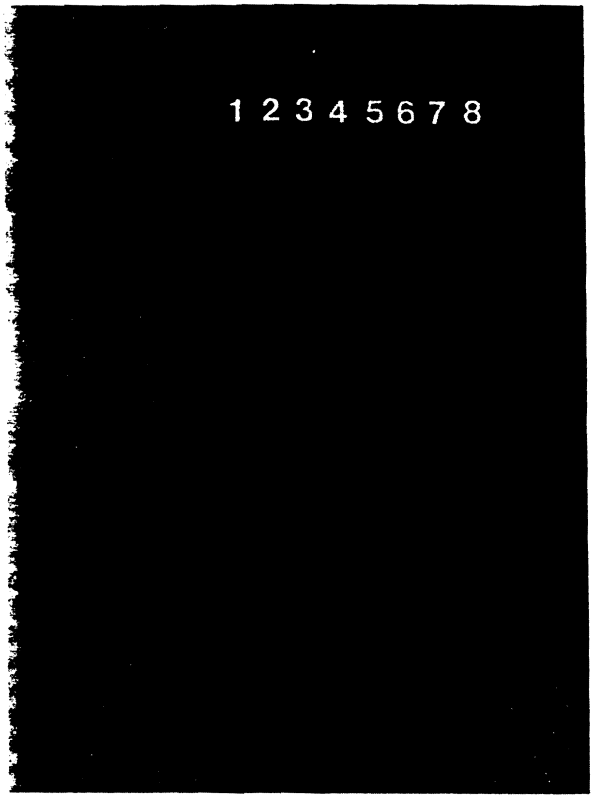

Fig 1. Autoradiograph for FKBP expression. FKBP gene expression was observed in both control (lanes 2–4) and FK 506-treated recipients (lanes 5–8) on all the days studied.

From the Department of Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania.

Address reprint requests to Prakash N. Rao, PhD, Department of Surgery, University of Pittsburgh, Falk Clinic 6E, Pittsburgh, PA 15213.

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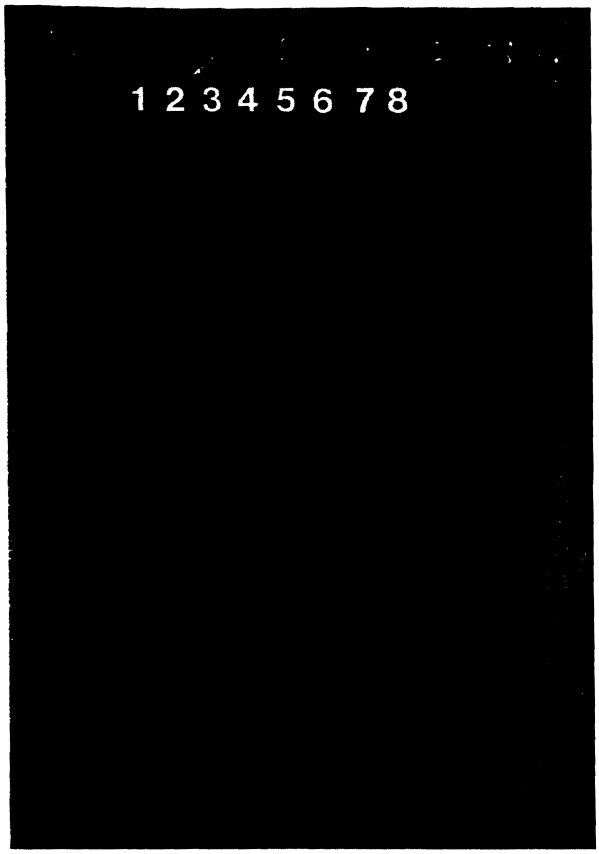

Fig 2. Autoradiograph for TGF-β expression. No evidence of TGF-β gene expression was observed in control livers (lanes 1–3). On the other hand, FK 506-treated livers showed an induction of TGF-β gene expression on days 3 and 6 (lanes 4 and 5), which tapered off on days 9 and 12 (lanes 6 and 7) following OLTx.

TGF-β gene expression was noted in untreated control livers. The immunoregulatory properties of TGF-β include inhibition of cytokine production,[20] cytotoxic T-cell generation,[21] thymocyte proliferation,[22] and T cell/B cell generation.[23] It has been successfully used to prevent allorejection in murine cardiac and islet transplantation[12–15] and in murine models of autoimmune disease.[24,25] It is therefore interesting to speculate that FK 506 may be exerting its immunosuppressant action through the induction of TGF-β.

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