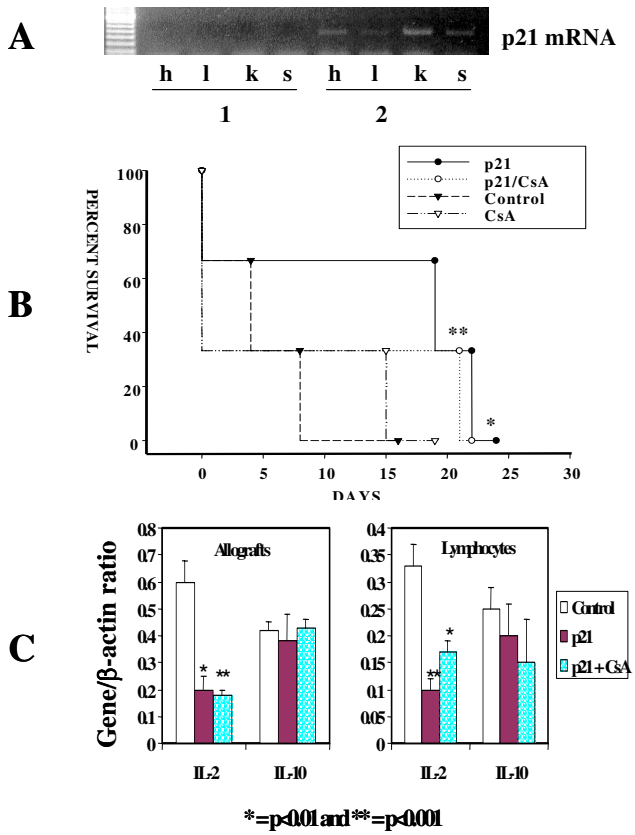

Figure 4

p21WAF1/CIP1 over-expression prolongs allograft survival: **A**: p21 Injection of p21 sense plasmid DNA injected mice (set 2not empty vector plasmid DNA induces p21 mRNA expression in heart (h), liver (l), kidney (k) and spleen (s). **B**: Kaplan-Meyer survival graph for rat cardiac transplant recipients. Significant difference in the survival of allografts in p21WAF1/CIP1 transfected recipients compared to controls (* = $p < 0.04$) and p21WAF1/CIP1 together with CsA (* * = $p < 0.005$) can be seen. **C**: Effect of p21WAF1/CIP1 over-expression and CsA treatment on mRNA expression of IL-2 in lymphocytes and allografts. A significant decreased expression of IL-2 mRNA expression in lymphocytes and allografts compared to controls is shown (* = $p < 0.01$) and (* * = $p < 0.001$).

To confirm that this effect was due to the inhibition of alloimmune activation, we studied the expression mRNA of IL-2 in lymphocytes isolated from spleens and heart allografts. We also examined the expression of IL-10 mRNA in lymphocytes and allografts. The results are shown in the Figure 4C. The expression of IL-2 mRNA both in lymphocytes and allografts was higher in animals injected with empty vector plasmid DNA demonstrating increased allo-immune activation. IL-2 mRNA expression decreased significantly in recipients treated with p21WAF1/CIP1 sense plasmid DNA alone or together with CsA. The expression of IL-2, correlated with rejection, which indicated an increased immune activity due to alloimmune response resulting in the rejection as compared to p21WAF1/CIP1 or p21WAF1/CIP1 /CsA treated recipients. We did not observe any significant changes in the expression of IL-10 mRNA in allografts, which decreased in animals treated with p21WAF1/CIP1 sense plasmid DNA alone or with CsA, however it did not reach a level of significance (Figure 4C).

Discussion

The experiments performed in this study were designed to understand the role of cyclins on mitogen and allo-stimulation of immune cells and also, if the inhibition of cyclins will correlate with pro-inflammatory cytokines. We also studied if p21WAF1/CIP1 modulation in recipients of cardiac transplantation modulates allo- and mitogenic stimuli and allograft survival. The results demonstrate that during lymphocyte activation, mRNA expression of cyclins and pro-inflammatory cytokines is significantly increased and CsA inhibited lymphocyte activation, mRNA expression of cyclins, pro inflammatory cytokines but induced p21 mRNA and protein expression.

Studies [9-12] have demonstrated that the expression of cyclin D3, cdk6, and cyclin E is activated in IL-2-stimulated T lymphocytes. However, the novel finding of this present study is that mRNA expression of cyclins in activated lymphocytes correlates with that of pro-inflammatory cytokines, and the expression of both the cyclins and pro-inflammatory cytokines is inhibited by immunosuppressive agent CsA. These are novel findings not demonstrated previously. Our results emphasize that the cell cycle progression and inflammation are concerted events thus regulation of cell cycle control could result in decreased inflammation.

Our *in vitro* findings on the increased expression of cyclins mRNA in activated lymphocytes were reproduced in our *in vivo* studies. The mRNA expressions of cyclin D3, G and E in lymphocytes (possible predominantly T cells, CsA inhibits proliferation of T lymphocytes) isolated from spleens from untreated recipients of rat cardiac transplant were significantly higher than those treated with cyclosporine. The increased expression of cyclins may represent an uncontrolled allo-immune activation in these rats. Since CsA treatment resulted in the inhibition of alloimmune activation and increased graft survival accompanied by a significant inhibition of mRNA expression of cyclins in CsA treated. This possibly was due to the CsA mediated inhibition of alloimmune activation. These results indicate the presence of an active cell cycle progression during allo-immune activation. Therefore, the control of cell cycle progression should prevent inflammation