leading to an improved graft survival. These results are supported by our studies with MLR cultures using lymphocytes from rat heart transplant recipients. An increased proliferation of lymphocytes accompanied increased expression of cyclins and pro-inflammatory cytokine mRNA when responders lymphocytes were used from untreated rats as compared to those from isografts or CsA treated rat heart transplant recipients. Again, these activated lymphocytes were possible predominantly T cells, T lymphocyte proliferation is a key component of allo-immune activation. Therefore, these results lend credence to our thinking that the inhibition of allo-immune activation accompanies decreased expression of cyclins and pro-inflammatory cytokines.

These results confirm that control of cell cycle progression plays a significant role in T cell proliferation/activation. Role of p21 in other aspects of lymphocyte proliferation has been studied. Studies of Balomenos et.al, [13] Santiago-Raber et al [14] and Brian et al [15] demonstrated that T lymphocytes from p21WAF1/CIP1$^{-/-}$ mice proliferated significantly more than from wild type mice upon stimulation. These results support our studies that p21WAF1/CIP1 modulation alters cell cycle progression and the immune system. Jackson et al [16] showed that increased levels of p21WAF1/CIP1 at the end of G (1) could prevent cdk-mediated entry into S phase, leading to proliferative unresponsiveness also found in our experiments with p21WAF1/CIP1 over-expressing Jurkat T cells.

The results from this study are of significance because p21 is one of the most potent regulators of the cell cycle and is known to inhibit cell proliferation in two different ways. p21 binds to Cdk2 and inhibits PCNA (proliferating cell nuclear antigen), which is an auxiliary protein in DNA polymerase needed for DNA synthesis and nucleotide excise-n- repair [17]. PCNA has 6 binding sites for p21 [18]. Studies also [19] demonstrated that the PCNA binding and inhibitory activities reside in the C-terminal domain of p21, compared to the location of the CDK inhibitory activity in the conserved N-terminal domain. The authors also concluded that the CDK and PCNA inhibitory domains prevented DNA replication suggested a dual function of p21 as a cell-cycle inhibitor in vivo. We conducted these studies exclusively with cyclin kinase inhibitor p21WAF1/CIP1, though p53 and cyclin kinase inhibitors (p27, p16) have been shown to inhibit cell cycle yet p16 and p21WAF1/CIP1 inhibit cell cycle progression through distinct mechanisms [20]. The specific target for p16 is the Cdk/4cyclin D complex and in a tumor model, p21WAF1/CIP1 and p16 did not show additive or synergistic effects [21]. Furthermore in contrast to p21WAF1/CIP1, the expression of p27 is not under transcriptional control and its mRNA expression remains unchanged during cell cycle [22]. Also, high levels of p27 but not p21WAF1/CIP1 are observed in most quiescent cells and the inhibition of p27 levels precedes the progression of cell cycle [23]. Though both p21WAF1/CIP1 and p27 are critical in the response of cells to mitogens, p21WAF1/CIP1 provides a better balance between cyclins and cyclin kinase inhibitors [24] stressing its significance in inhibition of proliferation/immunosuppression. It is therefore possible that p21WAF1/CIP1 overexpression could interrupt the cell cycle progress and also prevent inflammation. It is well known that during T cell activation, expression of pro-inflammatory cytokines IFN-γ, TNF-α and IL-6 is significantly increased. Since T cells are the key mediators of allo-immune activation, this increased expression of cytokines in organ transplant recipient results in graft rejection [25-27]. Our results demonstrate a parallel increase in the expression of cyclins and pro-inflammatory cytokines. Therefore an inhibition/regulation of cell cycle progression of immune cells by over-expression of cyclin kinase inhibitor p21WAF1/CIP1 would decrease both allo-immune activation and inflammation in transplant recipients.

We also demonstrate that rats transfected with p21WAF1/CIP1 plasmid DNA over expressed p21WAF1/CIP1 mRNA in different tissues. The recipients of cardiac allograft animals who received intra-muscular injection of p21WAF1/CIP1 sense plasmid DNA had significantly increased graft survival compared to the recipients transfected with empty plasmid DNA. These very preliminary studies suggest that p21 overexpression can prolong graft survival to a degree comparable to prolongation by CsA. Further studies will surely be required to confirm and quantify the effect of p21 on graft survival. We also present unique results that the expression of IL-2 mRNA was significantly decreased in both lymphocytes and allografts isolated from p21WAF1/CIP1 over-expressing recipients of rat heart transplants.

Our method of using plasmid DNA to obtain *in vitro* and *in vivo* transfection of p21WAF1/CIP1 is based on the data supporting the efficacy of intra-muscular injection of plasmid DNA for a number of genes [28]. A number of studies [29-32] have demonstrated that non-viral plasmid DNA provides a simple, safe, and viable alternative for gene therapy involving muscle tissue resulting in high level of expression. More significantly plasmids do not induce neutralizing immunity, which permits repeated administration. Rauh et al [33] tested the hypothesis that intramuscular injection of naked DNA could result in the distribution remote from the site of needle placement, facilitating intramuscular gene transfer. Using transcutaneous ultrasound imaging the authors demonstrated that a solution of plasmid DNA administered by direct intramuscular injection into the skeletal muscles of the limb is distributed well beyond the site of needle entry and