Cytotechnology **28:** 1–8, 1998.

Cell therapy in kidney failure

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Received 26 August 1998; accepted 26 August 1998

Key words: bioartificial organs, renal failure, stem cells, tubule cells

Abstract

Current therapy for acute renal failure continues to have an exceedingly high mortality rate, exceeding 50% even with dialytic or hemofiltrative support. Current renal replacement therapy in ARF only substitutes for filtration function of the kidney but not its cellular metabolic functions. Replacing these metabolic functions may optimize current therapy for this devastating disease process. In this regard, a renal tubule assist device (RAD) has been developed to be placed in an extracorporeal continuous hemoperfusion circuit in series with a hemofilter. The RAD consists of porcine renal proximal tubule cells grown as confluent monolayers in a multifiber bioreactor with a membrane surface area from 0.4 to 1.6 m^2. The cells along the inner surface of the hollow fibers are immunoprotected from the patient's blood by the hollow fiber membrane. *In vitro* experiments demonstrate that this device possesses differentiated renal transport, metabolic and endocrinologic properties. These properties, in fact, are responsive to normal physiological regulatory parameters. In preliminary experiments in uremic dogs, this device has also been shown to tolerate a uremic environment while providing reabsorptive, metabolic, and endocrinologic activity. Pilot human trials of the RAD are anticipated within the next year to improve current renal replacement therapy in this devastating disease process.

Introduction

The improved understanding of the cellular and molecular basis of organ function and disease has been translated during the last two decades into new diagnostic and therapeutic approaches to a wide range of disease processes including renal failure. A whole new biotechnology industry has evolved to translate this knowledge in basic biological sciences into more effective therapeutic and diagnostic modalities. Some notable successes have ensued. The most successful applications of biotechnology to date have been to apply recombinant genetic engineering to produce new pharmacological agents. This method consists of identifying a disease due to a lack of a single protein made by cells, isolating the gene for this protein, and finally using recombinant molecular biologic techniques to introduce the gene into an expression system to produce large amounts of the gene product. Both erythropoietin and human insulin have been produced in this manner. Although this approach has been proven successful, the opportunities to use such a strategy are limited, as many physiological responses are due to a complex interaction of a series of cell products rather than to a lack of one component.

In such situations, in which the desired effect is dependent on an array of cell products, a possible solution is the development of a more complex approach using 'cell therapy'. Cell therapy is based on the concept that specific cells can be cultured *in vitro* to perform differentiated biological tasks. The simplest manifestation of cell therapy is to implant living cells to produce a natural hormone or protein in short supply as a result of disease, usually with a regulatable element to a biological signal (Humes, 1997). Oxygen regulation of erythropoietin production and glucose control over insulin secretion are notable examples.

The use of cells as gene-product delivery vehicles is another application of cell therapy. The ability to

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grow and expand cells *in vitro* allows for efficient transfection or transduction of a targeted gene into a population of cells grown in tissue culture. The introduction of these transduced cells into a recipient allows for the production of a gene product that the patient lacks or that is present in an abnormal form. The development of immunocapsulation techniques will allow the use of heterologous cells (Tai, 1993). The genes for coagulation factors VIII and IX are two examples of the potential treatment of the genetic diseases of hemophilia A and B.

A final technology has been termed 'tissue engineering', a developing field in which techniques from the biological and engineering sciences are combined to create structures that mimic the functions of human organs (Langer, 1993). The majority of applications currently envisioned for tissue engineering involve placing modified animal or human cells within an artificial construct. Current efforts are focused on producing bioartificial organs using cells seeded on hollow-fiber bioreactors perfused in an extracorporeal circuit using modified dialysis equipment. Pilot human studies have already taken place, with an extracorporeal liver-assist device designed to replace hepatic function while waiting for the patient's own liver to regenerate after an acute insult (Sussman et al., 1994). Given the successes of renal replacement therapy in the last four decades, a natural application of tissue engineering is in the treatment of acute and chronic renal failure.

Acute renal failure

Acute renal failure (ARF) is a result of toxic or ischemic insults to the kidney. It is a common disorder affecting nearly 200,000 patients per year in the United States (Lake, 1994; Thadhani et al., 1996). It presents as a devastating clinical disorder with whole organ failure occurring within days of the initiating injurious event. The patients with this condition are gravely ill, requiring intensive care unit care.

Current therapy for ischemic or toxic acute renal failure, or acute tubular necrosis (ATN), is predominantly supportive in nature. The therapeutic goals are the maintenance of fluid and electrolyte balance, adequate nutrition, and, when present, treatment of infection and uremia. Uremia is treated with either intermittent hemodialysis or continuous hemofiltration. Although this approach has had substantial impact on this disease process over the past 40 years, patients with ATN still have an exceedingly high mortality rate of greater than 50%, even with dialytic or filtrative support. The precise reason for this mortality rate in the face of normal electrolyte and fluid balances, and a nonuremic condition, is unclear.

Perhaps an explanation for this high mortality rate resides in the recognition that hemodialysis or hemofiltration only substitutes for the filtration function of the kidney but does not replace the homeostatic, regulatory, metabolic, and endocrine functions of the kidney. Review of the causes of death in patients suffering from ATN demonstrates that the single factor most responsible for death was development of disseminated bacterial infection due to impairment of host defense (Lordon, 1972; Whelton, 1969). This impairment is likely the result of the loss of cellular metabolic function of the kidney rather than lost filtrative function. Accordingly, the development of cell therapy modalities replacing these reabsorptive, synthetic, metabolic, and endocrinologic functions of the kidney may add significant value to the current suboptimal supportive options available to treat established ARF. An approach to this form of therapy is the development of a bioartificial tubule to replace these functions and to optimize current treatment modalities.

Bioartificial renal tubule

Critical to providing organ function replacement through cell therapy is the need for the isolation and growth *in vitro* of specific cells from adult tissue. These cells are those that possess stem cell-like characteristics with a high capacity for self-renewal and the ability to differentiate under defined conditions into specialized cells to develop correct structure and functional components of a physiologic organ system (Hall, 1989; Potten, 1990). Recent data by our laboratory have demonstrated methodology to isolate and grow renal proximal tubule progenitor cells from adult mammalian kidneys (Humes et al., 1996; Humes, 1992). These studies were promoted by the clinical and experimental observations suggesting that renal proximal tubule progenitor cells must exist, as tubule cells have the ability to regenerate after severe nephrotoxic or ischemic injury to form a fully functional and differentiated epithelium.

In this regard, the adult mammalian kidney tubule epithelium exists in a relatively dormant, slowly replicative state, but has a large potential for regenerative

morphogenesis following severe ischemic or toxic injury. Under selective serum-free growth conditions, which included epidermal growth factor and retinoic acid, a subpopulation of renal proximal tubule cells isolated from adult rabbit kidney were grown in cell culture. This report defines conditions in which to selectively grow from adult mammalian kidney a subpopulation of cells with an ability to differentiate morphogenically and with a high capacity for replication. Under the growth conditions of these experiments, these cells were able to both differentiate individually into a renal proximal tubule cell phenotype with cell polarity, apical microvilli, and tight junctional complexes between cells along the luminal border and to pattern form collectively into cylindrical arrays of cell monolayers surrounding a centralized lumen. These cells also were shown to have a high capacity for self-renewal. Genetic marking of the cells with a recombinant retrovirus and dilution analysis demonstrated that *in vitro* tubulogenesis often arose from clonal expansion of a single genetically tagged progenitor cell. These tubules were derived from tubule cells grown in primary culture and serial passages (at least 4 replication cycles) before suspension into collagen gels. Because many tubules in the collagen gel contained as many as 150 cells (at least 7 replication cycles), these findings demonstrate that *in vitro* tubulogenesis arose from clonal expansion of a single cell with the ability to undergo at least 11 replication cycles. These results suggest that a population of proximal tubule progenitor cells exist within the adult kidney in a relatively dormant, slowly replicative state but with a rapid potential to proliferate, differentiate, and pattern form to regenerate the lining proximal tubule epithelium of the kidney following severe ischemic or toxic injury commonly seen in clinical situations.

The bioartificial renal tubule is clearly feasible when conceived as a combination of living cells supported on polymeric substrata. A bioartificial tubule uses epithelial progenitor cells cultured on water and soluble-permeable hollow fiber membranes seeded with various biomatrix materials, such that expression of differentiated vectorial transport, metabolic, and endocrine function is attained. With appropriate membranes and biomatrices, immunoprotection of cultured progenitor cells can be achieved concurrently with long-term functional performance as long as conditions support tubule cell viability (Humes, 1997; Cieslinski, 1994). The technical feasibility of an implantable epithelial cell system derived from cells grown as confluent monolayers along the luminal surface of polymeric hollow fibers has been recently achieved (McKay, 1998).

As a first step towards developing a tissue engineered renal tubule assist device, Madin-Darby Canine Kidney (MDCK) cells, a permanent renal epithelial cell line, were seeded into the lumen of single hollow fibers (McKay, 1998). Functional confluence of the cells was demonstrated by the recovery of intraluminally perfused ^{14}C-inulin, which averaged greater than 98.9%, versus less than 7.4% with the control non-cell hollow fibers under identical pressure and flow conditions. The baseline absolute fluid transport rate averaged 1.4 ± 0.4 μL 30 min^{-1}. To test the dependency of fluid flux with oncotic and osmotic pressure differences across the bioartificial tubule, albumin was added to the extracapillary space followed by addition of ouabain, an inhibitor of Na^+K^+ATPase, the enzyme responsible for active transport across the renal epithelium. Addition of albumin resulted in a significant increase in volume transport to 4.5 ± 1.0 μL 30 min^{-1}. Addition of ouabain inhibited transport back to baseline levels of 2.1 ± 0.4 μL 30 min^{-1}. These results were the first demonstration that renal epithelial cells could be successfully grown as a confluent monolayer along a hollow fiber and developed functional active transport capabilities.

Bioartificial renal tubule assist device: In vitro performance

The next step in the development of a bioartificial renal tubule assist device (RAD) is to scale up from this single hollow fiber renal tubule to a multifiber bioreactor with renal proximal tubule cells that maintain not only transport properties, but also differentiated metabolic and endocrine functions. To accomplish this next step, a reliable tissue source for renal progenitor cells is required. Although successful renal tubule progenitor cell expansion has been achieved with human adult kidneys, a nonhuman animal tissue source for tubule cells has been strongly considered. Because an expensive screening process for infectious agents must be accomplished to ensure the safety of a human donor source of tissue along with the lack of consistent access and procurement of human tissue, an animal tissue source for renal tubule cells for RAD construction has been elected to be developed. The short-term use of this device for acute therapy in the intensive care unit (ICU) setting allows a nonhuman tissue source as a preferred strategy. For economic and

safety concerns, pigs can be used as a tissue source for this extracorporeal short-term RAD. Because of its anatomic and physiological similarities with humans and the relative simplicity with which it can be bred in large numbers, the pig is currently considered the best source of organs for both human xenotransplantation and immunoisolated cell therapy devices (Cozzi, 1995; Cooper et al., 1991; Calne, 1970). Kidneys are taken from 4–6 week old Yorkshire breed pigs. A full clinical profile of each donor pig for pathogens and blood and tissue pathology is accomplished to ensure the safety and noninfectivity of donor tissue. From these kidneys, renal proximal tubule segments are isolated and renal tubule progenitor cells are expanded with techniques previously described (Humes et al., 1996; Humes, 1992).

Further experiments are now under way to scale up to a clinically applicable device with the use of commercially available high-flux hollow fiber cartridges. Preliminary experiments have tested transport and metabolic functions of these cells grown intraluminally within these cartridges with membrane surface areas of from 97 cm^2 to 1.6 m^2. Starting with a high flux hemofilter cartridge, the intraluminal surface of the hollow fibers were coated with laminin. Renal tubule cells were then seeded at a density of 10^5 cells mL^{-1} into the intracapillary space with four cell infusions separated by 30 min and a 90° rotation of the cartridge. The seeded cartridge was connected to the bioreactor perfusion system, in which the extracapillary space was filled with culture media and the intracapillary space perfused with similar media at a rate of 4–5 mL hr^{-1}. Culture media, both intracapillary and extracapillary, were changed every 2–3 days to maintain adequate metabolic substrates for growth. After 7 to 14 days of growth, the unit was studied. Preliminary *in vitro* experiments utilizing porcine renal proximal tubule progenitor cells have clearly shown differentiated transport and metabolic function of the RAD unit as summarized in Table I.

Of importance, the transport properties were inhibitable by specific inhibitors–ouabain for active sodium transport, phlorizin for active glucose transport, probenecid for para-aminohippuric acid (PAH) secretion, and acivicin for glutathione transport and metabolism. The metabolic processes of the RAD also demonstrated sensitivity to normal physiological variables: ammoniagenesis was pH sensitive and vitamin D_3 activation was PTH and phosphate sensitive. The absolute values of these various functions and responsivity to inhibitors and physiologic modulators are detailed in the attached data summary.

Ex vivo performance of the RAD

While assessing the functionality of a RAD unit *in vitro* is an important component of bioreactor design, the true test of functionality and utility comes in testing the device *in vivo*, or *ex vivo*. Before clinical trials can be undertaken with such a device, extensive testing must be done in large animals, where the system can be evaluated under physiologic conditions and can be optimized for functionality and ease of use. The renal assist device will be used as a component in an extracorporeal circuit, where care must be taken to ensure proper operating conditions. Conditions under operation should mimic, as best as possible, those that the cells in the device are accustomed to seeing physiologically or *in vitro*. Important parameters to monitor and adjust are, for example, flow rates, pressures, and temperature.

The bioartificial kidney set-up consists of a filtration device (a conventional hemofilter) followed in series by the tubule unit. Specifically, blood is pumped out of a large animal using a peristaltic pump. The blood then enters the fibers of a hemofilter, where ultrafiltrate is formed and flows to the RAD downstream to the hemofilter. Processed ultrafiltrate exiting the RAD is collected and can be discarded as 'urine.' The filtered blood exiting the hemofilter enters the RAD through the extracapillary space (ECS) port and disperses among the fibers of the device. Upon exit of the RAD, the processed blood is delivered back to the animal. This additional pump is required to maintain appropriate hydraulic pressures within the RAD. In this regard, the pressure of the blood and ultrafiltrate just before entry into the RAD are monitored. Heparin is delivered continuously into the blood prior to entering the RAD to diminish clotting within the device. The RAD is oriented horizontally and placed into a temperature controlled environment. The temperature of the cell compartment of the RAD must be maintained at 37 °C throughout its operation to ensure optimal functionality of the cells. Maintenance of a physiologic temperature is a critical factor in the functionality of the RAD.

The tubule unit is able to maintain viability, because metabolic substrates and low-molecular weight growth factors are delivered to the tubule cells from the ultrafiltration unit and the blood in the ECS. Fur-

Table 1. In vitro functional characteristics of the renal assist device (RAD)

Transport	**Metabolic**
Fluid reabsorption	Ammoniagenesis
Ouabain inhibition	pH responsive
Sucrose inhibition	
	Gluconeogenesis
Bicarbonate reabsorption	
Acetazolamide inhibition	Glutathione synthesis
Glucose reabsorption	
Phlorizin inhibition	**Endocrinologic**
PAH secretion	1,25-Dihydroxyvitamin D_3 activation
Probenecid inhibition	PTH enhancement
	Phosphate inhibition

thermore, immunoprotection of the cells grown within the hollow fiber is achieved due to the impenetrance of immunoglobulins and immunologically competent cells through the hollow fiber if the encapsulating membrane has a pore size which excludes compounds with a molecular weight greater than 150,000 Da. Rejection of these cells will, therefore, not occur. This arrangement thereby allows the filtrate to enter the internal compartments of the hollow fiber network, lined with confluent monolayers of renal tubule cells for regulated transport and metabolic function.

The use of such a device in uremic and non-uremic large animals has shown that inulin leak rates increased by a few percentage points from less than 5–10% to slightly greater than 10% immediately after use, but returned to pre-study values after being maintained in culture for two weeks, meaning cells remain viable and can continue growing. The use of scaled-up cartridges can increase metabolic production. A surface area expansion from 0.4 to 0.8 m^2 results in an increase in the number of cells from 2×10^9 to 4×10^9 cells. Of note, RAD cartridges have been maintained in culture in excess of two months after use in large animal studies.

With the development of this extracorporeal circuit and design considerations, preliminary studies have been completed. Dogs weighing approximately 25 kg were placed on a controlled, low protein diet five days prior to being made uremic by performing bilateral nephrectomies. A double lumen catheter was placed into the internal jugular vein, extending into the heart, and sutured into place on the skin to prevent dislodgment. After 24 hr of post-operative recovery, the dogs were treated either with hemofiltration and the RAD or with hemofiltration and a control cartridge containing no cells. Dogs were treated daily for either 7 or 9 hr for 3 days or for 24 hr continuously. Preliminary *ex vivo* experiments in these uremic dogs have demonstrated that the RAD performs differentiated transport, metabolic and endocrinologic function characteristic of the proximal tubule *in vivo*.

The successful completion of proven functional performance of the RAD in uremic dogs prepares for the progression of the testing of this device in human subjects. Upon Food and Drug Administration (FDA) approval for an IND, a phase I/II trial is planned for safety and toxicity studies followed by a full controlled phase III efficacy clinical trial. Before proceeding to human clinical trials, the FDA, the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) are currently developing new requirement before allowing human studies to proceed. The need for newer requirements and safeguards were mandated with the discovery that pigs, the favored donor animal, contain porcine endogenous retroviruses (PERV) which can, under selected conditions, infect human cells (LeTissier et al., 1997). The technology described in this report, however, has much less risk than xenotransplantation (animal-to-human solid organ transplantation) due to the short exposure time, the immunoisolation of the cells, and the use of the RAD in non-immunosuppressed patients. The ability to proceed with these clinical studies will

be dependent upon meeting the soon-to-be-established safeguards for animal-to-human cell therapy.

Added value of the RAD to current therapy

Current therapy for ischemic or toxic ARF is predominantly supportive in nature. The therapeutic goals are the maintenance of fluid and electrolyte balance, adequate nutrition, and treatment of infection and uremia when they are present. Uremia is treated with either intermittent hemodialysis or continuous hemofiltration. Although this approach over the last 40 yr has had substantial impact on this disease process, patients with ATN still have an exceedingly high mortality of greater than 50%, even with dialytic or hemofiltrative support (Thadhani, 1996; Humes, 1995). Perhaps an explanation for this high mortality is that hemodialysis or hemofiltration only substitutes for the filtration function of the kidney and does not replace the homeostatic, regulatory, metabolic, and endocrine functions of the kidney. Review of the causes of death in patients suffering from ATN demonstrated that the single factor most responsible for death was development of infection. These infectious complications, with resulting sepsis and septic shock, may develop in ATN due to the loss of the nonfiltrative and metabolic functions of the kidney.

The kidney not only is important as an excretory organ but provides important resorptive, homeostatic, metabolic, and endocrinological functions. There are multiple renal cellular functions that the RAD may provide to a patient with ATN which may be important in preventing the septic complications of this disorder. Three key examples can be provided to demonstrate this added value.

The loss of both glutathione synthetic function and production of key free-radical scavenging enzymes in renal failure undoubtedly diminishes host defense function (Dröge et al., 1994; Avissar et al., 1994) and contributes to the pathogenesis of septic shock (Kinscherf et al., 1994; Zimerman, 1995). Increasing evidence demonstrates that the redox state within immunocompetent cells critically regulates immunologically relevant genes so that the balance between glutathione (GSH) and glutathione disulfide (GSSG) regulates these processes. This evidence clearly supports the concept that GSH is a key limiting factor that determines the magnitude of immunologic functions both *in vitro* and *in vivo*, thereby playing an important role in host defense. GSH depletion may well be responsible for the induction of immunologic nonresponsiveness under conditions of antigenic stimulation (Dröge et al., 1994). Even a small depletion of intracellular glutathione pool alters dramatically the process of myeloblast transformation, proliferation, and cytotoxic T-cell generation (Kinscherf et al., 1994).

A variety of studies have clearly demonstrated that excessive free radical generation contributes to the vascular and tissue damage in sepsis. The sepsis syndrome triggers a complex series of coagulation, complement and cytokine cascades to defend against bacterial invasion (Zimerman, 1995). Overwhelming sepsis, however, results in excessive host defense responses, including neutrophil activation with excessive release of free radicals (Goode, 1993). Since the action of free radicals is normally limited by antioxidant defense systems, in which GSH/GSSG axis is central, it is not surprising that GSH was protective to oxidant injury and improved the hemodynamic and metabolic derangements in both experimental animal and clinical studies of sepsis (Goode, 1993; Lash et al., 1986).

In this regard, the kidney plays an integral role in glutathione metabolism of the body (Curthoys, 1983). The initial step in glutathione turnover is its release from the cell where it is transported to the kidney where it is almost completely extracted and degraded to its constituent amino acids, predominantly in the proximal tubules. Within these epithelial cells, glutathione is resynthesized and returned to the systemic circulation. This process is so dynamic that renal glutathione turnover rate has a half-life of only 30 min. The kidney, and specifically proximal tubule cells, are also the major source of synthesis of antioxidant glutathione-related enzymes (Mohandas et al., 1984; Avissar et al., 1994). The key role of the kidney in this GSH regulatory pathway is exemplified by the fact that renal failure patients have severely low plasma levels of GSH and glutathione peroxidases (Avissar et al., 1994; Ross et al., 1997) and therefore are at increased risk for oxidative stress, especially in bacterial infection.

The loss of key endocrinologic functions in ARF may also diminish host defense to infection in patients with this disorder. The 1-hydroxylation of 25-OH vitamin D_3 in the proximal tubule cells converts vitamin D_3 into its most active metabolite. Both acute and chronic renal failure results in declines in the circulating level of this active metabolite and leads to vitamin D deficiency. A number of studies have clearly

demonstrated that 1,25-dihydroxyvitamin D_3 plays an important role in the regulation of the immune system (Koren et al., 1992). High affinity receptors are found in peripheral blood lymphocytes and thymocytes and vitamin D deficiency impairs cell mediated immunity (Yang et al., 1993). Neutrophils from patients with vitamin D deficiency have abnormal motility and phagocytic ability (Bhalla, 1989). Administration of vitamin D_3 to patients on hemodialysis restores mitogen stimulated T cell responses to normal (Bikle, 1992). A critical role of cytosolic calcium in the oxidative burst of granulocytes has been acknowledged (Sullivan et al., 1989). The immune system has, therefore, been clearly recognized to be an important target tissue of this important hormone.

The kidney may play another role in host defense by participating in the complex and dynamic network of pro- and anti-inflammatory cytokines. The proximal tubule cells derive embryonically from mesodermal progenitors closely related to bone marrow precursor cells and have retained many elements of immunologically competent cells, including the ability for antigen presentation and production of a variety of immunologic active cytokines (Ong, 1994). Little has been explored into the role of the kidney in the sepsis syndrome, even though this disorder is a defining factor in the high mortality rate associated with ATN. Septic shock is an acute syndrome that is characterized by hypotension, coagulopathy, and eventual multiorgan failure primarily to ischemic tissue injury. This disorder is associated with dramatic elevations in inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 (Galley, 1996). This reactive and uncontrolled inflammatory response results in the adverse hemodynamic and metabolic disturbances in septic shock. More detailed understanding of the pathophysiology of this process, however, has suggested that the problem arises not from the expression of pro-inflammatory cytokines but the inadequate modulation by anti-inflammatory cytokines (Galley, 1996). One central anti-inflammatory mediator in this cascade is IL-10. IL-10 is a very proximal inhibitor of the cell-mediated (Th1-type) immune response, thereby promoting Th2-type humoral immune responses (Galley, 1996; Standiford, 1997). This compound has potent anti-inflammatory properties, including deactivation of neutrophils and macrophages, and diminishes the production of TNF-α, interferon-γ and members of both the C-X-C and C-C chemokine families. In fact, endotoxemia results in elevated plasma levels of IL-10 in response to endotoxin challenge in mice with lipopolysaccharide (LPS) but the source of this IL-10 in endotoxemia is not clear (Howard et al., 1993). The administration of IL-10 to mice challenged with LPS protects from lethal endotoxemia and neutralization of IL-10 increases lethality after LPS challenge, thereby demonstrating the important role of IL-10 in endotoxin shock (Standiford et al., 1995; Howard et al., 1993). The manner in which these insights relate to the kidney in ATN is suggested by the recent data from our laboratory that LPS exposure to proximal tubule cells (PTC) *in vitro* results in substantial production of IL-10 by PTC. This finding leads to the hypothesis that ATN, which is characterized by predominant PTC damage, may diminish the systemic response of increased IL-10 plasma levels in response to septic shock. This blunted IL-10 anti-inflammatory response could then lead to worsening hemodynamic and metabolic abnormalities in the sepsis syndrome, incremental multiorgan failure and mortality.

For clinical usefulness, these devices must be prefabricated with a defined and carefully screened porcine tissue source and held in storage in cell incubators. Upon request to treat a patient suffering from acute renal failure, which develops over a three to five day time course, the bioartificial device must be transported similar to a transplanted organ to the distant site for patient use. The logistics required to successfully achieve this effort are significant but may well be worth developing if the RAD has a significant positive outcome on the morbidity and mortality of acute renal failure. With the successful technological achievements with recent *in vitro* and *ex vivo* experiments with this device, initial clinical studies are planned in patients within the year.

As discussed, prior studies suggest that the kidney and its proximal tubule cells play a critical role in host defense and the immunologic processes responsible to combat bacteriologic and viral infections. Cell replacement therapy with an extracorporeal bioartificial renal tubule assist device (RAD), by providing critical metabolic, endocrinologic and cytokine factors during ARF, will hopefully result in a decrease in the mortality rate of this devastating disorder.

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