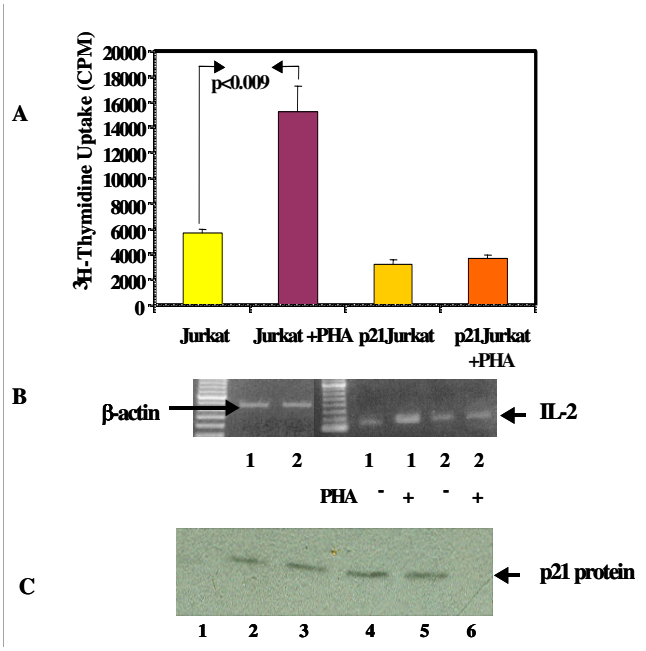

Figure 3

Over expression of p21WAF1/CIP1 inhibits proliferation and IL-2 mRNA expression in Jurkat T cells: **A**: A comparison of the proliferation of Jurkat T cells with and without p21 overexpression. **B**. Il-2 mRNA expression in PHA activated normal and p21 over-expressing Jurkat T cells. An identical expression of house keeping gene β-actin is also shown. **C:** p21 protein expression in four different clones of p21 overexpressing Jurkat T cells, Untreated (lane 1), p21 overexpressing (lane 2–5) and transfected with empty vector DNA (lane 6).

thymidine uptake assay. The results demonstrate that the control Jurkat cells, but not p21WAF1/CIP1 over-expressing Jurkat T cells, responded to mitogenic stimulation by PHA (Figure 3A). PHA stimulation resulted in an increased expression of IL-2 mRNA in Jurkat cells, not in p21WAF1/CIP1 over-expressing Jurkat cells (Figure 3B). These results indicated that the p21WAF1/CIP1 over-expression rendered Jurkat cells unresponsive to mitogenic stimuli, possibly p21WAF1/CIP1 over-expression did not allow increased cyclin expression, thereby preventing lymphocyte activation by PHA. An increased expression of p21 protein in four different sets of p21-overexpressing Jurkat T cells (lanes 2–4) compared to Jurkat T cells transfected with empty vector plasmid DNA (lane 1) is also shown (Figure 3C) suggesting the increased p21 protein expression in these p21 overexpressing cells.

Effect of p21WAF1/CIP1 over-expression on graft survival in a rat heart transplant model

p21WAF1/CIP1 over-expression in Rats

Encouraged by our studies with mice, which demonstrated that transfection with p21WAF1/CIP1 sense plasmid DNA resulted in decreased lymphocyte proliferation, we performed pilot experiments to determine if *in vivo* over expression of p21 will also result in improved graft survival in a rat cardiac transplant model. We injected (intramuscularly) either p21WAF1/CIP1 sense plasmid DNA or empty vector plasmid DNA (1 mg) to 4 rats in each group. Since in our experiments with mice we used 100 μg of DNA, based on difference in the average weights of a rat and mouse, we used 10 times more DNA in rats. Seven days after the injection, animals were sacrificed, RNA was prepared from heart (h), liver (l), kidney (k) and spleen (s), reverse transcribed to cDNA and amplified for p21WAF1/CIP1 mRNA. Results shown in Figure 4A demonstrate that injection with p21WAF1/CIP1 sense plasmid DNA but not with empty vector plasmid DNA resulted in an over-expression of p21WAF1/CIP1 mRNA. These results also demonstrate that p21WAF1/CIP1 transgenesis using intramuscular injection of plasmid DNA can be achieved in rats. Since during isolation of RNA the contaminating DNA is treated with DNAse, the amplification of injected p21 sense plamsid DNA can be ruled out. The expression of p21WAF1/CIP1 protein using western blot was also detected in spleens, which was the only tissue analyzed (results not shown).

We then studied the effect of modulation of p21WAF1/CIP1 on alloimmunity in a rat heart transplant recipients. We have extensive experience using the completely MHC mismatched WF ($RT1^u$) into LEW ($RT1^l$) strain combination and have well defined thresholds of cyclosporine-based immunosuppression. In this model, animals reject within 7 to 10 days in the absence of immunosuppression and as late as 180 days with immunosuppression (CsA 2.5 mg/kg). A total of 12 rat transplants divided into four groups (**A-D**) were performed. Recipients in **Group A** were given one intramuscular injection of empty vector plasmid DNA (1 mg); **Group B** rats were given a daily dose of CsA (2.5 mg/kg). Rats in **Group C** rats received three weekly injections of p21WAF1/CIP1 sense plasmid DNA (0.5 mg); and **Group D** rats were given one intramuscular injection of p21WAF1/CIP1 sense plasmid DNA and a daily injection of CsA (2.5 mg/Kg). The allografts were followed by palpitation, and an arbitrary scale of 1–4 was used to rate the heartbeat to determine the time of graft rejection. The rats were sacrificed when a heartbeat of 1–2 was recorded, which was considered as a cutoff for rejection. Though the number of transplants is low, yet as shown in Figure 4B, p21WAF1/CIP1 alone ($*p < 0.04$) or in combination with CsA ($**p < 0.005$) significantly prolonged the graft survival.