Bacterial Endotoxin in Human Disease

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How advances in understanding the role of Gram-negative bacteria and endotoxin in infectious diseases and complications may improve the development of diagnostic and treatment options

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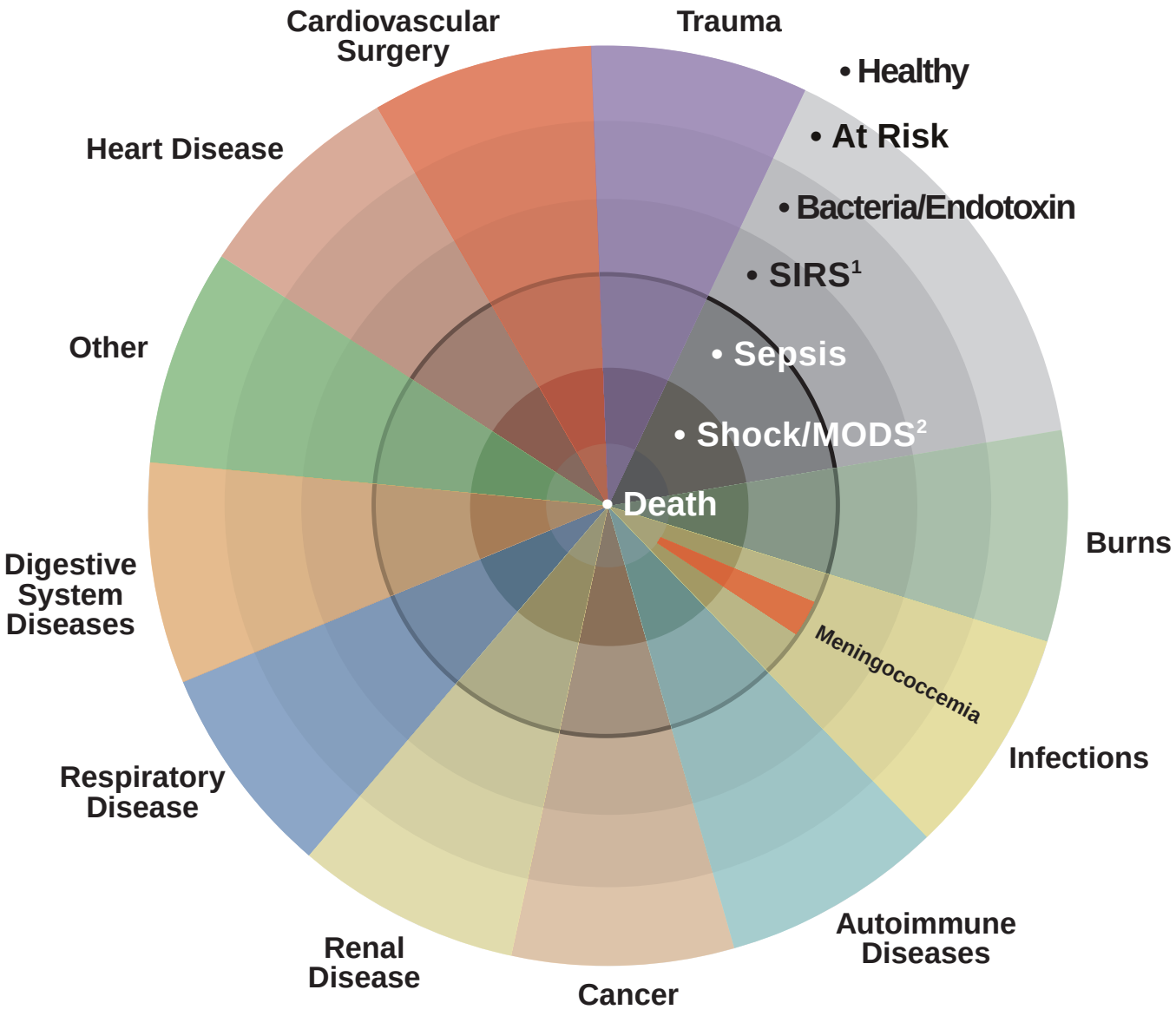
Figure 1: A Model for Diseases Potentially Associated with Bacteria/Endotoxin



1 Systemic Inflammatory Response Syndrome

2 Multiple Organ Dysfunction Syndrome

Preface

Gram-negative bacteria and their endotoxins may be a causal or complicating factor in many serious diseases. The syndromes most commonly connected with bacterial endotoxins are sepsis and septic shock, which are systemic complications of many diseases. Systemic infections (septicemias) caused by invasive Gram-negative bacteria are a well known source of endotoxin exposure. Less well-recognized, although perhaps of greater importance, are infectious complications (such as those following trauma or surgery) that may be initiated by exposure to endogenous Gram-negative intestinal bacteria.

Whatever the source, exposure to endotoxin induces a systemic inflammatory response (also called the inflammatory or sepsis cascade) that involves many interconnected cellular and plasma mediators. The inflammatory response manifests in such clinical signs as fever, increased heart and respiratory rates, and other systemic symptoms. These may be self-limiting, or the cascade can proceed to shock, organ failure and death. Currently available treatment efforts are limited to antibiotics and, in serious cases, supportive intensive medical care.

Advancements in efforts to prevent or treat the systemic inflammatory response to endotoxin have been hampered by several factors. The lack of a consistent and rapid diagnostic for endotoxin exposure is one. The choice of a clinically-relevant therapeutic target is another. Halting the ongoing endotoxin stimulation of the inflammatory response at the source would seem to be more effective than inhibiting any individual component. Nevertheless, most investigational therapies have targeted individual mediators within the inflammatory cascade. A third factor is the conceptual lock that sepsis has had on pharmaceutical development. In the past eight years, at least 13 different products have failed to show the required survival benefit in sepsis trials. Surprisingly, these failures have, with rare exceptions, not stimulated radical change in pharmaceutical companies' or regulators' approach to clinical trial design or disease targets.

This paper challenges the current understanding in this field. In the old model, sepsis was viewed as a unique clinical syndrome, difficult to treat, but the obvious target for therapy. The new model (see Figure 1) incorporates sepsis, but as a late-stage syndrome on a continuum of endotoxin-related diseases. The new map encompasses the entire inflammatory cascade and its clinical manifestations. Understanding such a new paradigm may open the way to improved diagnostic and therapeutic approaches that can identify at-risk patients and treat them at the appropriate stage in the inflammatory process, within the context of each patient's underlying disease.

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Summary

- Endotoxins are complex lipopolysaccharides (LPS), major cell wall components in all Gram-negative bacteria. LPS has two regions: polysaccharide and lipid A. Lipid A, highly conserved across bacterial families, is the primary toxic component.
- Endotoxin can enter the blood (causing endotoxemia) in two ways: 1) through local or systemic infection by exogenous Gram-negative bacteria, and 2) by translocation of endogenous Gram-negative bacteria or fragments across the intestinal membrane when permeability is increased following systemic insults such as trauma.
- Circulating endotoxin can induce an overwhelming inflammatory host response (the "systemic inflammatory response" or "sepsis cascade"). Mediated initially by LPS binding to lipopolysaccharide binding protein (LBP), the LBP-LPS complex stimulates reactions from immune system and tissue cells and activates various interrelated non-cellular cascades. The resulting clinical syndrome is known as "sepsis" when an infection can be detected (e.g., meningococcemia), and as the systemic inflammatory response syndrome (SIRS) in the absence of documented infection.
- Sepsis can progress to septic shock, a catastrophic syndrome characterized by refractory hypotension and multiple organ failure. With perhaps half a million patients annually affected in the U.S., fatality rates of up to 40%, and no approved pharmaceutical therapy, sepsis and septic shock remain a significant and growing unmet medical need.
- Endotoxin has also been associated with a myriad of diseases and syndromes although its clinical significance is not always clear. Examples include: complications associated with trauma, burns and invasive surgery, as well as organ-specific illnesses such as cystic fibrosis, inflammatory bowel disease, liver disease, kidney dialysis complications, asthma and autoimmune diseases.
- Technical and biological hurdles have prevented the development of reliable diagnostic tests for endotoxin. Outside of Japan, there is no approved diagnostic for endotoxemia. Even reliable tests might not detect transient but clinically-significant endotoxin exposure. The absence of good diagnostics has hampered the clinical development of therapeutic agents.
- The current treatment of septic complications is immediate resuscitation and administration of antibiotics, followed by stabilization and support of vital functions. There are no predictably effective pharmaceutical interventions for sepsis, probably in large part because it is a complication of so many different underlying diseases.
- Nevertheless, over the past decade, many products have been clinically tested in heterogeneous populations of sepsis and septic shock patients. None have succeeded. Despite the previous unsuccessful attempts, new therapeutic candidates continue to enter the clinic. As of this writing, at least a dozen Phase II and III clinical trials are underway for therapies targeting established or suspected sepsis mediators. Most of these therapies are directed at specific components of the sepsis cascade.
- Given what we now know about the inflammatory cascade, a more fruitful avenue of development could be to target endotoxin within the context of the underlying disease. In addition to targeting better characterized diseases, clinical trials could also focus on preventing, rather than treating, septic complications. A few agents now in clinical trials are indeed following this approach.
- A historical legacy of confusing terminology has impeded communication in this area. An American College of Chest Physicians Consensus Conference in 1992 clarified clinical definitions of sepsis and septic shock, but to date there is still no generally-accepted scientific terminology to describe the full landscape of endotoxin-associated disorders.

I. Introduction

A. Medical Importance of Endotoxin

Endotoxins are high-molecular weight complexes of lipopolysaccharides (LPS) that constitute the major cell wall component in all Gram-negative bacterial families (McCuskey). These molecules have been intensively investigated because of the increasing appreciation of their potentially pathogenic role in a wide variety of human disease states (Rietschel).

For example, endotoxin is now believed to be the primary trigger of Gram-negative septic shock, a catastrophic and frequently fatal systemic syndrome characterized by hypotension, inadequate organ perfusion and multiple organ failure. Sepsis and septic shock, which have been increasing in prevalence since the 1950s (ACCP, Glauser), cause an estimated 175,000-200,000 deaths annually in the United States (Hoffman, Ulevitch). The syndrome has been associated with Gram-negative bacterial infection and, therefore, with endotoxin, in 30 to 80% of patients in recent large studies (Glauser). The lack of a documented infection does not, however, exclude the possibility of endotoxemia induced by undetected Gram-negative bacteria.

Despite advances in medical therapy, sepsis carries a mortality rate of 10-15% in children and up to 40% in adults (Carcillo). According to the Centers for Disease Control and Prevention (CDC), septicemia (the term CDC uses) is the 12th leading cause of death in the United States. Nationwide, sepsis is estimated to cost $5-10 billion annually (Bone). Septic shock is the leading cause of death in hospital intensive care units, where the incidence is often 2-5 times higher than in other hospital departments (Gasche). The incidence of sepsis and its associated morbidity and mortality is rising with the growing use of invasive techniques, immunosuppression and cytotoxic chemotherapy, and concomitant increases in nosocomial infections (Lamy).

Bacterial endotoxin can enter the bloodstream, causing endotoxemia, in two ways. The most well-recognized is infection by exogenous Gram-negative bacteria (infection from without). The second potential source is the population of endogenous Gram-negative bacteria that inhabit the gastrointestinal tract (infection from within). The translocation hypothesis postulates that in a wide variety of surgical and medical situations, gut bacteria and their endotoxins leak through an abnormally permeable intestinal wall (Deitch, Hecht, van Leeuwen) and enter the bloodstream. Translocation has been demonstrated in various animal models and is implicated in a variety of clinical situations, but its clinical relevance remains controversial (Lemaire).

In addition to its causal role in documented Gram-negative sepsis and related syndromes, endotoxin has been associated, with varying degrees of supporting evidence, with numerous other clinical syndromes. While some diseases, such as meningococcal septicemia (meningococcemia) (Brandtzaeg) and enteropathogenic *E. coli* syndromes (Kaplan), involve documented infection by exogenous Gram-negative bacteria, other conditions do not. For example, blunt trauma (Brathwaite, Hiki, Langkamp-Henken, Reed), burns (Jones II [a], Jones II [b]) and cardiac (Anderson, Casey, Martinez-Pellus, Riddington, Rocke, Taggart) or vascular (Baigrie, Roumen and Frieling) surgery have all been associated with endotoxemia and sepsis, presumptively attributed to translocation of bacteria or endotoxin from the gut.

There is abundant evidence that in the circulation, endotoxin on or released from bacteria activates a systemic inflammatory reaction, a complex of cellular and humoral responses in the host (Bone, Glauser, Rietschel, Remick). Endotoxin stimulates reactions such as the release of cytokines and arachidonic acid from monocytes, neutrophils, and vascular endothelial cells. Activated humoral (non-cellular) pathways include the complement and coagulation cascades. These responses of the normal components of the host-defense system reflect the host's best effort to battle a systemic infection without antibiotics to reduce the bacterial load. However, if continually stimulated by the pathologic presence of endotoxin (as in an ongoing infection or intestinal translocation), the prolonged inflammatory response may damage or kill the host.

Because of the increasing incidence and lack of new treatments for sepsis and related syndromes, as well as a growing appreciation for the protean manifestations of endotoxin-related diseases, the medical community is taking renewed interest in the clinical significance of endotox-

in and endotoxemia. In the past decade, new research has contributed to a growing understanding of the mechanisms underlying these illnesses. This article reviews the molecular biology and pathophysiological mechanisms of endotoxemia, sepsis, septic shock, and other diseases and complications associated with endotoxin. The article also presents some information about current and potential future treatments of endotoxin-related disease.

B. Terminology and Approach

Conflicting terminology and confusing semantics have long impaired communication in this field. The problem has become more acute as clinical trials multiply and investigators, reviewers and regulators need a common language to aid design, analysis and evaluation of trials. A 1992 American College of Chest Physicians (ACCP) Consensus Conference report developed a conceptual framework and a limited set of definitions in an attempt to standardize terminology and facilitate communication.

These definitions, however, still depend on clinical diagnoses based on signs and symptoms. There is at present no laboratory or other test to determine cause (other than identifying particular infectious agents) and prognosis in sepsis patients. Furthermore, the ACCP definitions do not encompass milder or localized manifestations of exposure to endotoxin, endotoxemia or the inflammatory response.

Because of the long-standing confusion of terminology in this area, this paper uses a consistent common lexicon throughout. The accompanying Glossary (see page 21) defines terms central to the concepts presented here, as well as those that have the greatest potential for ambiguity or misinterpretation. While some of these terms have been defined in the literature, many have not, and suffer multiple and sometimes conflicting connotations from paper to paper. We have used the ACCP definitions when possible and have tried to be consistent with their conceptual framework in defining other terms.

Because language drives perception, we have constructed the Glossary with the intent of focusing the terminology on two central themes of this article. First, sepsis (and therefore septic shock) are not diseases in their own right, but clinical syndromes which complicate the course of patients with pre-existing illnesses. Second, the systemic effects of bacterial endotoxin exposure lie on a continuum from asymptomatic Gram-negative bacteremia and endotoxemia to a systemic inflammatory response syndrome (SIRS) that may progress to sepsis and the multiple organ dysfunction syndrome (MODS), through septic shock, organ failure and death (Rangel-Frausto) (see Figure 1, inside front cover).

Inherent in this continuum model is an additional key concept: there is a point in the progression where irreversible clinical effects occur. Therefore, future specific therapies should be aimed at aborting the progression of events before this point of no return. There is some evidence to suggest that intervention with an endotoxin-neutralizing therapy can reverse the course of events even in septic shock patients (Giroir). In general, however, early intervention is better than late and interventions aimed at bacteria and endotoxin should have more effect than those targeting individual components of the sepsis cascade.

II. Basic Biology of Endotoxin

A. General Features

Bacterial endotoxins were first described at the end of the last century after the recognition that, in addition to then-known secreted toxins (exotoxins), certain types of bacteria also produce biologically active, heat-stable molecules associated with the bacterial cell itself (Rietschel). Initially found in *Vibrio, Salmonella*, and *Serratia* species, endotoxins are now known to be a component of the outer membrane of all Gram-negative bacteria (Carcillo, Glauser, Rietschel, Remick, Ulevitch).

Purified endotoxin is a complex glycolipid composed of a biologically active lipid (lipid A) linked to a polysaccharide region (Glauser, Rietschel, Ulevitch). In addition to LPS, other endotoxic cell membrane molecules have been identified, including lipooligosaccharide (LOS, a short-chain endotoxin) and certain lipoproteins.

As depicted schematically in Figure 2 (opposite), the basic endotoxin molecular structure consists of two distinct regions: a hydrophilic carbohydrate (polysaccharide) portion which includes an O-specific side chain and an inner and outer core region, and the hydrophobic toxic lipid A component (Glauser, Rietschel). Although the general

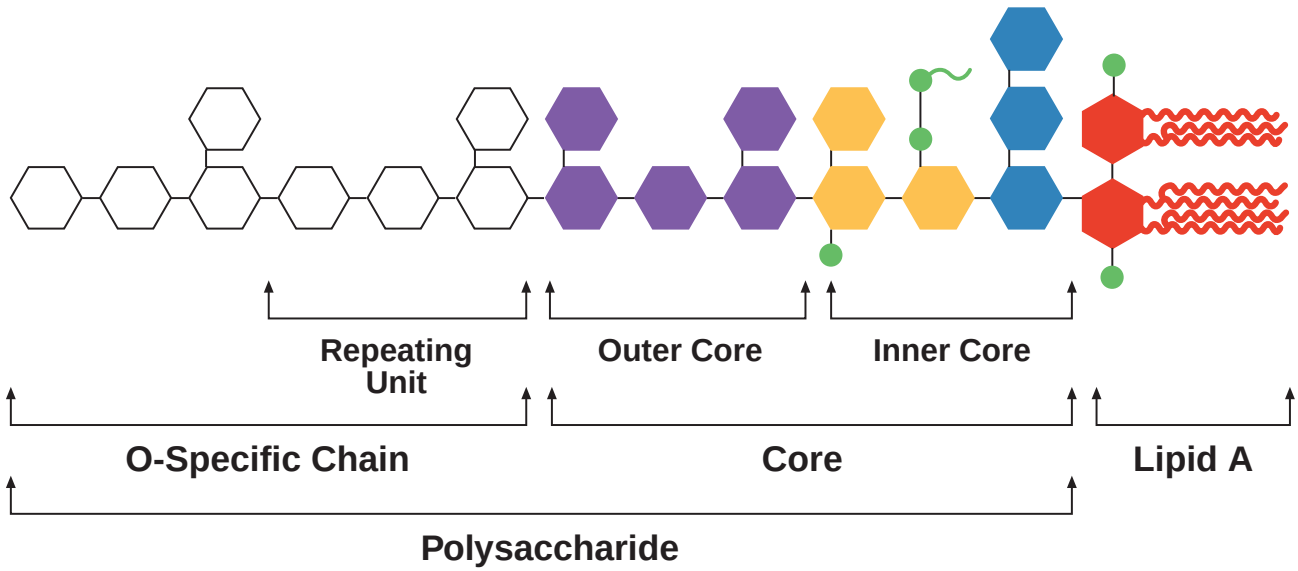
structure is highly conserved among Gram-negative bacteria, there is considerable structural variability at the O-specific chain between bacterial species.

Since the O-specific chain is enzymatically constructed by the sequential addition of oligosaccharides, the endotoxin of a given bacterium at a given point in time is a heterogeneous mixture of molecules with short, intermediate, and long O-specific chains. Thus, there is no precise or standard way to measure molecular composition or molecular weight of endotoxin. Evolutionary pressure exerted by phagocytic cells and macrophages on Gram-negative bacteria may account for some of this heterogeneity (Nikaido), since the O-specific chain confers resistance to phagocytosis and bactericidal agents such as complement (Rietschel).

Structural variation in the O-specific side chains produces two distinct morphological types of Gram-negative bacterial growth in culture, the “rough” (or short O-specific chain-containing LPS) and the “smooth” (long chain-containing LPS) variants. This variation is more than a microbiological curiosity because of its impact on the disease-causing potential of the organism. In laboratory and in animal experiments, the smooth phenotype emerges as an important virulence factor, conferring resistance to complement mediated serum killing of bacteria (McCallum). Smooth *Salmonella* strains demonstrate accelerated rates of proliferation and mortality in mouse models of infection (Lyman). There also appear to be crucial human therapeutic implications of the smooth/rough dichotomy, in that anti-endotoxin antibodies have shown much lower binding to smooth Gram-negative bacteria than to rough strains (Siegel).

The lipid A component of endotoxin is highly conserved from one Gram-negative bacterial family to another and gives the endotoxin molecule its toxicity (Rietschel), whether as a component of a viable microorganism or when shed from the cell wall. The most powerful evidence implicating lipid A as the biologically-active portion of endotoxin involves studies using synthetic molecules. These show that lipid A, independent from all carbohydrate constituents, is as toxic as its naturally-occurring endotoxin counterpart (Ulevitch). The actual endotoxic activity of LPS is believed to be dependent upon the specific conformation of the lipid A portion of the molecule. At high concentrations this conformation appears to be a three-dimensional nonlamellar structure (Schromm). It is

Figure 2: Bacterial Lipopolysaccharide (LPS)



Adapted from Rietschel, *et al., Progress in Clinical & Biological Research* 189:31-51, 1985

believed that this conformation enables endotoxin to maximally interact with specific humoral and cellular host factors, triggering the inflammatory cascade.

B. Circulating Endotoxin (Endotoxemia)

In blood or serum, Gram-negative bacteria may release endotoxin in a variety of forms, such as membrane fragments, blebs, and vesicles, in combination with bacterial phospholipids (Parker). Once in the circulation, LPS may be bound by a large number of serum constituents, some of which enhance its pathogenicity while others act to neutralize its effects. In the former category are LBP and soluble CD14 (Ulevitch), which are discussed in a subsequent section (see "Activation of Cellular Mediators", page 5).

Among the best-described natural binding ligands for LPS is bactericidal/permeability-increasing protein (BPI), a polypeptide found in neutrophil azurophilic granules (Elsbach & Weiss). Besides having bactericidal activity, (as its name implies), BPI binds with high affinity to LPS and neutralizes it as well as facilitating its clearance from the circulation. BPI and LBP are closely related, and show more distant sequence homology to two lipid transport proteins, cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP), suggesting common mechanisms of lipid binding in all proteins (Beamer). Interestingly, LPS is also bound by other normally circulating lipoproteins, including high-density, low-density, and very-low density lipoproteins and chylomicrons (Parker).

III. Pathophysiology of the Endotoxin-Induced Inflammatory Response

A. Routes of Exposure to Endotoxin

Humans may be exposed to endotoxin via two routes. The first and most widely appreciated is systemic or localized Gram-negative bacterial infection of exogenous source. As a consequence of infection by a specific pathogen, bacteria and bacterial cell wall fragments can cause local or systemic inflammatory responses. Current therapeutic efforts are primarily directed against the underlying infection—viz., antibiotics. However, even when the bacteria are successfully killed, their residual endotoxin can continue to fuel an inflammatory response.

It has been put forward that antibiotic treatment of Gram-negative bacterial infections may, ironically, increase endotoxin load and exacerbate the inflammatory response (Prins). Some animal and human studies suggest that antibiotic-induced bacteriolysis liberates LPS (Jackson, Prins, Shenep). Antibiotic classes may vary in this respect; one study in surgical intensive care patients found that ceftriaxone and cefotaxime were associated with greater endotoxin levels than were tobramycin, vancomycin, ciprofloxacin, and imipenem (Holzheimer). But other studies have found no significant difference between imipenem and ceftriaxone (Prins). Antibiotic-associated endotoxin release remains unproven and its clinical significance is unknown. Nevertheless, further research may lead to new approaches to patient management, such as more selective antibiotic choices (Prins) and synergistic use of endotoxin binding and neutralizing agents in conjunction with antibiotics (Elsbach & Weiss).

The second, less well-recognized route of endotoxin exposure is bacterial translocation from the gut (Lemaire, van Leeuwen). The gastrointestinal tract normally contains a population of nonpathogenic bacterial flora, primarily Gram-negative anaerobes. Outer-membrane fragments of Gram-negative bacteria, including endotoxin, are continuously produced within the normal gut, without apparent harm to the host (van Leeuwen). In fact, there is considerable evidence showing that minute amounts of endotoxin are constantly being shed into the portal circulation by the healthy gut and cleared by cells in the liver (Jacob, Mathison, Nolan, Ruitter). During this process, LPS is normally taken up by endothelial and Kupffer cells, and possibly hepatocytes, via pinocytotic or receptor-dependent mechanisms (van Leeuwen), and is thereby rendered harmless to the host before reaching the systemic circulation.

Pathologic translocation may lead to endotoxin-related illness when one or more of the natural host controls of this process fails, allowing excessive quantities of bacteria or endotoxin to exit the gut via lymphatic or vascular channels (Deitch, van Deventer, van Leeuwen). When this occurs, high concentrations of endotoxin can be found in the mesenteric lymph nodes, liver, spleen and general circulation. While the precise methods by which endotoxin can overwhelm physiologic host barriers are unknown, two

mechanisms have received the most attention: breakdown in gut mucosal integrity that allows bacteria and endotoxin to "flood" the bloodstream, and/or impairment of the liver's normal clearance mechanisms. With respect to the first hypothesis, numerous investigators have postulated that a variety of insults that impair normal blood flow and lead to varying degrees of gut ischemia can compromise the integrity of the gut mucosal barrier and allow "leakage" of endogenous LPS (Antonsson, Baigrie, Brewster, Casey, Martinez-Pellus, Riddington, Roumen & Freiling). This general mechanism has been invoked to explain the occurrence of SIRS or sepsis in many clinical situations where a primary site of infection may not be obvious (e.g., trauma or burns, as discussed in detail below).

The second mechanism by which translocated endotoxin can lead to SIRS or sepsis is hypothesized to be impaired liver clearance of LPS. This concept is documented in literature which shows that elevated circulating endotoxin levels occur in patients with cirrhosis, alcoholic liver disease and other conditions that result in hepatic failure (Bode, Fukui, Schafer). More specifically, these high LPS levels in the blood and lymphatic system have been attributed to decreased clearance of LPS from the portal circulation caused by intra-hepatic vascular shunts (which allow the LPS to circumvent the liver entirely), endothelial dysfunction, and the Kupffer cell/reticuloendothelial system (RES) impairment which is known to occur in chronic liver disease. The extent to which these elevated LPS concentrations promote systemic illness is unclear, but at least one investigator has suggested that they may contribute to the occurrence of hepatic encephalopathy (Odeh), perhaps explaining the reported beneficial effects of non-absorbable antibiotics in this syndrome.

While clinical sources and abundant animal data demonstrate that both increased gut permeability and translocation of endotoxin occur in association with a variety of systemic insults (van Deventer), data demonstrating that translocated LPS is directly pathogenic in humans are scant (Lemaire). Nevertheless, continued interest in bacterial translocation as a source of disease is fueled by the well-recognized occurrence of SIRS and shock in patients without culture-positive bacteremia or other clinically apparent sources of infection but with morbidity and mortality rates equivalent to those of culture-positive sepsis and septic shock patients (Rangel-Frausto). Clinical settings in which this occurs include immunosuppression, starvation, thermal and radiation injuries, hemorrhagic shock, blunt trauma, chemotherapy, and cardiac and vascular surgical procedures. These conditions have all been associated with gut ischemia and/or impaired RES function and may therefore predispose to bacterial/ endotoxin translocation, which may result in endotoxemia and sepsis (Brathwaite, Saadia, van Leeuwen).

B. Host Interactions with Endotoxin

Endotoxin exerts its highly complex array of pathophysiologic effects by interacting in the host with a panoply of naturally-occurring cellular and humoral elements (Bone, Glauser, Rietschel, Remick, Ulevitch); see Figure 3 [inside back cover]. These elements routinely mediate the normal host response against infectious insults. In SIRS or sepsis, however, the host's normal homeostatic mechanisms break down and the inflammatory response manifests as fever, vascular leakage, myocardial depression and shock (Rietschel, Suffredeni).

1. Activation of cellular mediators—LBP and CD14

Endotoxin interacts with virtually all components of the cellular immune system. It is taken up by neutrophils, leading to cell activation and the subsequent enhancement of the phagocytic ability of these cells. Further, it may activate neutrophils to express cell adhesion molecules which mediate neutrophil-to-neutrophil, neutrophil-to-vascular endothelial cell and neutrophil-to-tissue binding, causing local inflammation and vascular leakage (Glauser, Rietschel). LPS also appears to affect various populations of lymphocytes, stimulating B-cell proliferation and antibody production, activating T-cells to secrete cytokines, and down-regulating T-suppressor cells (Rietschel).

The most widely studied and probably the most significant cellular effects of endotoxin involve the interaction with cells of the monocyte/macrophage lineage (Glauser, Rietschel, Remick), which express a membrane receptor known as CD14 (Remick). Circulating LPS is bound by a glycoprotein serum factor, LBP, which facilitates binding of LPS to its principal cellular receptor, the CD14 molecule (Ulevitch). The importance of this interaction is demon-

strated by experiments in which preventing LPS-LBP binding to monocytes blocks the activity of endotoxin (Remick). Binding of LPS-LBP to CD14 induces monocytes to produce and secrete a myriad of pro- and anti-inflammatory cytokines, including interleukins (IL-1, IL-6, IL-8, IL-10), macrophage migration-inhibitory factor, and tumor necrosis factor (TNF) (Rietschel, Remick, Ulevitch). While numerous other humoral mediators are induced by LPS (see below), the overwhelming systemic production of the inflammatory cytokines appears to be a central component of the life-threatening organ failure and shock that characterizes endotoxin-related SIRS and sepsis (Rietschel, Remick).

2. Role of cytokines in SIRS and sepsis

The cytokines TNF, IL-1, IL-6 and IL-8, when expressed in limited quantities and confined to a local area, play a beneficial role in stimulating the host defense system to destroy invading microorganisms (Mastroeni). Yet, in endotoxemia, they are released systemically and in that setting may become crucial mediators accelerating the progression to SIRS and sepsis (Bone, Froon, Glauser, Gardlund, Rietschel). (See Figure 3, inside back cover.)

This sometimes sinister role of cytokines in sepsis is supported by several lines of evidence, among which are: injections of TNF and IL-1 into animals induce physiological responses that are similar to those of patients with septic shock (Remick); inhibition of TNF and IL-1 prevents organ damage and death in animals with septic shock (Opal, Rydberg); and plasma levels of TNF in certain sepsis patients have sometimes been shown to correlate with prognosis (Brandtzaeg, Gardlund, Roumen & Hendriks). Moreover, TNF, IL-1 and IL-6 stimulate receptor-carrying cells, resulting in the intensification of the host response to circulating LPS (Rietschel). When so activated, monocytes and macrophages are induced to release biologically-active molecules such as platelet activating factor, leukotrienes, prostaglandins, oxygen radicals, and pro-inflammatory proteases (e.g., elastase and collagenase), the primary mediators of the tissue damage characteristic of a systemic inflammatory response (Bone). In addition, cytokine overstimulation, endothelial dysfunction and damage caused by nitric oxide release, leukocyte adherence to vessel walls and consumptive coagulopathy (discussed below) lead to vasodilatation, vascular leakage and refractory hypotension.

Many of these humoral mediators act as endogenous pyrogens, causing fever. They also stimulate the release of ACTH, cortisol, and macrophage migration inhibitory factor and induce the liver to produce acute-phase proteins (Baumann, Steel), such as LBP (described above) (Glauser). Circulating TNF, in conjunction with the cytomodulators produced by activation of the complement system (discussed below), causes leukocytes to adhere to endothelial cells, resulting in leukopenia, and blood vessel and capillary injury (Rietschel).

3. Activation of other humoral mediators

Gram-negative bacteria activate the complement system through two separate pathways: bacteria and bacterial cell wall components complexed with antibodies activate the classical (antibody-dependent) complement pathway intended to kill the bacteria, while the bacteria and endotoxin directly activate the alternative (non-antibody) pathway (Glauser). The resulting complement cascade induced by LPS produces, among other mediators, the anaphylotoxins C3a and C5a, which contribute to vasodilatation, increased vascular permeability, and circulatory collapse. In addition, complement components induce adhesion and activation of platelets and neutrophils, stimulating the secondary events of platelet aggregation, release of lysosomal enzymes and arachidonic acid metabolites, and microvascular injury. The damage from inappropriate (pathologic) complement consumption is interlinked with overproduction of cytokines.

The precise role of arachidonic acid pathway mediators in sepsis and related syndromes is still under investigation. LPS-induced activation of susceptible cells, such as neutrophils, leads to the release of prostaglandins, leukotrienes, and other agents with vasoactive and pro-inflammatory effects. Presently, the extent to which these well-known molecules contribute to shock, or provide therapeutic targets in the treatment of endotoxin-induced syndromes, remains to be elucidated.

Endotoxin is the most potent known exogenous activator of the coagulation system. As with its effects on comple-

ment and cytokines, endotoxin converts the normally beneficial coagulation system into a pathological cascade. Factor XII (Hageman factor) of the coagulation cascade plays an important role in the pathogenesis of shock. When activated by LPS or lipid A, Factor XII triggers the intrinsic coagulation pathway by activating Factor XI and by stimulating endothelial cells and macrophages to produce tissue factor. The process of activating the intrinsic coagulation pathway also activates the extrinsic coagulation pathway, and both participate in the development of disseminated intravascular coagulation (DIC) (Glauser). Tissue factor, readily induced by endotoxin, may be responsible for stimulating DIC, since antibodies to tissue factor can prevent this in rabbits given endotoxin (Warr). LPS-activated Factor XII also contributes to hypotension and shock by converting prekallikrein into kallikrein, which in turn cleaves kininogen to release the potent hypotensive agent bradykinin (Colman).

The host response to LPS also includes the production and release of the potent vasodilator nitric oxide. Nitric oxide is a vascular relaxing agent produced by macrophages and endothelial cells (Palmer). When released, it induces vasodilation which, when extensive, results in hypotension. LPS-induced nitric oxide release by macrophages takes up to several hours, whereas its release by endothelial cells takes only a few minutes. Although the role of nitric oxide (NO) in the acute inflammatory cascade is still being investigated, its rapid release by endothelial cells may be the cause of the precipitous drop in blood pressure associated with septic shock (Vane). However, clinical trials of NO inhibitors have not shown a survival benefit.

The role of endogenous opioids in septic shock is poorly understood. The concept that endorphins may contribute to shock stems from two findings: opioid peptide secretions can be induced by endotoxin; and the opioid antagonist, naloxone, has some activity in reversing endotoxin-induced hypotension (Hackshaw), but has shown no survival benefit in the clinic. More research is needed to elucidate what role, if any, endorphins have in the development of sepsis.

C. Summary: Pathogenesis of the Inflammatory Cascade

A multifaceted host response can be triggered by endotoxin. Possible sources of endotoxin include exogenous Gram-negative bacterial exposure or gut translocation and failure of hepatic uptake and clearance mechanisms. Uncleared endotoxin not neutralized by naturally-occurring cellular components such as BPI in polymorphonuclear leukocytes (PMNs) will interact with LBP. The LBP/LPS complex, which binds selectively to soluble and membrane-bound CD14, is the primary trigger of the production and release of crucial humoral mediators (TNF, interleukins) by monocytes/macrophages, endothelial cells, granulocytes and lymphocytes. As outlined above, when homeostatic mechanisms break down, the interconnected cellular and humoral inflammatory cascades can lead to shock and organ failure. As is easily discernible from Figure 3 (inside back cover), this process is enormously complex and involves multiple simultaneous and self-propagating cascades.

For example, TNF, IL-1 and IL-6 induce fever and cause the liver to release acute phase proteins which may lead to the release of additional TNF (Baumann, Geller, Glauser, Steel). Blood clotting factors are consumed and the complement system is activated to produce additional cytomodulators which, in conjunction with TNF and leukocyte aggregation, can lead to leukopenia, blood vessel injury and capillary leakage. Endothelial cells are stimulated to produce nitric oxide, which causes vasodilation, hypotension and increased cardiac output. T cells release colony-stimulating factor and interferon γ, causing leukocytosis and stimulating macrophages to produce humoral mediators and further perpetuate the inflammatory response (Glauser, Rietschel).

If the normal host response spins out of control, the resulting catastrophic clinical illness, known as sepsis when associated with an identified infectious process, (or as SIRS, when no infectious agent is identified), is characterized by fever, chills, leukopenia or leukocytosis, and hypotension despite tachycardia and marked increases in cardiac output. The overwhelming systemic inflammation may lead to DIC and hemorrhage, the vascular leakage described above, and pulmonary edema progressing to the acute respiratory distress syndrome (ARDS). Refractory

hypotension ("septic shock" in the presence of infection) progresses to life-threatening decreases in organ perfusion, manifested as metabolic acidosis, renal insufficiency, obtundation and coma, intestinal ischemia, and, ultimately, terminal respiratory failure and/or cardiovascular collapse (Glauser, Parillo, Rietschel).

Recent investigations have added a new dimension to the picture of the sepsis cascade. A decade ago, sepsis was felt to be a "horse-out-of-the-barn" phenomenon—in other words, once endotoxin initiates events, the pathogenic cascade is self-perpetuating and unstoppable. The current view, however, posits that the continuous presence of endotoxin is necessary to maintain the SIRS in addition to simply initiating it (Dedrick, Huang). This concept brings a strong note of optimism to newer therapeutic efforts because it suggests that even late in the clinical course, specific endotoxin-neutralization therapy may be able to abort established but still-reversible SIRS, sepsis and septic shock (Giroir).

D. Diagnostic and Prognostic Markers of SIRS and Sepsis

Given the heterogeneous clinical presentations of sepsis and septic shock, the difficulty in making a definitive diagnosis with currently available technology, and the urgency of instituting potentially life-saving therapy, it is no wonder that numerous attempts have been made at developing laboratory techniques to detect endotoxemia. To date, however, there is no reproducible, validated laboratory test for documenting endotoxemia; all attempts thus far have been plagued by methodological problems, assay variability, and lack of clinically useful specificity and sensitivity. As a result, clinicians have neither a laboratory tool for making an unequivocal diagnosis nor a prognostic marker to identify those patients who might benefit from specific therapeutic interventions (Goldie).

Many attempts have been made to utilize serum endotoxin assays as a diagnostic test. Although endotoxin has been found in the circulation in about two thirds of septic patients in several trials, there is as yet no consensus about its utility as a clinical marker. While some groups have reported a good correlation between whole blood endotoxin levels and clinical course in sepsis and septic shock patients (Danner, Ng), most other authors have been unable to demonstrate either diagnostic or prognostic value of blood endotoxin assays (Elin, Engervall, Goldie, Guidet, Rintala, Stumacher). Thus, the practicality and usefulness of measuring circulating endotoxin in the clinical setting remain to be demonstrated.

There are numerous technical and biological issues which hamper the development of clinically-useful endotoxin diagnostic methods. Endotoxin release is episodic and circulating LPS has a short half-life (minutes), obscuring the value of a single determination. In the bloodstream, LPS is distributed in varying proportions bound by LBP, micelles, chylomicrons, and various other lipid fractions; some of these bound moieties are biologically inactive, but most assays measure all LPS whether biologically active or not—again, making the relevance of the results uncertain. Further, assays are measured against positive controls, yet, as discussed earlier, each bacterial species has different LPS composition, and the most appropriate control strain has never been defined. Finally, false positive results can lead to unwarranted and dangerous treatment while false negative results could be catastrophic for the patient, particularly if effective anti-endotoxin therapy was available.

Additionally, all currently available LPS/endotoxin assays are different, and results from one cannot be correlated to results from another. Therefore, they share one paramount clinical characteristic: all are unsuitable for use as diagnostic, prognostic, or patient monitoring tools. These limitations have contributed to the lack of regulatory approval for such assays for diagnostic use in patients in all countries other than Japan.

As the limitations of direct measurement of endotoxin levels have become apparent, the search for other clinically useful biological markers of exposure to endotoxin has been extended to cytokines and other inflammatory modulators—again, without notable success. Among the cytokines evaluated are TNF, IL-1, IL-6, and IL-8, with generally inconclusive results (Goldie, Rhodes). Early suggestions that elevated IL-6 levels, when combined with assays of endotoxin and/or phospholipase-A2, are useful in the management of febrile, neutropenic cancer patients await further confirmation (Engervall, Rintala), as does the

observation that circulating IL-6 levels >3000 pg/mL predict mortality in intensive care patients (Goldie).

A variety of other naturally occurring serum proteins have been advanced as candidates for the laboratory diagnosis of SIRS and sepsis, but as yet none have been subjected to rigorous field testing, independent validation, or clinical confirmation. In a comprehensive study of 146 patients, circulating endotoxin, TNF, IL-1β, and IL-6, and two forms of soluble TNF receptor, IL-1 receptor antagonist, and anti-endotoxin core antibodies were evaluated. The authors suggested that low concentrations of IgG anti-core antibodies correlated with mortality (Goldie). Phospholipase-A2, a marker of neutrophil activation and a key enzyme in the arachidonic acid inflammatory cascade, appeared to be a sensitive marker of acute lung and other organ injury, and perhaps sepsis (Rae, Rhodes). And although much recent attention has been focused on the use of procalcitonin as a marker of systemic inflammation and a predictor of outcomes in septic shock, external confirmation is still needed (de Warra, Meisner, Rhodes).

Because of its critical position early in the inflammatory process, it has been suggested that LBP may be a better target than the cytokines for diagnostic and prognostic development. LBP is a plasma protein produced in the liver in response to endotoxin exposure. Plasma levels of LBP are elevated in patients with diseases characterized by exposure to Gram-negative bacteria and endotoxin, such as Gram-negative sepsis, abdominal infections, meningococcemia, Crohn's disease, and ulcerative colitis (Carroll). Cystic fibrosis patients, who suffer recurring bouts of lung infections, usually by Gram-negative *Pseudomonas* bacteria, also have elevated LBP levels. Furthermore, while plasma LBP levels were low in hemorrhagic trauma and liver surgery patients immediately after trauma or surgery, sequential sampling showed that LBP levels become highly elevated within several days of the original insult. This not only provides support for bacterial translocation, but also suggests that LBP itself may be a useful diagnostic and prognostic marker across a range of septic and non-septic complications.

Although this is beyond the scope of this review, there is also an extensive literature evaluating a myriad of physiologic parameters as prognostic markers in sepsis and septic shock. These have included blood pressure, vascular resistance, cardiac index and other derived hemodynamic parameters, systemic oxygen delivery and tissue oxygen consumption, anion gap, and gastric tonometry (Rhodes). As with laboratory tests of LPS and other chemical markers, there is as yet no consensus about the utility or specificity of any of these measures for diagnosing sepsis or SIRS, predicting outcomes, or guiding therapeutic decisions. Research continues in the hope of identifying individual or combination markers that can improve treatment strategies and, thereby, prognosis (Rhodes).

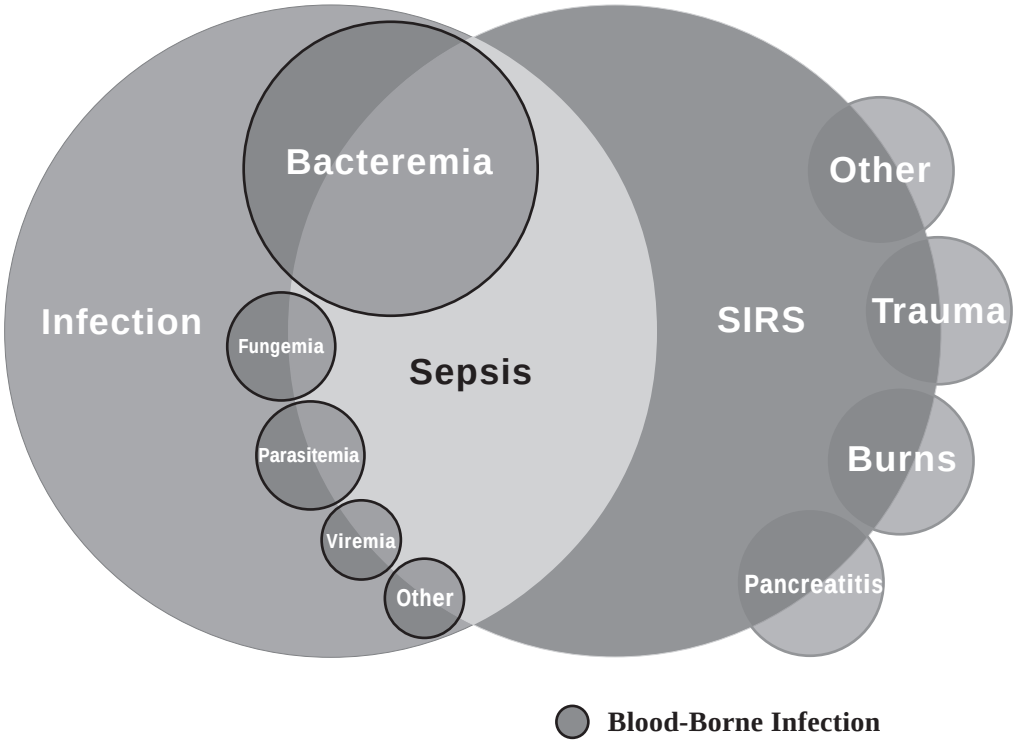
IV. Endotoxin-Related Clinical Syndromes

Sections IV and V will review the clinical consequences of endotoxin exposure. Section IV starts with definitions of sepsis, SIRS and associated severe organ dysfunction syndromes and ends with a discussion of meningococcemia, a unique Gram-negative bacteremic syndrome. This is followed by a summary of syndromes that may be associated with gut translocation of Gram-negative bacteria and/or LPS. Finally, Section V presents a series of more localized illnesses whose pathogenesis may, in part, be related to LPS. (These illnesses are summarized in Table 1, pp 12-13.)

A. Sepsis, Septic Shock and SIRS

Conflicting terminology and confusing semantics have long impaired communication in the field of sepsis. The 1992 ACCP Consensus Conference report developed definitions, built upon a body of previous epidemiological and clinical observations (Bone & Balk, Rangel-Frausto) in an attempt to standardize terminology and facilitate communication. Resolving these issues is particularly important as clinical trial activity grows and clinical investigators, reviewers, and regulatory authorities require a common language for assessing clinical presentations, trial entry criteria, subpopulation analysis and outcome measures. At the same time, the ACCP's definitions point out that the diagnosis of SIRS, sepsis and septic shock remain based upon a characteristic constellation of clinical signs and symptoms. There is, at present, no single diagnostic criterion that establishes the presence of SIRS, sepsis or septic shock.

Figure 4: An Earlier Proposed Interrelationship Between Systemic Inflammatory Response Syndrome (SIRS), Sepsis, and Infection



Roger C. Bone and the ACCP/CCCM Consensus Conference Committee, Chest, 101:6,1644-1655.

The ACCP introduced the term "systemic inflammatory response syndrome" (SIRS) to describe the general systemic inflammatory process independent of cause. It is symptomatically characterized by hyper- or hypothermia, tachycardia, hypoventilation and/or leukocytosis. The term "sepsis" is now defined as the "systemic inflammatory response to infection," that is, SIRS plus a culture-documented infection. "Septic shock" is considered a subset of sepsis (and therefore requires a documented infection) and is defined as "sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction" (ACCP). (See Figure 4 above)

More specifically, the diagnosis of sepsis is defined by the ACCP as a systemic response to a culture-documented infection consisting of two of the following four criteria:

- Temperature > 38°C or < 36°C
- Heart rate > 90 beats/minute
- Respiratory rate >20 breaths/minute or a $PaCO_2$ < 32 torr
- White blood cell count >12,000 cells/mm^3, < 4000 cells/mm^3, or > 10% immature forms.

Patients who demonstrate identical clinical findings but who do not have a detectable infection are classified as having SIRS.

Septic shock represents an extreme manifestation of the sepsis syndrome, and includes patients meeting the above-

mentioned criteria (including the presence of documented infection) who also demonstrate refractory (in the absence of medical intervention) arterial hypotension and clinical manifestations of hypoperfusion, such as lactic acidosis, oliguria, or acutely depressed mental status.

SIRS (no detected infection) with shock should therefore be referred to as "SIRS plus shock", "shock of unknown origin" or "suspected or presumed septic shock", but common usage is still not consistent in this regard. The absence of a documented infective source does not necessarily eliminate the possibility of exposure to infectious agents including Gram-negative bacteria translocated from the gut.

As it progresses, the end stages of septic or SIRS-related shock may include other clinical syndromes distinct enough to warrant specific names, such as the various organ dysfunctions: acute renal failure (ARF), acute respiratory distress syndrome (ARDS), hepatobiliary dysfunction (HBD), central nervous system dysfunction (CNSD), or disseminated intravascular coagulation (DIC). The presence of more than one of these organ dysfunctions comprises multiple organ dysfunction syndrome (MODS). Like septic shock, this syndrome is associated with poor prognosis and with increased mortality rates (ACCP). The ACCP did not specifically define the organ dysfunctions (i.e, ARF, ARDS, HBD, CSND and DIC) associated with SIRS. However, they recommended using the term "multiple organ dysfunction syndrome (MODS)", defined as refractory inability to maintain organ homeostasis in the absence of medical intervention. This would replace the dichotomous term "organ failure" with a continuum of functional derangements.

ARDS refers generically to the most severe form of acute lung injury, characterized by diffuse pulmonary inflammation, increased lung capillary permeability, pulmonary edema and respiratory insufficiency (Lamy). Primary ARDS is caused by direct lung injury (for example, drowning or toxic inhalation); secondary ARDS occurs commonly through indirect, systemic mechanisms such as those that characterize septic shock. As a late stage complication of septic shock, ARDS may occur alone, or as the pulmonary component of MODS. Studies of trauma patients reveal that the lungs are the first organ system to fail in the presence of endotoxin (Welbourne), and many investigators feel that understanding and preventing the effects of endotoxin on the lung may be crucial to preventing the failure of additional organs.

When multiple organs (such as the heart, lungs, and kidneys) become dysfunctional—as defined by the inability to maintain normal homeostasis in the absence of medical intervention—the resulting clinical picture is termed MODS (ACCP). MODS was recently clarified and defined by the ACCP according to the circumstances surrounding its onset. Primary MODS occurs in response to a direct insult to an organ and includes such events as pulmonary contusion or coagulopathy due to multiple transfusions (ACCP). On the other hand, secondary MODS is a consequence of an abnormal and overwhelming host inflammatory response. Secondary MODS occurs with some latency after the acute insult, involves distant organs in the systemic inflammatory response, and is most frequently observed as a complication of severe infection (ACCP). In the context of sepsis, MODS is typically characterized by pulmonary and/or renal failure, circulatory failure, and hepatic, gastrointestinal and central nervous system dysfunction. Organ failure, like septic shock, is diagnosed using clinical criteria; the definitions of any of these organ dysfunctions are not universally agreed upon.

Sepsis and septic shock are not syndromes that develop in healthy persons; rather, they occur in patients already suffering acute catastrophic illness, severe underlying disease, or major trauma (Bone). Likewise, patients at greatest risk of dying are those with pre-existing physiologic compromise, such as advanced age, malignancy, immunosuppressed status, or major organ dysfunction. It has been postulated that these conditions predispose to sepsis and mortality because they independently induce the release of the same pro-inflammatory activators that mediate septic shock (Bone).

B. Meningococcemia

Neisseria meningitidis is a Gram-negative organism (meningococcus) whose natural habitat is the human nasopharynx. It is most commonly associated with epidemic or sporadic meningitis in children and young adults. The cell wall in *N. meningitidis* bacteria incorporates an endotoxin (lipooligosaccharide, LOS) that may be responsible

Clinical Condition	US Incidence
Sepsis/septic shock	500,000 cases/yr[1] (hospitalized)
SIRS	unknown
Meningococcemia	< 3000 cases/yr[2]
Trauma/hemorrhagic shock	> 250,000 cases/yr • < 40% complications
Burn injuries	50,000 cases/yr[1] (hospitalized)
Cardiovascular surgery (Cardiopulmonary bypass; aneurism repair)	600,000 surgeries/yr[3]
Liver surgery/ transplant	< 4000 transplants/yr[4] • < 50% complications
Liver disease (hepatitis B & C, cirrhosis etc.)	> 4,000,000 chronic cases/yr[4] • 26,000 deaths/yr
Acute pancreatitis	80,000 cases/yr[5]
Inflammatory Bowel Disease	1-2 million cases/yr[5]
Necrotizing Enterocolitis	2000-4000 cases/yr[11] • 1000 neonate deaths
Periodontal disease	7.5 million/yr[7] treated for ~ 50 million affected
Pneumonia	1.2 million in hospital/yr • 82,000 deaths/yr[2]
Lung infections in Cystic Fibrosis patients	30,000 people with CF [6]
Asthma	14.5 million new cases/yr[2]
Coronary Artery Disease	1.1 million heart attacks/yr[3] • 481,000 deaths/yr[3]
Congestive Heart Failure	400,000 new cases/yr • 43,000 deaths/yr[3]
Complications of renal dialysis	181,000 renal dialysis patients[5]
Hemolytic Uremic Syndrome (*E. coli* O157:H7)	27,000 cases[8]
Autoimmune diseases	500,000 rheumatoid arthritis/yr[9]
Cancer	575,000 in chemotherapy/yr[10]

1. National Center for Health Statistics, Bethesda, MD
2. Centers for Disease Control (CDC)
3. American Heart Association
4. American Liver Foundation
5. National Institute of Diabetes and Digestive and Kidney Diseases (NIH)
6. Cystic Fibrosis Foundation
7. American Dental Association

Conditions

Possible Mechanisms of Endotoxin Exposure	Comments
various identified infectious agents: bacteria, fungi	many predisposing conditions; elevated LPS & LBP
no documented infectious source	inability to culture organism does not rule out infection
Neisseria meningitidis bacteremia	classic example of fulminant sepsis; elevated LPS & LBP
intestinal translocation related to blood loss	elevated LBP, LPS levels variable
infection and/or translocation	sepsis is a frequent complication
intestinal translocation associated with ischemia	infectious complications frequent
gut translocation plus impaired liver clearance of LPS	complications include pneumonia and sepsis
impaired liver clearance of LPS	may lead to endotoxemia
similar to post-trauma and -surgical sepsis	elevated LPS
gross impairment of gut integrity may lead to translocation	exacerbations often complicated by SIRS/sepsis; elevated LBP, variable LPS
focal or systemic infection, gut translocation	elevated LPS, cytokines
LPS from infecting bacteria	clinical significance unknown
exogenous infection/shock-related translocation	like sepsis, a frequent complication of trauma, surgery
colonization by Gram-negative bacteria	elevated LBP, CF predisposes to recurring infection
LPS-containing allergenic dusts that trigger inflammatory response	
infection and/or translocation	controversial
translocation due to hypoperfusion	
nosocomial infections	
disruption of GI tract by infection by exotoxic enteropathogens	
LPS induction of inappropriate immune response	
chemotherapy-induced translocation and neutropenia	elevated LPS, septic complications

8. Healthway Online (www.healthanswers.com)
9. CIBC Oppenheimer figures for Rheumatoid Arthritis (~ 1/2 of all cases of autoimmune disease)
10. Medical and Healthcare Marketplace Guide, 1998
11. National Institute of Child Health and Human Development

for some of the nervous system tissue damage that occurs during meningitis.

In some patients, the primary manifestation of meningococcal infection is meningococcal septicemia or meningococcemia, a characteristic acute systemic inflammatory syndrome marked by meningococcal bacteremia, septic shock, and high mortality (Brandtzaeg). Meningococcemia is a unique and well-characterized example of Gram-negative bacteremia, and can be viewed as a particularly fulminant form of Gram-negative sepsis.

Unlike most episodes of septic shock, where an underlying medical problem predisposes the patient to infection and sepsis, meningococcemia unexpectedly strikes previously healthy children and young adults. The illness is characterized by rapid onset; patients may progress from undifferentiated flu-like symptoms to a septicemic purpural rash and shock within hours. As with septic shock, hypotension and ARDS are common, as well as renal failure, adrenal infarction and acute adrenal crisis. Severe coagulopathy (DIC) is particularly noted in meningococcemia. Even in cases where the illness is not fatal, it may result in serious morbidity such as stroke or tissue necrosis that requires extremity amputations.

Systemic meningococcal disease is marked by some of the highest levels of circulating endotoxin documented in any illness, and mortality appears to correlate with the endotoxin (and bacterial) load (Brandtzaeg). Although appropriate antibiotic therapy is largely effective in eradicating the causative organism (penicillin-resistant strains are emerging, however), the endotoxin-related complications can still progress. Thus, current investigational efforts to treat this devastating illness include using a recombinant BPI protein to eliminate the bacterial endotoxin as well as the *N. meningitidis* bacteria. (Giroir). Other investigational approaches target the coagulopathy (activated Protein C) or individual mediators in the sepsis cascade (TNF, etc.).

V. Other Clinical Conditions

A. Complications That May Be Associated With Gut Translocation

Clinicians have long recognized that patients without definitive proof of either local or bacteremic Gram-negative infection suffer SIRS and other complications that are presumptively of infectious origin. This problem (which reflects the diagnostic gap discussed above) in part fuels the interest in translocation. This section summarizes those clinical syndromes in which this mechanism has the most theoretical appeal and/or experimental support: trauma, cardiovascular surgery associated with intestinal ischemia, and burns. Interestingly, these are also conditions in which the normal host response to bacteria or endotoxin may be impaired—by immunosuppression or RES dysfunction, for example—thereby facilitating the consequences of bacterial and endotoxin translocation. (Hiki, Rocke).

Trauma

Many patients suffering from trauma have the course of illness complicated by SIRS, organ dysfunction or sepsis. When severe blood loss associated with trauma causes hemorrhagic shock, the results may include splanchnic ischemia and the subsequent release of endotoxin from the gut (Saadia). Secondary endotoxemia appears to be a plausible link between translocation and post-trauma organ failure (Moore). The emergence of infectious complications post-trauma has been associated with multiple blood transfusions (Agarwal). Various investigations have demonstrated increased intestinal permeability in trauma victims (Reed, Faries), the post-trauma appearance of IgM anti-endotoxin antibodies in one small series (Hiki), and the microscopic demonstration of bacterial translocation to mesenteric lymph nodes in trauma patients (Langkamp-Henken).

While none of these studies have demonstrated a causal relationship between translocation and septic complications, an intriguing recent investigation of patients suffering severe trauma found strong positive correlations between increases in intestinal permeability post-trauma and four separate clinical parameters: the severity of trauma (based on standard assessment scales); multiple organ dysfunction

scores; the ultimate development of SIRS; and the incidence of infectious complications (Faries).

A recent study in 400 trauma patients who received more than two units of blood showed an association between blood loss/replacement and infectious complications, including pneumonia, bacteremia and DIC (Smith). That same study also showed a statistically-significant reduction in pneumonia and ARDS incidence in patients receiving an endotoxin-neutralizing BPI-derived drug.

Cardiovascular Surgery

In adults and children undergoing cardiovascular surgery, endotoxin, presumptively derived via gut translocation, may trigger an abnormal inflammatory response, delayed recovery and other post-operative complications, and perhaps even sepsis (Andersen, Baigrie, Casey, Martinez-Pellus, Riddington, Rocke, Roumen & Freiling, Watarida). Studies have shown the presence of circulating endotoxin and TNF in pediatric patients following open-heart surgery (Casey), and circulating endotoxin in adults undergoing cardiopulmonary bypass surgery (Andersen, Rocke). Investigators have postulated that these findings relate to the decrease in mesenteric circulation that occurs during aortic cross-clamping and cardiopulmonary bypass, and have suggested that measures which protect the gut mucosa could prevent perioperative endotoxemia (Riddington, Watarida). Although endotoxemia has not been unequivocally implicated as a cause of morbidity in cardiac surgery (Oudemans-van Straaten), one group has reported that low preoperative titers of endogenous anti-endotoxin antibodies (which may reflect impaired host immunity to endotoxin) may predict adverse outcomes (Bennett-Guerrero).

Similarly, abdominal aortic surgery is associated with multiple events that can impair splanchnic perfusion during surgery, cause gut ischemia and consequently induce systemic bacterial translocation (Baigrie, Taggart). Such events include hypotensive episodes during the surgery, the use of aortic cross-clamping procedures that reduce blood flow to the mesenteric circulation, and reperfusion following ischemia which may release oxygen free radicals and exacerbate the mucosal damage (Baigrie, Rocke, Saadia). Elevated cytokines (IL-1β, IL-6, TNFα) have been observed in patients undergoing elective abdominal aortic repair as well as in those suffering hemorrhagic shock caused by ruptured abdominal aortic aneurisms, but the correlation with endotoxemia was unclear. (Roumen *et al*.) Despite a number of published observations about these phenomena, it is still unclear whether they represent clinically relevant occurrences of endotoxin-associated disease in these patients or whether they are "experiments of nature" in which to study endotoxemia during highly controlled episodes of gut hypoperfusion.

Burns

In the case of burns, most occurrences of Gram-negative bacterial sepsis can be attributed to contaminated burn wounds (Saadia). Yet many burn-related sepsis cases occur without an apparent external source of infection, leading investigators to postulate translocation as causal (Jones II [a]; Jones II [b]). Severe fluid loss or shock associated with an extensive burn may cause intestinal ischemia and thus impair the normal gut barrier to translocation. Animal experiments have shown translocation of microorganisms and endotoxin from the gut to the mesenteric lymph nodes, distant organs and the systemic circulation after burn injury (Hansbrough). In addition, burns can lead to the activation of neutrophils capable of injuring endothelial cells and damaging the gut mucosa, further facilitating bacterial or endotoxin translocation (Jones II [b]; Hansbrough).

Summary

The phenomenon of gut translocation is well established in animal and human studies. Various animal models suggest that when a focal or systemic infection is not apparent, bacterial translocation is the most likely source of endotoxin. However, there is not yet a consensus on its clinical relevance in humans. Further research is needed to clarify the clinical significance of translocation.

B. Local and Organ-Specific Diseases

As indicated above, the past two decades have seen significant effort directed to the understanding and treatment of life-threatening systemic illnesses caused by Gram-negative organisms and their endotoxins. More recently, however, there has been a growing appreciation of the potential of endotoxin to cause or contribute to the pathogenesis of a heterogeneous group of other illnesses. In some cases, these are illnesses of obscure cause; in others, the cause is

fairly well understood. Most are local rather than systemic diseases, or at least have predominantly localized manifestations. In general, the literature about endotoxin association in these diseases is more preliminary than the established consensus that exists in the field of sepsis. Additional research will be needed to clarify clinical significance. Nevertheless, as more sophisticated investigational, diagnostic, and therapeutic techniques emerge, these illnesses may represent a new frontier for therapeutic intervention directed against possible endotoxin-related human pathology.

The sections that follow summarize selected literature in this emerging area, with disease states grouped by the organ system most prominently affected.

1. Digestive

- **Inflammatory Bowel Disease (Crohn's Disease and Ulcerative Colitis)**
  The precise pathogenic role of systemic endotoxemia in inflammatory bowel disease remains unclear. Circulating endotoxin has been detected in patients with Crohn's disease and ulcerative colitis (Gardiner, van Deventer, Wellman). One study also found antibodies to endotoxin as well as endotoxin itself in ulcerative colitis and Crohn's disease patients (Aoki). It is not yet known if circulating LPS has a pathogenic role in these illnesses and their systemic manifestations, or if its presence is simply the consequence of damaged intestinal mucosa.

- **Neonatal Necrotizing Enterocolitis (NEC)**
  As more and smaller premature infants survive, NEC is emerging as a significant cause of morbidity and mortality in neonatal intensive care units (Kliegman). Premature and low birth weight infants are at risk for this disease, which is a frequent cause of death (10-50% mortality) usually as a result of peritonitis and sepsis. The pathogenic role of endotoxin has been investigated in this disease that, like inflammatory bowel disease, involves a gross impairment of the gut mucosal barrier. Endotoxemia has been noted in NEC infants, with an additional association with thrombocytopenia (Scheifele). Three potential sources of endotoxin have been identified in NEC patients: focal infection (usually peritonitis), bacteremia, and the gut itself (Scheifele). Various animal models suggest that when a focal or systemic infection is not apparent, bacterial translocation is the most likely source of endotoxin. The risk factors for bacterial translocation are also risk factors for NEC, namely, impaired gut mucosal barrier function, intestinal bacterial overgrowth and impaired (or premature) host immune defenses (Deitch). An additional study examined inflammatory mediators in NEC and demonstrated elevated TNF and PAF (platelet-activating factor), although neither predicted disease severity or outcome (Caplan). As with IBD, the actual role of endotoxin remains to be fully elucidated.

- **Acute Pancreatitis**
  Most of the mortality in this disease (5-40%) is caused by septic complications. These are clinically similar to those seen after burns, trauma and surgery. Therefore, intestinal bacterial translocation has been considered as a possible cause of infectious complications in pancreatitis. Alterations in levels of antibodies to lipid A and high levels of circulating endotoxin also suggest that endotoxin may be a factor in the pathogenesis of acute attacks (Curley). The role of bacteria and endotoxin in pancreatitis is still under investigation.

- **Liver Disease**
  Clinical observations of elevated LPS levels in patients with cirrhosis, alcoholic liver disease, obstructive jaundice, and other hepatic conditions have led many investigators to suggest that endotoxin may be a factor in the development of numerous liver diseases and/or their complications (Bode, Fukui, Schafer), and that treatment to eliminate circulating LPS may have therapeutic benefit (Liehr). Patients with non-alcoholic and alcoholic liver disease frequently have depressed Kupffer cell function and/or the presence of vascular shunts that enable endotoxin to pass into the systemic circulation (Odeh). One investigator has hypothesized that the resulting systemic endotoxemia may trigger the release of TNF and leukotrienes, which can cause liver damage above and beyond that caused directly by alcohol or other toxic metabolites (Fukui). Another group has

reported that circulating endotoxin may contribute to renal impairment occurring in patients with either cirrhosis or obstructive jaundice (Wilkinson). And while speculative, endotoxemia and excess TNF in patients with acute and chronic liver diseases have also been postulated to cause or exacerbate hepatic encephalopathy (Odeh; Wellman).

- **Periodontal Disease**
  Endotoxin has been implicated as one of several potential bacterial mediators in the development of periodontal disease (Loesche). Gram-negative bacteria and endotoxin that accumulate on the tooth surface can penetrate the crevicular fluid and gingival epithelium. The ensuing local inflammatory response, in synergy with other bacterial toxins and destructive enzymes, can result in the soft tissue loss and bone destruction that characterize severe periodontitis (Loesche, Wilson). Periodontal disease has also been associated with cardiovascular disease, presumably through inflammation triggered by the gram-negative organisms entering the general circulation from the gums (Genco). The role of bacterial endotoxin in periodontal disease remains under investigation.

2. Respiratory

- **Cystic Fibrosis**
  Cystic Fibrosis (CF) patients have a genetic defect of the calcium channel that produces abnormally viscous mucus in the digestive system and the lungs. This predisposes these patients to recurring (and eventually fatal) lung infections, usually caused by Gram-negative bacteria such as *Pseudomonas aeruginosa.* Recent studies suggest that endotoxemia may be associated with acute exacerbations of CF. LPS and IL-1 appear in the plasma of CF patients during acute episodes of increased pulmonary inflammation, and elevated LPS levels decreased significantly following two weeks of intravenous antibiotic therapy (Wilmott).

- **Asthma**
  The observation that endotoxin exposure can cause wheezing and other respiratory symptoms in sensitive individuals has led several investigators to suggest that it may play a role in the onset or worsening of asthma (Dubin, Jagielo). One theory proposes that LPS, LBP and soluble CD14 are all present in the airways of asthmatic patients in low concentrations. An antigen challenge may lead to increased levels of LBP and CD14 and therefore trigger a magnified inflammatory response to inhaled LPS (Dubin). Additional studies of asthma and endotoxin exposure report that aerosolized endotoxin exposure correlated with decreases in pulmonary function in fiberglass wool factory workers (Milton), and that allergenic pollen may be contaminated with bacterial endotoxin in the absence of viable bacteria (Spiewak). Endotoxin has also been detected in asthma-associated house dust (Michel).

3. Cardiovascular

- **Coronary Artery Disease**
  Two Gram-negative human pathogens, *Helicobacter pylori* and *Chlamydia pneumoniae*, that bear LPS-like antigens, have been epidemiologically associated with the presence of coronary artery disease; seropositivity to either microorganism is an independent risk factor for electrocardiographic abnormalities (Patel). Since LPS exposure induces the production of inflammatory mediators that have been implicated in atherogenesis (Marcus), endotoxin may therefore be linked to atherosclerosis, although this hypothesis is at present very controversial.

4. Renal

- **Hemolytic Uremic Syndrome**
  Escherichia coli O157:H7, the enteropathogenic organism associated with several recent national outbreaks of serious illness and death, causes a life-threatening hemolytic uremic syndrome (HUS) characterized by acute renal failure, hemolytic anemia, thrombocytopenia and hemorrhagic colitis (Kaplan). Animal models have shown that LPS and Shiga-like toxins, both produced by this strain of *E. coli*, may contribute to nephropathology, gastrointestinal abnormalities, and death (Karpman). Furthermore, the presence of anti-*E. coli* LPS antibodies in patients with HUS is frequent enough (>70%) to have been proposed as a diagnostic tool in this illness (Greatorex). Intriguingly, similar antibody

responses have been observed in patients with HUS caused by non-O157 *E. coli* strains (Ludwig).

- **Complications of Renal or Peritoneal Dialysis**
  Patients undergoing renal or peritoneal dialysis may be exposed to microbial contaminants, such as Gram-negative bacteria and their components, in dialysis fluids or on dialysis membranes (Bottalico, Perez-Garcia). One study found that circulating TNF and IL-6 levels were highest in patients on chronic hemodialysis and receiving contaminated dialysate (Perez-Garcia). It has been hypothesized that cytokine release, possibly due to this presence of Gram-negative bacteria and/or endotoxin in the dialysate, may contribute to long-term complications in patients on hemodialysis or chronic peritoneal dialysis (Sundaram).

5. Immunologic

- **Autoimmunity and Rheumatoid Arthritis**
  The ubiquity of endotoxin, which is present in food, water, and certain vaccines—not to mention its constant production in, and shedding from, the gastrointestinal tract—has led to speculation that LPS may play a role in the development or continuation of autoimmunity. The lipid A component of endotoxin, when free, can be adsorbed onto phospholipid cell membranes. The resulting novel configuration may induce the production of autoimmune IgM antibodies against surface antigens, including phospholipids (van Rooijen). In rheumatoid arthritis, synovial fibroblasts exhibit increased sensitivity to LPS in the presence of soluble CD14 and LBP (Yu).

6. Cancer

- **Cancer and Chemotherapy**
  Immunocompromised cancer patients are at high risk of infectious complications, including gram-negative sepsis (Easson). Both radiation and chemotherapy treatment have direct cytotoxic impact on the GI tract, suggesting that bacterial translocation may be implicated in the development of septic complications. Investigations in specific cancers show that hepatic, gastrointestinal and hematological cancers show a strong association with elevated endotoxin levels (Easson, Yoshida, Amati, Engervall), especially in patients receiving chemotherapy or radiation. In patients with chemotherapy-induced neutropenia and fever, elevated endotoxin was detected in 60% of patients, with the highest values in patients with Gram-negative bacteremia (Engervall). DIC in cancer patients is most frequently found in biliary, gastric, hepatic and pancreatic cancer; endotoxemia was more frequently detected in those who received chemotherapy (Okubo). At present, while there is evidence of an association between cancer and endotoxin, it remains to be shown what the clinical significance of that association is.

VI. Current Treatment of Sepsis

Returning to the most dramatic and challenging of the endotoxin-related syndromes, Carcillo and Cunnion have presented a conceptual framework for the treatment of sepsis and septic shock in both children and adults (Carcillo). Early recognition depends upon a high index of suspicion and a clinical appreciation of common and uncommon diagnostic findings, so that early treatment (i.e. antibiotics) may be initiated and later complications avoided. Once septic shock occurs, however, these authors divide therapy into two phases: immediate resuscitation and stabilization.

The objective of immediate resuscitation is to control the patient's acute clinical condition and allow time for diagnosis, more sophisticated treatment, and eventual long-term stabilization. The airway must be secured, which can include intubation and ventilation in the case of respiratory failure or severe acidosis. Volume resuscitation is aimed at restoring blood pressure, and may involve crystalloids, colloids, and/or blood products for both children and adults. When the response to fluids is unsatisfactory, invasive hemodynamic monitoring is indicated and treatment with vasopressor and inotropes, such as dopamine, norepinephrine, or dobutamine, is begun. And, of utmost importance, antibiotic therapy is initiated urgently. Most often, patients are started on an empiric, broad-spectrum antibiotic regimen; however, if the source of infection and responsible organism are already known, specifically-targeted antibiotic therapy can be given (Carcillo, Ognibene).

With resuscitation underway, attention is then turned to preventing, or at least ameliorating, the potentially devastating downstream complications of shock. Respiratory and cardiovascular status is fine-tuned, and blood gases and blood pressure are optimized to the extent possible. Cultures and sensitivity determinations, invasive diagnostic procedures and even surgical exploration, if necessary, are performed to confirm the presence and type of infection and to direct definitive antibiotic therapy. Renal failure, if not prevented by aggressive fluid therapy, is managed as appropriate (e.g., peritoneal or hemodialysis), while due consideration is given to the patient's electrolyte balance and nutritional status (Carcillo).

In summary, the contemporary care of the patient with septic shock is primarily supportive; although antibiotics are always used in those cases with diagnosable infection and frequently used even if an infecting organism cannot be identified (i.e., fever of unknown origin, SIRS with shock). In a sense, current therapeutic options address only the extremes of the syndrome: antibiotics target the initiating event (bacterial infection), while supportive care deals with the end stages (shock and organ failure).

With the increasing incidence of SIRS, sepsis and septic shock, and the growing knowledge of the pathogenic events underlying these syndromes, numerous laboratories and investigators around the world are attempting to develop innovative and targeted therapies to address the cascade of events that occurs between infection and shock.

VII. Future Directions in the Treatment of Sepsis and Septic Shock

For more than a decade, research and clinical investigators have attempted to develop a new generation of therapeutics that go beyond the current nonspecific regimens of broad-spectrum antibiotics and physiologic support. These newer products, rather than treating advanced, or even end-stage, clinical manifestations of sepsis, are aimed much more selectively at proximate steps in the pathogenic pathway. Agents undergoing development at present are, for the most part, specific inhibitors, blockers or neutralizers of biochemical mediators of the systemic inflammatory response, using the rationale that interrupting the events of the inflammatory cascade will help prevent or ameliorate catastrophic sepsis.

Although a vast array of pathways have also attracted the attention of the pharmaceutical and biotechnology industries, the most frequently addressed targets for drug intervention thus far have been the LPS molecule itself, and two of the major effectors induced by LPS, TNF and NO (Baumgartner, Cavaillon, Verhoef). Unfortunately, to date, none of those attempts to block the systemic inflammatory cascade have succeeded in demonstrating a survival benefit in clinical trials. The lack of successful drug development in the medical arena stems from two general issues:

1) The redundancy of the cascade makes interfering with a single downstream mediator such as TNFa, IL-1, IL-8, NO, etc. (the focus of numerous drug development programs) an unlikely means of altering the complex course of the syndrome.

2) Appropriate clinical trial design remains problematic because of several critical, but to date uncontrollable, variables in trial conduct, including:

- Heterogeneous patient populations with many different underlying and overlying diseases with their own intrinsic mortality rates.
- Various (and not necessarily documentable) infectious sources, including Gram-positive and fungal infections, the presence of neither of which eliminates the possibility of a concomitant cryptic Gram-negative infection (e.g., gut translocation).
- The time lag between the actual onset of the inflammatory response, clinical diagnosis and initiation of therapy, superimposed on variable rates of sepsis progression from patient to patient.
- The use of a 28-day, all-cause mortality endpoint based on old study designs that have become dogma with investigators and regulators.

Of course, for treating endotoxemia, the most compelling therapeutic strategy would seem to be eliminating endotoxin itself such that the induction and maintenance of the systemic inflammatory response and the catastrophic cytokine avalanche can be avoided or substantially ameliorated.

To date, attempts to detoxify endotoxin have been done using monoclonal anti-LPS antibodies (E-5® and HA-1A or Centoxin®). However, in large placebo-controlled trials, both antibodies failed to consistently reduce 28-day mortality in patients with sepsis, including septic shock (Bone, McCloskey). The negative HA-1A experience has also been confirmed in a retrospective observational study (National Committee for Evaluation of Centoxin). Many attribute these failures not to a focus on the wrong target but rather to flaws, perhaps unavoidable, in the clinical trial designs. Furthermore, while these early antiendotoxin agents assisted in clearance, they did not neutralize the biological properties of LPS *in vivo.*

One of the most important downstream mediators of sepsis, TNF, has also been heavily targeted for therapeutic intervention. At least six recent human clinical trials have studied various forms of anti-TNF monoclonal antibodies in sepsis, all with the same disappointing results (Ognibene). Although these antibodies were fairly effective at neutralizing the target cytokine, in general, 28-day all-cause mortality rates were equivalent in the treated and placebo groups, again highlighting the methodological difficulties which plague the anti-endotoxin trials.

Taken together, these results demonstrate that sepsis, as a complication of many diseases, is an extremely complex area both medically and in terms of clinical investigation. To put some perspective on the difficulty of developing new therapies for sepsis and septic shock, one author has calculated that there have been at least 13 failed Phase III trials of various agents, in an aggregate population of 10,864 patients (Scannon).

Fortunately, past experience has not totally constrained the development of new therapies. Perhaps the most important lesson from the failed studies is that there is a great need for standardization in the conduct of clinical trials in sepsis. Standardization of patient populations, disease definitions, outcome variables, study designs and statistical approaches are crucial to bringing much-needed new therapeutics to market, as is a fundamental understanding of a therapeutic molecule's strengths and weaknesses. Certainly, the lessons learned during the early trial failures will provide a better road map for future clinical investigations. The time may soon come when treatments for sepsis are far more sophisticated and specific than antibiotics and supportive care.

VIII. Conclusion: Beyond Sepsis

Equally important in fostering the seeds of optimism in this area are emerging concepts which may alter the way sepsis and related diseases are viewed and approached by the clinician and by the pharmaceutical researcher. For example, to the extent that bacterial endotoxin not only induces an inflammatory response but must remain present to perpetuate the cascade, focus should turn to earlier recognition and specific endotoxin-neutralization therapy. The development of better diagnostics could facilitate earlier, better-targeted treatment not only for sepsis, but for the many diseases that predispose for septic complications. Likewise, the appreciation that septic shock lies at the far end of a continuum of clinical manifestations of the inflammatory response to bacteria and endotoxin will direct attention to preventative measures to treat at-risk patients. New paradigms may lead to new approaches to clinical strategy, such as performing smaller clinical studies in well-characterized patient populations (e.g., severe pediatric meningococcemia rather than all-comers sepsis) to provide meaningful early data on the efficacy of new drugs.

Beyond bettering the ongoing search for an approvable sepsis product is a whole frontier of pharmaceutical research that investigates therapies in a broader category of systemic inflammatory reactions to bacteria and endotoxin. New therapeutic approaches to treating earlier-stage infectious complications such as those that follow surgery and trauma may prevent at-risk patients from progressing to sepsis at all. In addition, patients suffering from a number of other diseases in which Gram-negative bacteria and endotoxin are found to be important primary or complicating factors could benefit greatly from new treatments that target inflammatory mediators or endotoxin itself, in addition to the standard treatment of antibiotics and medical support. The most fruitful approach may therefore be to redefine the medical targets altogether, instead of attempting to treat complex multifactorial syndromes with a "one size fits all" approach.

Glossary

Acute Renal Failure (ARF): sudden and severe failure of kidney function, one of several organ failures associated with later stages of **sepsis.** Treatment of ARF requires careful metabolic support, up to and including dialysis. ARF may be a manifestation of the **Multiple Organ Dysfunction Syndrome.**

Acute Respiratory Distress Syndrome (ARDS): severe respiratory dysfunction, characterized by lung inflammation, increased permeability of lung capillaries, pulmonary edema and inadequate pulmonary gas exchange. It may be caused by direct lung trauma (e.g., drowning, inhalation of toxins) or by indirect systemic mechanisms such as inflammatory processes related to infections or sepsis. ARDS may be a manifestation of the **Multiple Organ Dysfunction Syndrome.**

arachidonic acid: a phospholipid fatty acid molecule, released from membranes of white blood cells and other cells active in host defense. Arachidonic acid and many of its metabolites, including prostaglandins and leukotrienes, mediate local and systemic inflammatory reactions.

bacteremia: literally "bacteria in the blood"; a bacterial infection originating in or spreading to the bloodstream.

BPI (bactericidal/permeability-increasing protein): a host-defense protein produced by polymorphonuclear leucocytes. It binds to **endotoxin** on or from **Gram-negative bacteria**, killing the bacteria. It neutralizes endotoxin by binding to **LPS** or **LOS** molecules with higher affinity than **LBP** (with which it shares considerable homology).

CD14: a receptor produced by monocytes and displayed on cell membranes as well as being shed into the bloodstream. The CD14 molecule binds the **LPS-LBP** complex and initiates the **inflammatory cascade.**

cascade: a complex sequence of events characterized by multiple positive and negative feedback loops of biologically active molecules (such as **cytokines, complement,** and **coagulation** factors). Each component of the cascade is activated and/or suppressed by previous components, and in turn activates and/or suppresses later components.

coagulation cascade: the series of tissue-based and circulating molecules which are activated in sequence to cause blood clotting (coagulation).

complement, complement cascade: a system of at least 15 naturally-occurring plasma proteins (complement proteins) which play a role in host defense and mediate a number of inflammatory reactions.

cytokines: signaling chemicals involved in inflammation, such as interleukins (e.g., IL-1, IL-6), interferons, and tumor necrosis factor (TNF). Cytokines are released by macrophages, lymphocytes, and other cells in response to pro-inflammatory stimuli such as infectious organisms or **endotoxin.**

disseminated intravascular coagulation (DIC): a syndrome of generalized activation of **coagulation** factors, leading to clinical manifestations of inappropriate clotting, bleeding, and shock.

endotoxin (lipopolysaccharide, LPS): lipopolysaccharides are complex molecules composed of "fat plus many sugars". The LPS molecule is a structural part of the cell wall in Gram-negative bacteria. Endotoxin initiates a potentially catastrophic **inflammatory cascade** that can lead to **sepsis,** shock, organ failure and death. Additional endotoxic membrane molecules include **lipooligosaccharide (LOS)** and lipoproteins.

endotoxemia: the detectable presence of **endotoxin** in the bloodstream.

exotoxin: pathogenic chemicals secreted by bacteria such as the Shiga-like toxin produced by *E. coli* O157:H7 or the toxic shock molecule produced by certain strains of *Staphlococcus aureus.*

Glossary

Gram-negative bacteria: the Gram stain is a microscopic viewing technique that distinguishes two types of bacteria. Gram-positive bacteria stain blue when prepared with Gram stain. Gram-negative bacteria do not take up the stain and appear pink or red under the microscope because their outer membrane, with its **lipopolysaccharide (LPS)** structural component, is resistant to absorbing the stain. Gram-negative bacteria are frequently implicated infectious agents in various local and systemic infections, including **sepsis.** They are also the primary microbial population in the intestines and are therefore a potentially complicating factor in other infections as well as in trauma and surgery (see **intestinal translocation**).

inflammation: a multifactorial cellular and humoral host defense response classically characterized by redness, swelling, warmth, and pain when confined to a local area. Systemic activation of the inflammatory response, however, can manifest as fever, shock, organ dysfunction, and even death (see **sepsis** and **Systemic Inflammatory Response Syndrome**).

inflammatory cascade: the specific systemic **cascade** induced in response to infection or exposure to infectious organisms. Clinical signs range from none through fever, malaise, hypo- or hyper-tension, and tachycardia, to shock, multiple organ failure and death. See also **Systemic Inflammatory Response Syndrome (SIRS)**.

intestinal translocation: the transfer of bacteria or their breakdown products, including endotoxin, across the mucosa of the gastrointestinal tract to the systemic circulation. When normal host mechanisms which modulate the process fail, bacteria and/or their endotoxins may trigger a systemic inflammatory response.

lipid A: the lipid portion of **endotoxin** that is responsible for its toxicity by binding to **LBP** and inducing the inflammatory cascade.

lipooligosaccharide (LOS): short chain **endotoxin**; found in *N. meningitidis* bacteria that cause meningococcemia.

lipopolysaccharide (LPS): long chain **endotoxin**; the most common form in **Gram-negative bacteria**.

lipopolysaccharide binding protein (LBP): An acute-phase plasma protein that binds to endotoxin. The LBP-LPS complex binds to the **CD14** receptor on macrophages, triggering cytokine release and initiating the **inflammatory cascade**.

Multiple Organ Dysfunction Syndrome (MODS): in acutely ill patients altered function of multiple organs—such as the kidneys, lungs, liver, and central nervous system—as defined by the inability to maintain normal homeostasis in the absence of medical intervention. Primary MODS is the result of a direct insult to the organs. Secondary MODS is the consequence of the systemic inflammatory response.

sepsis: a systemic response to infection; a syndrome characterized by a culture-documented infection and two or more of the following signs and symptoms: hyper- or hypothermia, tachycardia, hyperventilation and leukocytosis or leukopenia.

septic shock: septic shock refers to the most severe form of sepsis, characterized by refractory hypotension, clinical evidence of organ hypoperfusion, and/or organ dysfunction in the presence of a documented infection.

SIRS (Systemic Inflammatory Response Syndrome): the systemic inflammatory response, to a variety of severe clinical insults. Characterized by fever or hypothermia, tachycardia, tachypnea, abnormal white cell count. Differs from sepsis only in that the term SIRS is used in the absence of a documented infection.

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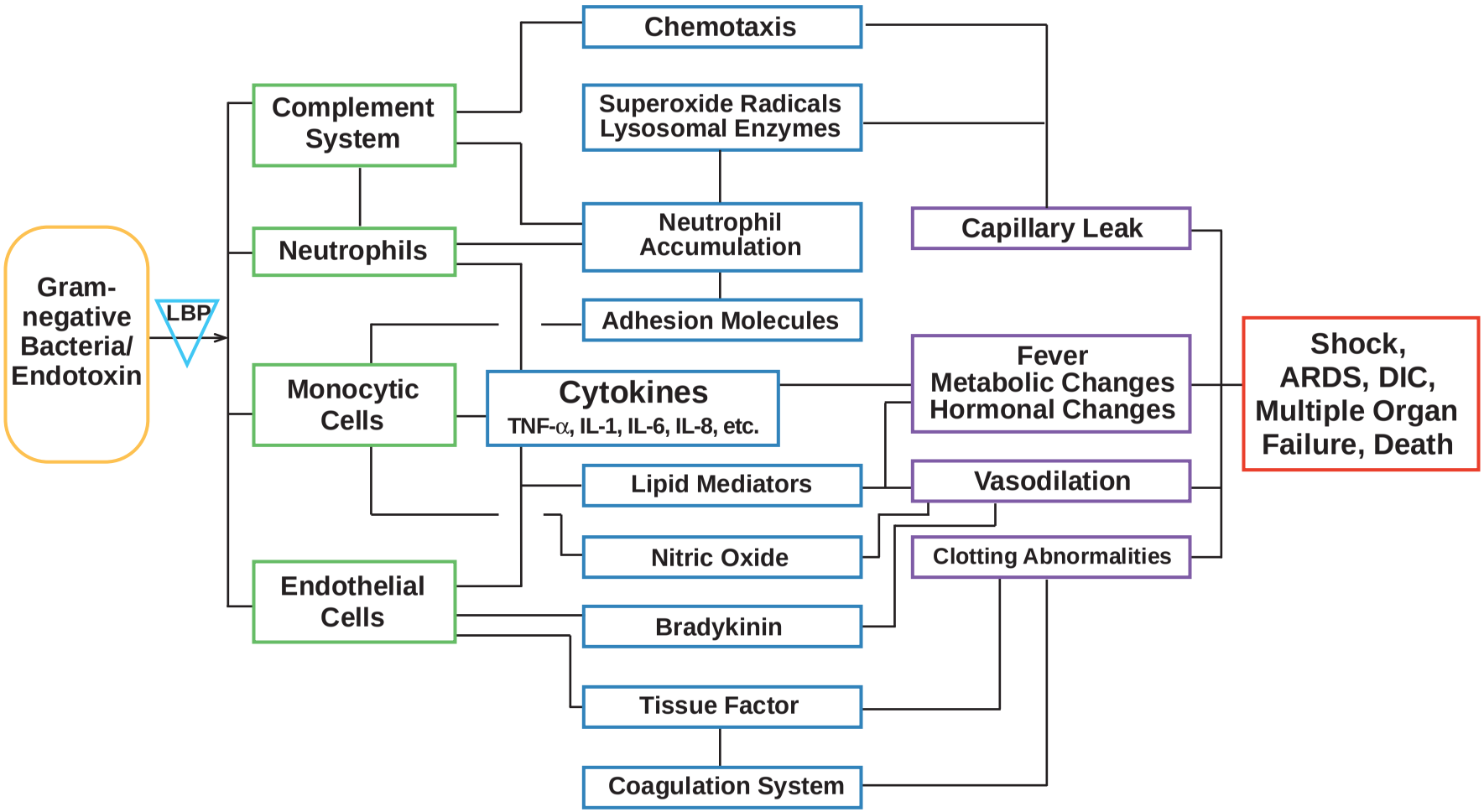
Figure 3: The Systemic Inflammatory Cascade



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