Evolving strategies in the treatment of sepsis and systemic inflammatory response syndrome (SIRS)

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Summary

In recent years, much basic science research has investigated the predisposing factors, initiation, propagation, and resolution of Gram-negative sepsis, endotoxaemic shock, and the newly defined entity of systemic inflammatory response syndrome (SIRS). A major cause of morbidity and mortality in the post-surgical, neonatal, and geriatric hospital population, sepsis has proven itself notoriously resistant to classical modes of therapy, including antibiotics, fluid/pressor and respiratory support. Recently, the widespread nosocomial isolation of new antibiotic-resistant strains of endotoxin-producing bacteria has further complicated management. For these reasons, there is much interest in alternative treatment modalities which focus upon the endotoxin molecule itself and the systemic inflammatory response it provokes via the cytokine, complement, and coagulation cascades. In this review, recent experimental approaches to the therapy of sepsis and SIRS are discussed in light of each step in the complex inflammatory cascade and in comparison to traditional approaches to prevention and therapy of Gram-negative bacteraemia and septic shock.

Introduction

Gram-negative sepsis and endotoxic shock are complicated pathophysiological entities which have defied effective medical treatment for centuries and are still major sources of morbidity and mortality in the elderly, the immunocompromised, and in the post-operative setting. Recent studies have estimated a mortality of close to 40% in patients with severe sepsis and related haemodynamic compromise.1,2 Past therapeutic research has focused primarily upon treating Gram-negative bacteraemia and, in so doing, indirectly blocking the formation and activity of the primary deleterious bacterial product, endotoxin or lipopolysaccharide (LPS) and its associated proinflammatory cytokines (of which tumour necrosis factor alpha and interleukin–1 are probably the most important).3 The systemic inflammatory response syndrome (SIRS), or the inflammatory cascade associated with sepsis and other conditions, is the dreaded complication which far outweighs the direct toxicity of the bacterial infection itself in clinical importance.4 Endotoxin, and the SIRS it provokes, are the foci of the development of new antisepsis agents and the subject of this review.

Both Gram-positive organisms such as Staphylococcus aureus and Gram-negative species such as Bordetella pertussis also elaborate proteinaceous exotoxins which appear to act through a similar molecular pathway to that of the much better understood endotoxin;4–6 indeed, a recent study suggested that mortality in Gram-positive septic shock may in fact be higher.7 The clinical and laboratory parameters of Gram-positive and Gram-negative infection are not sufficiently different to allow reliable separation by these findings; definitive identification of the organism by serology or culture of infected fluids or tissue is required.8,9
With wider understanding of the role of endotoxin in the aetiology of septic shock, the vast majority of research into therapeutics has focused on this bacterial product rather than the other infection-related toxins. For this reason, this review focuses upon anti-endotoxin therapy specifically and, necessarily, also discusses anti-inflammatory therapy which would theoretically counteract the SIRS associated with all forms of septic shock, regardless of aetiology.

While the development of new antibiotics directed against the most common aetiological organisms in sepsis has proceeded apace in recent years, the emergence of new pathogens in the nosocomial setting and the increase in antimicrobial resistance among previously susceptible organisms has frequently produced frustrating treatment failures. As such antimicrobial therapy has often proved unsatisfactory in the face of overwhelming sepsis, the direct antagonism of endotoxin and inflammatory cytokines in vivo has received much recent attention, and may yet prove the most effective modality in the prevention and treatment of sepsis and its complications.

**Endotoxaemia and the immune response**

Sepsis presents in the patient as the consequence of a complex cascade of mediators secreted by immune cells in response to the LPS (lipopolysaccharide cell wall, or endotoxin) produced by Gram-negative infection. Free intravascular LPS is produced from degradation of bacterial cell walls; once in the circulation, LPS can bind to a protein, LPS-binding protein (LBP), to form a complex. This complex then binds with high avidity to a monocyte/macrophage cell surface receptor, CD14, and the result is cellular activation and secretion of inflammatory cytokines. Similarly, soluble CD14 can also bind LPS/LBP and can thus activate endothelial cells, which do not express surface CD14.10

Prominent among the mediators produced in response to LPS activation of CD14 is tumour necrosis factor (TNF), along with IL-1b and IL-6, eicosanoids, intercellular adhesion molecules, platelet activating factor, oxygen free radicals, and nitric oxide. These primary factors, and many others interact with each other and adjacent cells, setting into motion a complex series of local and systemic inflammatory responses. The secretion of TNF (primarily from macrophages) in particular, is very strongly stimulated by LPS, and it is believed that this mediator, (and not LPS directly), is one of the primary agents which sets in motion the exaggerated cellular, metabolic, and vascular responses of septic shock.11

TNF and its attendant cytokine system exert myriad effects at the cellular and molecular level, which include: (i) hypermetabolism, manifested by decreased lipogenesis and increased lipolysis, active osteoclastic bone resorption, hyperglycaemia, and fever (due to direct action of TNFz and IL-1b on the hypothalamus);12 (ii) coagulopathy, manifested by widespread microvascular endothelial damage secondary to microthrombus formation (due in part to the stimulated secretion of procoagulant mediators and the direct activation of the extrinsic pathway by TNF);13 (iii) increased vascular permeability due to arachidonic acid metabolites, serotonin, and histamine release;14 (iv) increased leukocyte priming by complement components C3a and C5a for oxygen radical formation, with release of associated collagenases and enzymes which contribute further to the vascular compromise and widespread tissue damage;15,16 (v) systemic vasodilation (with subsequent hypotension) due in part to stimulation of nitric oxide (NO) release by the endothelium of the microvasculature;17 (vi) increased leukocyte adhesiveness and transmigration with destruction of the microvasculature; and (vii) decrease in the ability of the end-organ to utilize oxygen (with an ever-increasing reliance on anaerobic metabolism), the mechanism for which is poorly understood.18

When considering potential approaches to antisepsis therapy, it is important to realize that TNF and the attendant byproducts of the cytokine cascade are, in moderate amounts, beneficial and accelerate recovery from systemic bacterial infection. The microvascular changes induced with infection in an immunocompetent individual increase blood flow to foci of infection, promote leukocyte adhesion, transmigration, and bactericidal activities, and activate the complement cascade to ultimately clear infection. In addition, the complementary array of endogenous anti-inflammatory cytokines are simultaneously produced, including IL-4, IL-10, transforming growth factor beta, and also specific inhibitors of the cyclooxygenase and coagulation cascades. Ideally, these factors act in concert with pro-inflammatory mediators to eradicate infection and then return to an immunological steady state. However, when infection is overwhelming or external defenses are continuously compromised (e.g. with extensive burns, chronic instrumentation, or immunosuppression), pro-inflammatory cytokines may be secreted in abundance, to the point at which the damage to the microvasculature may be irreversible by endogenous counterinflammatory systems. This imbalance worsens as the growth of infectious organisms outpaces host immune response and the exogenous administration of antibiotics. Ultimately, as immunological ‘exhaustion’ sets in, the patient suffers worsening systemic compromise and ultimately, multiorgan system failure and death.19

Other effects of endotoxin in septic shock can
further complicate the generalized inflammatory state: fever, coagulopathy, decreased haematopoiesis, accelerated metabolism and hyperglycaemia, in conjunction with the hyperadrenergic ‘stress’ state of the patient, all contribute to the excessive cardiovascular and neurological excitation induced by sepsis, and can accelerate death. Therefore, in addition to the antibiotic treatments specifically directed against the infecting organism, and the supportive measures of crystalloid fluid resuscitation, colloid infusions, and inotropic/antiarhythmics agents which have been used to control the symptoms of sepsis, much attention has been turned in recent research to therapy which is directed against the primary culprit—the endotoxin product itself and the cytokine cascade it invokes.

Outlining an effective antisepsis strategy

The treatment of sepsis has traditionally relied upon the management of systemic bacterial infections through the use of antibiotic therapy directed against the infecting organism. Research concerned with anti-endotoxin agents at first focused upon standard antibiotic therapy, in the hopes that any given antibiotic could act prophylactically to eradicate foci of Gram-negative infection before large amounts of the endotoxin could be released and thus induce a clinical state of sepsis. This was the rationale behind preoperative doses of antibiotics before select surgeries, such as colectomy, or universal coverage with antibiotics in individuals deemed at greatest risk for septic complications. While this was modestly effective at first, the increasing incidence of multi-drug-resistant Gram-negative organisms necessitates increasingly powerful and expensive regimens, and has diminished the attractiveness of this approach. In addition, even large doses of antibiotics specific for the infecting organism causing a septic event will have no effect on the already circulating endotoxin, which continues to exert its immunological effects upon the patient. Further, antibiotic administration to patients unlikely to clear an infection completely with such treatment (e.g. due to insufficient dosing, insufficient spectrum of antibiotic coverage, or partial resistance of organism) may actually worsen the systemic inflammatory response. This paradoxical reaction is thought to be secondary to increased endotoxin release with limited bactericidal activity of the antibiotic and disruption of microbial cell walls containing LPS. Although important in the therapy of any patient with active infection, a discussion of the current thinking regarding antibiotic therapy in Gram-negative sepsis is beyond the scope of this document; the reader is directed to several excellent recent reviews on this subject.

As the intricacies of the actions of endotoxin and the related cytokine cascade have become clearer in recent years, investigators have turned their attention to specific points in the cascade where antagonists, either endogenous or synthetic, could potentially play a role in slowing, stopping, and even reversing the inflammatory sequelae of sepsis. The septic inflammatory cascade, with potential points of action of antagonists, is shown in Figure 1. The following discussion of these points of intervention follows the time-course of events in a ‘standard’ septic episode.

Anti-endotoxin therapy

Lipopolysaccharide (LPS), or endotoxin, is a Gram-negative bacterial product which is localized to, and emanates from, the cell wall of these organisms. This substance is the primary inciting mediator of the inflammatory response to these organisms in the host, and is composed of three primary components: the O (outer) polysaccharide, the core, and the lipid component (Lipid A). These components, while displaying some degree of heterogeneity when compared across genera and species of bacteria, do share certain homologous sequences which have been seized upon as potential targets of therapy, particularly in the lipid A component. Lipid A has in fact been shown to be the portion of LPS which exerts the toxic effects and incites the inflammatory response of the host.

The earliest therapeutic investigations involved the administration of polyclonal convalescent sera to septic animals, and later, patients in controlled clinical trials. Although initial reports using hyperimmune sera were promising, subsequent attempts to purify a monoclonal antibody (Mab) with broad-spectrum reactivity across bacterial genera proved daunting. HA-1A (a human Mab) and E5 (a mouse Mab), both with reported specific activity against the Lipid A region of the endotoxin produced by the J5 strain of Escherichia coli, were shown in multiple studies to have unsatisfactory binding and neutralizing capability of LPS both in vitro and in vivo, and little or no effect on the outcome of Gram-negative sepsis in the clinical setting. Recently-completed phase III clinical trials with EDOBACOMAB (a murine IgM anti-Lipid A Mab) have demonstrated a similar lack of efficacy, despite encouraging results in animal models. Despite such findings, continuing studies with HA-1A in particular have demonstrated recently in a murine model that anti-endotoxin antibodies may be of some benefit in specific clinical instances, such as in severe burns and pre-invasive bacterial overgrowth in the gut, to prevent or delay the onset of sepsis. Similarly, two
clinical trials with E5 did not show improved survival in septic shock; however, a meta-analysis combining these trials did demonstrate more rapid recovery and improved survival in a subset of patients who were not in refractory shock at the time of treatment. A subsequent trial demonstrated no decrease in overall mortality for septic patients who were not yet in shock, but did show prevention of acute respiratory failure and CNS sequelae of sepsis, and more rapid resolution of multisystem organ failure in survivors. The efficacy of this agent in sepsis remains controversial, and further trials are underway. Other monoclonal antibodies under investigation for anti-LPS activity include those directed against the outer, or O polysaccharide portion of the molecule; others or absent production of IL-8 in presence of LPS) have proposed development of antibodies specific to the most common organisms producing lipopolysaccharide, such as Pseudomonas spp, Klebsiella spp, and others. Blockade of LPS binding and activation of mononuclear cells offers another potential approach. Immunophenotypic studies of circulating monocytes have demonstrated that CD14 is the membrane receptor for LPS, and that binding of this ligand to the cell surface initiates part of the cellular cascade which culminates in sepsis. Extracellular soluble CD14 (sCD14) and a similar agent, LPS-binding protein, are formed endogenously and thus serve as natural scavengers for excess LPS in the circulation and in tissue fluid surrounding foci of infection. High serum levels of endogenously produced sCD14 in both Gram-negative and Gram-positive sepsis have been correlated with a poor outcome, implying that this factor must act in accordance with other antimicrobial agents to neutralize LPS, and may not be effective in isolation. The LPS blocking function of sCD14 (i.e. decreased or absent production of IL-8 in presence of LPS) has been demonstrated convincingly in human subjects and has been inhibited by mAbs specific for the LPS-binding portion of the molecule. Therapeutic applications of sCD14 and other LPS-binding proteins are being investigated.

An agent homologous to LPS-binding protein, bactericidal/permeability-increasing protein (BPI) is a naturally occurring compound within the body with
natural activity against endotoxin. This highly cationic protein has been isolated in the azurophilic granules of neutrophils and has been shown in vitro to bind to several different types of lipopolysaccharide (a negatively charged compound) with high affinity via the lipid A portion of LPS, to prevent neutrophil activation by LPS, and also to inhibit LPS-related tumour necrosis factor release both in vitro and in vivo.\(^{47}\)

Recent research has further identified the specific region of the BPI molecule responsible for its endotoxin activity. A 23 kDa fragment (rBPI) was cloned from the N-terminal portion of the molecule, and, when expressed, demonstrated anti-endotoxin activity in vitro equal to that of the native product. A fusion product incorporating portions of BPI and LPS-binding proteins with affinity for LPS has also been produced.\(^{48}\) Furthermore, a macrophage cell line, RAW 264.7, with the capability to produce a fusion product of BPI and IgG, demonstrated marked resistance to LPS-induced production of TNF and LPS; infusion of these cell lines or introduction of the transcript via a gene vector may prove effective antisepsis therapy in animal models in the near future.\(^{49}\)

Other substances have shown some specific anti-endotoxin activity. Polymyxin B, an antibiotic with affinity for Lipid A, has been shown to protect mice following a Gram-negative bacterial challenge\(^{50}\) and has been used successfully in an extracorporeal circuit to remove LPS from serum in plasmapheresis in an animal model;\(^{51}\) lipoproteins (LDL, HDL, VLDL, and chylomicrons) have been shown to block LPS-induced TNF-\(\alpha\) secretion; indeed, hypolipoproteinaemia, especially HDL, is a common feature in sepsis, and controlled trials of administration of parenteral lipoprotein solutions high in HDL in the septic postsurgical population have been promising.\(^{52}\)

Species-specific forms of Lipid A itself are being evaluated for the potential production of therapeutic monoclonal antibodies; lipid A from Rhodobacter capsulatus (E5531), for example, has shown potent anti-LPS activity in vitro, and was protective against septic challenge in a murine model.\(^{53}\) Another intriguing approach uses an endotoxin-neutralizing protein produced by the Limulus polyphemus spp. horseshoe crab; a recombinant form of this product decreased the rise in serum endotoxin and improved survival in rabbits with \(E.\ colli\) sepsis.\(^{54}\)

Mechanical removal of endotoxin, bacteria, and cytokine byproducts directly from blood using haemodialysis and related techniques has been attempted, but the results so far have been disappointing. Although the molecular size of endotoxin and its products would not theoretically prevent filtration using standard haemodialysis filters, the yield from such use has been unimpressive and the fixation of LPS-specific antibodies in these filters has not improved the outcome of this therapy.\(^{55}\)

Plasma exchange procedures to remove such toxins had some success in older studies involving septic neonatal dogs and burn patients (as part of multicomponent therapy).\(^{56,57}\)

Lastly, the phenomenon of induced tolerance to endotoxin and the molecular constituents has been the subject of some recent study. Research has shown that some animals and human subjects challenged with LPS will display tolerance (i.e. downregulation of cytokine release) upon rechallenge.\(^{58}\) Challenge with the purified monophosphoryl lipid A portion of LPS has shown similar effects in a double-blind human trial.\(^{59}\) Investigation into these intriguing findings continues.

**Inhibition of secondary mediators: TNF-\(\alpha\), IL-1, and the cytokine cascade**

While several agents with specificity for the LPS molecule itself are under investigation, much attention has also been brought to bear on the modulation and arrest of the secondary cascade of cytokines, coagulation activation, and microvascular compromise which contributes significantly to the morbidity and mortality of the primary infective process. Initial attempts at cytokine modulation utilizing corticosteroids were disappointing, with an overall increase in mortality;\(^{60}\) currently, systemic corticosteroids are contraindicated in sepsis. Opiate antagonism (i.e. naloaxone) was similarly disappointing in animal models.\(^{61}\)

Modulation of the cytokine cascade in sepsis has focused primarily upon those agents which sit at the head of the pathway, TNF-\(\alpha\) and IL-1. Investigation into anti-TNF antibodies has prompted a large controlled clinical trial, the INTERSEPT study group. The anti-TNF antibody, BAY \(\times\) 1351, exhibited some efficacy in reversal of shock over placebo groups in the study; further clinical trials are underway.\(^{62}\) Soluble TNF receptors, p55 and p75, which are endogenously produced in septic states and block binding of TNF to cells bearing the surface form of the TNFR. While secretion of these agents is increased in inflammatory states, they are secreted independently of serum TNF-\(\alpha\) concentrations, indicating a constitutive, ‘scavenger’ role for these molecules.\(^{53}\) Other studies have shown that p55 and 75 may also enhance TNF function by stabilizing the active TNF isoform.\(^{64}\) The significance of this finding for therapeutic investigation is unclear; other studies indicate that p55/75 levels can predict outcome in septic animals and increases in serum levels 1000-fold may prevent sepsis.\(^{65}\) Workers have also shown that a fusion product of p55 and the Fc portion of IgG demonstrates improved clearance of
bound TNF from serum and prevents sepsis; a similar effect for p75 was not demonstrated.\textsuperscript{66} Also, drugs such as rolipram, milrinone, and nitraquazone suppress TNF and IL-1b production from the monocyte by inhibiting several classes of phosphodiesterase which function in such secretion.\textsuperscript{67}

Other agents along the cytokine cascade downstream from TNF have been investigated. Rises in endogenously-produced soluble IL-1 receptors in serum are seen in humans challenged with endotoxin, suggesting a potential therapeutic role for this product or a monoclonal antibody directed against IL-1,\textsuperscript{68} and the production of sufficient quantities of IL-1 receptor antagonists in serum will block cellular binding of IL-1 in rats and preserves microvascular integrity and function in sepsis.\textsuperscript{69} Endogenous anti-inflammatory cytokines may also play a role; monoclonal antibodies directed against IL-10 in endotoxaemic mice causes a rapid upsurge in LPS induction of TNF-\textit{x} and IL-1 secretion, as well as the pro-inflammatory cytokine, gamma interferon.\textsuperscript{70} Monoclonal antibodies or other antagonists to proinflammatory agents in sepsis (IL-6, IL-8) may also prove of interest.\textsuperscript{71,72}

**Phosphodiesterase inhibitors**

A class of agents, phosphodiesterase inhibitors, exhibit significant anti-inflammatory effects in sepsis without significant detriment to endogenous antimicrobial activity; these agents also preserve endothelial cellular function in the microvasculature via an unclear mechanism (possibly maintenance of endothelial cell-derived nitric oxide production). The best characterized of these agents, pentoxifylline, has exhibited numerous beneficial effects in sepsis including downregulation of TNF, IL-6, and endothelin-1 production by macrophages, improvement of end-organ oxygen extraction from blood, preservation of microvascular blood flow, and reduction of the typical weight loss and muscle wasting seen in overwhelming sepsis. Improved survival in septic piglets and premature septic human infants has been noted in controlled trials;\textsuperscript{73,74} further detailed clinical testing is needed before this intriguing agent can be employed as potential therapy. A similar xanthine derivative, HWA 138, demonstrated improved survival in septic rats over that of pentoxifylline in a recent study.\textsuperscript{75} Another agent, amrinone, has demonstrated significant benefit as an inotropic aid in hypodynamic cardiac failure.\textsuperscript{76} Similar to pentoxifylline, amrinone inhibited much of the significant muscle wastage seen in sepsis by preventing TNF-induced abnormalities in skeletal muscle pyruvate dehydrogenase activity and high plasma lactate concentrations.\textsuperscript{77}

**Coagulation, DIC, and arachidonic acid metabolites**

One of the more devastating secondary cytokine effects of endotoxaemic shock is a state of hypercoagulability. LPS and TNF act together to promote loss of capillary endothelial integrity and chemotaxis of neutrophils to the vessel walls with attendant degranulation and endothelial damage. This damage promotes coagulation overlaying denuded subendothelial surfaces. Such microvascular thrombosis in the setting of sepsis can rapidly evolve into disseminated intravascular coagulation (DIC) with rapid consumption of platelets and clotting factors, resulting in a paradoxical bleeding tendency in the setting of diffuse microvascular thrombosis. In combination with the already dire effects of LPS and the cytokine cascade on endothelial integrity and peripheral oxygen utilization, the prognosis associated with DIC can be dismal. Investigators have initially focused upon upregulation of endogenous anticoagulant formation and, failing this, exogenous anticoagulation.

Apart from the standard clinical support of coagulation in DIC with fresh frozen plasma (which has also shown beneficial endotoxin-neutralizing effects in early septic human subjects),\textsuperscript{78} laboratory isolation and purification of coagulation mediators such as antithrombin III and proteins C and S has made these agents available for the first time in forms which avoid the infective risk inherent in prior pooled preparations derived from human donor plasma.\textsuperscript{79} Apart from their better-known functions as regulators of the coagulation cascade, these agents may also have direct cytokine modulatory function, and arrest many of the secondary sequelae of septic shock. Activated protein C has demonstrated the ability to block leukocyte accumulation and vascular injury in the lungs of septic rats, presumably by inhibiting the generation of chemotactic factors in coagulation,\textsuperscript{80} protein S may function in a similar fashion. Hirudin, a specific thrombin inhibitor, was coadministered with tobramycin to rats with Klebsiella spp. sepsis, and led to an improvement in many DIC parameters such as fibrinogen and platelet count, and improved liver function and overall survival over that of controls with sepsis-related multi-organ failure.\textsuperscript{81} Along these same lines, the widespread intravascular coagulation typifying DIC in sepsis requires diffuse microvascular endothelial damage by the infecting organism or an agent produced or promoted by response to the organism; the body will respond to this microvascular damage through activation of the ‘contact system’ or kallikrein/kinin pathways which promote vasodilation and coagulation at the site. Inhibitors of this system have been proposed for use in the treatment or prevention of DIC. Agents such
as C1-esterase inhibitors, monoclonal antibodies to factor XII and tissue factor, and protease inhibitors such as aprotinin may serve this purpose in combination with other anticoagulant therapy.\textsuperscript{82–84}

Also of interest in the coagulation cascade is the role of platelet activating factor (PAF) in haemodynamic and microvascular compromise in sepsis, and the therapeutic potential of PAF inhibitors. PAF is an inflammatory lipid involved in platelet activation at sites of endothelial injury, neutrophil infiltration and production of oxygen radicals, and tissue damage in sepsis.\textsuperscript{85} Terpenes, natural compounds with some limited ability to inhibit PAF activation of neutrophils, have been evaluated, but effects of the agents are apparently transient.\textsuperscript{86} Several synthetic PAF inhibitors are currently under study; one, TCV-309, was shown to improve hemodynamic parameters (including elevation of mean aortic pressure, cardiac output, left ventricular stroke work index and increased urine volume) in septic beagles.\textsuperscript{87} It also suppressed the LPS-associated rise in TNF and the subsequent cytokine cascade in septic chimpanzees.\textsuperscript{88} Human trials are currently underway.

Certain prostaglandins (particularly thromboxane A2) and bradykinin contribute to the pathophysiology of septic shock through their effects on the clotting cascade, microvascular integrity, and vasoconstriction/dilatation. Leukotrienes, on the basis of a recent study, were noncontributory to these effects.\textsuperscript{89} Prostaglandin classes which counter these effects, (such as PGE2 and PG12) and cyclooxygenase inhibitors (such as ibuprofen) have been studied largely in animals and demonstrate some utility in symptomatic treatment and leukocyte inhibition in clinical studies, but do not in themselves prevent the onset of shock.\textsuperscript{90–92} A recent trial using a novel bradykinin antagonist, CP-0127, or Deltibant, demonstrated statistically significant improvement in survival after a 28-day interval when administered to a small subset of patients with septic inflammatory response syndrome (SIRS), or clinically-diagnosed sepsis; the remainder of the study group demonstrated no benefit, and the efficacy of this agent is still in question.\textsuperscript{93}

Imidazole compounds such as ketoconazole have demonstrated inhibitory activity against thromboxane synthetase,\textsuperscript{94} but recent in vitro studies, while confirming the beneficial effects of this agent on haemodynamics in sepsis, was unable to confirm the precise mechanism; thromboxane synthetase activity was unchanged following administration of the imidazole in the study.\textsuperscript{95}

**Complement, NO, and the contact system**

Inhibition of the complement cascade has been investigated, considering its central role in tissue damage and chemotactic recruitment of leukocytes. C-1 esterase inhibitors have been used, as mentioned previously in conjunction with arrest of the contact system, and preliminary results in humans suggest some ability to reverse septic hypotension.\textsuperscript{96} Production of monoclonal antibodies to complement component and chemotactic factor C5a has shown inhibition in reperfusion injury in pigs with myocardial infarction, and may have similar effects in prevention of chemotaxis of leukocytes and tissue damage in sepsis.\textsuperscript{97} Purification and production of a soluble form of the C5a receptor on the leukocyte may be similarly efficacious.\textsuperscript{98} As mentioned above, endothelium modulates host response to LPS largely through the release of nitric oxide (NO). NO, while improving blood flow and leukocyte migration in localized infection, is deleterious to microvascular integrity if not adequately controlled endogenously, and results in significant morbidity if activated on a systemic scale. The vasodilation and hypotension incurred by this agent induces reactive hyperdynamic cardiac function; further large-scale production of NO induces microvascular hyporesponsiveness to vasopressors such as epinephrine. The investigation and production of NO synthase inhibitors has also been encouraging. In rats, such an inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), restored pulmonary vascular responsiveness 16 h following caecal ligation and puncture with subsequent sepsis.\textsuperscript{99} A similar effect was noted in ovine sepsis, with restoration of vascular response to epinephrine.\textsuperscript{100}

**Inhibition of leukocyte activity**

Through control of the complement cascade and the contact/coagulation cascade in the microvasculature, many of the destructive secondary sequelae of sepsis can be avoided. In addition to the agents previously discussed, several others are in development which act at the ultimate level of microscopic tissue damage due to leukocyte migration, adhesion, transmigration, and production of proteases and oxygen radicals. The CD11/18 complex, members of the integrin family of adhesion molecules, are receptors on the surface of leukocytes (predominantly neutrophils) which allow adhesion to the endothelial surface and subsequent transmigration. They are under some scrutiny as potential therapeutic agents;\textsuperscript{101} a recent study of monoclonal antibodies against CD18 (CL26) in human subjects following myocardial infarction demonstrated a marked decrease in neutrophil adhesion, transmigration, and reperfusion injury to the myocardium.\textsuperscript{102} Similarly, improvement in function of the reperfused paediatric transplanted heart following ischaemic cold storage was noted following administration of an antibody directed against the CD11b portion of the integrin complex.\textsuperscript{103} These
agents have also demonstrated antirejection effects via a similar mechanism in dog lung. On the endothelium, molecules such as E-selectin act as receptors specific to leukocytes which stabilize adhesion achieved during margination. Endogenous production of soluble E-selectin and other endothelium-derived adhesion molecules may serve both as a serum marker of the extent of systemic endothelial damage and necrosis, but may also serve to block further leukocyte adhesion by binding to these cells in the circulation.

Blockade of E-selectin and P-selectin in burned rats using a monoclonal antibody, CY-1747, demonstrated significant inhibition of leukocyte margination and sequestration in lung and liver. The possibility of such activity in the setting of sepsis-induced microvascular damage is intriguing; a recent study in septic swine used EL-246, a monoclonal antibody with dual activity for E- and L-selectins, produced significant protection against acute lung injury by blocking neutrophil infiltration and the subsequent oxidative burst, but failed to attenuate haemodynamic derangements associated with sepsis. Studies with similar agents are ongoing.

Blockade of neutrophil activation is of interest to researchers; the role of NADPH oxidase as a priming influence in neutrophil and monocyte activation and degranulation is a potential target for such a blockade. Two agents with the ability to block neutrophil activation in murine arthritis, diphenylene iodonium chloride (DPI) and staurosporine, may have adjunctive functions in sepsis treatment. In a study involving rabbits, DPI demonstrated the ability to inhibit lung NO generation and block hypoxia-induced vasoconstriction in the pulmonary par enchyma.

Antioxidants and free radical scavenging

Following transmigration and activation, the production of oxygen radicals via the oxidative burst of the infiltrating neutrophil is one of the ultimate causes of tissue damage in sepsis, as noted above. Agents with the ability to inhibit this burst, or its products, are being investigated for clinical use in this setting. Other sources of oxygen radical species include activated macrophages, and various extracellular molecular processes such as arachidonic acid metabolism and xanthine dehydrogenase oxidation are noted in sepsis and ischaemia. Antioxidants such as superoxide dismutase (SOD) and catalase have been evaluated as prognostic serum markers in septic patients, patients with sepsis have low concentrations of these and other antioxidants. SOD, in conjunction with diltiazem, was shown to decrease febrile response and mortality in septic male Sprague-Dawley rats. Catalase, in conjunction with SOD, has demonstrated activity against CNS vasodilation and pathological increases in intracranial pressures in septic rats. Ascorbic acid (vitamin C) loading in septic patients has demonstrated a rapid consumption of this agent over controls, presumably secondary to radical scavenging activity; however, vitamin C has also been associated with pro-oxidant activity through its potentiation of iron-catalyzed reactions. Thus, the clinical utility of vitamin C is unclear at this point. Vitamin E, or alpha-tocopherol, has demonstrated benefit in septic rats, with inhibition of lipid peroxidation by oxygen radicals, improvement in end-organ oxygen utilization, and improved survival. Other anti-oxidant agents, known as lazaroids, have been shown to decrease oxygen radical lipid peroxidation in the septic rat kidney, reducing preglomerular vasoconstriction and hypoperfusion without affecting central haemodynamics; these agents may be useful for maintaining kidney function in cases of mild sepsis in the susceptible patient. N-acetylcysteine (NAC) decreases neutrophil-induced damage in the pulmonary vasculature of septic rats and decreases the overall incidence of alveolitis in this setting, with overall improvement in oxygenation and clinical outcome. An intriguing class of synthetic compounds known as ‘nitrones’ have the ability to bind covalently to large amounts of free radicals in the circulation; while their use has been investigated most intensively in the area of post-ischaemic brain damage, its function as a radical ‘trap’ may have obvious applications as an adjunct in the therapy of sepsis. The ability of nitrones to trap NO in the microcirculation of the CNS has also been documented, and could potentially be used in conjunction with the NO synthetase inhibitors mentioned previously.

Miscellaneous therapies

Several agents which have shown beneficial effects in sepsis act systemically on many different portions of the LPS inflammatory cascade, or by a poorly understood mechanism, and do not fit conveniently into the above categories. Chlorpromazine, a neuroleptic antipsychotic with blockade of serotonin reuptake as its primary mode of function in the CNS, was coadministered with dexamethasone and N-acetylcysteine to septic rats, which showed amelioration of pulmonary oedema and improved survival; anxiolysis and modulation of the pituitary-adrenal corticosteroid axis may be contributory, although its effects are difficult to separate from the other agents in the study (NAC, as discussed above, is an oxygen radical scavenger). Therapy using variant steroids, though controversial in the setting of overwhelming infection, has produced some interesting findings. For example, while prednisone definitely downregul-
ates the immune system and is contraindicated in sepsis, therapy with dehydroepiandrosterone has demonstrated an upregulatory effect in burned rats with gut sepsis; rats treated with DHEAS demonstrated no effect on neutrophil transmigration of gut wall, but markedly improved efficiency of PMN bacterial killing and improved time to recovery and overall survival over groups treated with prednisone and those receiving no treatment. Investigation into other hormone therapies in sepsis discovered a protective effect of the estrogen agonist estriol in murine sepsis, largely secondary to its regulation of TNF-α, IL-1, and IL-6 production. An interesting synthetic agent, liposome-encapsulated haemoglobin (under current consideration as a blood substitute), also has significant anti-inflammatory and anti-endotoxin properties which were unsuspected, and have been the subject of several recent animal studies. Its use in septic mice demonstrated modulation of the cytokine release by monocytes/macrophages, possibly by trapping LPS and presenting it for phagocytosis by the reticuloendothelial system before it can bind and activate cytokine release. Infusion of this agent well before LPS challenge was protective against sepsis. Thalidomide, controversial due to its significant teratogenic effects, has demonstrated the ability to block TNF-α production and improve survival in septic rats; its use in human subjects is unclear at this point.

Lastly, several new approaches involving hormonal and cellular manipulation at even the molecular level are under investigation. The use of recombinant granulocyte-colony stimulating factor in children with sepsis is now a viable adjunct to prophylaxis and care in these populations, via direct infusion in vivo, or in vitro stimulation of granulocytic cell lines, which are then infused to prevent infection in neutropenic individuals or combat overwhelming infection in septic patients. Gene therapy may also have something to offer in the near future; the actions of endotoxin in an animal model of acute lung injury were inhibited by introducing a liposomal vector carrying the gene for prostaglandin synthase. The gene was incorporated into alveolar epithelium, and resulted in increased production of prostaglandin E2 and prostacyclin. It is speculated that genes for anti-oxidants and protease inhibitors could be similarly introduced to combat sepsis.

**Conclusions**

Recent years have seen a flurry of basic science research into the dizzyingly complex pathogenesis of Gram-negative sepsis and the equally daunting inflammatory cascade left in its wake. The significant advances in our understanding of these basic mechanisms of the systemic inflammatory response syndrome (SIRS) has been parlayed into the investigation and development of a multitude of agents and potential therapies for sepsis based on both in vitro and in vivo animal models. Despite some encouraging results in several animal studies of sepsis ranging from mice to baboons, detailed, controlled human data is still largely lacking. Indeed, only a handful of the agents discussed have shown a sufficiently documented survival benefit in septic patients, despite this evidence in animal models. This implies that: (i) animals are not sufficient models for the unique human response to sepsis, given variances in immunity and host flora in each organism; (ii) dosages, timing of administration, and duration of benefit are only now being defined in the animal models, and very little human data of this sort are available; and (iii) while antagonism documented for an agent against a specific inflammatory mechanism may imply clinical benefit, it is impossible to distill such effects from that upon the entire inflammatory and immune response, and any individual antagonistic action within a single step of the inflammatory pathway may in fact be detrimental to the organism as a whole. For example, corticosteroids, currently contraindicated in sepsis due to potentiation of growth and invasion of infectious organisms, would misleadingly appear to benefit the patient when evaluated in the isolated context of their ability to circumvent much of the inflammatory cascade which complicates sepsis and ultimately causes death.

As research continues and more controlled clinical trials are underway, we may yet encounter an agent with proven benefit in sepsis and specific action against its sequelae. At present, however, the paucity of well-controlled human data forces the health-care practitioner to focus on prevention, and early intervention with antibiotics, in the clinical settings where patients are at particular risk for developing sepsis. In those patients suffering from overwhelming septic shock and the associated systemic inflammatory response syndrome (SIRS), a treatment protocol consisting of antibiotics, fluid and pressor support, oxygenation and other respiratory support, and appropriate support of coagulation if DIC ensues (a very common complication of sepsis) is recommended.

It is likely that, as human trials produce more reliable data, a therapeutic approach to sepsis may be devised which is both specific and global in approach. Specific agents may be directed against single pro-inflammatory factors in sepsis, and inhibit the cascade at defined points which may be tailored to the individual patient’s needs (e.g., focusing on antithrombin III in septic DIC patients or on throm-
boxylogans in patients with pronounced pulmonary vascular compromise and bronchoconstriction. Along the same lines, if sepsis is detected early and pre-emptive intervention is still an option, therapy directed against the endotoxin molecule itself or those cytokines (TNF-α and IL-1) which induce the rest of the cascade, thereby short-circuiting the entire process, and demoting the disease to one treatable with standard antibiotic therapy and symptomatic support.

References


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