Therapeutic Perspective

CLINICAL INVESTIGATION

Strategies to treat sepsis: old and new

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Despite advances in critical care, the incidence and mortality from severe sepsis remains unacceptably high. Numerous strategies focusing on the pathophysiology of severe sepsis are being pursued. Bacteria and bacterial products that trigger sepsis are being targeted via antibodies, signaling antagonists and filtration columns. Excessive inflammation is being addressed with the use of specific blocking antibodies for injurious cytokines as well as devices and molecules that address the proinflammatory cytokine pathways. Injury to the gut and the endothelial lining that occur during sepsis and propagate inflammation and organ dysfunction is being addressed with protective molecules. Replacement strategies for protective molecules exhausted during the sepsis episode are being examined. Finally, strategies to reverse the immunosuppression of sepsis are being revived.

Keywords: complement cascade • immunosuppression of sepsis • severe sepsis • superantigens • TNF-α

Current state of treatment for severe sepsis

Severe sepsis, defined as a systemic inflammatory response due to infection with associated organ dysfunction, continues to be a considerable cause of morbidity and mortality worldwide. Epidemiologic studies have estimated an annual incidence of approximately 750,000 cases in the USA with a hospital mortality of approximately 30% [1]. From 1979 to 2000, Martin and colleagues demonstrated an annual increase of 8.7% in the number of cases [2]. Over this period, patients with severe sepsis were on average older, had more comorbidities and more organ dysfunction making their care more challenging.

An abundance amount of research over the past 20 years has led to improvements in the care of patients with severe sepsis. Kumar and colleagues examined the importance of the timeliness of appropriate antimicrobial therapy in the outcome of patients with septic shock [3]. In their study, every hour delay in appropriate antibiotic therapy from the onset of septic shock was associated with a 7.6% increase in mortality. Rivers and colleagues protocolized a treatment algorithm for the treatment of patients with septic shock and tissue hypoxia that involved targeting a mixed venous oxygen saturation of 70% with use of fluids, vasopressors, red blood cells and inotropes. This strategy termed Early Goal Directed Therapy (EGDT) was associated with a 42% relative risk reduction in in-hospital mortality [4]. Whether the remarkable results of this strategy can be replicated in larger studies at other centers remains to be demonstrated. In patients with acute respiratory distress syndrome, the use of lower tidal volume ventilation (6 ml/kg) and targeting lower inspiratory plateau pressures below 30 cm H_2O was associated with an improved mortality and fewer ventilator days compared with traditional tidal volumes of 12 ml/kg [5].

A number of therapies for the treatment of patients with severe sepsis remain somewhat controversial. A study by Annane and colleagues suggested a benefit of corticosteroids dosed hydrocortisone at 200 mg/day in patients with septic shock

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who had an impaired adrenal response to adrenocorticotropic hormone challenge [6]. A larger follow-up Hydrortisone Therapy for Patients with Septic Shock (CORTICUS) study did not confirm the benefit seen in the Annane study but did show a faster resolution of shock in the corticosteroid-treated group [7]. This study suggested that the role of corticosteroids is limited to the small population with refractory hypotension despite fluid resuscitation and vasopressors. Recombinant human-activated protein C (rhAPC), a molecule with anticoagulant, anti-inflammatory and profibrinolytic properties was associated with a 6% absolute reduction in mortality in patients with severe sepsis with the benefit largely confined to the sickest patients with acute physiology and chronic health evaluation (APACHE II) scores above 25, septic shock and two or more organ dysfunctions [8,9]. This agent was approved for use in these populations but an absence of benefit with this agent in a pediatric study and in these subgroups in a follow-on trial prompted the European regulatory agency to require a new placebo-controlled trial. An initial study in surgical patients indicated a benefit with intense glycemic control attempting to keep blood glucose below 110 mg/dl [10]. The Intensive versus Conventional Glucose Control in Critically Ill Patients (NICE-SUGAR) study did not confirm this benefit and instead revealed a worse outcome with a greater incidence of hypoglycemic events with this target glucose [11]. A target mean glucose of 140 mg/dl has been found to have a better outcome without hypoglycemic events.

The Surviving Sepsis Campaign has been an attempt to decrease the mortality from severe sepsis by protocolizing the care of patients. Care is divided into a resuscitation bundle to occur within the first 6 h of sepsis, which includes EGDT, antibiotic therapy and source control, and a management bundle to occur in the first 24 h that includes low tidal volume ventilation and glucose control. Individual hospitals are also directed to develop a policy on the use of rhAPC and corticosteroids [12]. Studies performed in Spain and globally demonstrated an increased compliance with these bundles over time with the use of these bundles being associated with a decrease in mortality [13,14]. The global study demonstrated a reduction in mortality over time from 37 to 30.8% [14]. The mortality from sepsis, however, remains unacceptably high and will require additional adjuvant therapies to lower mortality further. The remainder of this article will focus on old and new experimental strategies currently being investigated (Box 1).

Box 1. Experimental strategies for sepsis.

Targeting bacterial pathogens
- Antistaphylococcal monoclonal Abs
- Antipseudomonas monoclonal Abs
- Lytic bacteriophage against *Pseudomonas aeruginosa*

Targeting endotoxin
- Eritoran
- TAK-242
- Alakaline phosphatase
- Immobilized polymyxin B filter

Targeting superantigens
- Superantigen antagonists

Targeting signal transduction & inflammatory response
- Polyclonal TNF Fab fragments
- Vagal nerve stimulation
- α7-nicotinic acetylcholine receptor agonists
- HMGB-1 inhibitors
- Anti-RAGE Abs
- Suppressors of cytokine signaling
- High-volume hemofiltration
- PPARγ agonist
- Statins

Targeting the gut
- Bovine lactoferrin
- Recombinant human lactoferrin

Targeting coagulation cascade/endothelium
- Transgenic antithrombin
- Activated protein C
- Activated protein C variants
- Recombinant soluble thrombomodulin

Targeting complement
- Anti-C5a antibodies

Repletion of protective molecules
- Inter α inhibitor proteins
- Estrogen receptor binding agonists

Targeting immunosupression
- Inhibitors of lymphocyte apoptosis:
  - siRNA against Fas and BIM
  - HIV protease inhibitors
  - IL-7 and -15
  - Anti-PD-1 Abs
- Reversing monocyte hyporesponsiveness:
  - GM-CSF
  - IFN-γ

Abs: Antibodies; HMGB: High mobility group box; PD: Programmed death; PPAR: Peroxisome proliferator-activated receptor; RAGE: Receptor for advanced glycation end products.

Targeting bacterial pathogens, products & mediators

Antistaphylococcal & antipseudomonal monoclonal antibodies

Human monoclonal antibodies offer an attractive therapeutic option for targeted biologic antimicrobial therapy in sepsis. These antibodies are obtained by cloning antibody genes of human donor B-lymphocytes using hybridoma technologies, then screening for separate

monoclonal antibodies that bind the target antigen. Two clinically important pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa* currently have monoclonal antibodies in clinical development. These two pathogens have the remarkable capacity to acquire antibiotic resistance genes and cause invasive disease in normal hosts and immunocompromised patients [15].

Immune-based strategies against staphylococci are focused on preventing staphylococcal adherence and colonization factors, such as clumping factors A and B and microbial surface components recognizing adhesion matrix molecules [16–18]. Immunotherapy directed against known virulence factors during invasive staphylococcal infections are also potential targets. Polyclonal or monoclonal antibodies to staphylococcal capsular antigens [15], lipoteichoic acid [19,20], α toxin [21] and quorum-sensing molecules [22] are all under investigation. Tefibazumab (Aurexis®), a humanized monoclonal antibody, binds to clumping factor A, a virulence factor in *S. aureus* [16]. A Phase II clinical trial of patients with *S. aureus* bacteremia treated with tefibazumab yielded reduced nasal colonization in the treated group, but no difference in clinical outcomes. Monoclonal antibodies to *S. aureus* α-hemolysin, a pore-forming cytotoxin essential for its virulence in pneumonia have recently been discovered to antagonize toxin activity, protect experimental animals against lethal *S. aureus* pneumonia and prevent human lung cell injury *in vitro* [21]. A chimeric monoclonal antibody to lipoteichoic acid, known as pagibaximab, has been extensively studied in both high-risk neonates and adults [19,20]. The results thus far suggest some possible benefit, but the data are inconsistent and not convincing as yet. Further clinical studies with antibodies to α toxin and quorum-sensing regulators are planned in the future [15,21,22].

Despite its therapeutic rationale and clinical appeal, variable titers of protective human anti-Pseudomonas antibodies against a variety of molecular targets have made the monoclonal antibody approach to *P. aeruginosa* a difficult task to accomplish [23]. Antibodies directed against alginate capsules [24], O-antigens [25,26], flagellin [27], type III secretion systems [28] and quorum-sensing molecules [29] all appear promising in experimental models of *P. aeruginosa* infection. The use of Ig-inactivated transgenic mice reconstituted with human immunoglobulin loci to generate human Mab against various serotypes of *P. aeruginosa* lipopolysaccharide (LPS) O-specific side chains and recombinant human IgG1 in transgenic tobacco has proven to be useful [26]. Previous studies have noted tolerability and increased opsonophagocytic activity for pseudomonas in humans [23]; however, efficacy trials in human sepsis are still needed. A type-specific, human, IgM monoclonal antibody to *P. aeruginosa* serotype 011 is now in early clinical trials [25].

■ Lytic bacteriophages against *Pseudomonas aeruginosa*

The progressive development of antibiotic resistance among common bacterial pathogens has rekindled interest in novel non-antimicrobial alternatives to eradicate invasive bacterial infections. One of the more intriguing strategies to resurface recently is the use of lytic bacteriophage biotherapy against bacterial infection. Bacteriophage therapy was attempted in the preantibiotic era with variable results, but this approach was supplanted by the introduction of sulfa drugs and penicillin by the middle of the 20th century. A controlled clinical trial with direct instillation of a pseudomonas-specific phage into the ears of patients was recently reported in patients with refractory infection with multidrug resistant *P. aeruginosa* chronic otitis [30]. Phage therapy was well tolerated, improved symptoms and significantly reduced the quantity of *P. aeruginosa* in ear samples in treated patients compared with the standard care control group. Desperate measures, such as systemic administration of phage therapy, might be a consideration for severely septic patients with multidrug-resistant strains of *P. aeruginosa*.

Targeting microbial mediators

■ Endotoxin

Eritoran

Eritoran (E5564) is a semisynthetic lipid A antagonist derived from an unusual LPS molecule found in *Rhodobacter spp.* that functions as a competitive inhibitor at the cell membrane level. The LPS-MD2-Toll-like receptor (TLR)4 complex is now recognized to be the major LPS (also known as endotoxin) receptor in vertebrate animals including humans [31]. The lipid A component bacterial LPS is a potent activator of the innate immune response and inhibitors of LPS have been sought as therapy for sepsis for over a century [32]. E5564 is a tetra-acylated lipid A structure that binds to the hydrophobic pocket of MD2 and sterically inhibits the ability of hexa-acylated active forms of lipid A from pathogenic Gram-negative bacteria to bind to this essential receptor signaling complex [33]. E5564 contains an unusually long fatty acid (C-18) adjacent to unusually short fatty acids (C-10) rather than the standard 12–14 carbon fatty acids found in highly active forms of lipid A. E5564 binds to MD2 but fails to link to TLR4. Thus, E5564 blocks the final step of dimerization of TLR4-intracellular domains essential for signal transduction. When sufficient quantities of E5564 are available, LPS signaling is completely shut off [34,35]. Moreover, E5564 also appears to inhibit signaling from other endogenous danger-associated molecular pattern molecules that signal innate immune responses via TLR4 (e.g., high mobility group box-1 [HMGB-1]

and S100a proteins). This lipid A antagonist is highly active in preclinical models and in several Phase I human endotoxin volunteer studies. A 293 patient, multicenter, Phase II sepsis trial with eritoran has just been published with promising results [36]. The lipid A antagonist has been investigated in a recently completed Phase III, international, clinical trial in severe sepsis. The results of this study should be available in early 2011.

It should be noted that several other anti-TLR4 inhibitors are in various levels of preclinical development including specific monoclonal antibodies to MD2 and TLR4, soluble decoy receptor constructs and signal transduction inhibitors. Further progress with many of these other TLR4 inhibitor strategies is likely follow after the results of the clinical trial with eritoran are reported.

TAK242

Small molecule inhibitors that block the intracellular signaling induced by LPS attachment to MD2–TLR4 dimers have been developed and tested in human sepsis. One such molecule is known as TAK242. This molecule blocks LPS signaling at the level of engagement of the intracellular domain of the TLR4 receptor and its intracellular adapter molecules, including MyD88 [37,38]. TAK242 has proven effective in a variety of preclinical models of LPS-mediated cytokine expression and in animal models of sepsis [38]. This signal transduction inhibitor has undergone extensive testing in clinical sepsis with a Phase II clinical trial demonstrating promise with favorable trends (but not statistically significant) in mortality reduction in treated patients versus placebo-control standard therapy [39]. The 28-day all-cause mortality rate was 24% for placebo, 22% for low-dose TAK242 (1.2 mg/kg/day) and 17% for high-dose TAK242 (2.4 mg/kg/day) However, the study noted some toxicity with dose-related increase in methemoglobin levels and failed to reduce proinflammatory cytokine levels, the primary end point of the Phase II trial. Other similar small molecule signal transduction inhibitors of the TLR4 pathway are in early preclinical development with better safety profiles. Some of these inhibitors might be brought into clinical investigation in the near future.

Recombinant human alkaline phosphatase

Alkaline phosphatase is best known as a marker for intra- and extrahepatic biliary obstruction. Elevated alkaline phosphatase levels are frequently observed in the cholestasis of sepsis and yet the physiologic role of alkaline phosphatase has never been clearly defined. Alkaline phosphatase has the capacity to cleave phosphate groups from the lipid A portion of bacterial endotoxin [40]. Lipid A is biphosphorylated and removal of one or both of the phosphate moieties greatly diminishes or ablates the toxicity of lipid A structures [41]. Administration of human recombinant alkaline phosphatase has proven to be efficacious as an antiendotoxin strategy in a number of animal models [42,43]. In a small randomized, placebo-controlled clinical trial the intravenous administration of alkaline phophatase was associated with reduced renal inducible nitric oxide synthase expression and improved renal function compared with placebo [44].

Polymyxin B immobilized hemofiltration columns

The polymyxins (B and E) are cyclic cationic polypeptide detergents, first introduced into clinical medicine as antibiotics for Gram-negative bacterial infections in the early 1950s. Their clinical indications were severely limited due to their limited antimicrobial activity, nephrotoxicity and neurotoxicity. However, they have seen a renewed interest recently as a result of progressive antibiotic resistance to standard antimicrobial agents in Gram-negative bacteria, particularly *P. aeruginosa* and *Acinetobacter baumannii*. Polymyxin B is an antibacterial agent that possesses potent LPS-binding capacity via its cationic amino acids and alternating hydrophobic amino acids [45]. To avoid nephrotoxicity and neurotoxicity, polymyxin B has been immobilized onto hemofiltration cartridges for extracorporeal use as an endotoxin removal device. There is extensive clinical experience with this endotoxin adsorption strategy in Japan. These polymyxin B-immobilized columns may remove other potentially injurious components of septic plasma such as HMGB-1 and other negatively charged, bioactive lipid molecules [46,47]. A number of other types of hemoperfusion columns with many different polymers and physiochemical properties are also under clinical investigation at the present time.

Numerous clinical trials with polymyxin columns suggest therapeutic benefit in severely septic patients and a recent meta-analysis of these studies supports their clinical value [45]. A recent, randomized, double-blind study comparing polymyxin B immobilized columns with a dummy cartridge hemoperfusion system in septic shock patients due to intra-abdominal infection demonstrated statistically significant improved cardiovascular function and a trend towards improved survival with polymyxin B columns [48]. A large, randomized, placebo-controlled, Phase III trial is now underway in North America to determine if this survival advantage can be confirmed in a larger patient population with severe sepsis.

■ Superantigen antagonists

The superantigens are a family of streptococcal and staphylococcal exotoxins that induce profound activation of the immune system without the need for initial antigen processing and presentation. Superantigens bind

to the MHC class II molecules on antigen-presenting cells and link with specific β loops on the variable end of the T-cell receptor (Vβ) commonly expressed on a substantial percentage (5–20%) of $CD4^+$ T cells. This linkage endows superantigens with the capacity to activate both T cells and monocytes with the generation of excessive quantities of proinflammatory cytokines and chemokines. These superantigens are responsible for the molecular pathogenesis of streptococcal and staphylococcal toxic shock syndromes [49].

Inhibition of superantigens can be accomplished through adsorption, antibody clearance or the use of specific peptide inhibitors of superantigen activity. Passive immunotherapy with human immunoglobulin remains a standard adjuvant treatment for toxic shock syndromes, despite their uncertain efficacy. Improved superantigen antagonists are under development, which may prove to be of clinical value for toxic shock syndrome states and perhaps more general use in a variety of other bacterial infections [50].

Targeting signal transduction & the inflammatory response

■ Ovine anti-TNF-α polyclonal Fab fragment (CytoFab™)

TNF-α is a potent and early proinflammatory cytokine released from myeloid cells in response to pathogen-associated molecular patterns such as endotoxin, lipoteichoic acid, bacterial lipopeptides and peptidoglycan. This cytokine can be lethal at high concentrations by receptor-mediated damage to endothelial tissues, activation of the clotting cascade, expression of acute phase proteins and induction of nitric oxide synthesis. CytoFab™ is an ovine anti-TNF-α polyclonal Fab fragment antibody preparation produced by immunizing sheep with human TNF-α. While early attempts using anti-TNF-α antibodies have failed; the advantage of this strategy from an immunologic perspective is the ability of a polyclonal antibody preparation to bind to multiple epitopes on the TNF protein. Moreover, the Fab fragments provide a reduced risk of immunotoxicity due to absence of a Fc antibody tail (prevents complement fixation and prevents against antibody-dependent cellular cytotoxicity). The Fab fragments have a larger volume of distribution owing to its smaller size and more rapid clearance. CytoFab has been studied in a Phase IIb randomized double-blind placebo-controlled trial in 81 septic patients with shock or two organ dysfunctions. CytoFab increased mean ventilator-free days (15.0 vs 9.8; p = 0.040) and ICU-free days (12.6 vs 7.6; p = 0.030) compared with placebo. The all-cause 28-day mortality rates were 26% in the CytoFab group versus 37% in placebo patients [51]. The polyclonal antibody preparation is currently undergoing another and larger Phase II study.

■ Vagal nerve stimulation & α7 nicotinic acetylcholine receptor agonists

Stimulation of the vagus nerve results in decreased production of proinflammatory cytokines from monocytes. This cholinergic anti-inflammatory reflex occurs via the action of the vagus' principal neurotransmitter, acetylcholine, on the α7 acetylcholine receptor on monocytes. Electrical stimulation of the vagus nerve in a lethal rat model of endotoxemia decreased hepatic TNF-α production and prevented the development of shock. Animals that underwent a vagotomy had an increased production of TNF-α and a shorter time to the development of shock [52]. This reflex is primarily accomplished by vagal actions on monocyte/macrophage populations residing in the spleen. Animals that received vagal nerve stimulation had less activation of the clotting cascade, less inhibition of the fibrinolytic system and smaller decreases in other endogenous anticoagulant levels [53]. Implantation of a vagal nerve stimulator in severely septic patients could be a safe and effective method in the future to control the inflammatory and coagulopathic response.

Pharmacologic intervention to induce this anti-inflammatory reflex mechanism is also a potentially novel therapeutic strategy for sepsis. Mice given nicotine before an intraperitoneal challenge with *Escherichia coli* experienced diminished inflammatory cell infiltrates within the peritoneum and decreased cytokine levels. In the cecal ligation and perforation (CLP) model in mice (a commonly used model causing intra-abdominal sepsis in mice, that involves ligating then puncturing the mouse cecum), nicotine treatment was able to improve survival even when delivered 24 h after the infectious insult. Treatment with nicotine also reduced HMGB-1 levels, a proinflammatory molecule and late mediator of sepsis [53]. Highly selective α7 nicotinic acetylcholine receptor agonists to activate this pathway are currently under development [54].

■ HMBG-1 protein inhibition

High mobility group box-1 is a nonhistone, nuclear and cytoplasmic protein that binds to DNA and regulates its transcription. HMGB-1 is now recognized as a danger-associated molecular pattern molecule. HMGB-1 is released into the extracellular fluid in response to a systemic inflammatory process and by necrotic cells. In low concentrations, HMGB-1 can stimulate chemotaxis, by facilitating binding of neutrophils to the endothelium and through the release of TNF-α and IL-1. In high concentrations, HMGB-1 disrupts the endothelial barrier, enterocyte stability and can lead to acute lung injury [55]. HMGB-1 is an attractive target for therapeutic intervention compared with many other proinflammatory mediators as it is a late mediator of

lethality. Release of HMGB-1 occurs approximately 20 h after monocytes are challenged with endotoxin. In patients with severe sepsis, high levels of HMGB-1 are readily detected as much as 7 days after onset, at a time when other proinflammatory cytokines have peaked and are undetectable [56].

Anti-HMGB-1 antibodies given to mice as late as 24 h after an endotoxin challenge or cecal ligation and puncture-induced peritonitis are protective [55]. These findings indicate that anti-HMGB-1 therapies might be efficacious even when administered well after the onset of established severe sepsis. The HMGB-1 protein contains two DNA binding regions, the 'A Box' and 'B Box' near the carboxy-terminus. The B Box component of the molecule stimulates TNF-α release from monocytes and mediates lethality; the A Box portion can competitively inhibit the inflammatory actions of the HMGB1 molecule. Passive treatment with the A Box 24 h postendotoxin challenge and cecal ligation and perforation is also protective in mice. Nicotine and other selective agonists of the 7α acetylcholine receptor, transcutaneous vagus nerve stimulation, green tea and lysophosphatidyl choline can decrease the production of HMGB-1 from LPS-challenged monocytes [55]. Human trials of molecules that specifically target HMGB-1 have yet to be performed.

■ Anti-RAGE therapies

The receptor for advanced glycation end products (RAGE) is ubiquitously expressed on immune effector cells, epithelial cells and mesenchymal cells of the human body [57]. RAGE is of special interest as it is upregulated in acute and chronic inflammatory states while most other cytokine receptors are downregulated in acute inflammation. RAGE recognizes a wide array of endogenous ligands of potential significance in septic shock, such as HMGB-1, S-100a and other calgranulins. RAGE is also a counter receptor for the neutrophil adhesion molecules β-2 integrins. Inhibition of RAGE by soluble decoy receptors or anti-RAGE antibodies has beneficial effects in clinically relevant, experimental models of sepsis [58,59]. RAGE inhibitors can be given as a salvage therapy in established sepsis, at least in animal models of intra-abdominal sepsis [60]. Clinical trials with RAGE inhibitors are currently under consideration as a treatment strategy for severe sepsis.

■ Suppressors of cytokine signaling

Suppressors of cytokine signaling (SOCS) are a family of eight endogenous, intracellular, signaling proteins that regulate cytokines, chemokines, interferon and growth factor signaling pathways. They primarily act upon the JAK–STAT pathways. SOCS-3 is prominently expressed in myeloid cells and its presence limits excess cytokine generation [61]. SOCS-1 is primarily found in T cells and prevents excess apoptosis of activated T cells during acute inflammatory states. Therapeutic agents that promote the expression of SOCS proteins could provide survival advantage in humans, as has been demonstrated in experimental animals [62]. Preclinical work on SOCS continues, but no clinical trials with specific SOCS activator molecules in human sepsis have been undertaken thus far.

■ High-volume hemofiltration systems

High-volume hemofiltration (HVHF) is designed as a blood purification technique by using membranes with high porosity characteristics with high flow rates to efficiently clear middle-size molecules, including proinflammatory cytokines, vasoactive peptides and chemokines that are felt to be central mediators in the pathogenesis of septic shock [63]. The major advantage of this technique is the simultaneous removal of a myriad of inflammatory molecules, thereby attenuating the deleterious systemic inflammatory response. Local and likely beneficial extravascular inflammatory reactions at the site of infection are left unchecked by this technique [63,64]. In addition to the hemodynamic and technical issues that arise when employing HVHF in septic shock patients with sepsis, there is a possible concern over the concomitant loss of coagulation regulators, anti-inflammatory cytokines and other counter-regulatory molecules that are found in the plasma at the later stages of sepsis. Patients are usually given an albumin infusion to maintain oncotic pressure while using this technique. Pulse dosing of HVHF and various techniques of heme absorption to remove specific inflammatory cytokines are also under study [64,65].

The clinical efficacy of this HVHF technique in septic shock remains a subject of considerable debate. Initial studies with HVHF with continuous venovenous hemofiltration appeared to provide survival benefits in severe sepsis if filtration rates were maintained at above 45 ml/kg/h [65,66]. Several other clinical studies with HVHF have shown inconsistent results. The completion of several, large prospective clinical trials should finally determine the safety, feasibility and efficacy of this strategy for severe sepsis [63,64].

■ Peroxisome proliferator-activated receptor-γ agonists

Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the nuclear receptor family and has numerous desirable attributes that might be useful as a therapy for sepsis. PPARγ agonists have been found to regulate inflammation and support mitochondrial number and function. When PPARγ is engaged by ligands it forms a heterodimer with retinoic acid receptor and blocks coactivators of proinflammatory genes [67]. PPARγ

activation attenuates the cytokine and hemodynamic response to sepsis in experimental models of sepsis and reduces neutrophil infiltration into vital organs. Commonly prescribed thiazolidinediones, such as the glucose-lowering agent pioglitazone, are ligands for PPARγ and activate this molecule. Other ligands include cyclopentenone prostaglandins and nonsteroidal anti-inflammatory drugs [68]. In murine models of sterile inflammation and multiple organ dysfunction, PPARγ agonists decreased inflammation and organ injury. Human trials with specific agonists for PPARγ are now under consideration as a novel treatment for sepsis.

■ Hydroxymethyl methylglutaryl–coenzyme A reductase inhibitors (statins)

Hydroxymethyl methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors are effective in lowering cholesterol through their involvement in the mevalonate pathway. These agents also have potent anti-inflammatory properties and limit lipid raft formation. Large observational studies have shown a decreased mortality rate in patients with bacteremia who are receiving statins as lipid-lowering agents [69]. Statins have decreased mortality in animal models of sepsis and maintain blood pressure and decreased proinflammatory cytokine production. When healthy human volunteers are given statins before endotoxin challenge, they experience improved blood pressure responses, decreased monocyte tissue-factor expression, decreased inflammatory markers and decreased TLR4 expression on their monocytes [70]. A variety of HMG-CoA reductase inhibitors are currently in clinical trials of abdominal sepsis, severe influenza pneumonia, early sepsis and septic shock. The results of these studies will determine if these statin agents are valuable as therapies for severe sepsis/septic shock.

Targeting the gut

The GI tract has often been implicated as the driving 'motor' for the systemic inflammation seen in sepsis. Splanchnic hypoperfusion is associated with epithelial damage and altered intestinal permeability. Increased intestinal permeability can lead to the translocation of bacterial products including endotoxin that augment the inflammatory response. Ischemia to the gut also leads to the release of proinflammatory cytokines from gastrointestinal epithelial cells [71]. Apoptosis of lymphocytes in the Peyer's patches of the small bowel has also been detected during sepsis leading to a state of immunosuppression [72]. All of these features suggest that the gut is a prime target for sepsis therapy.

Lactoferrin, a neutrophil granular protein and secreted protein in breast milk, has many important gut protective mechanisms of action when administered orally. Orally administered lactoferrin has essentially no bioavailability in the systemic circulation [73]. Lactoferrin was able to reduce nonsteroidal anti-inflammatory drug-induced gut permeability when administered orally compared with placebo [74]. In a rat model lactoferrin prevented the translocation of *E. coli* and endotoxin from the bowel following artificial instillation [75]. In a rat LPS model of sepsis, oral lactoferrin decreased mortality and abrogated the rise in pro- and anti-inflammatory cytokines compared with placebo [Malik R, Pers. Comm.]. Studies using human recombinant lactoferrin have indicated that it can modulate both the Th1 response (proinflammatory) or Th2 response (anti-inflammatory) based on host response, potentially making it a more sophisticated sepsis therapy than a strict anti-inflammatory molecule [76].

Lactoferrin has been studied in two human sepsis studies. Bovine lactoferrin was given orally in a placebo-controlled trial of very low-birth-weight neonates to look at the incidence of late-onset sepsis. The incidence of late-onset sepsis was significantly lower in the bovine-derived lactoferrin group (9/153 [5.9%]) compared with the placebo group (29/168 [17.3%]; p = 0.002) [77]. Recombinant human lactoferrin (talactoferrin) was studied in a placebo-controlled study of 190 adult patients with severe sepsis. Talctoferrin was given as a 1.5 g dose orally every 8 h during the ICU stay for up to 28 days. The 28-day, all-cause mortality was 14.6% in the talactoferrin arm and 26.6% in the placebo arm with a p-value of 0.057 when adjusted for the presence of cardiovascular dysfunction. The incidence of adverse events was similar in the two treatment arms [78]. A Phase III trial of talactoferrin is currently being planned.

Targeting the coagulation cascade/endothelium

Perturbations to the endothelium, activation of the coagulation system and inhibition of the fibrinolytic system have been well described during sepsis. Proinflammatory cytokines, including TNF-α and IL-1 are capable of triggering the expression of tissue factor on the surface of the endothelium and monocytes ultimately leading to the downstream production of thrombin and fibrin. Thrombin, in itself, is a proinflammatory molecule. Thrombin signaling through the protease-activated receptor (PAR)-1 receptor perpetuates inflammatory signaling in sepsis. Inhibition of normal fibrinolysis occurs during sepsis due the production of plasminogen activator inhibitor (PAI)-1. Counter-regulatory endogenous anticoagulants including antithrombin, activated protein C and tissue factor pathway inhibitor (TFPI) are poorly produced during sepsis and are rapidly consumed [79]. The endothelial lining is damaged during sepsis resulting in the capillary leak syndrome that is characteristic of patients with sepsis [80].

The perturbations to the endothelium, coagulation system and fibrinolytic system have presented many potential treatment strategies for sepsis. Anticoagulant therapy for sepsis has been studied extensively. Retrospective studies and subgroup analyses from major trials of endogenous anticoagulants suggested a clinical benefit to heparin in patients with severe sepsis. The recently completed, placebo-controlled, Randomized Clinical Trial of Unfractionated Heparin for the Treatment of Sepsis (HETRASE) Study did not reveal any benefit with unfractionated heparin compared with placebo with respect to 28-day all-cause mortality [81]. TFPI, a molecule that prevents thrombin generation at multiple coagulation steps similarly did not demonstrate a benefit in patients with severe sepsis or in a recently completed trial in severe community-acquired pneumonia [Wunderink R, Pers. Comm.] [82]. Heparin and TFPI are no longer being studied for sepsis but an anti-tissue factor monoclonal antibody is under evaluation in acute lung injury/ARDS.

Anticoagulant drugs that are still being investigated for sepsis are antithrombin (AT), rhAPC, recombinant soluble thrombomodulin (rSTM). AT is an endogenous anticoagulant that inactivates multiple clotting enzymes including Xa, IXa, XIa and tissue factor: VIIa complex while also inhibiting thrombin by forming thrombin-AT complexes. Furthermore, AT has anti-inflammatory activity by inhibiting proinflammatory cytokine release and limiting white cell-endothelial interactions [83]. Plasma derived AT was tested in a large, placebo-controlled trial in patients with severe sepsis. No treatment benefit was observed with AT in this trial [84]. Potential reasons for failure of this study included a failure to achieve the supraphysiologic levels of AT of 200% necessary to achieve a clinical response as well as the allowance for heparin coadministration, which blunts the anti-inflammatory activities of AT and resulted in a greater incidence of bleeding events [83]. A transgenic AT molecule produced in goats is now available for clinical testing in humans. Any future trials of AT should target higher blood levels than those reached in previous trials and should avoid the concomitant use of heparin.

Activated protein C is an endogenous anticoagulant that remains under intense investigation. As stated previously, while the recombinant form of this molecule (rhAPC) is a currently approved therapy for severe sepsis, skepticism over its efficacy has prompted a worldwide, confirmatory placebo-controlled trial in patients with septic shock (PROWESS Shock). Multiple sources of data suggest that the benefit of APC may be caused by the maintenance of endothelial integrity (the protein C cytoprotective pathway) rather than its anticoagulant effects. In the PROWESS trial, the 28-day all-cause mortality in patients with septic shock was 29.3% in rhAPC-treated patients compared with 37.6% in placebo-treated patients [85]. In a human endotoxin challenge model, rhAPC-blocked endotoxin induced changes in blood pressure but had little effect on coagulation or inflammatory parameters [86]. *In vitro* studies have demonstrated that APC in concert with the endothelial protein C receptor results in the cleavage of PAR-1. PAR-1 activation by APC results in sphingosine-1-phosphate receptor crossactivation stabilizing the endothelial barrier [87]. Mutants of APC with less than 10% anticoagulant activity compared with the wild-type molecule but with intact PAR-1 signaling have been created and been found to be protective in an LPS challenge model of sepsis [88]. Further testing of these so called 'APC mutants' as well as sphingosine-1-phosphate agonists in humans is warranted.

Soluble thrombomodulin is a molecule involved in the protein C pathway that is being investigated in sepsis-induced disseminated intravascular coagulation. Native thrombomodulin plays a role in concert with thrombin and the endothelial protein C receptor in the conversion of protein C to APC [89]. The nonanticoagulant lectin domain of thrombomodulin has anti-inflammatory properties, including the blockade of HMGB-1 and inhibitory effects on complement [90,91]. A recombinant soluble thrombomodulin (ART-123) was tested against a heparin comparator in patients with DIC in Japan. ART-123 was associated with a 6.6% reduction in 28-day all-cause mortality in the patients with infection-induced DIC (95% CI: -246–11.3) [92]. Results are eagerly awaited on a recently completed, randomized, placebo-controlled study of 750 patients with sepsis-induced DIC. Much like activated protein C, thrombomodulin variants with an active anti-inflammatory lectin domain and little by way of anticoagulant activity should also be studied.

Targeting the complement cascade

The complement cascade is an arm of the innate immune system directed at destroying invading pathogens. The pathway can be triggered by preformed antibodies, bacterial components, such as endotoxin or the recognition of bacterial or fungal carbohydrates by endogenous lectins. The cascade consists of a sequence of consecutively activated proteins that converge at the level of C3 convertase with the production of the effector molecules C3a, C3b, C5a, C5b and C5b-9 [93]. C3b is essential to opsonization while C5a is essential for chemotaxis. C5b-9 is the terminal membrane attack complex that can directly lyse bacterial cell walls of Gram-negative organisms [93].

The vast amount of interest in targeting complement as a therapeutic strategy has focused on the C5a molecule. Other than serving as a chemotactic factor C5a leads to many events, which in excess can lead to pathologic consequences [94]. Excessive C5a production can lead to inflammation through increased production of the proinflammatory mediators TNF and IL-1, coagulation through the elaboration of tissue factor on the endothelial and monocyte surfaces and myocardial depression by its deleterious effects on cardiac myocytes. Excessive C5a can lead to immunosuppresion by causing defective neutrophil function and oxidative burst, and lymphocyte apoptosis.

The consequences of C5a blockade have been examined in a number of animal models of sepsis. In a rat cecal ligation and perforation model, polyclonal anti-C5a antibody administration was associated with improved survival, improved H_2O_2 production by neutrophils and decreased bacterial counts compared with rats treated with preimmune IgG. Improved survival occurred when therapy was initiated up to 12 h following the insult [95]. In this same model, blockade of C5a was associated with less activation of coagulation cascade and inhibition of fibrinolysis compared with preimmune IgG-treated rats [96]. A decrease in thymocyte apoptosis was observed in this model with anti-C5a administration [97]. In a porcine model of *E. coli* sepsis, anti-C5a antibodies were associated with improved oxygen utilization and attenuated lactate levels compared with control animals [98]. Flierl and colleagues recently demonstrated the prevention of the breakdown of the blood–brain barrier with anti-C5 antibodies in an animal model of sepsis [99]. While anti-C5 molecules are in clinical development; none have been tested in septic humans as yet.

Repletion of protective molecules

■ Inter-α-inhibitor protein

Inter-α-inhibitor protein(s) (IαIp) belong to a family of endogenous plasma antiproteases, including the serine protease inhibitors. These proteins circulate in high concentration in both health and disease and defend the host against excess systemic protease activity. IαIp is a complex protein that circulates as a covalently linked set of two heavy chains and one light chain held together with a glycosaminoglycan, known as chondrotin sulfate [100]. The active site is located on the light chain and contains two kunitz domains essential to its enzymatic inhibitory function. IαIp is a broad spectrum protease inhibitor with substrates that include typsin, fibrin, granzymes, clotting enzymes and activated complement components [101].

Longitudinal studies in severely septic patients reveal a progressive loss of IαIp levels as a result of both decreased hepatic synthesis and accelerated degradation and clearance [100]. Decreased IαIp levels are associated with poor prognosis and repletion of IαIp using purified plasma-derived human IαIp results in improved outcomes in experimental models of sepsis [100,101]. Phase II testing in China with the light chain of IαIp, known as urinary thrombin inhibitor, was associated with improved outcomes in severely septic patients [102,103]. Follow-up clinical testing in North America using human plasma-derived IαIp are planned in the near future.

■ Estrogens & estrogen receptor binding agonists

Women have a decided advantage in epidemiologic studies with respect to reduced incidence of septsis compared with men, and female animals fare better than their male counterparts in experimental sepsis systems. This protective effect is mediated primarily by the sex sterols and in particular estrogens [104]. The major estrogenic compound in humans is estradiol and administration of estradiol has multiple salutary effects that contribute to host defenses against the development of sepsis. Estrogens have two major cytoplasmic and nuclear receptors known as estrogen receptor (ER)-α or -β. ERα primarily mediates female secondary sex characteristics while ERβ is expressed both in males and females and mediates a myriad of extragonadal functions, including maintenance of the splanchnic microcirculation during hypotensive stress, attenuation of cytokine synthesis, mucosal stability and epithelial membrane barrier function, antiapoptotic activity, mitochondrial stability and cardiac myocyte protection [105]. The benefits attributable to estradiol therapy are principally related to its ability to ligate with ERβ and activate these functions [106]. High affinity, nonsteroidal agonists of ERβ have been developed and these agonists have proven to be highly effective in animal models of sepsis in both males and females [107,108]. These agents have been studied extensively in patients for other indications. It is anticipated that ERβ agonists will be studied as a therapy for sepsis in the near future.

Targeting the 'immunosuppression' of sepsis

The vast majority of experimental treatment strategies for severe sepsis have focused on attenuating an overexuberant inflammatory response. Laboratory and autopsy studies have indicated that a state of immunosuppression or 'immunoparalysis' can develop during the course of the septic episode. This immuoparalyzed state is believed to develop due to both lymphocyte apoptosis and monocyte hyporesponsiveness [109]. While these observations are not recent, there is a renewed interest in targeting these states with therapeutics.

The development of lymphopenia is a consistent finding during human sepsis. Autopsy studies have demonstrated excessive lymphocyte death in the spleen and the gut-associated lymphoid tissue (GALT) as well as peripheral lymphopenia in patients dying from sepsis compared with patients dying from other nonseptic causes of critical illness [72]. This lymphopenia has been found to occur through apoptosis or programmed cell death [110]. Apoptosis can be triggered by the proinflammatory cytokines TNF-α and Fas via caspase 8 or molecules including reactive oxygen intermediates and corticosteroids via caspase 9. Both of these caspases activate caspase 3 resulting in DNA fragmentation and cell death [111].

A number of strategies aimed at preventing the excessive lymphocyte apoptosis of sepsis have been tested in animal models. siRNA molecules developed against the apoptotic factors Fas and BIM have been protective in the cecal ligation and perforation model of sepsis [112,113]. HIV protease inhibitors are associated with decreased HIV-induced CD4 T-cell apoptosis as well as decreased lymphocyte apoptosis to stimuli such as pancreatitis. HIV protease inhibitor administration in the cecal ligation and perforation model of sepsis was associated with improved mortality, an increase in Th1 proinflammatory cytokines and a decrease in TH2 anti-inflammatory cytokines [114]. IL-7 and IL-15 have also been demonstrated to limit lymphocyte apoptosis and improve lymphocyte function [115]. Recently the negative costimulatory molecule programmed death-1 (PD-1) has been demonstrated to be upregulated following a septic challenge and to be associated with lymphocyte apoptosis, and a decrease in proinflammatory cytokine production. In the cecal ligation model anti-PD-1 antibodies were associated with improved survival, prevention of the loss of splenic lymphocytes and improved IL-6 production [116].

A state of monocyte hyporesponsiveness whereby lymphocytes lose the ability to generate inflammatory cytokine release has been found to occur during sepsis. The expression of human leukocyte antigen DR on monocytes (mHLA-DR) has been determined to be a marker of the capacity of monocytes to generate proinflammatory cytokines in response to a stimulus such as LPS [117]. Prolonged periods with decreased mHLA-DR expression have been associated with a poor outcome in sepsis [118]. Studies dating back to 1997 by Docke and Kox demonstrated the ability to reverse monocyte deactivation in patients with mHLA-DR expression under 30% with the administration of subcutaneous IFN-γ-Iβ [119,120]. Large multicenter trials of IFN-γ did not take place due to the lack of a standardized assay for mHLA-DR expression from laboratory to laboratory. The type of flow cytometer as well as its settings and the specimen handling were found to dramatically affect the mHLA-DR expression.

The development of a standardized assay for monocyte HLA-DR expression has opened the door for testing agents that could potentially reverse the monocyte deactivation of sepsis. The Becton Dickinson Quantibrite™ assay, which utilizes an anti-HLA-DR antibody conjugated 1:1 with phycoethryin, allows the measurement of antibodies bound per cell (AB/cell) and is not affected by the type of flow cytometer or settings [120]. Studies with this assay in patients who have undergone cardiopulmonary bypass demonstrated that under 5000 AB/cell was predictive of patients who developed infectious sequelae while normal controls often have approximately 13,000–21,000 AB/cell [121]. Lukaszewitz and colleagues demonstrated in a critically ill population that a slow recovery slope mHLA-DR expression was associated with the development of secondary infection [122]. GM-CSF was examined in a pilot study of severe sepsis patients with under 8000 AB/cell for two consecutive days [123]. GM-CSF administered subjects compared with placebo-treated subjects had statistically significant increases in mHLA-DR expression, proinflammatory cytokine production and trends toward improvement in ICU and hospital stay as well as duration of mechanical ventilation. Larger trials of GM-CSF and IFN-γ should be performed using this assay with examination of different cut-off values for mHLA-DR expression.

Future perspective

The current treatment of severe sepsis largely consists of appropriate antibiotic therapy, source control of infection and support of failing organs. rhAPC is the only approved adjuvant agent for severe sepsis but its use is not widespread due to bleeding risk and uncertainties over its efficacy and protective mechanism of action. The future of sepsis therapy will involve the use of a menu of treatment modalities that address the numerous pathways involved in the septic response as well as a means to determine in real time which of these pathways are at play in a given individual at a given point of time in the illness. Gene expression and proteomic methods can monitor multiple pathways in an individual simultaneously. The net state of inflammation or immunosuppresion will be able to be monitored allowing a physician to prescribe a pro- or anti-inflammatory regimen or the use of a device to adjust the balance of mediators. The ability of real-time detection of bacteria in the bloodstream as well as bacterial mediators would allow the use of monoclonal antibodies against specific pathogens, superantigen antagonists, endotoxin antagonists and hemofiltration systems. Disruption of

Executive summary

Current state of treatment for severe sepsis

- The incidence and mortality of severe sepsis remain unacceptably high despite improvements in critical care.
- Current therapy of severe sepsis includes early appropriate antibiotic therapy, source control of infection, reversal of tissue hypoxia, low tidal volume ventilation for ARDS and reasonable glucose control.
- The roles of recombinant human-activated protein C in severe sepsis and corticosteroids in septic shock remains unclear.

Targeting bacterial pathogens, products & mediators

- Monoclonal immunoglobulins and bacteriophage are being created against the common etiologies of sepsis *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Targeting microbial mediators

- Endotoxin signaling through the Toll-Like receptor 4 can be blocked by eritoran and TAK242.
- Polymyxin B-immobilized columns can remove endotoxin from the circulation and have shown clinical benefit in multiple small studies of patients with septic shock.
- Superantigens released by Gram-positive organisms can result in a massive proinflammatory cytokine release via T-cell and monocyte activation.

Targeting signal transduction & the inflammatory response

- An ovine polyclonal TNF Fab fragment preparation has demonstrated an improvement in mortality and ventilator-free days in a preliminary Phase II study.
- Pharmacologic or electrical stimulation of the vagus nerve can elicit a cholinergic anti-inflammatory reflex.
- High mobility group box-1 is a late proinflammatory mediator of sepsis that can be targeted via multiple approaches.
- Commonly prescribed drugs including statins for hypercholesterolemia and peroxisome proliferator-activated receptor-γ agonists for glucose lowering have anti-inflammatory activity that may benefit sepsis patients.

Targeting the gut

- Lactoferrin, a neutrophil granular protein secreted in breast milk, has been shown to decrease gut permeability in response to injury, decrease endotoxin and bacterial translocation across the gut, and modulate cytokine release.
- The oral administration of bovine lactoferrin was associated with a decreased incidence of late-onset sepsis in very-low-birth weight neonates.
- Administration of oral recombinant human lactoferrin was associated with a trend towards decreased mortality compared with placebo in adults with severe sepsis.

Targeting the coagulation cascade/endothelium

- Severe sepsis is associated with the disruption of the endothelial barrier, activation of the coagulation cascade, inhibition of normal fibrinolysis, and the depletion of the endogenous anticoagulants antithrombin and protein C.
- Administration of heparin, recombinant tissue factor pathway inhibitor and antithrombin have not improved outcome in severe sepsis.
- Nonanticoagulant-activated protein C variants with enhanced endothelial barrier protective function warrant further study.
- Recombinant soluble thrombomodulin, a cofactor in the activation of protein C with additional anti-inflammatory benefits, may have benefits in patients with sepsis-induced disseminated intravascular coagulation.

Targeting the complement cascade

- The complement cascade, an arm of innate immunity, is involved in bacterial lysis, normal opsonization and white cell chemotaxis.
- In animal models of sepsis, C5a blockade has been associated with improved survival, decreased coagulation activation, improved oxygen utilization, decreased thymocyte apoptosis and protection of the blood–brain barrier.

Repletion of protective molecules

- Inter-α-inhibitor proteins (IαIp) are plasma proteins that have broad spectrum protease inhibitor functions.
- Depletion of IαIp is associated with poor outcome in septic humans and repletion of depleted IαIp has been demonstrated to improve outcome in animal models of sepsis.
- Estradiol decreases systemic inflammation and has protective effects on the microcirculation, cardiac myocytes and mitochondria.

Targeting the 'immunosuppression' of sepsis

- A state of immunosuppression develops during sepsis and is thought to be due to excessive lymphocyte apoptosis and monocyte hyporesponsiveness.
- Numerous targets responsible for lymphocyte apoptosis including Fas, BIM and PD-1 are being targeted with siRNA and antibodies.
- Decreased expression of monocyte HLA-DR is a marker of monocyte hyporesponsiveness.
- GM-CSF and IFNγ have been demonstrated to be well tolerated and capable of increasing monocyte HLA-DR expression in septic humans.

the endothelium will be able to be monitored with biomarkers allowing the use of a protective strategy, such as an APC variant. Biomarkers will also serve as theragnostics for detecting and replacing depleted protective molecules, such as IαIP. In short, sepsis therapy in the future will become increasingly sophisticated through the use of multiple biomarkers and a cocktail of therapies adjusted to the individual subject's response.

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