CHAPTER 197

Cell-Based Therapies

H. David Humes, Lenar Yessayan, and Balazs Szamosfalvi

OBJECTIVES

This chapter will:

1. Present the scientific rationale for renal stem cell and progenitor cell therapy.
2. Describe an extracorporeal approach to renal cell replacement therapy.
3. Present the scientific basis of continuous cell processing in an extracorporeal device.
4. Summarize preclinical and clinical data on these cell-based approaches.

Acute and chronic solid organ failures are costly disease processes with high mortality rates. Inflammation plays a central role in acute and chronic organ failure, including heart, lung, and kidney. In this regard, new therapies for these disorders have focused on inhibiting the mediators of inflammation, including cytokines and free radicals, with little or no success in clinical studies. Recent novel treatment strategies have been directed to cell-based rather than mediator-based approaches, designed to immunomodulate the deleterious effects of inflammation on organ function. One approach, cell therapy, replaces cells that were damaged in the acute or chronic disease process with stem/progenitor technology, to rebalance excessive inflammatory states. As an example of this approach, the use of an immunomodulatory role of renal epithelial progenitor cells to treat acute renal failure and multiorgan failure arising from acute kidney injury, is reviewed. A second therapeutic pathway, cell processing, removes and modulates in situ the primary cellular leukocyte components of inflammation, which promote worsening organ tissue injury. The use of an immunomodulating leukocyte selective cytopheretic inhibitory device (SCD) also is reviewed as an example of this cell processing approach. Both of these unconventional strategies have shown early clinical efficacy in pilot clinical trials and may transform the therapeutic approach to organ failure disorders.

Loss of immunoregulation results in a propensity to develop systemic inflammatory response syndrome (SIRS), sepsis, multiple organ failure (MOF), and a high risk of death because of systemic immunologic or inflammatory imbalance. In acute kidney injury (AKI), activation and release of inflammatory proteins from circulating activated leukocytes, and imbalance between pro- and antiinflammatory proteins are provoked and aggravated by kidney cell injury. These conditions play a central role in the proinflammatory state in AKI with SIRS and/or MOF. SIRS is a catastrophic consequence of a variety of clinical insults and is usually present with AKI.

Growing evidence suggests that AKI is not merely a surrogate marker for severity of disease but also an independent predictor of death and a separate pathogenic entity, even when nearly physiologic levels of small-molecule clearance are administered. This possibility gives rise to the hypothesis that the native kidney has clinically

important functions that are not replaced by dialysis or hemofiltration. These functions may include synthesis of cytokines,[1–4] antigen presentation, reclamation of glutathione, synthesis of glutathione reductase, oxidative deamination and gluconeogenesis, 1,25-dihydroxyvitamin D_3 hydroxylation, trace mineral and element reclamation, and other, as-yet-undiscovered entities.[5]

RENAL TUBULE ASSIST DEVICE

Human renal cells have been isolated from cadaveric kidneys and cultured for the purpose of integrating them within a filtration device to provide more complete renal replacement.[6,7] These tubule cells, obtained from adult tissue and having stem cell–like characteristics, are grown in confluent monolayers along the inner surface of the hollow fibers in a conventional hemofiltration cartridge. The resulting construct containing these living cells is called a *bioartificial renal tubule assist device* (RAD).[8] The RAD is clearly feasible when conceived of as a combination of living cells supported on polymeric substrata acting as scaffolds for the cells. The renal tubule progenitor cells were cultured on the biomatrix-coated, hollow-fiber membrane of a standard high-flux hemofiltration cartridge. The membrane is both water and solute permeable, allowing for differentiated vectorial transport and metabolic and endocrine activity. Immunoprotection of cultured progenitor cells is achieved concurrently with long-term functional performance so long as conditions support tubule cell viability.[9]

BIOARTIFICIAL KIDNEY IN SERIES WITH RENAL REPLACEMENT THERAPY

The bioartificial kidney therefore can be fabricated with a filtration device (a conventional high-flux hemofilter) connected in series to the RAD (Fig. 197.1). Blood pumped out of the patient enters the fibers of the hemofilter, where ultrafiltrate is formed and delivered into the fibers of the tubule lumens within the RAD, downstream of the hemofilter. Processed ultrafiltrate exiting the RAD is collected and discarded as "urine." The filtered blood exiting the hemofilter enters the RAD through the extracapillary space port and disperses among the fibers of the device. Upon exiting the RAD, the processed blood is returned to the patient's body.

Studies have shown that the bioartificial kidney using a RAD consisting of either porcine or human cells replaces filtration, transport, metabolic, and endocrine functions of the kidney in acutely uremic dogs after bilateral nephrectomies.[10,11]

Further experiments demonstrated a survival advantage in large animals developing AKI.[12,13] These encouraging preclinical animal data led to FDA-approved clinical trials in intensive care unit (ICU) patients with AKI/ARF requiring ontinuous renal replacement therapy (CRRT).

Favorable phase I and II trial results[14] led to an FDA-approved, randomized, controlled, open-label phase II investigation at 12 clinical sites to determine whether this cell therapy approach alters patient mortality. This phase II study involved 58 patients, of whom 40 were randomized to receive RAD therapy and 18 made up a control group with comparable demographics and severity of illness.

Renal cell therapy improved the 28-day mortality rate from 61% in the conventional hemofiltration treatment control group to 34% in the RAD treatment group. This survival impact continued through the 90- and 180-day follow-up periods ($p < .04$), with the Cox proportional hazard ratio indicating that the risk of death was 50% of that observed in the conventional CRRT group.[15]

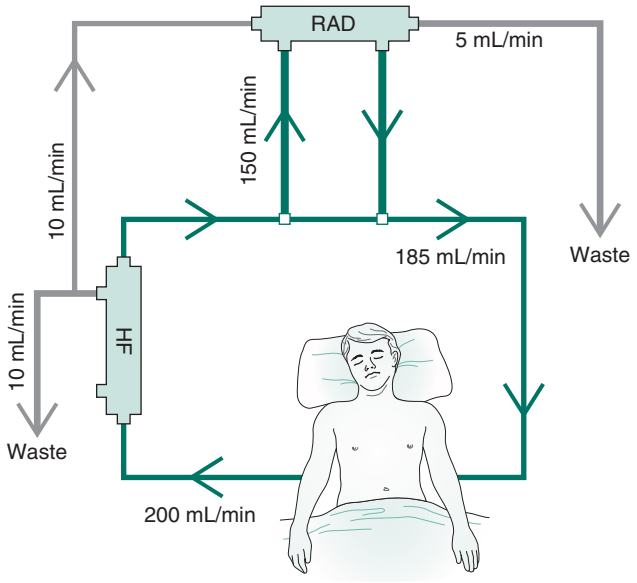

FIGURE 197.1 Schematic of the extracorporeal circuit for perfusion of the bioartificial kidney that was used in the phase I/II clinical trial described in the text. *HF,* Hemofilter; *RAD,* renal tubule assist device. (Redrawn from Humes HD, Weitzel WF, Bartlett RH, et al. Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure. *Kidney Int.* 2004;66: 1578–1588.)

LEUKOCYTE CELL PROCESSING IN SITU

This innovative approach to the treatment of AKI with renal progenitor/stem cells has been currently postponed because of a serendipitous discovery that led to a simpler, but elegant, approach to modulate the innate immunity response to inflammation. This approach uses biomimetic membranes in an extracorporeal blood circuit containing an SCD.[16]

This device preferentially binds activated leukocytes (LE). In the low calcium environment afforded by regional citrate anticoagulation (RCA), the bound LE are reset from an inflammatory phenotype to a more reparative phenotype.[17,18] The blood flow path results in low shear forces similar to capillary shear, so the membrane has selectivity to bind activated LE. This continuous cell processing activity results in measurable diminution of excessive inflammatory responses in a variety of clinical disorders. This approach was developed based upon the increasing understanding that inflammation is central to acute and chronic organ dysfunction. Inflammation, triggered when innate immune leukocytes sense infection or tissue injury, normally is locally focused and helpful. However, when circulating leukocytes (neutrophils and monocytes) become highly activated in inflammatory conditions, injury to normal tissue occurs. Uninterrupted, this exuberant inflammation resulting from activated leukocytes progresses to MOF, including AKI, with documented increases in morbidity and mortality in critically ill patients. Thus the SCD delivers its therapeutic

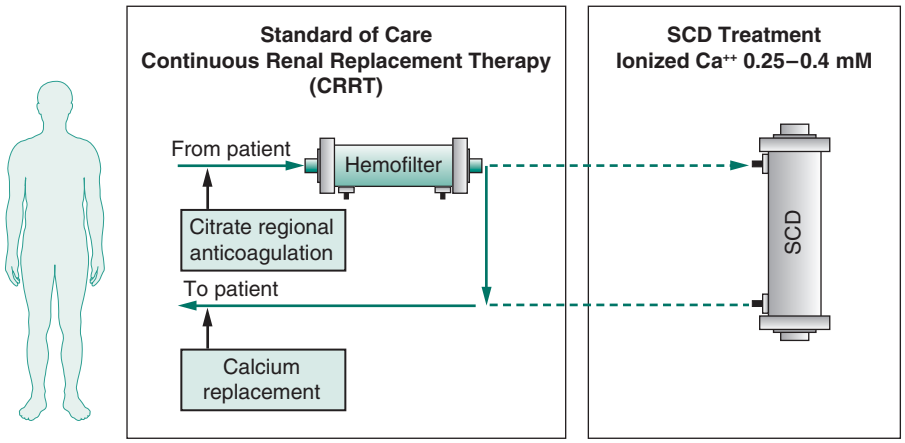

FIGURE 197.2 Selective cytopheretic inhibitory device use in continuous renal replacement therapy system. *SCD,* Selective cytopheretic inhibitory device.

benefit by using in situ cell processing to attenuate the exuberant inflammatory response of circulating activated leukocytes.

The mechanism of action (MOA) of the SCD consists of four steps within the SCD and its extracorporeal blood circuit: (1) binding activated leukocytes on the SCD biomimetic membrane; (2) deactivating the activated leukocytes by lowering blood ionized calcium (iCa) to 0.25 to 0.4 mM within the SCD using well-accepted RCA protocols; (3) deactivated leukocytes then release from the SCD membrane and return to circulation; (4) replacement calcium (Ca++) is infused into the blood circuit exiting the SCD so that blood returning to the patient maintains a normal calcium level in the patient.

CLINICAL ASSESSMENT OF THE SELECTIVE CYTOPHERETIC DEVICE

This approach has been evaluated initially in ICU patients with acute kidney injury (AKI) and multiorgan failure requiring CRRT. These trials were initiated after preclinical experiments in a porcine model demonstrated that SCD therapy had a significant ameliorative effect on sepsis-induced organ dysfunction, including cardiac, pulmonary, and renal parameters along with an immunomodulatory effect on circulating LE. The clinical evaluation of SCD therapy has been completed in three exploratory clinical trials in ICU adult patients with AKI requiring CRRT and multiorgan dysfunction.[19–21] These trials have demonstrated an excellent safety profile and suggested efficacy impact. Leukopenia and sustained thrombocytopenia were not observed; accelerated renal recovery with RRT discontinuation and an approximately 15% to 20% or greater improvement in survival rates has been observed compared with conventional RRT. This device is added in series to the CRRT after the standard HD filter (Fig. 197.2). In all three trials SCD therapy was well tolerated, without significant effects on hematologic parameters, including white blood counts (WBC) and platelet counts, and with no unanticipated serious adverse events related to SCD therapy. Because of these favorable results in three trials, a Phase III controlled, randomized, multicenter clinical trial was undertaken. The control group received standard CRRT with RCA and the SCD treated group received up to 7 days of device therapy. An analysis of per-protocol patients demonstrated no device-related serious adverse events. The SCD therapy group had a 60-day mortality rate of 16% compared with 41% in control. The SCD therapy group had no patients; dialysis dependency at day 60 compared with 23% of control patients. The composite end point of either death or dialysis dependency at day 60 was 16% for SCD therapy vs. 58% for control ($p = .01$). The analysis of this Phase III trial revealed a critical role of maintaining the iCa within the perfusion circuit at recommended levels below 0.4 mM.[22] This observation mandates the development of RCA protocols and sensors to measure in real time the iCa levels in the extracorporeal circuit to ensure therapy mandated iCa.

RCA FOR CONTINUOUS RENAL REPLACEMENT THERAPY WITH THE SELECTIVE CYTOPHERETIC DEVICE

In the absence of RCA best technical practice guidelines, each center has its own citrate anticoagulation protocol while protocol design features are critical to keep circuit iCa levels below 0.4 mM and to deliver RCA safely with the SCD device in patients with severe sepsis. A very low blood flow of 60 to 80 mL/min and avoidance of predilution helps achieve the high first-pass citrate removal on the dialyzer essential to prevent citrate accumulation even in patients with severely impaired citrate metabolism. A high concentrated citrate flow to blood flow ratio ensures iCa less than 0.3 mM with strong anticoagulant effect in the circuit. The use of 0 Ca dialysate preserves the low circuit iCa after first-pass citrate removal. Finally, calcium dosing based on plasma Ca clearance and albumin level results in normal circuit return plasma iCa regardless of systemic calcium levels.[23]

RCA-CRRT protocol with predictable electrolyte profile is essential for patient safety and optimal SCD operation. Full automation of such a system for early deployment in the emergency room or operating room will require the development of an online filter effluent Ca- and citrate-sensor for verification of predicted CRRT circuit Ca- and citrate mass balance and for indirect measurement of systemic plasma total Ca- and citrate levels. This is clearly a clinically relevant and surmountable technologic challenge in the next decade.

CONCLUSION

New therapies directed toward treating inflammation in the past focused on interrupting the excessive levels of inflammatory cytokines (cytokine storm) or the activation of the coagulation system during sepsis, with little or modest effects on this disease process when tested clinically. As activated leukocytes are central to the pathogenesis and progression of sepsis and other clinical inflammatory disorders, new therapeutic approaches are being considered to limit the deleterious clinical effect of activated leukocytes that result from a dysregulated immune response to sepsis. The SCD is a synthetic, biomimetic membrane that binds and sequesters activated leukocytes from the systemic circulation along an extracorporeal blood circuit resulting with RCA in an immunomodulatory effect. This treatment approach changes systemic neutrophil kinetics and release of neutrophils from stored sites. Other novel, device-based therapies for systemic inflammation include the RAD, a cell bioreactor, which leverages metabolic and synthetic functions of stem-progenitor cells of the kidney directed toward a renal tubule cell fate in a multifactorial approach to the treatment of immune dysregulation and organ failure. Cell processing and cell therapy for the treatment of systemic inflammation are clinically promising approaches to combat sepsis and multiorgan failure.[24,25]

Key Points

1. Cell therapy has the potential to provide critically important functions of the native kidney that are not replaced by dialysis or hemofiltration.
2. A renal tubule assist device has been developed using either adult human or porcine renal tissue.
3. A bioartificial kidney consisting of the renal tubule assist device (containing either human or porcine cells) and a conventional filtration device has been found to replace filtration, transport, metabolic, and endocrine functions and adds value to current renal replacement therapies in both preclinical and clinical testing.
4. An extracorporeal biomimetic membrane device, called selective cytopheretic device (SCD), is a novel extracorporeal approach for in situ cell processing of activated circulating leukocytes.
5. The SCD has proven to be a platform technology to treat systemic and localized inflammatory disorders in preclinical animal models as well as clinical trials.

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