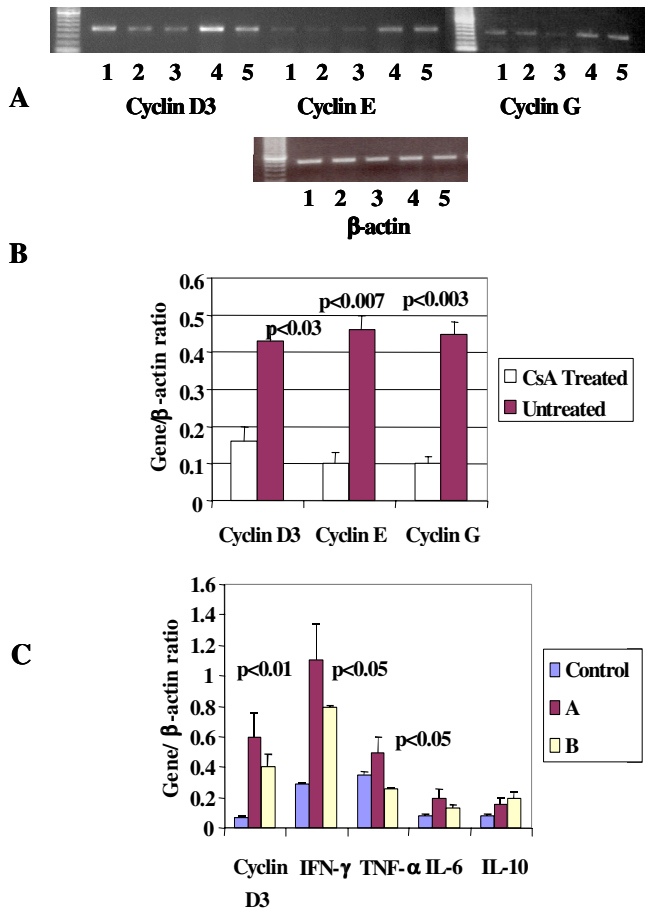

Figure 2

***In vitro* and *in vivo* mRNA expression of Cyclins relates to alloimmune activation**: **A:** Cyclins; D3, E and G and β-actin mRNA expression in lymphocytes isolated from spleens of rat heart transplant recipients. Untreated (lanes 4,5), CsA treated (lanes 1,2,3). **B:** Mean ± SEM of the ratio cyclins with β-actin, p values are calculated between densitometric numbers of untreated and CsA treated transplant recipient. **C:** Cyclin D3 and pro-inflammatory cytokines mRNA expression in lymphocyte from MLR assay using stimulants from donor strain with responders from donor strain (Control) untreated rejecting transplant recipients (A) and CsA treated non rejecting transplant recipients (B). The p values represent the statistical significance between ratio of densitometric numbers for each gene with β-actin from untreated (**Group A**) vs CsA (**Group B**) treated transplant recipients.

0.02 vs 0.1 ± 0.03, p < 0.003) and cyclin E (0.46 ± 0.03 vs 0.1 ± 0.04, p < 0.007). These results (Figure 2B) obtained from a semi-quantitative PCR analysis demonstrate that the aberrant alloimmune activation responsible for graft rejection is associated with the increased expression of cyclins. More significantly, CsA treatment significantly decreased mRNA expression of cyclins, alloimmune activation and prolongation of graft survival.

Correlation of the expression of cyclins and pro-inflammatory cytokines in allo-immune

To confirm that the increased expression of cyclins in lymphocytes from rejecting rats was due to alloimmune activation, we performed mixed lymphocyte reaction (MLR) using spleen cells from donor animals (WF) as stimulators and splenocytes from recipients (LEW) as responders. Three groups were studied; isografts (control), untreated allografts (A), and CsA-treated allografts (B). After five-day MLR lymphocytes were harvested and washed. RNA was prepared; reverse transcribed to cDNA, and was amplified by RT-PCR for cyclin D3, IFN-γ, TNF-α, IL-6 and IL-10 mRNA. As shown in the Figure 2C the mRNA expression of cyclin D3 was higher in lymphocytes from untreated allografts **group A** as compared to lymphocytes from isografts **group B**. This increased expression of cyclin D3 correlated with the pro-inflammatory cytokines IFN-γ and TNF-α mRNA expression. The expression of IL-6 and IL-10 mRNA was not statistically significant between groups A and B. CsA treatment decreased cyclin D3 expression by 50% and inhibited statistically significant (p < 0.03) IFN-γ mRNA expression. Lymphocyte activation in MLR assay was quantified by ^{3}H-thymidine uptake assay. Proliferation of lymphocytes from untreated allografts was significantly higher (two tailed p value = 0.02) compared to CsA- treated allografts (Mean ± SEM of counts per minute, n = 3, 11796 ± 728 vs 7575 ± 360). These results support our conclusions from rat transplant studies that the alloimmune results in increased expression of cyclins mRNA that correlates with production of pro-inflammatory cytokines. Allo-immune activation is demonstrated by increased lymphocyte proliferation from MLR assay, which decreased in CsA treated animals.

p21WAF1/CIP1 over-expression, lymphocyte proliferation and IL-2 expression

To confirm our previous *in vitro* and *in vivo* studies that p21WAF1/CIP1 over-expression will inhibit lymphocyte proliferation and IL-2 expression, we conducted studies using Jurkat T cells. Jurkat T cells were transfected with empty vector plasmid DNA (control DNA) and p21WAF1/CIP1 sense plasmid DNA. We used Jurkat T cells for these experiments because of the ease with which these cells can be transfected as compared to primary T cells; these p21WAF1/CIP1 over-expressing Jurkat T cells are described previously [7]. No differences were observed in the proliferation of normal Jurkat cells and Jurkat cells transfected with empty vector DNA. Control and p21WAF1/CIP1 over-expressing Jurkat T cells were activated with PHA (2 μg/ml) for 4 h for IL-2 mRNA expression studies and 24 h for proliferation studies using ^{3}[H]-