

the packaging sequence (105, 106). Deletion of not only the E1 region but also the E4 region of the adenovirus decreases expression of viral proteins, and reduces apoptosis of hepatocytes leading to blunted immunoreactions (107).

Due to the relative short duration of transgene expression, adenovirus appears to be a promising vector for transient gene therapy (173, 174), and appropriate for acute inflammatory processes, such as sepsis.

2. Specific aims of the project

The focus of my research in the recent years has been aimed at elucidating the pathophysiological mechanisms of the systemic inflammatory response syndrome in severely injured and septic patients (108-113).

Since treatment of sepsis and septic shock remains a clinical conundrum, and recent prospective trials aiming at modifying the inflammatory response have shown only modest clinical benefits, my interest has shifted towards therapies aimed at reversing the accompanying periods of immune suppression. Recent studies in experimental animals and critically ill patients have suggested that increased apoptosis of lymphoid organs and some parenchymal tissues may contribute to the immune suppression, anergy and organsystem dysfunction and appear to contribute to adverse mortality. Animal studies have demonstrated that blocking apoptosis can improve outcome in experimental models of severe sepsis. Although clinical trials with anti-apoptotic agents remain distant, mainly due to present technical difficulties associated with their administration and tissue targeting, inhibition of lymphocyte apoptosis represents an attractive therapeutic target for the septic patient.

In this light we focused on modifying the immune suppression by the help of gene therapy. We investigated the feasibility of gene therapy using an adenoviral construct for the treatment of acute inflammation induced by cecal ligation and puncture (114-117).

The specific aims were:**1) Feasibility to transfect the lymphocyte rich thymus by gene therapy**

- a. Which cells are transfected *in vivo*?
- b. Are these cells able to produce the implanted transgene?
- c. What is the host immune response to the virus?
- d. Are we able to block T-cell apoptosis during inflammation by gene therapy?

2) The biological effect of gene therapy in an acute inflammation model

- a. Does inhibition of thymocyte apoptosis improve outcome in a model of generalized peritonitis?
- b. Is there a difference between local and systemic application of gene therapy?
- c. What is the mechanism of blocking T-cells apoptosis?

3) Clinical approach of targeting dendritic cells in an acute inflammation model

- a. Do *in vivo* transfected dendritic cells via footpad application of the vector travel to the nearest lymph node?
- b. Do we have an impact on dendritic cell function as well as T-cell activation in the lymph node?
- c. Can we improve outcome in a generalized peritonitis model by footpad gene therapy?

4) Evaluation of reinjection of ex vivo transfected dendritic cells into an acute inflammation model

- a. What effect does transfection of dendritic cells with a viral vector have in regard to activation, maturation, endocytosis?
- b. What effect do these transfected dendritic cells have on T-cell function?
- c. Can we improve outcome in a generalized peritonitis model by reinjection of transfected dendritic cells?