# 5. Summary and Conclusion

As outlined in the introduction, sepsis remains a significant clinical conundrum, and recent clinical trials with anti-cytokine therapies have produced only modest positive results. In this light studies in experimental animals and critically ill patients have demonstrated that increased apoptosis of lymphoid organs and parenchymal tissues contribute to suppression of the immune system, anergy, and organ system dysfunction. In addition, animal studies have demonstrated that blocking apoptosis can improve outcome in experimental models of severe sepsis. Moreover, overexpression of the anti-apoptotic Bcl-2 protein in T- and B-lymphocytes improved survival following a cecal ligation and puncture, as well as prevented apoptosis in lymphoid organs. Therefore, my aim was to locally manipulate inflammatory cells which induce immunesuppression by inhibiting T-cell apoptosis through the methods of gene therapy.

We first investigated the feasibility of gene therapy using an adenoviral construct to transfect T-cells locally. We showed that we are not able to transfect T-cells, but we were capable of transfecting dendritic cells *in vivo*. Moreover, we demonstrated that nearly 50% of the total thymic dendritic cells could be transfected with a first-generation adenoviral construct following intrathymic injection, and that these transfections were associated with only modest inflammation. Additionally, we showed that thymic cells transfected with adenoviral recombinants are able to express an intracellular protein (β-galactosidase, green fluorescent protein), as well as secrete human interleukin 10 (hIL-10) in the local compartment. Since IL-10, an anti-inflammatory cytokine, has been previously shown *in vitro* to reduce T-cell apoptosis in part through up-regulation of Bcl-2, it served as our transgene in a bystander effect to manipulate T-cell apoptosis. In this line we showed that forced expression of hIL-10 decreases thymic apoptosis during acute bacterial peritonitis. Furthermore, we showed that inhibition of thymocyte apoptosis by targeted adenovirus-induced thymic expression of human interleukin-10 reduced blood bacteremia and prevented mortality in sepsis. In contrast, systemic administration of adenovirus expressing IL-10 was without any protective effect. Improvements in survival were associated with significant increases in Bcl-2 expression, and

reductions in caspase-3 activity and thymocyte apoptosis, demonstrating that thymic apoptosis plays a critical role in the pathogenesis of sepsis.

Knowing that we can not treat septic patients by intrathymic injection but that we are able to easily transfect dendritic cells we chose foot pad injection as a more clinical approach to treat acute inflammation by gene therapy. Dendritic cells are the most potent antigen presenting cells of the immune system, capable of stimulating naive T cells to proliferate and differentiate into effector T cells. We demonstrated that footpad injection of a recombinant adenovirus transfects myeloid and lymphoid DCs in the draining popliteal lymph node, but not in other lymphoid organs. Popliteal DCs transduced with an empty recombinant adenovirus underwent maturation, as determined by high MHC class II and CD86 expression. However, transduction with vectors expressing human IL-10 limited DC maturation and associated T-cell activation in the draining lymph node. The extent of IL-10 expression was dose dependent; transduction with low particle numbers ($10^5$) yielded only local expression, while transduction with higher particle numbers ($10^7$, $10^{10}$) led additionally to IL-10 appearance in the circulation.

Furthermore, local DC expression of human IL-10 at low particle numbers ($10^5$) significantly improved survival following cecal ligation and puncture, suggesting that compartmental modulation of DC function profoundly alters the sepsis-induced immune response.

In further studies we investigated the influence of gene therapy on dendritic cells *ex vivo*. Control of dendritic cell maturation and function is critical for strategies to modulate innate and acquired immune responses. We examined whether transduction of murine DCs with adenoviral vectors expressing IL-10 could alter their responsiveness to inflammatory stimuli. Murine bone marrow-derived DCs were transduced *in vitro* either with an empty adenoviral vector, or one expressing gfp, or human IL-10. *In vitro* transduction of immature DCs with adenovirus expressing gfp resulted in dose-dependent maturation. Transduction with adenovirus expressing human IL-10 (Adv/IL-10) maintained DCs in an immature state with low MHC class II, CD86, and IL-12 expression. Although these DCs were responsive to additional maturation stimuli, the resulting DCs were phenotypically unique, characterized by a continued ability to endocytose antigen, a reduced production of IL-12, and the failure

to drive Th1 or Th2 responses. Administration of these DCs to mice prior to a generalized peritonitis improved their outcome.

In conclusion, these studies provide new targets within the pathophysiology of sepsis and demonstrate the feasibility of gene therapy for the treatment of acute inflammation. Moreover, these studies provide strong evidence that lymphocyte apoptosis plays a critical role in sepsis, indicating a potentially new therapeutical approach for treatment of sepsis syndromes. Furthermore our data demonstrate that gene targeting of DCs *in vivo* as well as *ex vivo* with IL-10 is useful for the treatment of acute infectious diseases, such as sepsis syndromes. Modifying DCs by *in vitro* adenoviral expression of IL-10 creates a hybrid DC that may provide a novel reasonable clinical approach to modulating acute and chronic inflammatory diseases.

**Although the mechanism of action has not been completely resolved, compartmentalized expression of IL-10 in DCs clearly alters the response to sepsis and improves outcome. Further studies will be required to more fully delineate the cellular mechanisms by which modification of DC function by gene therapy impacts host immunity and improves outcome in sepsis. These studies will further evaluate the feasibility of gene therapy for the treatment of acute inflammation.**