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The Systemic Inflammatory Response Syndrome (SIRS): Immunology and Potential Immunotherapy

Summary: Despite widespread advances in intensive care practices, and more potent and effective antimicrobials, septic shock continues to have a mortality rate of greater than 40%. Although antimicrobials can treat the etiologic organism, they do not alter the host response. It is becoming clear that invading organisms and other insults induce the release of cytokines and secondary mediators by the host. These mediators produce alterations in cellular, metabolic and physiologic functions producing the clinical picture of septic shock. Recent advances in cellular and molecular biology have permitted the identification of some of the mediators involved in this inflammatory cascade. Potential therapies are being developed which block or interrupt their activity. Treatment populations must be meticulously defined if we are to extract useful information concerning the efficacy of these new treatment modalities. In the following, proposed definitions for clinical patterns seen in patients with sepsis, and their inherent problems when applied to pediatrics are discussed. The pathophysiology of sepsis is discussed, and specific therapies designed to interrupt the inflammatory cascade are examined.

Zusammenfassung: *Das Syndrom der systemischen Entzündungsreaktion: Immunologie und Möglichkeiten der Immuntherapie.* Die Letalität des septischen Schocks liegt trotz vielfältiger intensivtherapeutischer Fortschritte und wirksamer antimikrobieller Therapien nach wie vor über 40%. Antimikrobielle Substanzen haben Einfluß auf den kausalen Erreger, aber sie ändern nicht die Reaktion des Wirtes. Es hat sich herausgestellt, daß eindringende Erreger und andere Schädigungen die Freisetzung von Zytokinen und sekundären Mediatoren durch den Wirtsorganismus in Gang setzen. Diese Mediatoren verändern zelluläre, metabolische und physiologische Funktionen und führen zum klinischen Bild des septischen Schocks. Einige der Mediatoren, die in diese entzündliche Reaktionskette eingebunden sind, konnten durch die neuesten Fortschritte auf dem Gebiet der Zellbiologie und Molekularbiologie identifiziert werden. Wenn es gelingen soll, diese neuen Behandlungsmodalitäten wirksam einzusetzen, muß die exakte Definition der therapeutischen Zielgruppen möglich sein. Im folgenden werden Definitionen für klinische Bilder, die bei Patienten mit Sepsis beobachtet werden, vorgeschlagen und die speziellen pädiatrischen Probleme diskutiert. Die Pathophysiologie der Sepsis und Therapie, die spezifisch in die Entzündungskaskade eingreifen, werden diskutiert.

Introduction

Sepsis is the 13th leading cause of death in the United States for persons older than 1 year of age, and accounts for five to ten billion dollars in medical costs annually [1]. For children 1 to 4 years of age, sepsis represents the ninth leading cause of death [2]. The prevalence of sepsis in hospitalized patients increased significantly in the last decade [1]. Advances in medical therapy, and increased use of invasive medical procedures and devices are factors contributing to a growing population of chronically ill, immunocompromised, and seriously ill patients at increased risk for sepsis.

Septic shock has a mortality rate of greater than 40%. Despite more potent and effective antibiotics, the use of combination antibiotic regimens, and widespread technological advances which support systemic hemodynamic performance and organ function, these rates have not changed significantly over the past 30 years. Although antimicrobials may effectively treat an underlying infection, they are insufficient to reverse the host's response to infection. The importance of the host response is suggested by the fact that many different types of infecting organisms, as well as non-infectious stimuli can produce the clinical picture of septic shock. It has become clear over the past ten years that the clinical syndrome of septic shock is the result of endogenous protein and phospholipid mediators secreted by the injured host. Other than initiating the production of inflammatory mediators, the infecting microbe plays a minor role. Recent advances in cellular and molecular biology have permitted the identification of mediators and mechanisms involved in producing the cellular, metabolic, and physiologic alterations associated with culture positive or negative sepsis. Therapies which aim to interrupt these cascades of cellular and physiologic alterations leading to septic shock are being developed, and some have already entered clinical trials.

This complex network of responses occurs in an extremely heterogeneous population of patients. Therefore, patients with septic shock can present with multiply different clini-

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[1] Supported in part by Bristol-Myers Squibb Company as a recipient of a Pediatric Infectious Diseases Fellowship Award.

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Table 1: Vocabulary.

Systemic Inflammatory Response Syndrome: A characteristic clinical response manifested by two or more of the following conditions:

Temperature > 38°C or <36°C (rectal)
Tachycardia*
Tachypnea+
WBC > 12,000 cells/mm^3, < 4,000 cells/mm^3, or > 10% immature (band) forms

Sepsis: The systemic inflammatory response due to infection.
Severe SIRS or Severe Sepsis: SIRS or sepsis associated with organ dysfunction, hypoperfusion, or hypotension.
Shock due to SIRS or Septic Shock: SIRS or sepsis associated with organ dysfunction or hypoperfusion abnormalities along with hypotension not responsive to fluid resuscitation. (Severe SIRS or Severe Sepsis and Hypotension). #

Multiple Organ Dysfunction Syndrome: State of physiologic derangements in which organ function is not capable of maintaining homeostasis.

* Tachycardia, infants heart rate > 160/min, children heart rate > 150/min.

\+ Tachypnea, infants respiratory rate > 60/min, children respiratory rate > 50/min.

\# Hypotension, infants systolic pressure < 65 mmHg, children systolic pressure < 75 mmHg or < 5th percentile for age.

cal patterns. Terms including septicemia, sepsis, sepsis syndrome, septic syndrome, and septic shock are among those used to describe these various clinical presentations. A consensus in terminology will be important for the accurate evaluation of sepsis clinical trials, and will be necessary if comparisons between studies are attempted. To this end, *Bone* et al. of the American College of Chest Physicians/Society of Critical Care Medicine held a consensus conference in August of 1991 at which new definitions for sepsis and the systemic inflammatory response syndrome were proposed (Table 1) [3]. Terminology adapted from that suggested by *Bone* et al. [3] has since been proposed for use in infants and children [4].

Vocabulary

The term "sepsis," in popular usage, implies a characteristic clinical pattern of hemodynamic and metabolic derangements arising from infection. A similar, or even identical clinical syndrome can arise from noninfectious causes such as trauma and tissue injury, pancreatitis, ischemia, hemorrhagic shock, and diseases of immunological dysfunction. Therefore, the phrase "systemic inflammatory response syndrome" (SIRS) has been proposed by *Bone* et al. [3] to describe this inflammatory process, independent of its cause. SIRS is manifested by two or more of the following conditions: a) hyperthermia or hypothermia; b) tachycardia; c) tachypnea; d) a pathologic alteration of the WBC count (Table 1).

When SIRS is the result of a confirmed infection, it is termed *sepsis*. Therefore, *sepsis* = SIRS due to infection [3]. SIRS and its sequelae represent a continuum of clinical and pathophysiologic severity which may result in multiple organ dysfunction and death. Despite this continuum of clinical presentation, *Bone* et al. [3] have attempted to define specific phases that characterize patients at increased risk of morbidity and mortality. They defined *severe* SIRS or *severe sepsis* as SIRS or sepsis "associated with organ dysfunction, hypoperfusion, or hypotension." Hypoperfusion abnormalities include lactic acidosis, oliguria, or an acute alteration of mental status.

In addition, these investigators defined *shock* associated with SIRS, or *septic shock* as "hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction." Patients who are on inotropic or vasopressor agents may not be hypotensive at the time they manifest hypoperfusion abnormalities or organ dysfunction, yet they would still be considered to have shock [3].

In pediatrics, hypotension is not a necessary component of shock, for decline in blood pressure is often a late and ominous terminal event during shock in children [5]. The characteristic hemodynamic alterations in early septic shock are a low systemic vascular resistance and a normal or supranormal cardiac output [6]. Patients in early septic shock often exhibit a normal systemic arterial blood pressure or a normal mean pressure with an increased pulse pressure. When compensatory mechanisms for diminishing vascular resistance are lost, or when sepsis-associated myocardial dysfunction ensues, the patient's status may rapidly deteriorate. Due to the rapidity of change and the possibility of catastrophic deterioration, identification of the early stage of shock is critical. *Shock* as defined in the *Textbook of Pediatric Advanced Life Support* is "a clinical state characterized by inadequate delivery of oxygen and metabolic substrates to meet the metabolic demands of tissue" [5]. This definition does not include "hypotension" as a prerequisite for shock. This definition includes patients in whom intrinsic compensatory mechanisms are able to maintain vital organ function, as well as those in whom compensatory mechanisms have failed, leading to organ dysfunction.

Despite deficiencies in the terminology proposed by *Bone* et al. when applied to pediatrics, there is an urgent need to define subsets of patients according to severity. At the present time there is no consensus of sepsis terminology established for pediatric critical care. Therefore, we will utilize adaptations of the definitions proposed by *Bone* et al. (Table 1) when describing the immunology and immunotherapy of this syndrome.

As newer technologies for the monitoring and support of patients surviving life-threatening critical illness have become established, it has become evident that a major threat to subsequent survival is not the underlying illness,

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or even a single complication thereof, but rather a process of progressive physiologic deterioration of interdependent organ systems. About 75% of deaths due to septic shock or shock associated with SIRS occur within hours to days of the onset of shock and are due to refractory hypotension. The other 25% of deaths occur days to weeks after patients have been successfully treated for hypotension and are associated with development of the *multiple organ dysfunction syndrome* (MODS), also defined by *Bone* et al. [3]. The terminology "dysfunction" is preferred to "failure," as it implies a continuum of physiologic derangements in which organ function is not capable of maintaining homeostasis. Early clinical studies identified occult infection as the most important cause of MODS [7, 8]. However, MODS can evolve in the absence of an untreated focus of infection [9], and can be reproduced experimentally by the infusion of host derived protein and phospholipid mediators of inflammation [10, 11]. Thus, MODS describes a pattern of multiple and progressive signs and symptoms representing the more severe end of a variable severity of illness that characterizes SIRS and sepsis [3].

Immunology of Severe Sepsis and Septic Shock

For many years, the general assumption was that microorganisms produced toxic substances which upon entrance into the circulation caused hypotension, decreased perfusion of vital organs, acidosis and death. Although the organisms primarily responsible for sepsis and septic shock vary among different groups of patients, the clinical picture is the same; evidence that the systemic response to invading organisms is independent of the type of organism, and the host dependent response is more important. In 1985, *Tracey* et al. demonstrated that passive immunization against an endogenous protein hormone, tumor necrosis factor (TNF), could protect mice from the lethal effect of endotoxin [12]. This experiment reinforced the importance of the host response in the pathophysiology of sepsis and indicated that TNF was a specific and essential mediator of endotoxin mortality. Further evidence supporting TNF as a major early determinant of septic shock came from studies in which recombinant TNF was administered to experimental animals. The effects of TNF mimicked the tissue injury and metabolic derangement witnessed in the setting of endotoxic shock [13, 14]. Although not consistently detected, high concentrations of circulating TNF have been found in humans with severe sepsis and septic shock, and these concentrations are inversely correlated with survival [15, 16].

In the eight years since TNF was initially purified, advances in biotechnology have led to a more complete understanding of the host response to infection. It currently appears that the cytokine class of mediators are nearly uniformly operative within the context of sepsis induced shock and tissue destruction. Novel therapies designed to interrupt the synthesis or toxicity of these inflammatory proteins have emerged with the hope of finally lowering the high mortality rates due to septic shock and shock associated with SIRS. The majority of these therapies are based on the molecular interactions which induce or regulate the systemic inflammatory response. In the subsequent discussion we have tried to provide a basis for understanding the cellular alterations that occur with sepsis, thereby laying a foundation for understanding the rationale of emerging therapies. Comprehension of the basic mechanisms involved in the host inflammatory response is necessary if clinicians are to make educated choices and decisions regarding these not-so-distant future therapies. We will divide the pathophysiology of sepsis into four phases, and examine specific therapies designed to act at each phase.

I. The Induction Phase

SIRS may be initiated by infection with bacteria, viruses, protozoa, and fungi, or by non-infectious causes such as trauma, autoimmunity, cirrhosis, and pancreatitis. Among the various etiologies, gram-negative bacterial infection is the most thoroughly studied and understood. For our purposes, gram-negative bacterial infection will serve as a model from which other infectious, as well as non-infectious etiologies of SIRS may be approached. In addition, neutralization of the lipopolysaccharide (LPS) component of gram-negative bacterial outer membranes represents the first attempt at anti-mediator therapy for sepsis (Figure 1).

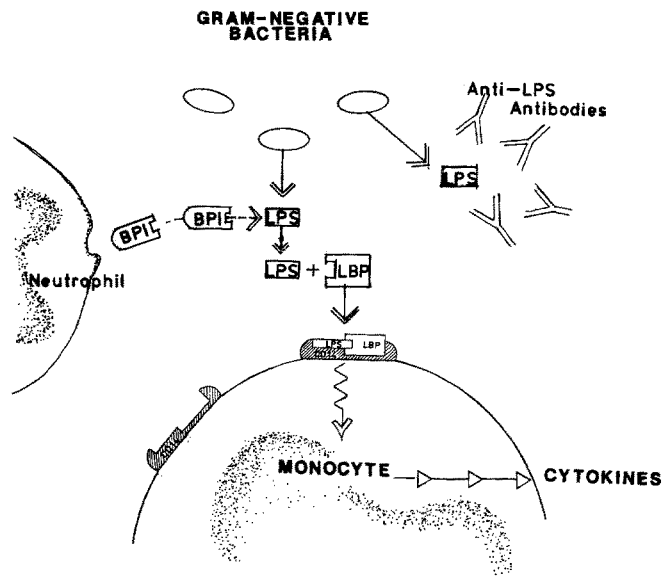

Figure 1: The induction phase of septic shock.
Experimental approaches for blocking this phase include: anti-LPS antibodies [17–30], soluble CD 14 receptors [34], anti-LBP [31–33], anti-CD 14 receptor antibodies, BPI [35].

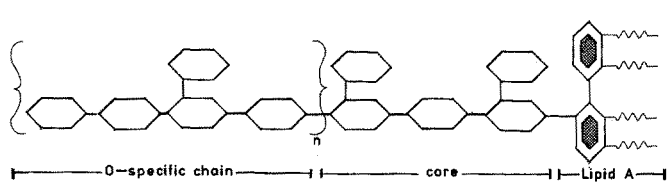

Figure 2: Diagramatic representation of bacterial lipopolysaccharides.

It is useful to briefly review the structure of LPS (Figure 2). LPSs are complex molecules composed of three major parts: a polysaccharide side chain (O-antigen), which is attached via a bridging (core) polysaccharide to a glucosamine-based phospholipid (lipid A).

The most variable part of the LPS structure is the O-antigen, responsible for the individual antigenic signatures of individual strains of gram-negative bacteria. Immunization with pathogenic gram-negative bacteria induces serotype-specific, anti-side-chain antibodies which are able to increase the opsinophagocytosis and intravascular clearance of both purified LPS and whole bacteria [17, 18]. The use of such anti-sera is highly protective in animal models of gram-negative sepsis, however; because of the extensive diversity of O-antigens among the numerous strains and species of gram-negative bacteria, the protection afforded by these antibodies is restricted to the strain used for immunization [19].

In contrast to the highly variable outer side chains of LPS, the lipid A and core regions are more conserved among gram-negative bacteria, with the lipid A moiety being the most highly conserved part of the structure. "Rough" mutant gram-negative organisms lack enzymes necessary to make complete LPS, so that the core and lipid A determinants are exposed. These rough mutants have been used as immunizing agents to elicit antibodies recognizing core and lipid A epitopes; antisera, polyclonal and monoclonal antibody preparations have been developed and tested in clinical trials. Although some of these trials have had positive results [20], others have not [21, 22].

Two prophylactic studies have been published comparing antiserum to preimmune serum: one in high risk surgical patients and one in neutropenic cancer patients; neither showed a reduction in gram-negative infections by the administration of antiserum [23, 24].

Recently murine and human monoclonal IgM antibodies have been developed with the *Escherichia coli* J5 mutant, and have been tested for the treatment of patients with gram-negative infections in prospective randomized, double-blind, multicenter trials [25–27]. In an initial study with E5 murine IgM monoclonal antibody [25], patients with suspected gram-negative sepsis were randomly assigned to receive either E5 (2 mg/kg/day for 2 days) or saline placebo. Although no decrease in mortality rates was observed for all patients, when results from subgroups of patients were analyzed, it appeared that there was a decrease in mortality rates in the patients without shock at the time of study entry ($p = 0.03$). Because of the improved survival with E5 therapy in the subgroup of patients without shock, a confirmatory multicenter trial was initiated [26]. This second study, consisting of 530 patients with gram-negative sepsis without shock, showed no improvement in survival with therapy.

The second antibody, HA-1A (human), was studied in 543 patients with a presumptive diagnosis of gram-negative sepsis [27]. The patients were randomized to receive either a single dose of 100 mg of HA-1A, or a similar volume of human albumin. Of the 543 patients who were treated, 200 (37%) had proven gram-negative bacteremia. In the overall population, treatment with HA-1A did not improve survival. In the 200 patients with gram-negative bacteremia there was a significantly improved survival at 28 days in those given HA-1A ($p = 0.014$) and this reduction in mortality was more pronounced in patients with shock compared with those not in shock.

The results of this study [27] prompted the release of this antibody to the market in some European countries. However, the HA-1A trial was recently reanalyzed according to the data presented at a Food and Drug Administration meeting and approval was denied pending further study [28]. Depending on the correction used for multiple subgroups and end points, analysis at day 28 reveals only a marginally significant decrease in mortality among the gram-negative bacteremic patients treated with HA-1A. Important concerns emerged: more patients in the placebo arm than in the HA-1A arm received inadequate antibiotic therapy and the former had considerably more risk factors at study entry.

The rationale behind the production of these immunological compounds was that antibodies developed against the conserved core and lipid A regions of rough gram-negative mutants could recognize epitope(s) that are exposed on pathogenic smooth gram-negative bacteria as well, and, therefore, would provide cross-protection. Of course, the exposure of the conserved innermost part of the core as antigenic determinants on pathogenic smooth gram-negative bacteria remains purely hypothetical. It has been difficult to demonstrate cross-reactivity in the case of polyclonal antisera to rough mutants, and divergent cross-reactivity results have been observed in the case of monoclonal antibodies, including HA-1A [29, 30]. In addition, it has not been shown that these antibodies participate in opsonic activity for bacterial or LPS clearance. It was hypothesized that these antibodies might neutralize endotoxin by steric hindrance of its toxic moiety. Thus far neutralization of the effect of endotoxin by anti-core polyclonal or monoclonal antibodies has not been described. Furthermore, experiments conducted on rodent models of *E. coli* sepsis [17] have demonstrated that HA-1A and other monoclonal core LPS antibodies do not diminish serum TNF or IL-6 levels compared to antibodies against the specific O side-chain. The theoretical premise that core LPS antibodies are cross-protective is attractive but requires further investigation.

The current limitations in early diagnosis of gram-negative infection in septic patients who require immediate treatment, combined with the fact that therapeutic benefit may be limited to only a subset of these patients will necessarily lead to overtreatment with these agents. The concerns about the efficacy of these antibodies are not trivial because the financial impact of the treatments is considerable.

Other Anti-LPS Therapies (LPS-Binding Protein, and Bacterial Permeability Increasing Protein)

In addition to anti-LPS antibodies, other therapies targeting LPS are in the early stages of development (Figure 1).

Many of these have come about because of recent discoveries pertaining to the fate of LPS released from bacteria. *Ulevitch* and his coworkers recently described a family of proteins possessing LPS-binding sites [31, 32]. These proteins have a striking homology in DNA sequence, but they have different functions. The first identified member of this family, LPS binding protein (LBP) is a 60 kD glycoprotein normally present in human serum, which increases 100-fold in concentration during an acute phase response due to infection [33]. LBP binds to the lipid A moiety of LPS, forming an LPS-LBP complex. The interaction of LPS with its receptor, CD 14, on myeloid cells is greatly enhanced by prior LPS-LBP complex formation. When LPS-LBP complexes interact with CD 14 receptors on monocytes and primed neutrophils, genes encoding cytokines are induced. Thus, LBP might be considered a warning allowing the detection of low levels of endotoxin, promoting an early response to gram-negative infection. Experiments are underway to investigate whether the modulation of this system with soluble CD 14 receptors, or with monoclonal antibodies to LBP or CD 14 can influence the inflammatory response. *Ulevitch* and coworkers have demonstrated that by either depletion of LBP in serum or by blocking CD 14 with specific antibodies, macrophage activation and TNF production in response to endotoxin is significantly diminished [33, 34].

In contrast to LBP, another member of the family of LPS-recognizing proteins, the bactericidal/permeability increasing protein (BPI), binds to LPS and prevents macrophage activation [33]. This protein is a human protein derived from neutrophil granules. It has been shown to be protective against otherwise lethal endotoxemia in rodents [35].

Modulation of the above mediators may well turn out to be beneficial for patients with sepsis due to gram-negative infection but, as stated earlier, severe SIRS and shock can occur due to a variety of other causes, infectious as well as non-infectious. Therefore, down-regulation of the synthesis and secretion of cytokines which are uniformly operative in severe SIRS and shock would benefit a wider spectrum of patients.

II. The Phase of Cytokine Synthesis and Secretion

Several potential regulatory sites exist for the synthesis and secretion of proteins by a cell: transcription of mRNA from DNA, mRNA processing, translation of RNA into protein, and post-translational processing and secretion of the protein. Each of these levels have become a target for potential immunotherapy of sepsis and SIRS (Figure 3).

In general, cytokines are not stored as pre-formed molecules and their synthesis is initiated by new gene transcription, or translation of preformed RNA. Also, cytokine genes are permanently inactivated in many cell types, perhaps as a result of the developmental process. Thus, cytokine genes are "accessible" for transcription in a limited number of tissues. Promoters for many cytokine genes contain putative binding sites for transcription activating proteins, an example being the transcription activating protein complex NF-κB. Activation of NF-κB by phosphorylation

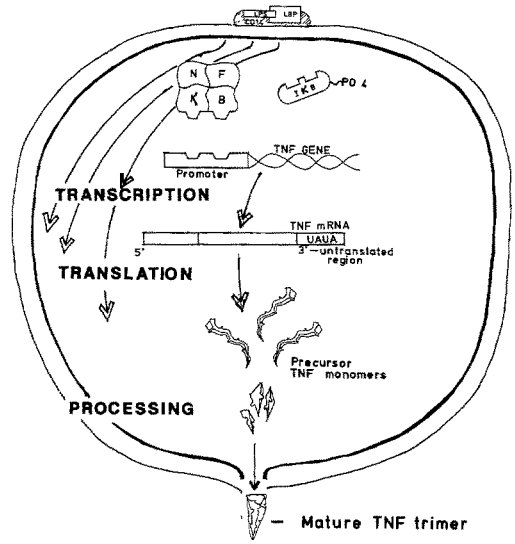

Figure 3: The phase of cytokine synthesis and secretion. (The TNF gene). Agents which block transcription include pentoxifylline [40–42] and amrinone [43]. Corticosteroids primarily block translation [44].

of the cytosolic inhibitor IκB seems to be a common feature of cell activation. But, some degree of selectivity is exercised, since not all cytokines are produced in response to an inflammatory signal.

Post-transcriptional control of cytokine biosynthesis is a very prominent method of regulation. Most cytokine mRNA molecules display a conserved UA-rich sequence within the 3'-untranslated region which has been associated with both translational activation [36], and mRNA stability [37], and may represent a common regulatory mechanism to more rapidly synthesize inflammatory proteins in response to acute infections [38].

The interaction of these molecular mechanisms of control is demonstrated by examining the molecular control of TNF synthesis in the macrophage. Following interaction of LPS-LBP complexes with CD 14, transcription of the TNF gene *in vitro* increases 3-fold; levels of TNF mRNA increase 100-fold, reflecting increases in the stability of transcribed TNF mRNA. When the inducing effect of the TNF promoter is combined with the inducing effect of the 3'-untranslated region, as much as a 4,000-fold induction has been observed [39].

Pretranslational Blockers: Pentoxifylline, Amrinone

Many studies have been performed demonstrating that there are several means of blocking cytokine synthesis, at least when LPS is the inciting stimulus. Agents which act at pretranslational levels include pentoxifylline, and amrinone. These agents are phosphodiesterase inhibitors which increase intracellular levels of cAMP. Increased intracellular cAMP levels presumably disrupt intracellular signaling through an as yet unknown mechanism, thereby decreasing the cellular response to LPS exposure [40, 41]. Pentoxifylline decreased TNF synthesis in a murine endotoxic shock model [42], and amrinone has been shown to be an

even more potent inhibitor of LPS-induced TNF production [43]. Of concern with these agents, however, is laboratory data which demonstrate that following abrupt discontinuation of either, a state of cellular hyper-responsiveness to LPS exists, potentialy sensitizing an individual to what otherwise would be insignificant endotoxemia [43].

Translational Blockade: Corticosteroids

Corticosteroids have been shown to primarily block translational activation of TNF mRNA in macrophages, thereby potently reducing the secretion of TNF in response to endotoxin [44]. The steroid effect is entirely preemptive; if administered after LPS, they are virtually without effect. This may explain their lack of benefit in published clinical trials when used as adjunctive therapy for septic shock [45, 46]. An important factor that could explain the failure of steroids in the recent clinical trials is the large steroid dosages that were used. Although the ideal dosage of steroids for therapy of sepsis in humans is unknown, there is good evidence in animal models that increasing steroid dosage beyond the optimum amount results in enhanced mortality [47, 48]. The use of low dose dexamethasone in pediatric patients with meningitis has been shown to decrease cytokine levels in the CSF and the incidence of severe hearing loss in survivors [49, 50]. The controversy surrounding the role of steroid therapy for sepsis continues.

Although TNF and other mediators can exert toxic effects which may lead to death, these mediators may also be important in mobilizing physiologic defenses to combat infection when antibiotic therapy is absent or insufficient [51]. For example, the LPS-insensitive C3H/HEJ mouse does not secrete TNF in response to exposure to LPS and thus survives lethal infusions of endotoxin. However, when these animals are infused with certain strains of bacteria, the animals die from inoculations which are survived by LPS-sensitive mice [52]. One could speculate that blocking TNF production may be most beneficial in cases of sepsis in which a majority of bacteria are rapidly killed by antibiotics, and where the inflammatory response can cause severe sequelae, for example, in meningococcemia or typical childhood meningitis. Whereas, in cases where sepsis is secondary to an occult infection not effectively treated by antibiotics, inhibiting inflammation may cause more risk than benefit. Future studies should consider the important variables of steroid dose and patient selection.

III. The Cytokine Cascade

Tumor Necrosis Factor alpha (TNF)

The cytokines, especially those of a more proximal and pro-inflammatory nature, have received much attention as mediators of severe sepsis and septic shock. TNF, or cachectin, is a protein hormone produced by a variety of cells in response to inflammatory stimuli. It exists in nature as a trimer and exerts its biological effects by cross-linking cellular TNF receptors. Tumor necrosis factor β, lymphotoxin, is produced by TH1 lymphocytes, displays 30% amino acid homology to TNF and binds to the same receptors.

Antigenic events initiating the appearance of TNF also promote the production of other cytokines, such as interleukin-1 (IL-1), and interleukin-6 (IL-6). A model of overwhelming bacterial sepsis in baboons defined the temporal nature of this cascade [53]. In this model, *E. coli* bacteremia was demonstrated and various cytokine levels were measured. TNF peaked after 1½ h, IL-1β at 3 h after bacterial challenge and γ-interferon (γ-IFN) 6 h later. Animals that were not passively immunized against TNF developed shock, MODS, and death. Passive immunization with monoclonal antibody to TNF 2 h before bacterial infusion dramatically conferred complete protection against both shock and death. Levels of IL-1β and IL-6 were attenuated in the immunized animals, suggesting that TNF is an essential stimulus for the release of these cytokines during septic shock.

Other evidence for TNF playing a primary role in severe sepsis and septic shock lies in the results of studies in which TNF has been exogenously administered to experimental animals [14]. Acute myocardial dysfunction, activation of coagulation pathways, increased release of neuro-endocrine stress hormones, as well as significant stimulation of immune function and metabolic regulation all occur within minutes to hours after the administration of TNF. Thus, pharmacological doses of TNF evoke the clinical events of severe SIRS, and yet circulating TNF is not consistently detected during conditions of clinical shock, infection or severe tissue injury. The timing of the measurement may be too late in many patients, or measurements of circulating TNF activity may be affected by circulating inhibitors of TNF. It is also possible that some or all of the TNF that is secreted never reaches the circulation [54, 55], but acts in a paracrine or autocrine fashion at the level of the tissue. The diverse tissue origins of TNF have recently been described through experiments on transgenic mice which bear a reporter gene construct in which the TNF coding sequence and introns are replaced by a chloramphenicol acetyltransferase coding sequence. In constrast to TNF, chloramphenicol acetyltransferase is stable and non-secreted, making tissue levels readily detectable. Experiments on this animal model have implicated a variety of organs (kidney, pancreas, lung, heart, spleen, and uterus) as important sources of TNF during endotoxemia *in vivo* [56]. In addition, the morbidity and mortality caused by TNF is synergistically enhanced by even low concentrations of IL-1 and γ-IFN, so that amounts of TNF that are untraceable *in vivo* may have profound effects on the host in the presence of other mediators [57].

Interleukin-1

IL-1 consists of two distinct molecules, IL-1α and IL-1β, that are structurally related polypeptides that show 25% homology at the amino acid level. Both are synthesized as 31 kDa precursor melocules, which are subsequently cleaved into 17 kDa forms. Most IL-1α remains in the cytosol of cells in its precursor form, or is associated with

the cell membrane. This membrane-associated precursor exerts biological activity. IL-1β on the other hand is cleaved by the IL-1β converting enzyme to its mature form within the cell, after which it is secreted.

There is much *in vivo* and *in vitro* evidence to support a crucial role for IL-1 as a co-mediator of severe SIRS. When administered to animals or humans it produces many of the same effects as exogenous TNF: fever, anorexia, sleep, increased, concentrations of colony-stimulating factors, IL-6, increased hepatic acute phase proteins, bone and cartilage resorption, the inhibition of lipoprotein lipase, the induction of PGE_2 and collagenase synthesis, capillary leak, and hypotension [58]. But, IL-1 has never been shown to be directly lethal to animals, as has TNF [14]. Thus, although exogenous IL-1 administration reproduces many of the acute hematologic and metabolic perturbations seen in severe sepsis, and is equally, if not more potent than TNF for the further induction of subsequent cytokines, the TNF component is necessary for induction of severe sepsis, septic shock, MODS and mortality.

Interleukin-6

IL-6 is a 26 kDa protein, which on the basis of its various activities, has been known as B-cell stimulatory factor, hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, and cytotoxic T-cell differentiation factor. The temporal relationship of IL-6 appearance within the cytokine cascade suggests a strong relationship to antecedant TNF or IL-1 stimulation during severe sepsis. Further, in sepsis models, when TNF or IL-1 activity is attenuated, the subsequent IL-6 response is decreased [59, 60]. Like TNF and IL-1, IL-6 is an endogenous pyrogen and an inducer of acute phase protein synthesis. Unlike TNF and IL-1, exogenous IL-6 administration does not cause hemodynamic compromise, regardless of the amount given to experimental animals [61]. IL-6 suppresses LPS-induced TNF production and LPS- and TNF-induced IL-1 production [62]. The spectrum of acute phase proteins induced by IL-6 includes many anti-proteases which possess anti-inflammatory properties. In general, it seems that Il-6 is an anti-inflammatory cytokine, yet evidence exists suggesting that IL-6 can play an adverse role during endotoxemia. Studies have shown that anti-IL-6 monoclonal antibodies protect mice from lethal *E. coli* infection as well as from administration of lethal amounts of TNF [63]. While the mechanism for such protection remains poorly defined, recent data suggest that TNF or IL-1 enhances the expression of a cell-associated glycoprotein, GP 130, that binds to the IL-6/IL-6 receptor complex, thereby enhancing IL-6 signal transduction [64]. Since GP 130 is not induced by IL-6 alone, antecedant TNF and IL-1 activity may be necessary for IL-6 to cause toxicity.

Blocking the Cytokine Cascade (Table 2)

The current wealth of pre-clinical data points to uncontrolled cytokine production as the cause of sepsis induced morbidity and mortality. Because cytokine activity is inducible by a wide variety of stimuli, therapies directed against the cytokine cascade rather than against a single inducing agent are appealing. Many of these therapies are now entering Phase III trials.

Table 2: Blocking the cytokine cascade.

Cytokine	Potential blocking agent
TNF	Anti-TNF antibodies [53] Soluble TNF receptors [65, 66] TNF-receptor-Fc chimeric proteins [67]
IL-1	IL-1RA [60, 68–70]
IL-6	Anti-IL-6 antibodies [63]

TNF Antagonism

Since TNF is a proximal mediator of septic shock, there are efforts to directly neutralize its toxic effects. No adequate clinical trial addressing the efficacy of anti-TNF antibodies in human sepsis has yet been published. Because antibody therapies in humans have their limitations, attention has been focused on the use of soluble receptors for TNF. These are naturally occurring proteins which represent the extracellular domains of the two TNF receptors. They are thought to act as natural TNF antagonists: their administration has prevented *E. coli* induced shock in baboons [65] and death in mice [66].

Chimeric molecules in which the soluble TNF receptor is linked covalently to the Fc portion of IgG, have been designed and produced [67]. The result is a specific inhibitor of TNF with the affinity of a natural receptor, but a half-life which approximates that of a naturally occurring antibody. In a guinea pig burn shock model, a one time dose of human TNF receptor-Fc chimeric protein significantly improves cardiodynamic function (*B. Giroir,* personal communication).

IL-1 Antagonism

The kinetics of TNF and IL-1 stimulation during sepsis suggest that any attempt to treat sepsis by modulating TNF production may have to occur soon after onset. At least in principle, however, one has hours in which to block the activities of IL-1. Support of this hypothesis lies in the results of a study in which interleukin-1 receptor antagonist (IL-1 RA) reduced mortality rates in a rabbit model of septic shock even when it was given after the onset of shock [68]. IL-1 RA is a naturally occurring protein which binds to the human IL-1 receptor but has no agonist activity [69, 70]. Therefore, it is a naturally occurring competitive antagonist of IL-1. It must be administered in very large molar amounts in order to block IL-1 activity. Unfortunately, a recently concluded phase III clinical trial with recombinant IL-1 RA for septic shock yielded disappointing results. This multi-center study which was conducted in

893 patients in some 60 centers and eight countries from around the world, showed no decrease in mortality in treated patients.

There exists ample evidence that TNF and IL-1 participate in the hemodynamic collapse attending overwhelming infection. Therefore, the acute blockade of these cytokines is a logical therapeutic goal. What remains to be seen, however, is if acute cytokine blockade will benefit patients with persistent inflammatory foci or repeated bouts of bacteremia.

IV. Secondary Mediators and End Products Causing Cellular Damage (Figure 4)

Recent data have shown a role for mediators other than endotoxin and cytokines in producing organ system dysfunction in experimental and clinical models of sepsis.

The endothelium plays an important role in this last phase of septic shock, both as a target for cytokines and as a source of additional mediators. Cytokines lead to the increased expression of adhesion molecules on both endothelial cells and neutrophils. The result is an increased migration and maintenance of activated cells in injured tissues.

Mediators and products released from activated neutrophils and endothelial cells include: arachidonic acid metabolites, free oxygen radicals, and nitric oxide. These appear to be direct mediators of the physiologic derangements seen in septic shock, and blocking their actions may prove to be an effective method of attenuating or preventing shock in patients with sepsis or SIRS. Recent evidence indicates that platelet-activating factor (PAF) interacts with cytokines and growth factors to either amplify or down-regulate mediator release, and specific PAF antagonists have been suggested to protect the tissue from microvascular failure and death in septic shock.

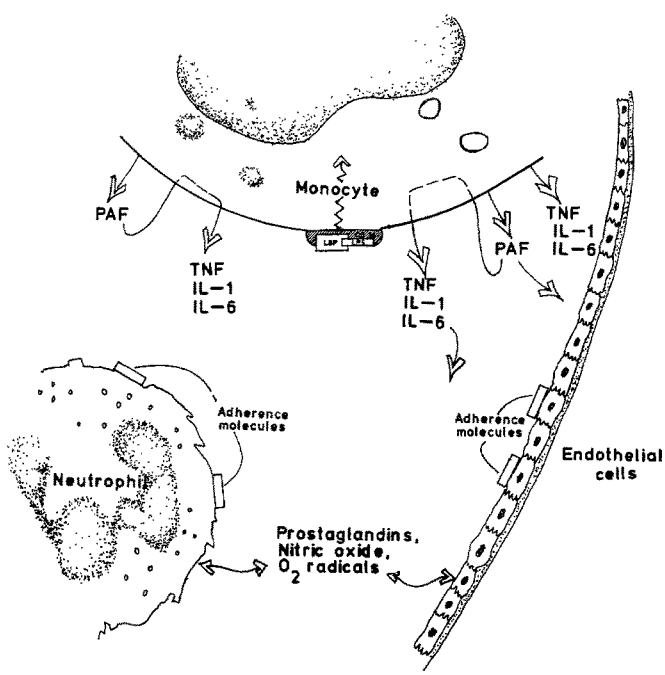

Figure 4: Secondary mediators and end products causing cellular damage.

Arachidonic Acid Metabolites

Products of the lipoxygenase and cyclooxygenase pathway have potent vasoregulatory effects and seem to play an important role in the low systemic vascular resistance and hypotension that occur in septic shock. LPS, TNF and IL-1 have all been shown to induce the release of prostaglandins from endothelial cells. The major prostaglandin produced by endothelial cells is PGI_2, which is a potent vasodilator. Indomethacin, given 1 h before or after a large intravenous dose of TNF has completely blocked metabolic acidosis, shock, and death in rats [71]. Elevated levels of PGI_2 have been found to correlate with the severity of septic shock in human patients [72]. Therefore, nonsteroidal anti-inflammatory agents may have beneficial effects in the treatment of sepsis. Animal studies have employed combination therapy with cyclooxygenase inhibitors and leukotriene receptor antagonists or lipoxygenase inhibitors. Combined blockade has proven to be more effective than single drug treatments in protecting animals from developing MODS [73, 74]. These studies provide the impetus for more extensive investigation of nonsteroidal anti-inflammatory agents in sepsis/SIRS.

Oxygen-Derived Free Radicals

When tissues are injured by ischemia or anoxia, as frequently occurs in sepsis, their ability to control the metabolism of oxygen is compromised [75]. Accordingly, reperfusion or reoxygenation causes enhanced free-radical production and associated tissue injury. The anions which are generated activate a superoxide-dependent chemoattractant [76]. This results in an influx of neutrophils which generate still more superoxide. Reactive oxygen species initiate lipid peroxidation, cause DNA strand breaks, and indiscriminantly oxidize organic molecules. A preliminary report of a randomized trial of *N*-acetylcysteine in patients with established sepsis-induced adult respiratory distress syndrome suggests that this antioxidant is useful [77].

Nitric Oxide

In 1980, *Furchgott* and *Zawadzki* [78] demonstrated the phenomenon of endothelium-dependent vascular relaxation. Seven years later, the mediator of this relaxation was identified as nitric oxide [79, 80]. Nitric oxide synthesized by constitutive nitric oxide synthase acts as a messenger molecule and appears to be the endogenous activator of soluble guanylate cyclase [81]. The resultant increase in cGMP is reponsible for nitric oxide-induced vasodilation [82], inhibition of platelet aggregation [83], modulation of leukocyte adhesion [84], and certain aspects of neurotransmission [85].

Upon stimulation with endotoxin or cytokines, an inducible form of the nitric oxide synthase enzyme is expressed in a variety of cells including endothelial cells, vascular smooth

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muscle cells, macrophages, and neutrophils [81]. The expression of inducible nitric oxide synthase leads to vascular relaxation and hyporesponsiveness to both vasoconstrictors and sympathetic stimulation. These effects can be reversed both *in vitro* [86] and *in vivo* by N_G-monomethyl-L-arginine [87], a nitric oxide synthase inhibitor. Early reports of the use of this drug in patients with septic shock seem encouraging [88, 89]. Concerns are that inhibition of nitric oxide synthase may lead to a degree of vasoconstriction detrimental to tissue perfusion. Indeed, animal studies have shown increased damage to vital organ systems when nitric oxide synthase inhibitors are given during endotoxic shock. Another concern is that this inhibition will lead to enhanced platelet activation in sepsis. Hopefully, *in vitro* investigation and studies in animals will identify which effects of increased nitric oxide production are beneficial, which are harmful, and whether the effects can be manipulated selectively.

Platelet Activating Factor (PAF)

Endotoxin induces the release of PAF from macrophages, polymorphonuclear leukocytes, platelets and endothelial cells. PAF is a potent phospholipid inflammatory mediator that increases cell adhesion and activates endothelial cells by direct effect or through the formation of toxic oxygen species and arachidonic acid metabolites. There is growing evidence that hematologic growth factors and cytokines interact with PAF leading to amplification of mediator release in septic shock, and that PAF mediates many of the toxicities associated with TNF and IL-1 [90, 91]. Specific PAF receptor antagonists provide protection against the fatal complications of endotoxic shock in animal models [92, 93]. Phase III clinical trials of PAF antagonists in septic shock are currently underway.

Clinical Strategies and Guidelines for the Use of Immunotherapeutic Agents

It is likely that a number of these individual therapies will prove beneficial for patients in septic shock or shock due to SIRS. The more difficult question will be which therapy or combination of therapies will provide the best outcome for a particular patient at a particular time during the course of illness.

Anti-LPS antibodies have proven to be an insufficient mode of therapy. In the first place, they are targeted for only a subset of patients with severe SIRS: those with gram-negative infection. In a recent review of septic shock in children, 25% of cases were due to gram-positive organisms [4]. Secondly, by the time the need for such intervention comes to the attention of the physician, the cytokine cascade is often already activated. These inherent problems, combined with high cost (~ $ 4000/dose), insufficient information about the mechanism of protection, and the conflicting results of clinical trials preclude any recommendations for clinical use of anti-LPS monoclonal antibodies in pediatric patients.

The recent advances in cellular biology have permitted the identification of mediators which play a more central role in sepsis and SIRS. It is now widely believed that TNF and IL-1 are the most promising candidates for anticytokine intervention during acute sepsis. However, as discussed earlier, neither TNF nor IL-1 is consistently demonstrable in patients with sepsis. The clinical spectrum encountered in infected and injured patients includes those with acute, abbreviated episodes of bacteremia or injury, to those with more complicated conditions elicited by repeated episodes of bacteremia or a persistent inflammatory focus. In many cases, the source of offending organisms remains elusive, and the late sequelae of such events include MODS and death.

There can be little doubt that proinflammatory cytokines participate in the acute hemodynamic collapse attending overwhelming bacteremia. But, the evidence that cytokines globally direct and mediate subacute and/or chronic inflammatory pathological states is largely indirect. It is likely that these proinflammatory mediators initiate the response, and secondary mediator pathways are necessary adjuncts for eliciting the full spectrum. We are currently far from a comprehensive understanding of the integrative biology of cytokine functions nor of their relationship with other signal pathways.

Many of the current treatment trials are designed to utilize dosing regimens derived from limited pre-clinical assessment of circulating cytokine levels. The possible existence of ongoing or recurring tissue cytokine production and the necessity to attenuate such influence over a longer treatment period need to be addressed. When administered prior to activation of the cytokine cascade, anti-cytokine therapies readily attenuate the symptoms of sepsis. Very few trials have evaluated the effects ot these agents when given after shock has developed.

There is evidence suggesting that a lack of cytokine activity may be detrimental [97, 98]. This observation has been made in stressed populations with progressive deterioration of nutritional status and organ system function [94]. It is possible that anti-cytokine therapies would be benefical only in the first few hours or days after insult. Whether this early intervention would compromise longer term inflammatory responses, or the later phase of tissue repair remains to be determined.

Some concerns must arise from the use of agents directed against any endogenously derived substance which is teleologically conserved. We lack sufficient knowledge of the probable roles that these mediators play in health and development. It has recently been demonstrated that TNF is spontaneously secreted in the thymus of developing animals, both prenatally and during early postnatal development [95]. TNF has also been identified in the placenta of animals from mid-gestation to parturition [96]. *Kossodo* et al. have also shown that treatment of neonatal animals with antibodies to TNF causes thymic involution, lymphoid hypoplasia, and profound disturbances in growth [97]. These data suggest that TNF serves an essential role in immune ontogeny or regulation during development, and that inter-

ruption of TNF activity in the newborn, even transiently, may have irreversible consequences. This raises concerns of what effects these therapies might have on certain age groups.

The era of cytokine response modification in the patient with severe sepsis has evolved with such rapidity that current interventional capacity is far ahead of our comprehension of the mechanisms involved in this clinical syndrome. The high mortality associated with severe sepsis and severe SIRS will undoubtedly lead to prompt and broad application of these therapies if they prove effective in the numerous clinical trials now in progress. In addition, these therapies will undoubtedly be of astronomical cost. Treatment populations must be meticulously defined if we are to extract useful information concerning the efficacy of these new treatment modalities.

Clinical trials in sepsis must address the complexity of this heterogeneous syndrome. Great care must be taken in selecting the patient population of interest and the entrance criteria for selection. Definitions allowing the classification of patients precisely and unambiguously must be stated. The question to be answered by the trial must be precise. A limited number of subgroups and endpoints must be prospectively defined, and the trial should be conducted under the most rigorous of guidelines. The history of research in this area, as demonstrated by the studies of anti-LPS antibodies, and the recent downfall of Synergen's IL-1 RA has been one of initial enthusiasm followed by sober recognition of problems. Strict guidelines must be followed if we are to avoid repeating past mistakes.

Acknowledgements

The authors are indebted to *Debra Fiser,* M. D., for her critical review of this manuscript, and to *Keith P. Miller* for his artistic additions.

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