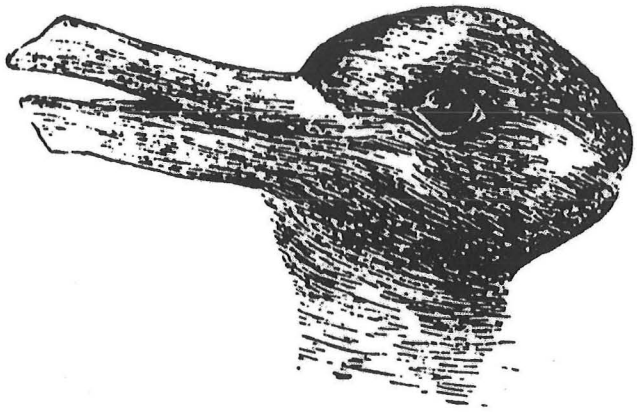
Evolution, Innate Immunity

and the Pathogenesis of Septic Shock



Internal Medicine Grand Rounds
Parkland Memorial Hospital
June 26, 2003

Robert S. Munford, M.D.
Infectious Diseases Division

This is to state that Robert S. Munford, M.D., has no financial interests or other relationships with commercial concerns related directly or indirectly to this presentation. He will be discussing off-label uses.

Robert S. Munford, M.D., is a Professor of Internal Medicine and Microbiology and the current holder of the Jan and Henri Bromberg Chair in Internal Medicine. He is in the Infectious Diseases Division. His research interests include the detoxification of bacterial endotoxin, treating inflammation using gene therapy, and the pathophysiology of sepsis.

Thanks to Dr. Tina Schade Willis, Department of Pediatrics, for letting me know about her interesting case and providing the image used in this presentation.

Introduction. This Grand Rounds has four parts. First, I'll briefly summarize the major observations and ideas that influenced how critical care and infectious disease physicians thought about sepsis during the last 3 decades. The second part will describe a different hypothesis for how the body responds to injury and infection. Third, I'll explain how this different perspective changes one's understanding of immunosuppression, bacteremia and septic shock. In the final part, I'll apply these ideas to two clinical scenarios.

I. Milestone observations that influenced how we think about the pathogenesis of severe sepsis and septic shock:

1. The discovery of "endogenous" pyrogens presaged the discovery of the pro-inflammatory, pyrogenic cytokines (IL-1β, TNF-α, IL-6, IL-12).
2. The demonstration that macrophages produce these cytokines and are required for mice to develop endotoxic shock established the primacy of the endogenous mediators and the cells that make them.
3. The report that a human antiserum to the J5 mutant of E. coli could rescue humans with septic shock due to gram-negative bacteria ignited interest in antiendotoxin therapies (1). (The strikingly positive results of this influential clinical trial were never confirmed, despite many attempts to do so.)
4. The discovery of TNF-α/cachectin's ability to induce septic shock in animals and the demonstration that TNF-α appears rapidly in the blood of humans challenged with endotoxin and can be found in the blood of many patients with septic shock. Similar results were obtained for IL-1β, which was shown to act synergistically with TNF-α.

These findings led most investigators to accept the following general sequence:

Gram-negative bacteria → LPS → monocyte-macrophages → TNF-α → severe sepsis/septic shock

(Gram-negative bacteria release LPS, which stimulates host cells [monocyte-macrophages] to produce TNF [and other pro-inflammatory mediators], which, if not controlled, induces severe sepsis/septic shock.)

One disease seemed to confirm the sequence. In patients with *fulminant meningococcemia*, high levels of circulating endotoxin and TNF-α were associated with a lethal outcome (2). It seemed likely that this uncommon disease was at one extreme of the clinical spectrum of Gram-negative sepsis...that its pathophysiology was generally representative of the septic process. In particular, the coagulopathy of fulminant meningococcemia was assumed to be present, usually with less severity, in all patients with sepsis and DIC. That meningococcemia is a good model for the septic state is still accepted today (3).

Although most patients with septic shock had positive blood cultures, many did not. It was argued that the culture-negative patients might have had bacteremia intermittently, or been treated with antibiotics before the cultures were drawn.

There were many attempts to interrupt this sequence, including monoclonal antiendotoxin antibodies and antagonists to TNF-α, IL-1β, PAF, bradykinin and other presumed mediators. Many of these interventions seemed promising at first but all of them failed to hold up when they were studied in subsequent trials.

Into the 1990's: SIRS, CARS, MARS, MODS

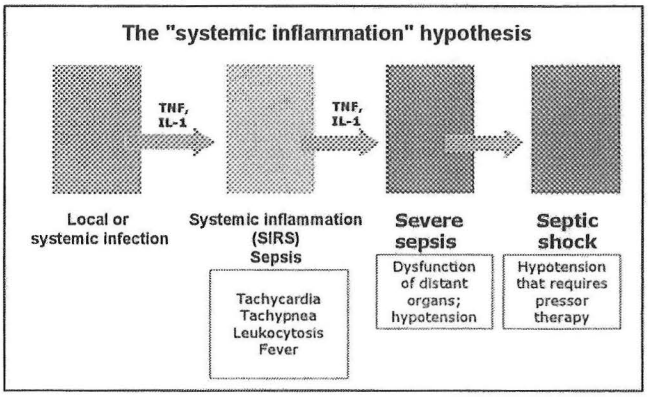
A notion that gained great popularity during the 1990's was the concept of "systemic inflammation." It has never been very clear what this term was intended to convey, but presumably it referred to the

activation of circulating leukocytes and the vascular endothelium by circulating pro-inflammatory mediators. ("We characterize SIRS as an **abnormal generalized inflammatory reaction** in organs remote from the initial insult.") (4) The term "Systemic Inflammatory Response Syndrome" (SIRS) was coined to describe the first stage in a presumed pathogenetic sequence:

SIRS → sepsis → severe sepsis → septic shock.

Definitions for these syndromes were recommended by a consensus panel in 1992 (5) and have been widely used, with some misgiving (6), ever since. In a prospective observational study, Rangel-Frausto et al. found evidence that SIRS, sepsis, severe sepsis and septic shock represent a continuum and that patients can progress from mild to severe over time (7).

According to this scheme, "sepsis" is SIRS due to a proven or presumed infectious etiology.



During the mid-1990's, however, several observations suggested that this scenario is too simple.

1. The blood of septic patients also contains numerous mediators that have anti-inflammatory actions. These include IL-10, IL-4, IL-1Ra, and soluble TNF receptors.
2. When it's tested for its ability to stimulate human cells ex vivo, the plasma of septic patients is anti-inflammatory – it inhibits the activation of these cells by LPS and other agonists (8).
3. Blood cells obtained from septic patients are 'reprogrammed' so that they produce anti-inflammatory mediators (such as IL-1Ra) when stimulated with LPS. Similar reprogramming occurs in the blood and thoracic lymph cells of patients undergoing surgery for non-infectious conditions (9).
4. Severe sepsis is associated with immunosuppression – skin test anergy, down-regulation of class II molecule expression on monocytes, greater susceptibility to CMV infection, etc.

To address these observations, Bone proposed a "counter-regulatory anti-inflammatory response syndrome" (CARS), in which systemic inflammation would elicit anti-inflammatory responses that balance, and ultimately reduce, the pro-inflammatory ones (4;10). He then went further to suggest a "mixed anti-inflammatory response syndrome" (MARS) as a manifestation of what he called "immune dissonance."

What's wrong with the SIRS-CARS-MARS model? First, it assumes that systemic inflammation is the body's normal response to infection. Second, it is "immunocentric" – it regards sepsis as an immunological phenomenon that occurs with little or no interference from the body's normal regulatory systems. Third, it assumes that sepsis is "special" – that the mechanisms that cause it are different from those that normally help us fight infection successfully.

Definitions

Inflammation is a response to injury, infection, or other stimuli that typically includes activation of leukocytes and vascular endothelium, transudation of fluid into tissue spaces, and homing of leukocytes (particularly neutrophils) to the affected site. It results in local hyperemia, warmth, edema and pain and promotes antimicrobial host defenses. Pro-inflammatory mediators include cytokines (TNF-α, IL-1ß, IL-12, IL-15, IL-18, interferon-γ), chemokines, bioactive lipids (leukotrienes, thromboxane, platelet activating factor), and reactive oxygen and nitrogen metabolites.

Anti-inflammation is a response that inhibits the production or action of pro inflammatory mediators, prevents or reduces phagocyte activation, and neutralizes the potentially toxic enzymes and metabolites produced during inflammation. Mediators with anti-inflammatory actions include cytokines (IL-4, IL-6, IL-10, IL-11, IL-13, transforming growth factor-ß), catecholamines, prostaglandin E_2, glucocorticoids, α-MSH, interleukin-1 receptor antagonist (IL-1Ra), and soluble TNF receptors. Antioxidants and protease inhibitors may also be anti-inflammatory.

In the rest of this presentation, I'll describe a somewhat different view—another hypothesis. It makes different assumptions. First, during evolution, advantageous adaptations should have helped animals destroy microbial invaders without doing harm to themselves. Second, the body is organized to confine both microbial invasion and inflammation to local tissues, where microbial invasion usually occurs; the bloodstream is meant to be sterile and inflammation-free. Third, although human lifestyles and medical practice have changed over the millennia, the body's adaptations to stress have not. Highly conserved elements of the stress response could be harmful to humans today.

II. Hypothesis: Innate immunity provides defense without self-destruction

Local defenses: walling off and killing invading microbes. When a bacterium breaches an epithelial barrier and enters the underlying tissue, it quickly encounters tissue-resident macrophages and dendritic cells. Before these cells engulf the bacterium and destroy it, they sense its presence. Their ability to sense nearby bacteria is conferred by host proteins that bind to highly conserved microbial molecules, usually lipids or sugars, and bring these molecules to the sentinel cells. The best understood system is that for recognizing bacterial lipopolysaccharide (LPS), but others exist for sensing the presence of bacterial peptidoglycan, DNA, lipopeptides, flagella, and other molecules. In the case of LPS, we have a 60 kDa protein called **LBP**, LPS-binding protein, that can transfer LPS from a bacterial membrane to various acceptor targets. The low concentrations of LBP that exist in tissue spaces probably transfer LPS to another binding molecule, **CD14**, that is abundantly expressed on the surfaces of DCs and macrophages. CD14, in turn, is part of a signaling complex that has two essential members: an extracellular protein called **MD-2** and the trans-membrane receptor element, **TLR4**. It is TLR4 that transmits the LPS recognition signal to the interior of the cell, where signal transduction and gene transcription pathways result in the production and/or secretion of numerous molecules that mediate the inflammatory response. These mediators include cytokines (in particular, TNF-α, IL-1β, IL-12, and interferon-γ), chemokines (IL-8), and lipid mediators, and they result in the familiar elements of local inflammation: **increased capillary permeability and blood flow, infiltration of neutrophils, and pain.** Local deposition of fibrin, initiated by the expression of tissue factor on activated macrophages and endothelial cells, helps wall off the infected tissue and provides an important impediment to bloodstream invasion. Inherited or acquired deficiencies in many of the local mediators and effectors (e.g., TNF-α, interferon-γ, IL-12; NADPH oxidase [chronic granulomatous disease]; CD11/CD18 [leukocyte adhesion deficiency]) have been associated with increased risk of serious infections and death.

Innate immunity : accepted elements

- Senses microbes through proteins that bind highly conserved microbial molecules (LPS, peptidoglycan, etc.)
- "Hard-wired" – in genome. Shaped by evolution. Does not change during an individual's lifetime.
- Responds rapidly.
- Elements: mannose-binding lectin (MBL), alternative complement pathway, 'natural' antibodies, pattern-recognition proteins (LBP, MBP, etc.), the "professional" phagocytes, NK cells, others.

Acquired immunity

- Recognizes microbial epitopes using T-cell and B-cell receptors → cellular and humoral immunity
- Requires gene rearrangements during the life of the individual
- Responds slowly to microbial invasion
- Protects the body from subsequent exposure to same and some related (cross-reactive) microbes
- Elements: antibodies, cytotoxic and helper T cells
- Is the basis for vaccine-induced immunity

In the usual scenario, the invaders are eliminated by phagocytes, complement, and other mechanisms, and the invaded tissue returns to normal. We imagine that this happens quite often during normal living, usually without our knowledge. These mechanisms evolved long ago, before the existence of *Homo sapiens* as a species.

Systemic responses: keeping infection and inflammation localized. How could systemic inflammation be advantageous to animals? What survival value would accrue from activating leukocytes, promoting leukocyte-endothelial adhesion and increasing vascular permeability in organs distant from a site of infection? Although the existence of a "systemic inflammatory response" has been widely accepted for many years, it's hard to imagine how animals would benefit from having it.

In fact, a lot of evidence supports the conclusion that the body's systemic responses to injury and infection and other stresses generally suppress inflammation within the bloodstream. This makes sense: preventing systemic inflammation would **support local defenses** by providing anti-microbial molecules (see Table) and phagocytes (neutrophils, NK cells), whereas it would **prevent systemic damage** by minimizing leukocyte-endothelial adhesion in uninvolved tissues. I think these responses are essential elements of innate immunity (11;12).

Homeland Security: if the enemy invades at Galveston, how would it help the national defense to tie up traffic in Chicago or disrupt telecommunications networks around the country?

The acute systemic response seems to have **four major arms** (see Table): **anti-infective, anti-inflammatory, pro-coagulant, and metabolic.**

A. Anti-inflammatory responses

Acute *leukocytosis*, which largely reflects the demargination of neutrophils, is brought about by epinephrine, cortisol, and possibly by IL-10 and other mediators. Leukocytosis contributes to two phenomena: first, neutrophils are mobilized to go to a site of infection, where they can adhere to activated endothelium and migrate into the infected tissue. Second, the ability of circulating leukocytes to adhere to uninflamed vascular endothelium is blocked, preventing unnecessary accumulation of neutrophils in uninfected tissues. Leukocytosis thus supports local inflammation while its underlying mechanism (diminishing PMN-endothelium adhesion) prevents systemic injury.

Normal systemic responses to infection and injury

Leukocytosis – mobilize neutrophils into the circulation
Tachycardia – increase cardiac output, hence blood flow to injured tissue
Fever – raise core temperature, shunt blood flow to injured tissue

Activation of the hypothalamic-pituitary-adrenal axis (ACTH, α-MSH, cortisol)
Activation of the sympathetic nervous system (epinephrine, norepinephrine)
Activation of the cholinergic anti-inflamammatory pathway (acetylcholine)

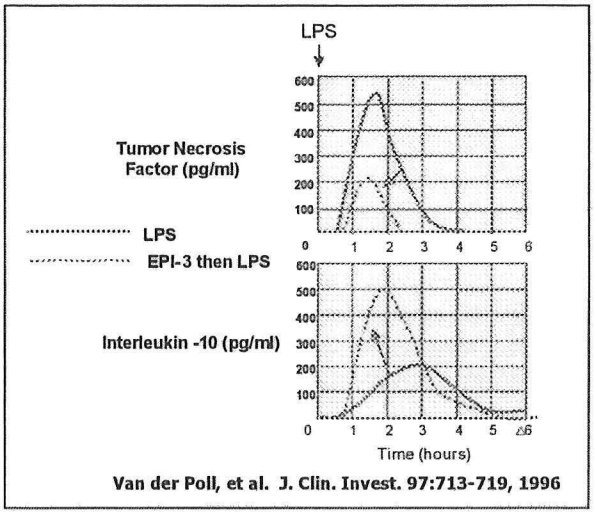
"Acute phase" responses, categorized according to possible roles in defense

- Anti-infective
  - Synthesis of complement factors, "microbe recognition" molecules (MBL, LBP, C-RP, others)
  - Sequestration of iron (lactoferrin) and zinc (metallothionein)
- Anti-inflammatory
  - Cytokine antagonists (IL-1Ra, sTNF-Rs)
  - Anti-inflammatory mediators (epinephrine, cortisol, IL-10, etc.)
  - Protease inhibitors
  - Anti-oxidants
- Pro-coagulant
  - Fibrinogen, PAI-1, C4b
- Metabolic – hyperglycemia, lipolysis, other changes
  - Epinephrine, cortisol, glucagon, cytokines
- Scavenging
  - C-RP, SAA

Other responses that seem to prevent inflammation within the bloodstream and in tissues distant from a site of injury/infection include increases in the blood levels of cytokine antagonists (IL-1Ra, soluble TNF receptors), mediators that inhibit inflammatory responses (epinephrine, cortisol, α-MSH, ACTH, IL-4, IL-10, TGF-β, C-RP), protease inhibitors, and anti-oxidants. In addition, circulating blood cells (as well as

the liver and spleen) are 'reprogrammed' so that they diminish their output of TNF in response to various agonists as they increase their production of IL-10 and IL-1Ra.

Evidence for immune-endocrine interaction. Van der Poll et al. infused a bolus of endotoxin into volunteers and measured their blood levels of TNF-α and IL-10 (13). In another group of volunteers, they infused epinephrine for 3 hrs prior to giving the endotoxin bolus. Epinephrine dramatically shifted the response from pro-inflammatory (TNF >> IL-10) to anti-inflammatory (IL-10 >> TNF). A similar (cAMP-mediated) 'reprogramming' of cellular responses to endotoxin can be demonstrated *ex vivo*; it can be caused by prostaglandin E_2 and other agonists that raise intracellular cAMP.



Van der Poll, et al. J. Clin. Invest. 97:713-719, 1996

This is perhaps the most straightforward demonstration in humans of how systemic responses can be modulated by the nervous system during periods of stress. Infusing hydrocortisone also has dramatic (though somewhat less predictable) effects on the cytokine response to an endotoxin bolus (14).

B. Metabolic responses

Hyperglycemia is often a feature of critical illness. It is usually attributed to the effects of epinephrine, cortisol, and other "counterregulatory" hormones on insulin release from beta cells, glycogenolysis, and gluconeogenesis. The same catabolic hormones induce lipolysis (so blood levels of FFA and glycerol increase) and muscle proteolysis. Insulin resistance reduces glucose uptake by muscle and contributes to muscle catabolism (15). Lipolysis, which occurs principally in adipocytes, involves activation of hormone-sensitive lipase by counter-regulatory hormones; lipoprotein lipase (in plasma) is inhibited during critical illness, accounting in part for the increase in circulating triglycerides that often occurs. Proteolysis in muscle releases amino acids (alanine, glutamine, others) that are used for hepatic gluconeogenesis and for producing acute phase proteins.

These changes are similar to those that occur during starvation. The accepted rationale is the requirement to maintain blood glucose levels at concentrations that can supply the brain until levels of ketone bodies, which can also be used by the CNS, rise later in the course of starvation (16). At least acutely, hyperglycemia may also increase glucose delivery to exercising muscle (for the "sprint across the savannah"). The modern treatment of critically ill patients with intravenous glucose solutions and parenteral and/or enteral feeding has largely supplanted the body's need to maintain euglycemia through insulin resistance and the other adaptive mechanisms discussed above.

"Counter-regulation"
Endocrinologists use this term to refer to hormones (catecholamines, cortisol, glucagon) that oppose the actions of insulin. In this presentation, catecholamines and cortisol are primary initiators of systemic responses that are themselves "counter-regulated" by MIF, HMGB-1, adrenomedullin, etc.

Insulin and glucose may have significant effects on immune function. Insulin has been called an "anti-inflammatory molecule" (17) for various reasons, while glucose can modulate monocyte-endothelial adhesion (18) and have other effects. In general, however, not much is known now about how these molecules influence host defenses.

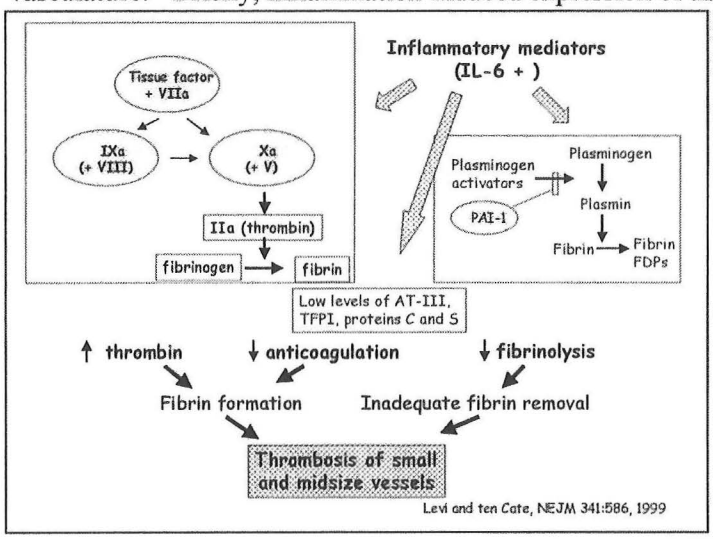
It's important to note that *the body's metabolic and systemic anti-inflammatory responses to injury and infection are largely regulated by the same molecules* – catecholamines and glucocorticoids. Cortisol's

effects on metabolism – promoting gluconeogenesis, glycogenolysis, insulin resistance, and lipolysis – are dose-related in a monogenic fashion. In contrast, its effects on various aspects of inflammation may be permissive (allowing acute phase protein synthesis) at low [normal, unstressed] concentrations and either suppressive (inhibiting cytokine and acute phase protein production) or stimulatory (increasing IL-10 production) at high concentrations within the physiological concentration range (19).

C. Procoagulant responses

Clotting is one of the oldest host defense mechanisms. It preceded complement and the acquired immune system during the evolution of animals. Inflammation-induced pro-coagulant responses contribute to abscess formation and delayed hypersensitivity reactions.

In individuals who have sustained physical trauma, activation of coagulation and inhibition of fibrinolysis occurs roughly in relationship to the severity of injury. In patients with severe sepsis, the pro-coagulant changes are so striking that one wonders what keeps the blood from clotting throughout the vasculature! Briefly, inflammation-induced expression of tissue factor on the surfaces of monocytes and endothelial cells is thought to initiate clotting via factor VIIa; depletion of natural anticoagulants (proteins C and S) removes the normal 'brake' on intravascular clotting; and increased production of plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis (20). Remarkably, there seem to be no "back-up" or "late" anticoagulants to limit clotting when proteins C and S are exhausted. Disseminated intravascular coagulopathy may ensue, as may limb-threatening arterial thrombosis. Excellent Dutch workers have concluded that IL-6 is the major pro-coagulant cytokine, whereas TNF-α is not pro-coagulant but activates fibrinolysis (21).



Activation of the so-called "intrinsic" clotting pathway seems to occur relatively late. It may be associated with activation of the kinin pathway that produces bradykinin, a potent vasodilator, and in a model of overwhelming bacteremia in baboons it may have contributed to hypotension (22).

Summary: ***the human body's acute systemic response to injury and infection has 4 major components: anti-infective, anti-inflammatory, pro-coagulant, and metabolic.*** All of these responses have been highly conserved during evolution, but some may not be advantageous to critically ill patients in today's ICUs.

Regulation, integration.

CNS regulation of systemic responses. Many essential responses are regulated by the hypothalamus and brainstem. In fact, the body's reactions to microscopic danger (microbial invasion) have a lot in common with its "flight or fight" responses to macroscopic threats.

Remarkably, the output of each of the three major CNS efferent pathways (the HPA, the sympathetic nervous system, and the parasympathetic nervous system) inhibits inflammation within the circulating blood. In contrast, some neurohormones may stimulate inflammation when they are produced in tissue beds – corticotrophin-releasing hormone, for example, and probably also catecholamines.

The nervous system's role in innate immunity has become appreciated only recently. It has both sensory and effector arms:

a. **sensory.** Traffic along *nociceptive fibers* alerts the CNS that local infection/ inflammation exists. Activation of the hepatic *vagus nerve* by circulating PGE2, IL-1β or endotoxin can induce fever in rodents, suggesting that the vagus may be a sensor for low blood concentrations of these agonists.

b. **effector.** The CNS effector response to infection has 4 major components:
1) the hypothalamic-pituitary-adrenal axis (ACTH, α-MSH, cortisol)
2) the sympathetic nervous system (catecholamines)(23)
3) the parasympathetic nervous system (via the vagus nerve, nicotinic receptors)
4) peripheral nerves that innervate infected tissues.

Whereas 1) – 3) exert largely anti-inflammatory influences within the splanchnic organs and bloodstream, the signals delivered by peripheral nerves to tissues can be pro-inflammatory, anti-inflammatory, or both.

The liver: essential roles in systemic responses to infection. The liver is anatomically situated to remove microorganisms that translocate across the gut mucosa and enter the portal circulation. Impairment of the hepatic filter (e.g., by cirrhosis) predisposes to bacteremic infections with *V. vulnificus* and certain other gut bacteria. Kupffer cells in the liver also play a major role in clearing endotoxin and bacteria that are injected into the bloodstream. The spleen is the major filter for opsonized microorganisms in the blood. The liver is also the site of most acute phase protein synthesis, and it's the body's principal regulator of energy metabolism. TNF-α, IL-6 and other cytokines may act on hepatocytes to induce many of the body's metabolic responses to injury and infection.

As noted above, the liver can also now be regarded as a key part of the ***sensory*** *system* that informs the CNS that microbes have invaded. In rodents, the ability of low doses of endotoxin or IL-1β to induce fever and activate the HPA axis can be blocked by cutting the hepatic branches of the vagus nerve. Higher doses can interact directly with the thermoregulatory center. Conversely, stimulation of the vagus nerve suppresses endotoxin-induced TNF-α production via a "*cholinergic anti-inflammatory pathway*" that involves inhibition of macrophage cytokine synthesis by acetylcholine (24).

The liver and spleen thus are extremely important for innate immunity – as blood filters that collect and kill blood-borne microbes, as "listening stations" that sense low concentrations of circulating cytokines and transmit this information to the CNS, as factories for the production of many (acute phase) elements of the systemic response, and as a major engine for infection-associated metabolic adaptations.

III. Evolution's gifts: possible consequences for patients today

A. Normal systemic responses to injury or infection may be immunosuppressive and predispose to infection with commensal microbes.

B. Regulated mechanisms limit systemic immunosuppression and prevent thrombosis. These may contribute to the pathogenesis of septic shock.

C. Limiting systemic immunosuppression may prevent infection.

D. Gene polymorphisms may influence both susceptibility to, and outcome from, serious infections.

A. Normal systemic responses to injury or infection may be immunosuppressive and predispose to infection with commensal microbes (25). Traumatic injury provides a useful opportunity to study the body's responses to stress, since it occurs at a discrete moment in time and often affects

previously healthy individuals. When humans sustain major trauma, a period of immunosuppression often ensues. During this period, which may last from days to weeks, patients typically develop skin test anergy (26), decreased expression of class II molecules on circulating monocytes (27), and high circulating levels of IL-6, IL-10, IL-1Ra, soluble TNF receptors (28) and other anti-inflammatory molecules. The ability of IL-12 to increase interferon-γ production by LPS-stimulated blood cells is diminished (29), as is its concentration in the blood. In general, these findings have correlated directly with risk of developing nosocomial infection and death. The investigators who have documented these changes have called them "immune paralysis", "immunoparesis", and other terms that suggest a pathological response to injury.

Jérome Pugin and I have suggested that post-trauma immunosuppression is actually an exaggerated expression of the body's *normal* systemic responses to injury, infection, and many other stresses (25). It's "endogenous" immunosuppression, induced when the body's normal mechanisms for preventing systemic inflammation are active for a prolonged time or to an extreme degree. We assume that the body's responses to less severe insults are qualitatively similar. In support of this assumption, a subset of the same changes occurs in the blood of humans challenged by minor surgery, strenuous exercise, or hypothermia. In addition, Rivera and others (Department of Surgery, UTSW) found that patients with acute appendicitis have high blood levels of IL-10 and low blood levels of IL-12, and that their plasma inhibits LPS-stimulation of reporter monocyte-macrophages (33); that these changes occur during a relatively mild and localized infection suggests, but obviously does not prove, that the systemic changes seen following major trauma may be an exaggerated expression of the same changes—the normal response to infection and injury.

Activity/condition	**Increased risk of infection**	Reference
Psychological distress	Rhinoviral infection HSV activation	(30)
Strenuous exercise	Upper respiratory infections	(31)
Intra-operative hypothermia	Post-operative infections	(32)

An important reservation: there is no single systemic response, or group of responses, that is definitely known to produce(s) immunosuppression. At the moment there is merely a pattern of responses that, collectively, seem likely to be immunosuppressive and have been associated temporally with increased risk of infection. How these changes in the blood interfere with local tissue defenses is not at all clear, yet they somehow impair the innate immune defenses that normally protect us from the microbes that make up our commensal flora.

In fact, many observers have found that the expression of CD11b, an important adhesion molecule, is increased on the neutrophils of patients after major injury, or during severe sepsis (34;35). This has been considered a 'proinflammatory' change but it is also consistent with the notion that circulating PMN are activated so that they will preferentially adhere to vascular endothelium at sites of infection—i.e., in the vascular beds where local inflammation is occurring and endothelial cell adhesion molecule expression is up-regulated.

We're able to observe invasive infections with commensal bacteria and fungi as a complication of post-trauma immunosuppression because modern technology allows humans to survive injuries that would have been lethal in earlier times. It's also noteworthy that our innate anti-viral defenses are relatively weak … most of the viruses that cause disease in humans were acquired after *Homo sapiens* began to domesticate (and live with) animals (36). In addition, the person-to-person transmission needed to perpetuate many of these viruses would have become possible only when humans began to live in large communities. This was long after the evolution of innate immunity seems to have finished – our "innate immunity molecules" and responses to injury are very similar to those observed in chimpanzees. When we get anxious or depressed and catch a cold, we may be experiencing the ability of the human body's stress response to produce immunosuppression – not enough to allow bacterial or fungal infection, since innate immunity evolved to deal with them, but sufficient to allow infection by a respiratory virus. Fortunately, our acquired immune mechanisms often do the job, at least the second time around.

B. Regulated adaptations may limit systemic immunosuppression...and prevent thrombosis? Is vasodilation a survival mechanism?

Speculation: to prevent harmful immunosuppression, there should be mechanisms that help restore normal immune balance. They should appear after the initial systemic responses have occurred. It might be sufficient for the "on" signals simply to decrease, but having discrete mechanisms that limit immunosuppression would probably be advantageous. This notion might account for two observed phenomena.

First, investigators have recently found that **proinflammatory mediators** appear in the blood late in the course of the normal response to severe infection.

a. One of these mediators is **macrophage migration inhibitory factor** (MIF), a macrophage product that is *induced by, and opposes the actions of, glucocorticoids* (37). Whereas MIF normally circulates at a low, basal level, its plasma concentration increases during infection and stress, and very high levels have been found in the plasma of patients with severe sepsis (38). The role played by MIF in endotoxin-induced sepsis is disputed (39).
b. A second "late" pro-inflammatory molecule is a transcription factor, **High Mobility Group Box-1** (HMGB-1), that appears in the blood several hours after infection begins, stimulates monocyte-macrophages (40) and contributes to death in a mouse endotoxin challenge model (41).

The second phenomenon is **tachyphylaxis** (desensitization) to the actions of catecholamines and other molecules. Since catecholamines have significant anti-inflammatory actions within the bloodstream, for example, down-regulation of β2-adrenergic receptors on monocyte-macrophages should reduce the anti-inflammatory milieu.

Evidence for *in vivo* tachyphylaxis to the anti-inflammatory effects of epinephrine comes from the important study by Tom van der Poll, Steve Lowry, and others cited above (13): when epinephrine was infused for 24 hours prior to administering i.v. endotoxin, the TNF-α response was somewhat less robust than that seen without epinephrine infusion, but the IL-10 responses with and without epinephrine were indistinguishable. After prolonged epinephrine exposure, there is evidently sufficient β_2-adrenergic receptor down-regulation to prevent the cAMP-mediated 'reprogramming' of pro- and anti-inflammatory responses to LPS. Desensitization to norepinephrine was also noted in a model that involved infusion repeated doses of endotoxin into a dorsal hand vein (42).

There is also evidence that critically ill patients can develop "partial" or "relative" **adrenal insufficiency.** Some have plasma cortisol levels that are lower than expected during severe stress, whereas others have high ACTH:cortisol ratios that suggest relative adrenal hyporesponsiveness (43). The underlying mechanism(s) are not known, and not all investigators have documented these findings (44). Using various diagnostic criteria, partial or absolute adrenal insufficiency has been found in 16 to 55% of adults with septic shock (45-47). In patients who survive, HPA axis function usually returns to normal (48;49). Other changes that might contribute to cortisol "deficiency" in sick patients include the appearance of MIF (see above) and down-regulation of glucocorticoid receptors in cells (50).

These "late" responses to infection should serve a protective function – when produced in the right amounts at the right time, they would prevent immunosuppression and help restore physiological homeostasis. Viewed in this way, they should have survival value. It's also quite possible that the late physiological changes—especially catecholamine tachyphylaxis, adrenal hyporesponsiveness, and vasopressin depletion—simply reflect exhaustion of physiological reserves at a time when the feedback

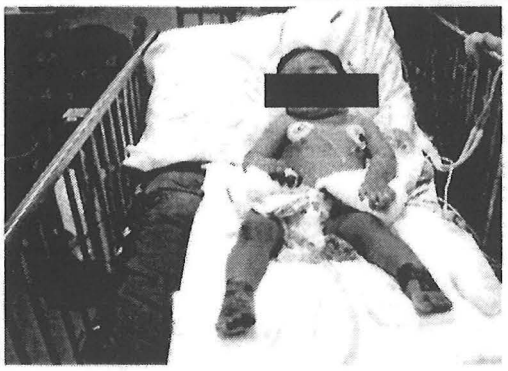
signals that would normally increase output are inoperative. Much more information is needed about this phase of the systemic reaction to infection.

Septic Shock. It's hard to imagine an Darwinian rationale for infection-induced hypotension, but several observations may be clues. In many patients, septic shock has two distinguishable phases. Vasoconstriction (cold shock) is quickly followed by vasodilation (warm shock). The vasodilatory phase of inflammation-associated shock is distinctive: it is not often seen with acute hemorrhagic or cardiogenic shock.

	Hemorrhagic Shock (acute)	Septic Shock	Cardiogenic Shock
SVR	High	Low	High
Cardiac output	High	High	Low
Plasma vasopressin	High	Normal /low	High
Associated coagulopathy	No	Yes	No

The factors that seem to contribute to inflammation-induced vasodilation include tachyphylaxis to catecholamines, which diminishes the sensitivity of vascular smooth muscle to catecholamines as pressors; the underproduction or ineffectiveness of glucocorticoids; the production of adrenomedullin, which has vasodilatory actions and inhibits aldosterone secretion while increasing renal blood flow (51); the release of nitric oxide from sites of inflammation (52); the absence of the normal baroreflex response that increases circulating vasopressin levels (and depletion of neurohypophyseal vasopressin stores) (53); the release of platelet-activating factor; the activation of K_{ATP} channels in arteriolar smooth muscle cells by hypoxia and lactate (54); and the generation of bradykinin.

Why should animals have a vasodilatory response to severe inflammation? An astute clinical observation may be a clue. A 16 month-old boy developed purpura fulminans as a complication of infection with an unknown bacterium (possibly Salmonella). He rapidly developed severe thrombotic occlusion of small arteries in three of his extremities. The unaffected limb was his left arm, which had been unusable since birth due to a brachial plexus injury. The authors found that his sympathetic reflexes in this arm were impaired and speculated that the limb may have been protected from thrombosis by an inability to vasoconstrict (55).



Willis et al. Pediatr.Neurol.24:379, 2001

Of the various measures reported anecdotally to benefit patients with inflammation-induced thrombosis, most should prevent or relieve vasoconstriction: sympathetic blockade, intravenous nitroprusside, topical nitroglycerine ointment, and treatment with an α-blocker (references in (56)). There is also evidence from studies in animals that catecholamines interact with inflammation-induced coagulopathy to produce thrombotic lesions. Administering epinephrine intradermally can sensitize rabbits to develop hemorrhagic necrosis following an i.v. dose of endotoxin (57), for example, while norepinephrine induces hypercoagulability in dogs and makes the endotoxin-induced generalized Shwartzman reaction worse (58).

Pro-coagulant intravascular responses to severe infection have been highly conserved, but significant thrombosis rarely occurs in infected humans. Could vasodilation have evolved as a mechanism to prevent thrombosis? Might vasodilatory shock have survival value for animals that can lie down and tolerate transient hypotension but would never survive the loss of a limb?

As noted above, a common feature of septic shock is a low (relative to the degree of hypotension) blood level of vasopressin. Since vasopressin is a potent vasoconstrictor, particularly for the intraabdominal vasculature, has a loss of the vasopressin response to hypotension been selected to favor peripheral vasodilatation while preventing GI ischemia? In keeping with this idea, Dünser et al. (59) found that

continuous vasopressin infusion for a median of 31 hrs to patients with septic shock was associated with significant elevations in transaminases and bilirubin.

It's interesting that the only known interventions that can rescue experimental animals from *established* endotoxic shock or septic peritonitis are antibodies to MIF (38) or HMGB-1 (41). (To be effective, all other interventions must be given before, or with, the challenge.) Hydrocortisone, which increases β-adrenergic receptor expression and can reverse catecholamine tachyphylaxis, can improve vasopressor responses to catecholamines in humans (60;61), and the results of clinical trials that used relatively low doses (50 to 100 mg q6-8h) to treat patients with septic shock have been promising (62-64). Since the ability of renin to stimulate aldosterone production may also be reduced in septic patients (65), addition of a mineralocorticoid to the hydrocortisone regimen may be useful (64). Vasopressin infusion may also raise blood pressure and reduce the requirement for pressor administration (53;59), but prolonged administration may have significant risks, as noted above (59).

These findings suggest that interventions designed to prevent the late, pro-inflammatory counter-reaction may be useful therapies for severe sepsis and septic shock.

C. Damping some systemic responses may prevent infection and/or the harmful reaction to it.

Modern medical practices have imposed stresses on the sick human body that could not have been anticipated during the evolution of innate immunity (intensive care, antibiotics, other drugs, etc.) yet they've also made the presumed evolution-based 'reasons' for some innate responses obsolete. Effective antimicrobial drugs have largely eliminated the body's need to wall off infection to prevent dissemination, for example, and nutritional support has supplanted the need to maintain plasma glucose concentrations through glycogenolysis, gluconeogenesis and insulin-resistance. If these responses aren't needed for survival yet they're potentially harmful, would preventing or dampening them be beneficial for hospitalized patients?

Can interventions that limit normal systemic responses be beneficial?

Adaptation to injury / infection	Presumed "Purpose"	Possible harm	Needed in ICU?
Metabolic	Preserve plasma glucose level	Immuno-suppression	NO
Pro-coagulant	Wall off invading microbes	DIC, thrombosis	NO
Anti-inflammatory	Prevent harm to uninfected tissues	Immuno-suppression	YES (but how much?)
Anti-infective	Prevent bloodstream invasion	None	YES

Prevent metabolic adaptations. Van den Berghe and others (66) tested the effects of administering intensive insulin therapy to critically ill patients in The Netherlands. Most of the patients had recently undergone cardiac surgery. When compared with patients who received conventional insulin treatment (to achieve blood glucose levels between 180 and 200 mg/dL), patients who received intensive insulin therapy (maintaining blood glucose between 80 and 100 mg/dL) were less likely to develop bacteremia (45% reduction) or critical illness polyneuropathy (44%), to require RBC transfusion (50%), or to die in the hospital (34%). Most of the deaths were from "multiple-organ failure with a septic focus." Multivariate logistic regression analysis revealed that a low blood glucose level, rather than the insulin dose, correlated with these beneficial effects (67). Insulin therapy had no effect on the requirement for vasopressor therapy.

In a retrospective analysis of the same clinical database (68), intensive insulin therapy was found to be associated with "anti-inflammatory" actions such as decreasing C-reactive protein and MBL levels and reducing the duration of leukocytosis/leukopenia and hypo/hyperthermia (data for the latter were not provided). Others have also noted insulin's "anti-inflammatory" properties (17). I think it's just as likely that insulin therapy (or maintaining normoglycemia) dampens the body's normal metabolic adaptations to major illness, thus preventing one aspect of the profound immunosuppression that predisposes critically ill patients to nosocomial infection. After all, elevations in C-RP and MBL, like leukocytosis and fever, are normal, anti-

inefective systemic responses to injury and infection. In keeping with this notion, low concentrations of insulin inhibit the transcription of genes for acute phase proteins in HepG2 cells in vitro (69).

In a multivariate analysis of data from the same trial, van den Berghe et al. found that "a high dose of insulin was associated with a worse outcome, and a lower blood glucose level was associated with a better outcome, suggesting that the latter had a crucial role (70)." It's also possible that controlling plasma concentrations of free fatty acids or other lipids might play a role. Since lower glucose levels (hence less insulin resistance and lower insulin requirement to maintain normoglycemia) would be expected in those individuals whose systemic response to injury was less severe, perhaps one message from this study is that it pays to have a modest systemic reaction to injury – enough to prevent systemic inflammation yet not enough to be immunosuppressive.

The often-stated rationale for stress-induced hyperglycemia is preservation of fuel for the brain until starvation-induced ketone body production provides an alternative energy substrate (16). Prolonged hyperglycemia, in contrast, seems to be harmful. This may be another example of the general phenomenon being discussed here: normal systemic responses to infection and injury can, when prolonged or intense, become harmful. Although hyperglycemia has been associated with increased infection risk in other clinical studies (71-73), its 'immunosuppressive' mechanism is not well established or understood. Fortunately, preventing it is unlikely to have a "down side" in patients whose nutritional needs are supported in the ICU.

A related approach is the administration of propanol, a β-adrenergic blocker, to prevent catecholamine-induced catabolic changes in severely burned individuals. Although the drug effectively inhibited hypermetabolism in burned children, it did not significantly influence plasma glucose levels and had no apparent effect on the (already low) incidence of pneumonia (74). Whether or not propanolol or other adrenergic blockers could be used to dampen systemic anti-inflammatory responses is not known; since epinephrine may have anti-coagulant actions (75), this approach could be risky.

Prevent immunosuppression. During the 1990's, several studies addressed the ability of interferon-γ to prevent severe sepsis in patients who had recently undergone major surgery or sustained major trauma. Unfortunately, the results of these studies didn't provide much encouragement that prophylactic administration of IFN-γ can reduce the incidence of nosocomial infection and severe sepsis (76;77), even though an impact of the drug on monocyte function was observed (78). In this instance, the goal was to prevent immunosuppression by providing a pro-inflammatory (Th1) cytokine; unfortunately, we don't know how important it is to maintain the balance of mediators that normally prevents systemic inflammation. If pro-inflammatory molecules could be provided at the local site of infection, rather than systemically, they might be safer and more efficacious.

One retrospective analysis found that patients who developed bacteremia while taking a statin were significantly less likely to die than non-statin-users (79). Although statins may have anti-inflammatory actions, a mechanistic basis for this unconfirmed report is not obvious.

Prevent coagulopathy. I found no clinical studies that tested the ability of low doses of anti-coagulants to *prevent* severe reactions to infection. However, the pharmaceutical industry's attention has recently focused on the coagulopathy and vascular endothelial injury that may accompany severe sepsis and septic shock. The guiding notion was that the inflammation-induced procoagulant changes contribute to organ dysfunction by forming thrombi that interfere with blood flow through the microcirculation. Although the loss of physiological function that takes place in different organs during severe sepsis is largely reversible and pathologists have found little evidence for sepsis-induced microthrombosis in muscle biopsies or at autopsy, it nonetheless seemed likely that the procoagulant changes are deleterious and that blocking them would be beneficial. Unfortunately, clinical trials of two recombinant anticoagulants (tissue factor pathway inhibitor [TFPI], antithrombin III (80)) showed no beneficial effect in patients with ongoing severe sepsis. A third anticoagulant, activated protein C, may have improved survival but there was no correlation between its apparent benefit and either pre-infusion activated protein C levels or post-infusion clotting

parameters (81). Although the drug's in vitro anti-inflammatory actions have been credited with causing its clinical success, two recent studies found that prior infusion of aPC has no effect on endotoxin-induced coagulopathy and plasma cytokine changes in volunteers.

(The dose of activated protein C used in the Prowess (phase III) trial was based on its anti-coagulant potency. If the drug's beneficial effect doesn't relate to its anticoagulant properties, perhaps a lower dose would improve survival with less risk of unwanted hemorrhage?)

Enhance anti-infective defenses. Another interesting approach to preventing infection and its complications would be the administration of mannose-binding lectin (MBL) to individuals with low-producing MBL alleles, since these polymorphisms seem to be associated with increased risk of infection and severe sepsis in ICU patients. In a related vein, one group reported that prophylactic use of IVIG protected patients from nosocomial pneumonia (82).

D. Gene polymorphisms may influence infection susceptibility and outcome. Numerous groups have now studied SNP-outcome associations in small groups of critically ill patients. Although there has been significant variability in the results of these studies, some SNPs do seem to have associations with susceptibility to gram-negative infection or with risk of developing severe sepsis and/or dying – at least in some ethnic groups. Although these studies are quite preliminary, it's remarkable that any of them have shown significant associations between genetic variability and outcome: the more severe the injury or underlying illness, the less one would expect the contribution of an individual gene to be evident.

Gene polymorphisms that have been associated with increased risk of infection include mannose-binding lectin (MBL; in press) and toll-like receptor 4 (for GN infections) (83). The same polymorphisms have been associated with increased risk of septic shock due to GN infection (84)(in press).

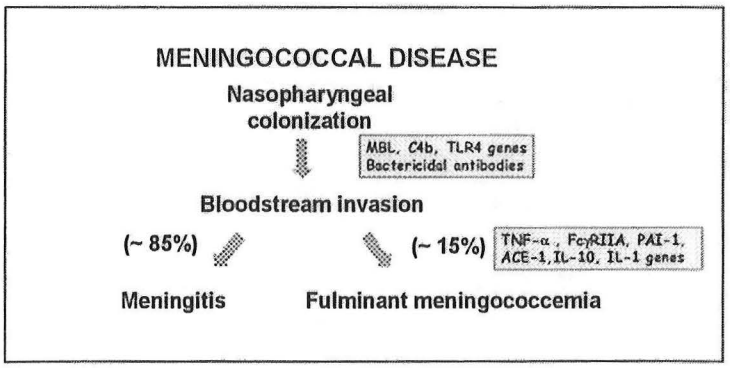
Polymorphisms associated with increased risk of severe sepsis in critically ill patients include (a) TNF –308, in France (85), Taiwan (86) and the U.S. (87), but not in Germany (88); (b) TNFB2, in Germany (88) but not in the U.S. or France.

These associations must be confirmed in larger populations and others must be sought. The obvious hope is that certain SNPs can be used to identify individuals at increased risk for immunosuppression, nosocomial infection, or an adverse outcome. A patient's genetic profile may someday be useful for guiding specific preventive or therapeutic interventions.

III. WHEN HOST DEFENSES FAIL – two examples

A. Fulminant Meningococcemia: Under the Radar

No disease has had a greater impact on how we think about sepsis than fulminant meningococcemia (FM). Not only is it the most dramatic bacterial disease of humans, but it's also been studied in great detail. It's important to consider here because its pathogenesis is very distinctive.



Meningococcal disease occurs almost exclusively in previously healthy children and young adults. It begins as an intravascular infection – patients with meningococcal disease rarely have signs of inflammation at the local site (the nasopharynx or oropharynx) where a bacterium invaded the bloodstream. Most individuals seem to tolerate low-level bacteremia long enough to seed their meninges and develop meningitis; in these patients, inflammation occurs in the CSF compartment, not in the blood. An unfortunate minority develops FM. In these individuals, the bacteria trigger an often-exposive inflammatory reaction within the vasculature.

The rapid course of FM is reflected in the observation that C-reactive protein levels are often normal when the sickest individuals present for medical care; it's as if the bacteria have entered the bloodstream without setting off the usual alarms. Then they've multiplied, infected vascular endothelial cells, released endotoxin, and initiated a remarkable disruption of normal physiology. The peripheral blood of patients with FM typically contains high levels of bacterial endotoxin and a large, complex mixture of pro- and anti-inflammatory mediators. Adrenal output of cortisol is often inappropriately low, due to direct adrenal injury (Waterhouse-Friderichsen) or other mechanisms (the ratio of ACTH to cortisol in plasma has correlated with severity of illness). Death occurs in approximately 10 to 20% of cases.

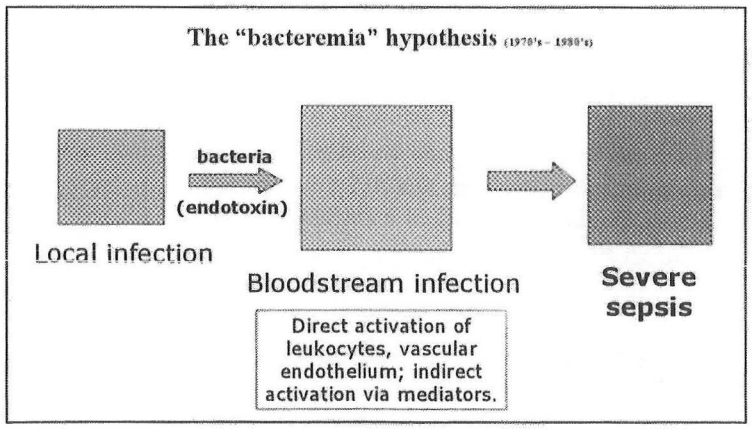
The telltale physical findings in patients with FM are the petechial and purpuric skin lesions. *N. meningitidis* bacteria differ from most other gram-negative bacteria by releasing large, endotoxin-containing membrane blebs as they grow; these negatively-charged blebs may serve as initiation sites for surface activation of coagulation. FM has been associated with abnormalities in numerous clotting pathway components; it has served many investigators as a model for understanding infection-associated DIC (89).

Just as certain gene polymorphisms seem to predispose exposed humans to develop meningococcal bacteremia, there seem to be important genetic influences on the subsequent course of the infection. More severe meningococcal disease has been associated with SNPs in genes that influence the phagocytosis of opsonized bacteria (FcγRIIA)(90;91), clotting (PAI-1)(92;93), and possibly the production of TNF-α (94;95), IL-1β (95) and IL-10 (96) . The course of meningococcal disease, like susceptibility to it, therefore seems at least partly determined by innate immunity genes.

Summary: In patients with FM, meningococci enter the bloodstream without eliciting local inflammation, then they and/or their products (endotoxin) probably directly activate vascular endothelium and initiate changes that can lead to coagulopathy and shock.

B. Nosocomial bacteremia with a commensal Gram-negative bacterium: Pickett's Charge?

Our surfaces are inhabited by vast numbers of bacteria that ordinarily don't cause disease. These are our *commensals,* our normal flora, the microbes that innate immunity evolved to confront. Their presence is beneficial to us, as they may carry out tasks that the human body cannot perform for itself. Remarkably, **over 80% of the bacteria found in blood cultures from septic patients today are commensals.** Severe sepsis due to a classical bacterial "pathogen" is actually a relatively unusual occurrence in academic hospitals in the U.S. today (97).



Individuals who develop serious disease due to a commensal bacterium generally have a significant defect in some innate immune defense – most often, epithelial barrier disruption (e.g., catheters, bites, cuts), immunosuppression (including neutropenia), or an inherited "susceptibility" allele. When hospitalized patients become infected with a commensal, they've usually been sick for some time. Their normal systemic responses have been activated for days, and they probably have some degree of endogenous immunosuppression. They may be unable to eliminate the invading microbes from the local site of infection and bacteremia may then occur. When severe sepsis or septic shock occurs in such a patient,

what is its pathogenesis? Do the circulating commensal bacteria directly trigger inflammation within the vasculature, or are they a sign that local infection is uncontrolled?

Several observations suggest that circulating Gram-negative bacteria can directly induce severe sepsis, presumably by activating vascular endothelial cells and/or circulating leukocytes. Blood cultures are positive more commonly in patients who have severe sepsis than in those with sepsis, for example, and the fraction that has a positive culture is even greater in those with septic shock (7;97). In patients with severe sepsis and documented infection, moreover, bacteremia has been associated with early mortality (98). On the other hand, many patients who meet clinical criteria for severe sepsis have sterile blood cultures, even when several specimens are obtained before initiating antimicrobial chemotherapy, and others have negative cultures from both blood and tissue sites (7;99). Developing the clinical picture of severe sepsis thus doesn't require the presence of circulating cultivatable microorganisms. The case-fatality rates for culture-positive and culture-negative patients with severe sepsis and septic shock are very similar (7;98;100), suggesting that bacteremia may contribute little to outcome. Finally, bacteremia can be transient, with few or no harmful consequences, particularly in children.

Several lines of evidence support the notion that circulating commensal Gram-negative bacilli are more often markers of uncontrolled local tissue infection/inflammation than inducers of severe sepsis. Since this conclusion is somewhat counter-intuitive yet consistent with the 'compartmentalization of inflammation" concept, I'll discuss this evidence in some detail.

1. Gram-negative bacteremia is usually transient. In their 1924 description of 28 patients with "Bacillus coli sepsis", a rare disease at that time, Felty and Keefer (34) noted "That the organisms disappear from the blood in many instances rather rapidly after the initial invasion is quite certain—an observation already made by many observers." In keeping with this conclusion, persistent Gram-negative bacteremia (defined as lasting 7 days or more despite appropriate antimicrobial therapy) has been reported only in patients with undrained abscesses or infected intravascular devices (101).

> "The body possesses remarkably efficient mechanisms for sterilizing the blood stream. Contrary to popular belief, most microorganisms are less capable of provoking disease when injected intravenously than when administered by any other route (2 ref.). With rare exceptions, living bacteria which enter the blood stream of animals or man disappear swiftly from the circulation."
> D.E. Rogers, Bacteriol.Rev. 24:50, 1960

In almost every study of bacteremia in experimental animals, bacteria have been introduced into the bloodstream by intravenous inoculation. Few investigators have attempted to study bacteremia that arises from a primary infection in an extravascular site. In an influential review of this subject, Rogers (102) nonetheless concluded that "The bulk of experimental evidence suggests that bacteremia persists or resurges only when there is an active seeding of the bloodstream." In other words, bacteria that are injected into the venous circulation are usually killed or cleared rapidly, so that persistent bacteremia requires re-seeding from outside the bloodstream. Intravascular killing is mediated by complement, activated by the the alternative, MBP or even classical pathways. The major site of clearance of Gram-negative bacteria is the liver, where the bacteria can be found in neutrophils and Kupffer cells. From the viewpoint of most commensal bacteria, bloodstream invasion may be a bit like Pickett's Charge on the 3rd day of the Battle of Gettysburg: a doomed advance against overwhelming odds.

2. The risk of developing severe sepsis does not correlate with bacterial density in the blood. If severe sepsis were triggered principally by circulating bacteria or their products, the risk of developing severe sepsis should be directly related to the concentration of bacteria in the patient's blood. No published study has formally addressed this relationship, but Du Pont and Spink performed quantitative blood cultures in hospitalized adults and found that the concentration of bacteria in venous blood correlated directly with mortality, but not with septic shock (103). In the 10-year experience with Gram-negative bacteremia reported by Kreger et al. (104), fewer than 10 cfu per ml blood were grown from 70% of the patients. Case-fatality rates were higher in patients whose blood cultures grew more cfu/ml, but only when underlying disease was not included in the analysis. In a more recent study, Kellog et al. found no relationship between

the fraction of eight blood cultures that were positive and the occurrence of sepsis syndrome (severe sepsis)(105).

On the other hand, there's little doubt that high bacterial density is associated with high complication rates in patients with *S. aureus* bacteremia (106), that high bacterial density is often seen in patients with fatal septicemic plague (107), melioidosis or *Vibrio vulnificus* bacteremia, or that septic shock occurs in asplenic patients who experience high-level pneumococcal, meningococcal or *H. influenzae* type b bacteremia. Occasionally, profoundly immunosuppressed (or neutropenic) patients may also have extremely high densities of circulating bacteria (108). When viewed in the overall context of bacteremia and severe sepsis as they occur today, however, these unusual experiences may have unduly influenced scientific opinion in favor of the "more circulating Gram-negative bacteria, worse outcome" notion.

3. Primary bacteremia does not often induce severe sepsis. Primary bacteremias originate in the bloodstream, either from a contaminated intravenous infusion or an infected intravascular catheter. Endocarditis is also a "primary" bacteremia. Unless there is a local catheter-site or other tissue infection, the circulating bacteria enter the bloodstream without activating many the body's usual immune defenses...they are "below the radar" of the innate immune mechanisms that normally defend our epithelial borders. On the other hand, most primary bacteremias occur in individuals who are stressed by illness, so the systemic reactions that prevent inflammation within the vasculature may be enhanced.

A. *Transfusion-related bacteremia.* When contaminated intravenous fluids are administered unintentionally to hospitalized patients, the initial responses to the bacteria should occur within the bloodstream and in the absence of infection or inflammation in a local tissue. In the most dramatic instance reported to date, infusion of heavily contaminated dextrose solutions induced "profound acute endotoxic shock" in 5 patients, 4 of whom died (109). The other published episodes of contaminated transfusions do not indicate how often the contaminated infusates induced severe sepsis or septic shock. If one uses mortality as a surrogate for septic shock, however, infusion-related bacteremia seems to be much less lethal than bacteremia that arise from a site of extravascular infection within the body. In the large outbreak of Gram-negative rod (Enterobacter sp.) bacteremia associated with contaminated intravenous infusion equipment described by Maki et al. in 1976, for example, the case-fatality rate was 13.4% overall and 7.4% in patients with non-fatal underlying diseases (110). These rates were substantially lower than the case-fatality rates for Gram-negative bacteremia found in contemporaneous studies of hospitalized patients with bacteremia (103;111;112). In other incidents of infusion-associated bacteremia, the case-fatality rates were 12.5% or less (0 or 1 of 8) (Pseudomonas thomasii)(113), 12% (3 of 26) (Serratia marcescens)(114), and none of 12 cases (Serratia marcescens)(115). As noted by Maki (111), patients who become bacteremic in infusion-related epidemics tend to be younger and less likely to have fatal underlying diseases than are patients who develop bacteremia from endogenous sites of infection, and the infusion is usually terminated promptly when contamination is suspected. In the only case-control analysis of risk, mortality in the infused patients was not different from that in the control population, and none of the deaths in the cases could be attributed directly to the infusion (B. Ostrovsky, personal communication)(114).

When assessing the impact of bacteremia, **mortality** is a weak surrogate for severe sepsis or septic shock. Bacteremia can contribute to a patient's demise without causing severe sepsis or septic shock, whereas most patients who develop severe sepsis will survive. Since the only adverse end-point reported in many publications on bacteremia is mortality, however, it is used as an approximate indicator of severe sepsis/septic shock. A low mortality is consistent with, but does not prove, a low incidence of severe sepsis, and vice versa. Bates et al. (116) found that the 1 year survival of bacteremic patients did not differ from that of control patients provided that underlying disease and major comorbidities were included in the analysis.

B. IV catheter-associated bacteremia. Patients who develop intravenous catheter-associated bacteremia offer another opportunity to assess the impact of bacteremia in the absence of an extravascular tissue infection. Case-control studies have found no outcome difference between ICU patients who experienced catheter-associated bacteremia and those who did not (117;118). Gram-negative bacteria were

isolated from very few (< 25%) of the cases in these studies, but I can find no evidence that the risk of developing severe sepsis during an episode of catheter-associated Gram-negative bacteremia is higher than the risk associated with coagulase-negative Staphylococci. The case-fatality rate for severely septic patients whose focus of infection is an intravenous catheter is substantially lower than that for patients who have pulmonary, abdominal, or unknown infection sites (98;119).

The direct introduction of Gram-negative bacteria into the bloodstream is obviously sufficient to trigger pyrogenic and other systemic reactions. The ability of directly introduced bacteria to induce severe sepsis or septic shock is less predictable; important factors include the size of the inoculum, the potency of the infused bacteria's endotoxin (or other agonistic molecules), the ability of the bacteria to survive and grow in human blood, the fitness of the host, and probably other variables. These factors converge to produce fulminant meningococcemia in some unfortunate individuals.

4. Bacteremia does not have distinctive clinical features. If circulating bacteria contribute directly to the pathogenesis of severe sepsis, patients with severe sepsis who are bacteremic might be distinguishable clinically from severely septic patients whose blood cultures are negative. Recognizing that early recognition of bacteremia should improve patient management and outcome, Bates et al. (99) performed a prospective cohort study of 1342 cases of severe sepsis in 8 academic medical centers in the northeastern United States. Of the factors that were associated with bacteremia in multivariate analysis, altered mental status and chills were the only indices of the host response; these were much less powerful predictors of bacteremia than were "suspected or documented focal infection" and "no antibiotics before onset." Peduzzi et al. (120) reviewed 465 cases of severe sepsis in a VA Cooperative Study trial and found that a classification model that included elevated temperature, low systolic blood pressure and thrombocytopenia could predict bacteremia, but misclassification was also high (sensitivity of 5%). These studies provide little support for the notion that bacteremia, per se, contributes in any distinctive way to the clinical manifestations of severe sepsis.

5. The risk of developing severe sepsis differs according to the site of the primary infection. If circulating bacteria were the dominant factor that provoked severe sepsis, the site of primary infection might not be very important. Having invaded the bloodstream, the bacteria would multiply, release their pro-inflammatory products, and trigger harmful systemic responses in various organs. In fact, the large study of bacteremic patients by Brun-Buisson et al. (121) found that the site of infection was the second most important variable for predicting risk of severe sepsis; only the patient's age was more significant. When infection site was included in the analysis, the kind of microbe isolated from the blood was not a significant determinant of risk. These data suggest that inflammation within a site of infection, and not the particular microbe isolated from the blood, is the driving force that triggers and sustains severe sepsis. In general agreement with this conclusion, Leibovici (122) found that an "unknown" source of infection was associated with septic shock, as were blood isolates other than viridans streptococci (in particular, anaerobes and polymicrobial isolates). In many earlier series, mortality was significantly higher in patients with bacteremia arising from pneumonia or abdominal infection than in those with urosepsis (119;123;124). The high mortality associated with polymicrobial bacteremia in many case series may reflect its origin, in most instances, in the gastrointestinal tract. Indeed, the importance of the primary infection site in the pathogenesis of sepsis was appreciated in the pre-antibiotic era by Felty and

Primary infection site	Severe sepsis (%)	Severe sepsis (multivariate Relative Risk)	Death (Multivariate Relative Risk)
Lungs	33	2.2 (1.5-3.5)	1.1
Abdomen	40	3.1 (2.1-4.6)	1.2
Skin, soft tissue	27		
GU tract	14	0.4 (0.3-0.6)	0.4
IV catheter	19	1	1.1

In bacteremic patients, the risk of developing severe sepsis differs according to the primary site of infection. The risk of dying does not. From Brun-Buisson et al., 1996.

Keefer, who concluded their discussion of 28 cases of *E. coli* bacteremia by stating that **"of chief importance in prognosis is not the sepsis itself, but rather the extent, severity and location of the primary focus."** Why should the site of infection be so important? Although there is no certain answer, bacteria may be more likely to re-seed the circulation from foci in the lungs or abdomen; alternatively, the inflammation that arises in these organs may be more intense, or more likely to elicit systemic reactions.

Compartmentalization of inflammation: experimental evidence. Remarkably clear evidence for the role of local cytokine production in systemic responses was recently published by Kurahashi et al. (125). These workers were unable to induce shock when they continuously infused rabbits with a virulent strain of *P. aeruginosa.* In contrast, when they introduced the same strain into the lungs, pneumonia developed and shock occurred well before bacteremia could be detected. An avirulent mutant caused pneumonia but it did not induce shock, nor did it allow movement of radiolabeled TNF-α from the lungs into the circulation. Shock could be prevented by intravenous administration of an anti-TNF monoclonal antibody. Kurahashi et al. concluded that the virulent strain caused sufficient alveolar epithelial cell injury to allow TNF-α to escape into the circulation. With the virulent strain, circulating bacteria contributed nothing to the pathogenesis of shock--bacteremia was just a marker for severe *P. aeruginosa* pneumonia.

The presence of bacteria in the blood may be insufficient to cause shock. Movement of inflammatory mediators from an inflamed tissue into the blood may be required. In these cases, bacteremia (and endotoxemia) may just be a marker for uncontrolled local infection/inflammation.

Compartmentalization of infection and inflammation may be important for therapy. During the 1990's, two monoclonal antiendotoxin antibodies were tested in phase III clinical trials (126;127). Neither antibody reproducibly salvaged patients with Gram-negative bacteremia. If the engine that usually drives the septic reaction is infection/inflammation in local tissues, and not circulating bacteria or endotoxin, a therapy intended to kill bacteria in the bloodstream and neutralize circulating endotoxin should not be successful. Small molecule endotoxin antagonists might fare much better. Recognition that positive blood cultures are often markers for unresolved tissue infection/ inflammation should also encourage clinicians to look harder to find the "source."

Summary: a comparison of FM and nosocomial Gram-negative bacteremia. The most important differences are the prior status of the host (healthy vs. sick), the primary site of infection (intravascular vs. extravascular) and the existence of prior infection/inflammation at a local tissue site. FM is a poor model for the pathogenesis of severe sepsis due to other Gram-negative bacteria.

The striking differences between these two diseases make the important points that **(1) severe sepsis and septic shock do not have a single pathogenetic pathway, and (2) different interventions may be needed for patients at risk in different clinical settings.**

	Fulminant Meningococcemia	Bacteremia with commensal GN bacillus
Previously healthy subject	Yes	No
Primary infection	Intravascular	Extravascular
High levels of endotoxin in blood	Yes	No
Endotoxin in blood is stimulatory?	Probably	Probably not
Fraction of cases of GN bacteremia seen today	< 5%	>95%

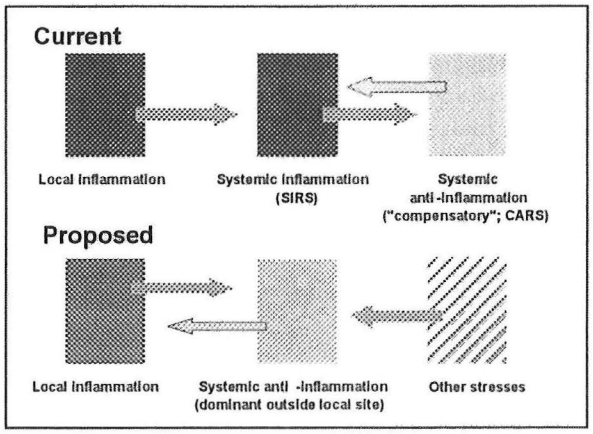
IV. Summary

The human body's innate immune responses to bacterial infection should be interpreted in light of (a) the physiological status of the host at the time infection occurs, (b) the nature of the infecting microbe, (c) the infection site, and (d) inherited variability in the local and systemic reactions to microbial invasion.

1. In general, the body's inflammatory responses to infection are compartmentalized. Local inflammation is accompanied by systemic changes that prevent inflammation elsewhere in the body.

2. The body's responses to microbial invasion are regulated by the hypothalamus and brain stem and integrated with responses to physical danger, starvation, and other stresses.

3. Normal systemic responses to injury, infection, and other stresses can be immunosuppressive.



4. There are normal mechanisms that limit these systemic responses, presumably to avoid life-threatening immunosuppression. These include "late" pro-inflammatory mediators, desensitization to catecholamines, damping the actions of glucocorticoids, and others. Exhaustion of the natural anticoagulants during the normal pro-coagulant response removes a major impediment to intravascular clotting, however, and no "late" anticoagulant appears to replace them; vasodilation may be a last-ditch mechanism for preventing arterial thrombosis.

5. In modern patients, dextrose infusion has effectively supplanted the evolution-based metabolic adaptations that maintain euglycemia in starving or stressed animals. Since hyperglycemia may be immunosuppressive, controlling it with intensive insulin therapy may reduce infection risk and improve survival from injury. Similarly, inflammation-induced pro-coagulant changes also serve no obvious function in most patients...might measures that blunt these changes without inducing hemorrhage help prevent severe sepsis in high risk patients?

6. When they've been activated by prior illness, the body's normal systemic responses limit the ability of circulating bacteria and their products to stimulate inflammatory reactions within the bloodstream. In patients whose blood cultures grow commensal Gram-negative bacteria, the bacteria often seem to be markers for uncontrolled infection in a local tissue site; inflammatory mediators arising in this site are the most likely stimuli for severe sepsis and septic shock, through currently unknown mechanisms. The best-understood exception is fulminant meningococcemia, an intravascular infection that occurs in previously well individuals with no local site of inflammation. Meningococcemia is a poor model for most other forms of Gram-negative bacteremia.

6. It's likely that genetic variability influences both local and systemic responses. Defining this variability should make it possible to apply preventive and therapeutic interventions more effectively.

Scientists ask questions at several levels. In biomedicine, "what" questions often lead to descriptions of newly-recognized phenomena. "How" questions explore mechanism, how things work, and prepare for new therapies. These are the stuff of serious investigation. "Why" questions are riskier, since answering them is more contextual and speculative. Nobody can pretend to understand why evolution occurred as it did, but one hopes that attempting to answer the "whys" of the human body will reward the foolhardy with insights that challenge existing dogma and, with luck, someday lead to new answers to "what" and "how."

(1) Ziegler EJ, McCutchan JA, Fierer J, Glauser MP, Sadoff JC, Douglas H et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant Escherichia coli. New Eng J Med 1982; 307:1225-1230.

(2) Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1987; 1:355-357.
(3) Cohen J. Meningococcal disease as a model to evaluate novel anti-sepsis strategies. Crit Care Med 2000; 28:S64-S67.
(4) Bone RC, Grodzin CJ, Balk RA. Sepsis: A new hypothesis for pathogenesis of the disease process. Chest 1997; 112:235-243.
(5) Cohen IL. Definitions for sepsis and organ failure: The ACCP/SCCM Consensus Conference Committee report. Chest 1993; 103:656.
(6) Vincent J-L. Dear SIRS, I'm sorry to say that I don't like you... Crit Care Med 1997; 25:372-374.
(7) Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). JAMA 1995; 273:117-123.
(8) Brandtzaeg P, Osnes L, Ovstebo R, Joo GB, Westvik A-B, Kieruf P. Net inflammatory capacity of human septic shock plasma evaluated by a monocyte-based target cell assay: identification of interleukin-10 as a major functional deactivator of human monocytes. J Exp Med 1996; 184:51-60.
(9) Lemaire LCJM, Van Lanschot JB, van der Poll T, Buurman WA, van Deventer SJH, Gouma DJ. Lymph of patients with a systemic inflammatory response syndrome inhibits lipopolysaccharide-induced cytokine production. J Infect Dis 1998; 178:883-886.
(10) Bone RC. Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med 1996; 24:163-172.
(11) Janeway CA, Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harbor Symp Quant Biol 1989; 54:1-13.
(12) Munford RS, Pugin J. The crucial role of systemic responses in the innate (non-adaptive) host defense. J Endotoxin Res 2001; 7:327-332.
(13) van der Poll T, Coyle SM, Barbosa K, Braxton CC, Lowry SF. Epinephrine inhibits tumor necrosis factor-α and potentiates interleukin 10 production during human endotoxemia. J Clin Invest 1996; 97:713-719.
(14) Barber AE, Coyle SM, Marano MA, Fischer E, Calvano SE, Fong Y et al. Glucocorticoid therapy alters hormonal and cytokine responses to endotoxin in man. J Immunol 1993; 150:1999-2006.
(15) Gore DC, Wolf SE, Herndon DN, Wolfe RR. Relative influence of glucose and insulin on peripheral amino acid metabolism in severely burned patients. JPEN J Parenter Enteral Nutr 2002; 26(5):271-277.
(16) Foster DW, McGarry JD. Glucose, lipid and protein metabolism. In: Griffin JE, Ojeda SR, editors. Textbook of Endocrine Physiology. New York: Oxford University Press, 2000: 393-420.
(17) Das UN. Is insulin an antiinflammatory molecule? Nutrition 2001; 17:409-413.
(18) Srinivasan S, Yeh M, Danziger EC, Hatley ME, Riggan AE, Leitinger N et al. Glucose regulates monocyte adhesion through endothelial production of interleukin-8. Circ Res 2003; 92:371-377.
(19) Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000; 21:55-89.
(20) Levi M, Ten Cate H. Disseminated intravascular coagulation. New Eng J Med 1999; 341:586-592.
(21) Levi M, van der Poll T, Ten Cate H, van Deventer SJH. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. Eur J Clin Invest 1997; 27:3-9.
(22) Pixley RA, De La Cadena R, Page JD, Kaufman N, Wyshock EG, Chang A et al. The contact system contributes to hypotension but not disseminated intravascular coagulation in lethal bacteremia. In vivo use of a monoclonal anti-factor XII antibody to block contact activation in baboons. J Clin Invest 1993; 91:61-68.
(23) Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 2000; 52:595-638.
(24) Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000; 405:458-462.
(25) Munford RS, Pugin J. Normal responses to injury prevent systemic inflammation and can be immunosuppressive. Am J Respir Crit Care Med 2001; 163:316-321.
(26) Christou NV, Meakins JL, Gordon J, Yee J, Hassan-Zahraee M, Nohr CW et al. The delayed hypersensitivity response and host resistance in surgical patients. 20 years later. Ann Surg 1995; 222:534-548.
(27) Livingston DH, Appel SH, Wellhausen SR, Sonnenfeld G, Polk HC, Jr. Depressed interferon gamma production and monocyte HLA-DR expression after severe injury. Arch Surg 1988; 123:1309-1312.
(28) Borrelli E, Roux-Lombard P, Grau GE, Girardin E, Ricou B, Dayer JM et al. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. Crit Care Med 1996; 24:392-397.
(29) Ertel W, Keel M, Neidhardt R, Steckholzer U, Kremer JP, Ungethuem U et al. Inhibition of the defense system stimulating interleukin- 12 interferon-gamma pathway during critical illness. Blood 1997; 89:1612-1620.
(30) Cohen S, Tyrrell DAJ, Smith AP. Psychological stress and susceptibility to the common cold. New Eng J Med 1991; 325:606-612.
(31) Nieman DC. Immune response to heavy exertion. J Appl Physiol 2000; 82:1385-1394.
(32) Kurz A, Sessler DI, Lenhardt R, for the Study of Wound Infection and Temperature Group. Perioperative normothermia to reduce the incidence of surgical wound infection and shorten hospitalization. New Eng J Med 1996; 334:1209-1215.
(33) Rivera-Chavez FA, Wheeler H, Lindberg G, Munford RS, O'Keefe GE. Regional and systemic cytokine responses to acute inflammation of the vermiform appendix. Ann Surg 2003; 237:408-416.
(34) Felty AR, Keefer CS. Bacillus coli sepsis. JAMA 1924; 82:1430-1433.

(35) Wakefield CH, Carey D, Foulds S, Monson JRT, Guillou PJ. Polymorphonuclear leudocyte activation. Arch Surg 1993; 128:390-395.
(36) Diamond J. Guns, Germs and Steel. New York: Norton, 1996.
(37) Bucala R. MIF rediscovered: cytokine, pituitary hormone, and glucocorticoid-induced regulator of the immune response. FASEB J 1996; 10:1607-1613.
(38) Calandra T, Echtenacher B, Le Roy D, Pugin J, Metz CN, Hültner L et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nature Med 2000; 6:164-170.
(39) Honma N, Koseki H, Akasaka T, Nakayama T, Taniguchi M, Serizawa I et al. Deficiency of the macrophage migration inhibitory factor gene has no significant effect on endotoxaemia. Immunology 2000; 100:84-90.
(40) Andersson U, Wang H, Palmblad K, Aveberger AC, Bloom O, Erlandsson-Harris H et al. High Mobility Group 1 Protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med 2000; 192(4):565-570.
(41) Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999; 285:248-251.
(42) Bhagat K, Collier J, Vallance P. Local venous responses to endotoxin in humans. Circulation 1996; 94:490-497.
(43) Van Woensel JBM, Biezeveld MH, Alders AMB, Eerenberg AJM, Endert E, Hack EC et al. Adrenocorticotropic hormone and cortisol levels in relation to inflammatory response and disease severity in children with meningococcal disease. Journal of Infectious Diseases 2001; 184:1532-1537.
(44) Schroeder S, Wichers M, Klingmüller D, Höfer M, Lehmann LE, von Spiegel T et al. The hypothalamic-pituitary-adrenal axis of patients with severe sepsis: altered response to corticotropin-releasing hormone. Crit Care Med 2001; 29:310-316.
(45) Soni A, Pepper GM, Wyrwinski PM, Ramirez NE, Simon R, Pina T et al. Adrenal insufficiency occurring during septic shock: Incidence, outcome, and relationship to peripheral cytokine levels. Am J Med 1995; 98:266-271.
(46) Oppert M, Reinicke A, Gräf K-J, Barckow D, Frei U, Eckardt K-U. Plasma cortisol levels before and during "low-dose" hydrocortisone therapy and their relationship to hemodynamic improvement in patients with septic shock. Intensive Care Med 2000; 26:1747-1755.
(47) Bollaert PE, Fieux F, Charpentier C, Levy B. Baseline cortisol levels, cortisol response to corticotropin, and prognosis in late septic shock. SHOCK 2003; 19(1):13-15.
(48) Marik PE, Zaloga GP. Adrenal insufficiency in the critically ill - A new look at an old problem. Chest 2002; 122:1784-1796.
(49) Briegel J, Schelling G, Haller M, Mraz W, Forst H, Peter K. A comparison of the adrenocortical response during septic shock and after complete recovery. Intensive Care Med 1996; 22(9):894-899.
(50) Molijn GJ, Koper JW, van Uffelen CJ, de Jong FH, Brinkmann AO, Bruining HA et al. Temperature-induced down-regulation of the glucocorticoid receptor in peripheral blood mononuclear leucocyte in patients with sepsis or septic shock. Clin Endocrinol (Oxf) 1995; 43(2):197-203.
(51) Nishio K, Akai Y, Murao Y, Doi N, Ueda S, Tabuse H et al. Increased plasma concentrations of adrenomedullin correlate with relaxation of vascular tone in patients with septic shock. Crit Care Med 1997; 25(6):953-957.
(52) Annane D, Sanquer S, Sébille V, Faye A, Djuranovic D, Raphaël JC et al. Compartmentalised inducible nitric-oxide synthase activity in septic shock. Lancet 2000; 355:1143-1148.
(53) Holmes CL, Patel BM, Russell JA, Walley KR. Physiology of vasopressin relevant to management of septic shock. Chest 2001; 120:989-1002.
(54) Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. New Eng J Med 2001; 345:588-595.
(55) Willis TMS, Hopp RJ, Romero JR, Larsen PD. The protective effect of brachial plexus palsy in purpura fulminans. Pediatr Neurol 2001; 24:379-381.
(56) Parmar MS. Symmetrical peripheral gangrene: a rare but dreadful complication of sepsis. Can Med Assoc J 2002; 167:1037-1038.
(57) Thomas L. The role of epinephrine in the reactions produced by the endotoxins of gram-negative bacteria. I. Hemorrhagic necrosis produced by epinephrine in the skin of endotoxin-treated rabbits. J Exp Med 1956; 104:865-880.
(58) Collins AD, Henson EC, Izard SR, Brunson JG. Norepinephrine, endotoxin shock, and the generalized Shwartzman reaction. Arch Pathol 1972; 93:82-88.
(59) Dünser MW, Mayr AJ, Ulmer H, Ritsch N, Knotzer H, Pajk W et al. The effects of vasopressin on systemic hemodynamics in catecholamine-resistant septic and postcardiotomy shock: A retrospective analysis. Anesth Analg 2001; 93:7-13.
(60) Annane D, Bellisant E, Sebille V, Lesieur O, Mathieu B, Raphael JC et al. Impaired pressor sensitivity to noradrenaline in septic shock patients with and without impaired adrenal function reserve. Br J Clin Pharmacol 1998; 46:589-597.
(61) Bellissant E, Annane D. Effect of hydrocortisone on phenylephrine-mean arterial pressure dose-response relationship in septic shock. Clin Pharmacol Ther 2000; 68:293-303.
(62) Bollaert P-E, Charpentier C, Levy B, Debouverie M, Audibert G, Larcan A. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. Crit Care Med 1998; 26:645-650.
(63) Briegel J, Forst H, Haller M, Schelling G, Kilger E, Kuprat G et al. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. Crit Care Med 1999; 27(4):723-732.
(64) Annane D, Sébille V, Charpentier C, Bollaert PE, François B, Korach JM et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002; 288:862-871.
(65) Zipser RD, Davenport MW, Martin KL, Tuck ML, Warner NE, Swinney RR et al. Hyperreninemic hypoaldosteronism in the critically ill: a new entity. J Clin Endocrinol Metab 1981; 53:867-873.
(66) Van den BG, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M et al. Intensive insulin therapy in critically ill patients. N Engl J Med 2001; 345(19):1359-1367.

(67) van den Berghe G, Wouters PJ, Bouillon R, Weekers F, Verwaest C, Schetz M et al. Outcome benefit of intensive insulin therapy in the critically ill: Insulin dose versus glycemic control. Crit Care Med 2003; 31(2):359-366.
(68) Hansen TK, Thiel S, Wouters PJ, Christiansen JS, Van den BG. Intensive insulin therapy exerts antiinflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. J Clin Endocrinol Metab 2003; 88(3):1082-1088.
(69) Campos SP, Baumann H. Insulin is a prominent modulator of the cytokine-stimulated expression of acute-phase plasma protein genes. Mol Cell Biol 1992; 12:1789-1797.
(70) van den Berghe G, Bouillon R, Lauwers P. Intensive insulin therapy in criticall ill patients. New Eng J Med 2002; 346:1587-1588.
(71) Gore DC, Chinkes D, Heggers J, Herndon DN, Wolf SE, Desai M. Association of hyperglycemia with increased mortality after severe burn injury. J Trauma 2001; 51(3):540-544.
(72) Zerr KJ, Furnary AP, Grunkemeier GL, Bookin S, Kanhere V, Starr A. Glucose control lowers the risk of wound infection in diabetics after open heart operations. Ann Thorac Surg 1997; 63(2):356-361.
(73) Rassias AJ, Givan AL, Marrin CA, Whalen K, Pahl J, Yeager MP. Insulin increases neutrophil count and phagocytic capacity after cardiac surgery. Anesth Analg 2002; 94(5):1113-9, table.
(74) Herndon DN, Hart DW, Wolf SE, Chinkes DL, Wolfe RR. Reversal of catabolism by beta-blockade after severe burns. New Eng J Med 2001; 345:1223-1229.
(75) van der Poll T, Levi M, Dentener M, Jansen PM, Coyle SM, Braxton CC et al. Epinephrine exerts anticoagulant effects during human endotoxemia. J Exp Med 1997; 185:1143-1148.
(76) Wasserman D, Ioannovich JD, Hinzmann RD, Deichsel G, Steinmann GG, for The Severe Burns Study Group. Interferon-γ in the prevention of severe burn-related infections: A European phase III multicenter trial. Crit Care Med 1998; 26:434-349.
(77) Dries DJ. Interferon-γ therapy for infectious complications of injury: A called third strike? Crit Care Med 1998; 26:419-420.
(78) Hershman MJ, Appel SH, Wellhausen SR, Sonnenfeld G, Polk HC, Jr. Interferon-gamma treatment increases HLA-DR expression on monocytes in severely injured patients. Clin Exp Immunol 1989; 77:67-70.
(79) Liappis AP, Kan VL, Rochester CG, Simon GL. The effect of statins on mortality in patients with bacteremia. Clin Infect Dis 2001; 33:1352-1357.
(80) Warren BL, Eid A, Singer P, Pillay SS, Carl P, Novak I et al. High-dose a randomized antithrombin III in severe sepsis - A randomized controlled trial. JAMA 2001; 286:1869-1878.
(81) Bernard GR, Vincent JL, Laterre P, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. New Eng J Med 2001; 344:699-709.
(82) Zanetti G, Glauser MP, Baumgartner JD. Use of immunoglobulins in prevention and treatment of infection in critically ill patients: review and critique. Rev Infect Dis 1991; 13:985-992.
(83) Agnese DM, Calvano JE, Hahm SJ, Coyle SM, Corbett SA, Calvano SE et al. Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. Journal of Infectious Diseases 2002; 186:1522-1525.
(84) Lorenz E, Mira JP, Frees KL, Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. Arch Int Med 2002; 162:1028-1032.
(85) Mira JP, Cariou A, Grall F, Delclaux C, Losser M-R, Heshmati F et al. Association of *TNF2*, a TNF- α promoter polymorphism, with septic shock susceptibility and mortality. JAMA 1999; 282:561-568.
(86) Tang GJ, Huang SL, Yien HW, Chen WS, Chi CW, Wu CW et al. Tumor necrosis factor gene polymorphism and septic shock in surgical infection. Crit Care Med 2000; 28:3090-3091.
(87) O'Keefe GE, Hybki DL, Munford RS. The G-->A single nucleotide polymorphism at the -308 position in the tumor necrosis factor-α promoter increases the risk for severe sepsis. J Trauma 2002; 52:817-826.
(88) Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-α concentrations and outcome of patients with severe sepsis. Crit Care Med 1996; 24:381-384.
(89) Faust SN, Levin M, Harrison OB, Goldin RD, Lockhart MS, Kondaveeti S et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. New Eng J Med 2001; 345:408-416.
(90) Van der Pol WL, Huizinga TWJ, Vidarsson G, Van der Linden MW, Jansen MD, Keijsers V et al. Relevance of Fcgamma receptor and interleukin-10 polymorphisms for meningococcal disease. Journal of Infectious Diseases 2001; 184:1548-1555.
(91) Domingo P, Muñiz-Diaz E, Baraldès MA, Arilla M, Barquet N, Pericas R et al. Associations between Fc gamma receptor IIA polymorphisms and the risk and prognosis of meningococcal disease. Am J Med 2002; 112:19-25.
(92) Westendorp RGJ, Hottenga JJ, Slagboom PE. Variation in plasminogen-activator-inhibitor-1 gene and risk of meningococcal septic shock. Lancet 1999; 354:561-563.
(93) Hermans PWM, Hibberd ML, Booy R, Daramola O, Hazelzet JA, De Groot R et al. *4G/5G* promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Lancet 1999; 354:556-560.
(94) Nadel S, Newport MJ, Booy R, Levin M. Variation in the tumor necrosis factor-α gene promoter region may be associated with death from meningococcal disease. J Infect Dis 1996; 174:878-880.
(95) Read RC, Camp NJ, Di Giovine FS, Borrow R, Kaczmarski EB, Chaudhary AGA et al. An interleukin-1 genotype is associated with fatal outcome of meningococcal disease. J Infect Dis 2000; 182:1557-1560.

(96) Keijsers V, Verweij C, Westendorp R, Gibson A, Kimberly R, Breedveld F et al. IL-10 promoter haplotypes: discrepancy between lack of functionality in transfection assays and skewed distribution in meningococcal disease. Genes and Immunity 1999; 1:78-79.
(97) Sands KE, Bates DW, Lanken PN, Graman PS, Hibberd PL, Kahn KL et al. Epidemiology of sepsis syndrome in 8 academic medical centers. JAMA 1997; 278:234-240.
(98) Brun-Buisson C, Doyon F, Carlet J, Dellamonica P, Gouin F, Lepoutre A et al. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults - A multicenter prospective study in intensive care units. JAMA 1995; 274:968-974.
(99) Bates DW, Sands K, Miller E, Lanken PN, Hibberd PL, Graman PS et al. Predicting bacteremia in patients with sepsis syndrome. J Infect Dis 1997; 176:1538-1551.
(100) Jones GR, Lowes JA. The systemic inflammatory response syndrome as a predictor of bacteraemia and outcome from sepsis. Q J Med 1996; 89:515-522.
(101) Harris JA, Cobb CG. Persistent gram-negative bacteremia. Observations in twenty patients. Am J Surg 1973; 125:705-717.
(102) Rogers DE. Host mechanisms which act to remove bacteria from the blood stream. Bacteriol Rev 1960; 24:50-66.
(103) DuPont HL, Spink WW. Infections due to Gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958-1966. Medicine 1969; 48:307-332.
(104) Kreger BE, Craven DE, Carling PC, McCabe WR. Gram-negative bacteremia. III. Reassessment of etiology, epidemiology and ecology in 612 patients. Am J Med 1980; 68:332-343.
(105) Kellogg JA, Bankert DA, Manzella JP, Parsey KS, Scott SL, Cavanaugh SH. Occurrence and documentation of low-level bacteremia in a community hospital's patient population. Am J Clin Pathol 1995; 104(5):524-529.
(106) Whimbey E, Kiehn TE, Brannon P, Benezra D, Armstrong D. Clinical significance of colony counts in immunocompromised patients with *Staphylococcus aureus* bacteremia. J Infect Dis 1987; 155:1328-1330.
(107) Butler T, Levin J, Linh NN, Chau DM, Adickman M, Arnold K. Yersinia pestis infection in Vietnam. II. Quantiative blood cultures and detection of endotoxin in the cerebrospinal fluid of patients with meningitis. J Infect Dis 1976; 133(5):493-499.
(108) Shenep JL, Flynn PM, Barrett FF, Stidham GL, Westenkirchner DF. Serial quantitation of endotoxemia and bacteremia during therapy for gram-negative bacterial sepsis. J Infect Dis 1988; 157:565-568.
(109) Meers PD, Calder MW, Mazhar MM, Lawrie GM. Intravenous infusion of contaminated dextrose solution: The Deveonport incident. Lancet 1973; 2(7839):1189-1192.
(110) Maki DG, Rhame FS, Mackel DC, Bennett JV. Nationwide epidemic of septicemia caused by contaminated intravenous products I. Epidemiologic and clinical features. Am J Med 1976; 60:471-485.
(111) Maki DG. Nosocomial bacteremia. An epidemiologic overview. Am J Med 1981; 70:719-732.
(112) Kreger BE, Craven DE, McCabe WR. Gram-negative bacteremia. IV. Re-evaluation of clinical features and treatment in 612 patients. Am J Med 1980; 68:344-355.
(113) Phillips I, Eykyn S, Laker M. Outbreak of hospital infection caused by contaminated autoclaved fluids. Lancet 1972; 1(7763):1258-1260.
(114) Ostrowsky BE, Whitener C, Bredenberg HK, Carson LA, Holt S, Hutwagner L et al. Serratia marcescens bacteremia traced to an infused narcotic. N Engl J Med 2002; 346(20):1529-1537.
(115) Grohskopf LA, Roth VR, Feikin DR, Arduino MJ, Carson LA, Tokars JI et al. Serratia liquefaciens bloodstream infections from contamination of epoetin alfa at a hemodialysis center. N Engl J Med 2001; 344(20):1491-1497.
(116) Bates DW, Pruess KE, Lee TH. How bad are bacteremia and sepsis? Outcomes in a cohort with suspected bacteremia. Arch Intern Med 1995; 155:593-598.
(117) Rello J, Ochagavia A, Sabanes E, Roque M, Mariscal D, Reynaga E et al. Evaluation of outcome of intravenous catheter-related infections in critically ill patients. Am J Respir Crit Care Med 2000; 162(3 Pt 1):1027-1030.
(118) DiGiovine B, Chenoweth C, Watts C, Higgins M. The attributable mortality and costs of primary nosocomial bloodstream infections in the intensive care unit. Am J Respir Crit Care Med 1999; 160(3):976-981.
(119) Weinstein MP, Murphy JR, Reller LB, Lichenstein KA. The clinical significance of positive blood cultures: A comprehensive analysi of 500 episodes of bacteremia and fungemia in adults. II. Clinical observations, with special reference to factors influencing prognosis. Rev Infect Dis 1983; 5:54-70.
(120) Peduzzi P, Shatney C, Sheagren J, Sprung C, VA Syst Sepsis Coop Study Group. Predictors of bacteremia and gram-negative bacteremia in patients with sepsis. Arch Intern Med 1992; 152:529-535.
(121) Brun-Buisson C, Doyon F, Carlet J, Bedock B, Annonay CH, Valente E et al. Bacteremia and severe sepsis in adults: A multicenter prospective survey in ICUs and wards of 24 hospitals. Am J Respir Crit Care Med 1996; 154:617-624.
(122) Leibovici L, Greenshtain S, Cohen O, Mor F, Wysenbeek AJ. Bacteremia in febrile patients. A clinical model for diagnosis. Arch Intern Med 1991; 151(9):1801-1806.
(123) Bryan CS, Reynolds KL, Brenner ER. Analysis of 1,186 episodes of gram-negative bacteremia in non-university hospitals: the effects of antimicrobial therapy. Rev Infect Dis 1983; 4:629-638.
(124) McCue JD. Improved mortality in gram-negative bacillary bacteremia. Arch Int Med 1985; 145:1212-1216.
(125) Kurahashi K, Kajikawa O, Sawa T, Ohara M, Gropper MA, Frank DW et al. Pathogenesis of septic shock in *Pseudomonas aeruginosa* pneumonia. J Clin Invest 1999; 104:743-750.
(126) Ziegler EJ, Fisher CJ, Jr., Sprung CL, Straube RC, Sadoff JC, Foulke GE et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin--A randomized, double-blind, placebo-controlled trial. N Engl J Med 1991; 324:429-436.
(127) Angus DC, Birmingham MC, Balk RA, Scannon PJ, Collins D, Kruse JA et al. E5 murine monoclonal antiendotoxin antibody in gram-negative sepsis - A randomized controlled trial. JAMA 2000; 283:1723-1730.