SEPSIS:
NEW BIOLOGY FOR AN OLD DISEASE

Lance S. Terada, MD
Internal Medicine Grand Rounds
University of Texas Southwestern
The Dallas VA
August 22, 2002

This statement acknowledges that Lance S. Terada, MD has served as an honoraria for Eli Lily Corporation. Dr. Terada will be discussing off-label drug uses in his presentation.

Lance S. Terada, MD
Professor of Internal Medicine,
Division of Pulmonary Medicine

Research Interests: Mechanisms of oxidant-related signal transduction in vascular endothelium, relevant to acute inflammation, HIV vasculopathic disorders, and angiogenesis.

I. Sepsis: Definitions determine scientific direction

By some reckoning, the modern field of sepsis biology started exactly 11 years ago in Chicago, August 1991, with the proposal of a consensus definition of sepsis and related conditions (bacteremia, severe sepsis, septic shock, multiple organ dysfunction syndrome, and a new term, systemic inflammatory response syndrome) [1]. The core definition of sepsis applied to infected patients with at least two of the hallmark clinical features; fever (or hypothermia), tachycardia, tachypnea (or hyperventilation), and leukocytosis (or leukopenia or bandemia). Although the definition invoked only clinical signs, a clear effort was made to provide a conceptual framework for defining sepsis as "the systemic inflammatory response to infection" [2]. Sharper resolution of human inflammation into the interlocking innate and adaptive immune responses led to the well-accepted mechanistic definition of sepsis as representing a broad dysregulation of innate immunity with consequent activation of systemic inflammatory signals (Figure 1). Sepsis research over the subsequent decade has been dominated by this central paradigm, begging the question: How helpful has this paradigm been in making inroads towards a reduction in sepsis mortality?

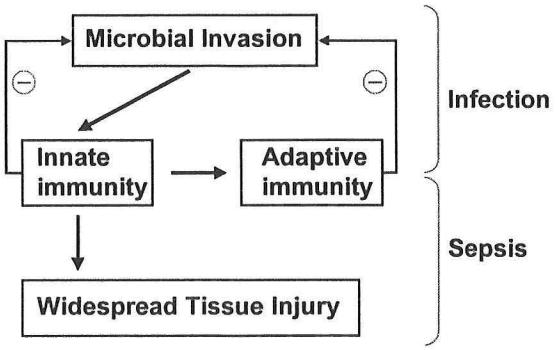

Figure 1: Current working model of sepsis etiology.

The innate immune response developed in primitive metazoans, presumably long before the appearance of adaptive immunity. The evolutionary roots of human sepsis can therefore at least in part be traced back to the innate response of the well-studied Dipterin, *D. melanogaster*. Upon invasion of this fly with a pathogenic microbe, at least three distinct pathways are initiated. The earliest response is the activation of two proteolytic pathways which lead to coagulation and melanization, respectively. Coagulation is thought to present a rapid containment mechanism which seals off the primitive hemolymphatic system from further microbial invasion, whereas activation of phenoloxidase generates microbicidal oxidants and melanin. Second, professional phagocytes are recruited and activated, and lastly, a battery of antibacterial and antifungal agents are synthesized *de novo*, largely in the fat body, a functional homolog of our liver.

In overall scope, the human innate response closely patterns that of the fly. Ligation of a limited number of pattern recognition receptors triggers the activation of two related proteolytic cascades, those of coagulation and complement (reviewed in [3]). While the adaptive value of coagulation may still lie in the sequestering of infecting microbes, a clearly pathologic vestige of this response occurs during disseminated intravascular coagulation (DIC). Similar to the *Drosophila* response, granulocytes and monocytes are recruited through specific adhesion molecules and are activated through both endogenous and microbial ligands. This cellular response defines acute inflammation, which again appears to be overactivated in the progression of sepsis. Lastly, transactivation of a large number of genes leads to the *de novo* production of microbicidal and intercellular signaling proteins, typified by cytokines and chemokines. Many if not most of these acute

phase genes are transactivated by NF-κB, receiving upstream signals from innate immunity receptors such as Toll-like receptor (TLR)-4 (endotoxin) and the IL-1 receptor [4]. Interestingly, the Drosophila orthologs of this pathway, leading from the Toll receptor to the Rel family member Dorsal, were originally identified by their ability to control dorso-ventral patterning of the developing fly larva. Only later were mutants of Toll identified which allowed normal development but abrogated the innate immune response to fungi [5], thus providing further evolutionary evidence for the prominence of NF-κB in innate immunity.

In complex vertebrate systems, perhaps more so than in the fly, the various arms of innate immune defense appear to feed back and regulate each other in both positive and negative fashion, hence presenting multiple opportunities for dysregulation with resultant emergence of sepsis. Conversely, intervention with one arm of innate immunity has been assumed to decrease activation of the other arms, thus forming the rationale for current therapeutic trials in sepsis.

II. Coagulation: Vicious cycle or local phenomenon?

In cases where infection leads to sepsis, coagulation is often triggered in many organs in a diffuse rather than localized pattern, leading to DIC. That DIC can result from activation of innate immunity is supported by studies of normal humans injected with the prototypical TLR4 ligand endotoxin. The response includes not only signs of sepsis (fever, tachycardia) and CNS depression (sleepiness, mild amnesia) but also activation of thrombin (a rise in circulating thrombin/antithrombin complexes and in the F1+2 prothrombin fragment) [6].

DIC affords an attractive target for clinical intervention for several reasons. First, its severity correlates roughly with the severity of sepsis, and secondly, DIC is a sustained rather than transient process, offering in theory a wide window of time for pharmacologic intervention (although in practice this does not appear correct). In addition, its final common mediator, thrombin, has direct proinflammatory effects independent of its ability to promote thrombosis. Thrombin, for instance, directly stimulates peripheral blood mononuclear cells and endothelial cells to release the potent neutrophil chemokine IL-8 and the procoagulant cytokine IL-6 [7], and stimulates endothelial cells to activate NF-κB with subsequent expression of leukocyte adhesion proteins such as ICAM-1 and E-selectin [8]. Thrombin also initiates rapid signaling in endothelial cells such as the translocation of P-selectin to the surface of cells and synthesis of platelet activating factor (PAF).

To directly stimulate immune-modulating cells such as mononuclear and endothelial cells, a thrombin receptor was hypothesized and subsequently cloned [9]. Eventually, a family of protease-activated receptors (PAR) was identified, with thrombin specifically activating PAR1,3, and 4 [10]. PAR1's amino-terminal exodomain was found to harbor a thrombin cleavage site, and crystallographic studies confirmed binding of thrombin's active site to this motif. Cleavage at this site released the N-terminal 41 residues, exposing a new N-terminal peptide with high affinity for the receptor-binding site. The formation of such a tethered ligand was found to be a general mechanism of receptor activation for the PARs, with downstream activation of various signaling pathways including p38 MAPK, PKC, DAG, and NF-κB [11].

The proinflammatory effects of coagulation, as typified by the effects of

thrombin on vascular and mononuclear cells, therefore suggested a "vicious cycle" effect whereby inflammation increased thrombosis, and thrombotic factors perpetuated inflammatory signaling. Several attempts have therefore been made to interrupt this cycle by blocking procoagulant factors.

One attempt has been to utilize the endogenous thrombin antagonist, antithrombin III (presently antithrombin, AT). AT targets endothelium-associated thrombin by simultaneous binding to cell-surface glycosaminoglycans or heparans and thrombin, thereby interrupting both fibrinogen cleavage and PAR signaling. AT has other effects *in vitro*, such as binding to the neutrophil receptor syndecan-4, reducing chemotaxis. Preclinical animal studies have generally supported the use of recombinant AT (rAT) in sepsis. For instance, rAT given 30 min prior to endotoxin improves lung injury and decreases TNF levels in rats [12, 13]. Oddly enough, rAT given 1 h prior to a lethal *E.coli* challenge in baboons improved DIC and mortality yet increased TNF levels [14], suggesting that coagulation and cytokine release are perhaps not so tightly coupled.

The use of rAT in sepsis was tested in the large phase III KyberSept trial, a randomized, placebo-controlled study of 2314 patients [15]. rAT treatment was initiated early and infused over 4 d, yet did not improve 28 d mortality (38.9% rAT, 38.7% placebo). A post-hoc analysis suggested a significant effect for patients not receiving exogenous heparin, raising concerns about the ability of exogenous heparin to delocalize rAT off of the vascular surface. However, this effect was not seen at 28 d (only 90 d), and the heparin-related effect has not been prospectively studied. Although rAT is presently FDA approved for AT deficiency, its off-label use in sepsis cannot be recommended at this point.

An alternative target for intervention in sepsis has been the tissue factor (TF) complex. Traditionally considered an extravascular protein, TF activates plasma factor VII when the vasculature is breached, to initiate coagulation through the extrinsic system. More recently, the TF/VIIa complex was found to activate factor IX, thus initiating the intrinsic pathway as well. A conceptual link to sepsis was established when TF was found to be induced on the surface of endothelial cells by a number of factors *in vitro* including endotoxin, IL-1β, aggregated IgG, and Ifn-γ [16-19] and in splenic microvascular endothelial cells in septic baboons *in vivo* [20]. In addition, inflammatory cytokines increase exocytotic budding of TF-rich microparticles from endothelial cells, platelets, and granulocytes. These microparticles circulate in meningococcal sepsis and initiate coagulation *in vitro* [21].

Like thrombin, the TF/VIIa complex initiates inflammatory signaling independent of its ability to propagate coagulation. Through the recruitment and activation of Xa, the cell surface-bound TF/VIIa/Xa complex recognizes, cleaves, and therefore activates a different PAR family member, PAR2 [22]. As with thrombin, TF/VIIa initiates a broad array of signaling events, including Ca^{2+} transients, MAPK activation, Src kinase family activation, and PI3K and Akt activation [18, 22-24]. Again, a "vicious cycle" is envisioned whereby cytokines increase TF production, which in turn activates inflammatory cells. Indeed, antibodies against TF improve mortality in the baboon *E.coli* sepsis model [25].

Accordingly, the endogenous TF antagonist Tissue Factor Pathway Inhibitor (TFPI) has been investigated as a potential anti-sepsis agent. TFPI is a serine protease inhibitor (serpin) which first binds Xa, then complexes with the TF/VIIa complex, and therefore

inhibits thrombin formation and presumably Xa-dependent PAR2 signaling. In preclinical animal studies, rTFPI appears to afford protection if given early. For instance, when given 30 min after *E.coli*, rTPFI greatly improved baboon mortality [26]. However, delaying rTFPI infusion to 4 h after bacterial challenge worsened mortality considerably. Likewise, the mortality of septic rabbits worsens considerably if rTFPI administration is delayed from 4 to 6 h post-infection [27]. Further, although rTFPI reduces TNF levels in endotoxemic rats [28], this antiinflammatory effect may not extend to humans. In normal volunteers, rTFPI completely blocks endotoxin-induced thrombin activation, yet has no effect on TNF, IL-8, MIP-1β, and MCP-1 levels, or markers of neutrophil or endothelial cell activation [29, 30]. Importantly, rTFPI also did not block the clinical signs of sepsis resulting from endotoxemia. Despite these observations, the potential of rTFPI to decrease inflammation during sepsis was strongly suggested by a large body of preclinical work, leading to its development as a pharmacologic agent. The phase II study or rTFPI in severe sepsis was not powered for mortality, but showed a nonsignificant 20% relative reduction in 28 d mortality [31]. However, the phase III trial completed last year apparently did not show a mortality benefit (Chiron press release, 11/01), and rTFPI is presently not recommended for sepsis.

A third anticoagulant developed for sepsis is activated protein C (APC). An early observation which suggested the utility of APC in sepsis was that dogs placed on ECMO developed an endogenous anticoagulant which imparted resistance to subsequent challenge with *E.coli* [32]. The anticoagulant was subsequently identified as APC. Upon thrombus formation, the inactive zymogenic protein C is normally recruited to the vascular surface through the Endothelial cell Protein C Receptor (EPCR), while thrombin localizes to the same site through the transmembrane protein thrombomodulin. A tetrameric complex then forms, allowing cleavage of protein C to APC [33], which in concert with protein S cleaves and inactivates factors VIIIa and Va and inhibits the antifibrinolytic agents PAI-1 and TAFI. Formation of APC therefore constitutes an important negative feedback loop for the coagulation pathway.

Other observations suggested a potential link between APC and outcome in sepsis. First, protein C deficiency was found to correlate with the onset of the highly lethal purpura fulminans in meningococcal sepsis, with shock in septic neutropenic patients, and with mortality in septic shock [34-36]. Second, in animal models of sepsis, a deficiency of APC worsens outcome. Heterozygous protein C knockout mice are more susceptible to endotoxin, for instance (M. Levi, unpublished) and impairment of activation of zymogenic protein C with antibodies against EPCR worsens outcome in the baboon *E.coli* sepsis model [37]. Finally, TNF decreases thrombomodulin expression by endothelial cells *in vitro* and *in vivo* in humans [38, 39], and both thrombomodulin and EPCR are decreased in immunostains of the endothelium in patients with meningococcal sepsis [40], suggesting impairment in the mechanism of activation of protein C.

Similar to thrombin and the TF/VIIa/Xa complex, APC appears to initiate endothelial and mononuclear cell signaling independent of its effects on the coagulation pathway. In contrast to thrombin and TF, however, the downstream effects of APC signaling appear to be antiinflammatory for the most part. APC inhibits endotoxin and Ifn-γ-induced production of TNF and assembly of Mac-1 in monocytes, and blocks TNF-induced activation

of NF-κB in monocytic and endothelial cells [11, 41, 42]. Gene profiling reveals a broad effect of APC signaling on endothelial cells, with a decrease in expression of adhesion proteins (ICAM-1, E-selectin) and immune modulators (MHC ClassI, LT-β, CX3C) and an upregulation of various antiapoptotic and prosurvival genes [11]. Recently, such antiinflammatory signaling was found to proceed through activation of the thrombin receptor PAR1 [43], raising interesting questions concerning the basis for signal specificity. *In vivo*, APC has been shown to decrease endotoxin-induced lung injury and TNF secretion [44, 45].

The ability of APC to decrease mortality in human sepsis was tested in the PROWESS study, which showed a significant improvement in 28-d mortality (30.8% placebo, 24.7% APC), supporting its use in severe sepsis [46]. Concerns over study procedural matters have clouded interpretation of the data, however. In particular, enrollment criteria were changed approximately mid-way through the study including institution of further exclusions. For example, "moribund" patients were now excluded. Although the intent was to minimize enrollment of patients dying from non-sepsis-related causes, the actual effects of the mid-term amendments are unclear. There are notable differences in 28-d mortality and the prevalence of metabolic acidosis between the pre- and post-amendment groups, for instance, whereas the difference between groups of non-sepsis-related deaths was minimal (5% vs 4%)[47].

Taken together, the anticoagulation strategies against sepsis have not proven to impart as substantial a mortality benefit as hoped for. While it is possible that the hypothesis is correct but that a series of unfortunate logistical problems have confounded analysis, a reevaluation of the extent to which a "vicious cycle" contributes to sepsis seems warranted. Does inflammation really augment DIC in a substantive way or does DIC reflect a more direct activation of coagulation by the innate immune system, as it does in flies? While abundant data support the activation of local coagulation by local inflammation, the evidence for a systemic connection is weaker. Primates injected with endotoxin, for instance, develop a prominent cytokine response and a DIC-like picture: antibodies against TNF decrease TNF, IL-6 and IL-8 levels but have no effect on thrombin generation and in fact suppress the fibrinolytic system, worsening the procoagulant profile [48]. Conversely, intradermal injection of TNF increases adhesion protein expression and leukocyte sequestration in humans, but does not elicit thrombosis [38]. Similarly, severely neutropenic patients, who by definition have little or no acute inflammatory response, develop severe DIC and robust thrombin activation [49], suggesting a separation of DIC from inflammation.

On the reverse side of the vicious cycle, does thrombosis perpetuate systemic inflammation? As mentioned above, rTFPI even given early completely blocks activation of the coagulation system by endotoxin in humans, yet has absolutely no effect on the release of a number of proinflammatory cytokines or markers of neutrophil and endothelial cell activation [29, 30]. Similar studies in endotoxemic chimpanzees demonstrate that antibodies against IL-6 potently decrease thrombin generation but have no effect on the release of TNF and IL-8 [50]. The difficulty encountered by groups trying to demonstrate a survival advantage with anticlotting agents may reflect a limited role for DIC in perpetuating many cases of sepsis. The case for treatment with such agents has often been developed using intensely DIC-driven

models such as baboon *E.coli* sepsis, which in some respects is akin to human meningococcemia. Ultimately, antithrombotic therapy may find maximum utility in the treatment of that part of the spectrum of human sepsis associated with such severe DIC.

III. Anti-leukocyte strategies: A double-edged sword

The histologic hallmarks of acute inflammation–congregation of inflammatory cells and tissue injury–reflect leukocyte recruitment and activation, respectively. To the extent that sepsis is considered an inflammatory disorder, leukocytes become potential pharmacologic targets. The science of leukocyte trafficking has advanced considerably in recent years, and the recruitment and activation of inflammatory cells is now understood to occur simultaneously. *In vivo*, the leukocyte must overcome tremendous shear and channeling forces to exit the vascular space. A rapid reduction in deformability facilitates entrapment in the narrow lung capillaries, but in most other organs low-affinity bonds form through selectin family interactions and mediate leukocyte rolling. This rolling behavior diminishes leukocyte velocity and also provides extensive opportunity for communication between leukocyte and endothelium. An example of such cross-talk occurs when endothelial cells expose the tethering molecule P-selectin and the leukocyte activator platelet activating factor (PAF) on their surfaces, thus leading to a juxtacrine activation of tethered leukocytes in part through activation of the innate response modulator NF-κB [51]. Oxidatively stressed endothelium, as occurs in abdominal sepsis, appears to precipitate a similar leukocyte response [52, 53], and endotoxin also invokes a collaborative recruitment and activation by P-selectin and PAF in rats [54]. Subsequent firm adhesion is then facilitated by β_2 integrins such as Mac-1.

In some experimental injury models, interference with leukocyte adhesion has been highly successful in reducing tissue injury. Most studies have employed blocking antibodies against the common β_2 integrin subunit CD18 in reperfusion or shock models [55]. However, sepsis models have been less encouraging. Although some reports suggest a decrease in lung injury or CNS inflammation in animal models of bacterial sepsis [56, 57], abrogation of β_2 integrin function in knockout mice worsens the mortality of pneumococcal sepsis [58]. Since directing leukocyte traffic into an infected region constitutes an action critical to the protective antimicrobial function of innate immunity, these results appear intuitive and seem to sound a clear warning against the use of anti-adhesion strategies in sepsis.

A reasonable alternate strategy, however, would be to interfere with the non-bacterial activation of leukocytes, as for instance through PAF. PAF is synthesized by both endothelium and platelets, and the PAF receptor is expressed by platelets, mononuclear cells, and granulocytes. Interestingly, autocrine signaling of platelets through PAF initiates *de novo* protein synthesis, including the cytokines IL-1β and IL-10 [59]. Gene profiling of the enucleate platelet reveals that a number of mRNA transcripts are preserved, ready to be translated into protein upon platelet stimulation. Local production of cytokines such as IL-1β in the region of a thrombus may then increase inflammatory cell recruitment and activation through its effects on the adjacent endothelium. Further evidence of a role for PAF in sepsis stems from the observation that the PAF receptor overexpressing transgenic mouse has

heightened lethality to endotoxin [60]. This mouse also displays sporadic hypermelanization, reminiscent of the ancient *Drosophila* phenoloxidase response to infection.

Despite these encouraging data, a phase III study of the PAF receptor antagonist BN 52021 failed to significantly improve 28 d mortality [61]. Although a post-hoc subgroup analysis suggested a significant beneficial response in gram negative sepsis, a subsequent prospective study failed to find benefit of BN 52021 in this septic subpopulation [62]. An alternative strategy presently in development is to use the endogenous PAF-catabolizing enzyme, PAF acetylhydrolase (PAF-AH). Recombinant PAF-AH decreases vascular leakage in inflammatory models [63] and improves mortality in rodent abdominal sepsis (G. Zimmerman, unpublished), and PAF-AH activity is significantly lower in septic patients [64]. A phase II trial of recombinant PAF-AH (Pafase) in severe sepsis was recently reported in abstract form, and demonstrated efficacy of the compound with improvement of 28 d mortality (placebo 19/43=44.2%, Pafase 1 mg/kg 9/42=21.4% $p<0.05$, Pafase 5 mg/kg 11/39=28.2% NS)[65]. An interesting dose effect, whereby protection was lost at higher dose, was also reflected in a nonsignificant worsening of mortality in a parallel trauma group with high-dose Pafase. The compound is presently undergoing phase III trials (ICOS).

IV. Anti-cytokine therapy: An example of complexity theory?

The induction of innate immunity response genes which are broadly anti-microbial includes the synthesis and release of cytokines. Overwhelming clinical and preclinical data support the participation of such cytokines in the pathogenesis of the sepsis response. The most intensely studied of these, TNF and IL-1β, have been targeted in clinical trials. Plasma TNF levels rise abruptly following endotoxin injection, and high levels predict mortality in sepsis [6, 66, 67]. TNF is a strong inducer of NF-κB activity, and initiates synthesis of adhesion proteins, cytokines, and chemokines. Although definitive proof of TNF's role in promoting human sepsis is lacking, genetic studies provide strong support for this contention. The TNF2 polymorphism (G-308A), for instance, which increases TNF expression [68, 69], is found in septic shock more frequently than in the general population (39% vs 18%)[70]. Likewise, homo- or heterozygosity for the TNF2 allele worsens mortality in septic shock by 3.75-fold [70]. Interestingly, another polymorphism in the adjacent gene for lymphotoxin-α (*TNFB*) is also associated with increased TNF levels in septic patients and is strongly associated with sepsis mortality [71]. Despite the wealth of basic and clinical data implicating TNF, however, all anti-TNF strategies, including both humanized monoclonal and TNFR-IgG fusion compounds, have failed to improve survival in sepsis [72-75].

A similar case can be made for IL-1β, which has numerous proinflammatory effects *in vitro* and *in vivo*. A recent genetic study also strongly implicated the IL-1 gene cluster as being linked to sepsis mortality. In this latter study, a restriction site polymorphism upstream of the IL-1β gene (-511 of *IL-1B*) correlated with mortality, with B2 allele homozygotes having an 81% mortality rate and B1 allele homozygotes bearing a 0% mortality [76]. An exonic polymorphism in the adjacent gene for the IL-1 receptor antagonist also strongly correlated with mortality. Again, however, efforts at blocking IL-1 in human trials, as with recombinant IL-1 receptor antagonist, have failed to improve mortality in sepsis [77].

The reasons for the failure of

anticytokine trials are not known, although it is clear that the biological effects of even a single cytokine in the setting of sepsis are extremely complex. For instance, the TNFRI knockout mouse displays reduced lung injury early after an endotoxin or pseudomonas challenge yet displays increased lung inflammation at a later time point [78], suggesting that TNF participates in both pro- and anti-inflammatory pathways, perhaps at different phases of sepsis. Compounding this level of complexity, the number of genes induced early in sepsis is enormous. Novel cytokines such as lymphotoxin and HMGB1 have recently been implicated in the sepsis response [79, 80]. Even in simple metazoans such as *Drosophila*, an infectious challenge is met with up- or down-regulation of at least 400 immune-regulated genes [81]. The breadth of this response is even greater in vertebrates, with thousands of genes showing induction or repression within hours after a septic injury. The expression profile of septic rats reveals patterns of time-dependence, organ specificity, and counterregulation [82]. As it is with other diseases, the genomic expression analysis of sepsis presently oustrips our capacity to relate these data to the physiologic syndrome of sepsis.

V. Sepsis Reconsidered

The past decade of sepsis research has seen enormous advances in our understanding of the human response to infection and the molecular and cellular events which accompany the development of the septic response. Despite the many clinical failures, several compounds resulting from these studies show promise. Still, the gap between this advancing knowledge frontier and the fundamental clinical features of sepsis (fever, tachycardia, tachypnea, leukocytosis) appears to be widening if anything. The basic physiologic abnormalities which lead to death in sepsis (e.g. metabolic acidosis and hypotension) have not been closely addressed.

From a bird's eye view, the prior decade of research has been heavily technology- and paradigm-driven. The technology has been one of biochemistry and cell biology, and now of genomics and proteomics, while the importance of sepsis physiology has receded. The paradigm has been one of inflammation and immunity. It has become almost axiomatic, for instance, that innate immunity causes inflammation and inflammation causes sepsis. While not incorrect, this view also seems incomplete. The C3H/HeJ mouse, which harbors a point mutation in the endotoxin receptor *Tlr4*, is highly resistant to endotoxin and consequently has a defective innate immune response. Why then is the phenotype of this mouse one of extreme sensitivity to gram negative sepsis, with as little as two *Salmonella* organisms causing death? Again, severely pancytopenic patients lack an appropriate acute inflammatory response, yet are at high risk for DIC and septic shock. When innate immunity falters and allows microbial outgrowth, the individual nonetheless succumbs to sepsis.

The physiologic profile of fatal sepsis generally includes tissue ischemia with lactic acidosis, in combination with refractory hypotension. It is therefore likely that the sepsis syndrome involves dysfunction of the primary physiologic regulators of cardiovascular function, such as the autonomic nervous system. Indeed, a novel hypothesis suggests a prominent role for the neuroendocrine system in the manifestation of severe sepsis [83]. Recent observations support this contention. As an example, *in vivo* imaging of the sublingual microvasculature reveals intense visceral vasospasm during sepsis, relieved rapidly with the anticholinergic

compound acetylcholine [84]. This finding is consistent with an important role for autonomic failure in the development of lactic acidosis. Another example of the importance of the neuroendocrine response to sepsis was suggested by observations that septic patients fail to appropriately secrete the hypothalamic hormone vasopressin, and that vasopressin infusion rapidly improves blood pressure in septic shock [85, 86].

Integration of lessons learned from the past decade with such new ideas about the pathogenesis of sepsis will likely require a reworking of fundamental assumptions and, therefore, of the working sepsis paradigm. Such change will be welcome.

REFERENCES

1.Bone, R.C., *et al.*, Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*, 1992. 101: 1644-55.

2. Matot, I. and C.L. Sprung, Definition of sepsis. *Intensive Care Med.*, 2001. 27.

3. Medzhitov, R. and C. Janeway, Jr., Innate immunity. *New Engl.J.Med.*, 2000. 343: 338-44.

4. Beutler, B. and A. Poltorak, Sepsis and evolution of the innate immune response. *Crit.Care Med.*, 2001. 29.

5. Lemaitre, B., E. Nicolas, L. Michaut, J.M. Reichhart, and J.A. Hoffmann, The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell*, 1996. 86: 973-83.

6. van Deventer, S.J., H.R. Buller, J.W. ten Cate, L.A. Aarden, C.E. Hack, and A. Sturk, Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. *Blood*, 1990. 76: 2520-6.

7. Johnson, K., Y. Choi, E. DeGroot, I. Samuels, A. Creasey, and L. Aarden, Potential mechanisms for a proinflammatory vascular cytokine response to coagulation activation. *J.Immunol.*, 1998. 160: 5130-5.

8. Rahman, A., K.N. Anwar, A.L. True, and A.B. Malik, Thrombin-induced p65 homodimer binding to downstream NF-kappa B site of the promoter mediates endothelial ICAM-1 expression and neutrophil adhesion. *J.Immunol.*, 1999. 162: 5466-76.

9. Vu, T.K., D.T. Hung, V.I. Wheaton, and S.R. Coughlin, Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell*, 1991. 64: 1057-68.

10. Coughlin, S.R., Sol Sherry lecture in thrombosis: how thrombin 'talks' to cells: molecular mechanisms and roles in vivo. *Arteriosclerosis Thrombosis & Vascular Biology*, 1998. 18: 514-8.

11. Joyce, D.E., L. Gelbert, A. Ciaccia, B. DeHoff, and B.W. Grinnell, Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. *J. Biol. Chem.*, 2001. 276: 11199-203.

12. Uchiba, M., K. Okajima, and K. Murakami, Effects of various doses of antithrombin III on endotoxin-induced endothelial cell injury and coagulation abnormalities in rats. *Thrombosis Research*, 1998. 89: 233-41.

13. Isobe, H., K. Okajima, M. Uchiba, N. Harada, and H. Okabe, Antithrombin prevents endotoxin-induced hypotension by inhibiting the induction of nitric oxide synthase in rats. *Blood*, 2002. 99: 1638-45.

14. Minnema, M.C., *et al.*, Recombinant human antithrombin III improves survival and attenuates inflammatory responses in baboons lethally challenged with Escherichia coli. *Blood*, 2000. 95: 1117-23.

15. Warren, B.L., *et al.*, High-dose antithrombin III in severe sepsis: a randomized controlled trial. *JAMA*, 2001. 286: 1869-78.

16. Cunningham, M.A., P. Romas, P. Hutchinson, S.R. Holdsworth, and P.G. Tipping, Tissue factor and factor VIIa receptor/ligand interactions induce proinflammatory effects in macrophages. *Blood*, 1999. 94: 3413-20.

17. Tannenbaum, S.H., R. Finko, and D.B. Cines, Antibody and immune complexes induce tissue factor production by human endothelial cells. *J.Immunol.*, 1986. 137: 1532-7.

18. Rottingen, J.A., T. Enden, E. Camerer, J.G. Iversen, and H. Prydz, Binding of human factor VIIa to tissue factor induces cytosolic Ca2+ signals in J82 cells, transfected COS-1 cells, Madin-Darby canine kidney cells and in human endothelial cells induced to synthesize tissue factor. *J. Biol. Chem.*, 1995. 270: 4650-60.

19. Crossman, D.C., D.P. Carr, E.G. Tuddenham, J.D. Pearson, and J.H. McVey, The regulation of tissue factor mRNA in human endothelial cells in response to endotoxin or phorbol ester. *J. Biol. Chem.*, 1990. 265:

9782-7.
20. Drake, T.A., J. Cheng, A. Chang, and F.B. Taylor, Jr., Expression of tissue factor, thrombomodulin, and E-selectin in baboons with lethal Escherichia coli sepsis. *Am.J.Pathol.*, 1993. 142: 1458-70.
21. Nieuwland, R., *et al.*, Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood*, 2000. 95: 930-5.
22. Camerer, E., W. Huang, and S.R. Coughlin, Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc. Natl. Acad. Sci. USA*, 2000. 97: 5255-60.
23. Versteeg, H.H., *et al.*, Factor VIIa/tissue factor-induced signaling via activation of Src-like kinases, phosphatidylinositol 3-kinase, and Rac. *J. Biol. Chem.*, 2000. 275: 28750-6.
24. Poulsen, L.K., *et al.*, Signal transduction via the mitogen-activated protein kinase pathway induced by binding of coagulation factor VIIa to tissue factor. *J. Biol. Chem.*, 1998. 273: 6228-32.
25. Taylor, F.B., Jr., Role of tissue factor and factor VIIa in the coagulant and inflammatory response to LD100 Escherichia coli in the baboon. *Haemostasis*, 1996. 1: 83-91.
26. Creasey, A.A., A.C. Chang, L. Feigen, T.C. Wun, F.B. Taylor, Jr., and L.B. Hinshaw, Tissue factor pathway inhibitor reduces mortality from Escherichia coli septic shock. *J.Clin.Invest.*, 1993. 91: 2850-6.
27. Camerota, A.J., A.A. Creasey, V. Patla, V.A. Larkin, and M.P. Fink, Delayed treatment with recombinant human tissue factor pathway inhibitor improves survival in rabbits with gram-negative peritonitis. *J. Infect. Dis.*, 1998. 177: 668-76.
28. Enkhbaatar, P., K. Okajima, K. Murakami, M. Uchiba, H. Okabe, K. Okabe, and Y. Yamaguchi, Recombinant tissue factor pathway inhibitor reduces lipopolysaccharide-induced pulmonary vascular injury by inhibiting leukocyte activation. *Am. J. Respir. Crit. Care Med.*, 2000. 162: 1752-9.
29. de Jonge, E., *et al.*, Tissue factor pathway inhibitor dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxemia. *Blood*, 2000. 95: 1124-9.
30. de Jonge, E., *et al.*, Tissue factor pathway inhibitor does not influence inflammatory pathways during human endotoxemia. *J. Infect. Dis.*, 2001. 183: 1815-8.
31. Abraham, E., *et al.*, Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study. *Crit.Care Med.*, 2001. 29: 2081-9.
32. Beller-Todd, B., L.T. Archer, and L.B. Hinshaw, Recovery from endotoxin shock after extracorporeal perfusion without anticoagulation. *Circulatory Shock*, 1979. 6: 261-9.
33. Esmon, C.T., *et al.*, Endothelial protein C receptor. *Thromb. Haemostasis*, 1999. 82: 251-8.
34. Powars, D., *et al.*, Epidemic meningococcemia and purpura fulminans with induced protein C deficiency. *Clin. Infect. Dis.*, 1993. 17: 254-61.
35. Mesters, R.M., *et al.*, Prognostic value of protein C concentrations in neutropenic patients at high risk of severe septic complications. *Crit.Care Med.*, 2000. 28: 2209-16.
36. Lorente, J.A., L.J. Garcia-Frade, L. Landin, R. de Pablo, C. Torrado, E. Renes, and A. Garcia-Avello, Time course of hemostatic abnormalities in sepsis and its relation to outcome. *Chest*, 1993. 103: 1536-42.
37. Taylor, F.B., Jr., *et al.*, The endothelial cell protein C receptor aids in host defense against Escherichia coli sepsis. *Blood*, 2000. 95: 1680-6.
38. Speiser, W., *et al.*, Effect of intradermal tumor necrosis factor-alpha-induced inflammation on coagulation factors in dermal vessel endothelium. An in vivo study of human skin biopsies. *Thromb. Haemostasis*, 2001. 85: 362-7.
39. Lentz, S.R., M. Tsiang, and J.E. Sadler, Regulation of thrombomodulin by tumor necrosis factor-alpha: comparison of transcriptional and posttranscriptional mechanisms. *Blood*, 1991. 77: 542-50.
40. Faust, S.N., *et al.*, Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *New Engl.J.Med.*, 2001. 345: 408-16.
41. Grey, S.T., A. Tsuchida, H. Hau, C.L. Orthner, H.H. Salem, and W.W. Hancock, Selective inhibitory effects of the anticoagulant activated protein C on the responses of human mononuclear phagocytes to LPS, IFN-gamma, or phorbol ester. *J.Immunol.*, 1994. 153: 3664-72.
42. White, B., *et al.*, Activated protein C inhibits lipopolysaccharide-induced nuclear translocation of nuclear factor kappaB (NF-kappaB) and tumour necrosis factor alpha (TNF-alpha) production in the THP-1 monocytic cell line. *Br. J. Haematol.*, 2000. 110: 130-4.
43. Riewald, M., R.J. Petrovan, A. Donner, B.M. Mueller, and W. Ruf, Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science*, 2002. 296: 1880-2.
44. Hancock, W.W. and F.H. Bach, Immunobiology and therapeutic applications of protein C/protein S/thrombomodulin in human and experimental

allotransplantation and xenotransplantation. *Trends Cardiovasc. Med.*, 1997. 7: 174-83.

45. Murakami, K., K. Okajima, M. Uchiba, M. Johno, T. Nakagaki, H. Okabe, and K. Takatsuki, Activated protein C attenuates endotoxin-induced pulmonary vascular injury by inhibiting activated leukocytes in rats. *Blood*, 1996. 87: 642-7.

46. Bernard, G.R., *et al.*, Efficacy and safety of recombinant human activated protein C for severe sepsis. *New Engl.J.Med.*, 2001. 344: 699-709.

47. FDA Briefing document, Drotrecogin alfa, September 12, 2001. *http://www.fda.gov/ohrms/dockets/ac/01/briefing/3797b1_02_FDAbriefing.pdf*, .

48. van der Poll, T., *et al.*, Differential effects of anti-tumor necrosis factor monoclonal antibodies on systemic inflammatory responses in experimental endotoxemia in chimpanzees. *Blood*, 1994. 83: 446-51.

49. Mesters, R.M., P.M. Mannucci, R. Coppola, T. Keller, H. Ostermann, and J. Kienast, Factor VIIa and antithrombin III activity during severe sepsis and septic shock in neutropenic patients. *Blood*, 1996. 88: 881-6.

50. van der Poll, T., *et al.*, Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. *J. Exp. Med.*, 1994. 179: 1253-9.

51. Weyrich, A.S., T.M. McIntyre, R.P. McEver, S.M. Prescott, and G.A. Zimmerman, Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. *J.Clin.Invest.*, 1995. 95: 2297-303.

52. Terada, L.S., J.J. Dormish, P.F. Shanley, J.A. Leff, B.O. Anderson, and J.E. Repine, Circulating xanthine oxidase increases following intestinal ischemia-reperfusion and mediates lung neutrophil sequestration. *Am.J.Physiol.*, 1992. 263: L394-L401.

53. Terada, L.S., B.M. Hybertson, K.G. Connelly, D. Weill, D. Piermattei, and J.E. Repine, XO increases neutrophil adherence to endothelial cells by a dual ICAM-1 and P-selectin-mediated mechanism. *J.Appl.Physiol.*, 1997. 82: 866-73.

54. Coughlan, A.F., H. Hau, L.C. Dunlop, M.C. Berndt, and W.W. Hancock, P-selectin and platelet-activating factor mediate initial endotoxin-induced neutropenia. *J. Exp. Med.*, 1994. 179: 329-34.

55. Mileski, W.J., R.K. Winn, N.B. Vedder, T.H. Pohlman, J.M. Harlan, and C.L. Rice, Inhibition of CD18-dependent neutrophil adherence reduces organ injury after hemorrhagic shock in primates. *Surgery.*, 1990. 108: 206-12.

56. Gao, X., N. Xu, M. Sekosan, D. Mehta, S.Y. Ma, A. Rahman, and A.B. Malik, Differential role of CD18 integrins in mediating lung neutrophil sequestration and increased microvascular permeability induced by Escherichia coli in mice. *J.Immunol.*, 2001. 167: 2895-901.

57. Tan, T.Q., C.W. Smith, E.P. Hawkins, and S.L. Kaplan, Anti-CD11b monoclonal antibody in an infant rat model of Haemophilus influenzae type b sepsis and meningitis. *J. Antimicrob. Chemother.*, 1997. 39: 209-16.

58. Prince, J.E., C.F. Brayton, M.C. Fossett, J.A. Durand, S.L. Kaplan, C.W. Smith, and C.M. Ballantyne, The differential roles of LFA-1 and Mac-1 in host defense against systemic infection with Streptococcus pneumoniae. *J.Immunol.*, 2001. 166: 7362-9.

59. Lindemann, S., N.D. Tolley, D.A. Dixon, T.M. McIntyre, S.M. Prescott, G.A. Zimmerman, and A.S. Weyrich, Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J.Cell Biol.*, 2001. 154: 485-90.

60. Ishii, S., *et al.*, Bronchial hyperreactivity, increased endotoxin lethality and melanocytic tumorigenesis in transgenic mice overexpressing platelet-activating factor receptor. *EMBO J.*, 1997. 16: 133-42.

61. Dhainaut, J.F., *et al.*, Platelet-activating factor receptor antagonist BN 52021 in the treatment of severe sepsis: a randomized, double-blind, placebo-controlled, multicenter clinical trial. BN 52021 Sepsis Study Group. *Crit.Care Med.*, 1994. 22: 1720-8.

62. Dhainaut, J.F., *et al.*, Confirmatory platelet-activating factor receptor antagonist trial in patients with severe gram-negative bacterial sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. BN 52021 Sepsis Investigator Group. *Crit.Care Med.*, 1998. 26: 1963-71.

63. Tjoelker, L.W., *et al.*, Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature*, 1995. 374: 549-53.

64. Graham, R.M., C.J. Stephens, W. Silvester, L.L. Leong, M.J. Sturm, and R.R. Taylor, Plasma degradation of platelet-activating factor in severely ill patients with clinical sepsis. *Crit.Care Med.*, 1994. 22: 204-12.

65. The Phase II Pafase ARDS prevention study group and ICOS Corporation. Recombinant platelet activating factor acetylhydrolase (PAFASE) decrease the incidence of acute respiratory distress syndrome and 28-day all cause mortality. *Intensive Care Med.*, 2000. 26: S321.

66. Brauner, J.S., L.E. Rohde, and N. Clausell, Circulating endothelin-1 and tumor necrosis factor-

alpha: early predictors of mortality in patients with septic shock. *Intensive Care Med.*, 2000. 26: 305-13.
67. Dofferhoff, A.S., *et al.*, Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute-phase proteins during the treatment of severe sepsis in humans. *Crit.Care Med.*, 1992. 20: 185-92.
68. Wilson, A.G., J.A. Symons, T.L. McDowell, H.O. McDevitt, and G.W. Duff, Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc. Natl. Acad. Sci. USA*, 1997. 94: 3195-9.
69. Kroeger, K.M., K.S. Carville, and L.J. Abraham, The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Molec. Immunol.*, 1997. 34: 391-9.
70. Mira, J.P., *et al.*, Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA*, 1999. 282: 561-8.
71. Stuber, F., M. Petersen, F. Bokelmann, and U. Schade, A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis. *Crit.Care Med.*, 1996. 24: 381-4.
72. Dhainaut, J.F., *et al.*, CDP571, a humanized antibody to human tumor necrosis factor-alpha: safety, pharmacokinetics, immune response, and influence of the antibody on cytokine concentrations in patients with septic shock. CPD571 Sepsis Study Group. *Crit.Care Med.*, 1995. 23: 1461-9.
73. Abraham, E., *et al.*, p55 Tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. A randomized controlled multicenter trial. Ro 45-2081 Study Group. *JAMA*, 1997. 277: 1531-8.
74. Fisher, C.J., Jr., *et al.*, Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N. Engl. J. Med.*, 1996. 334: 1697-702.
75. Reinhart, K., *et al.*, Randomized, placebo-controlled trial of the anti-tumor necrosis factor antibody fragment afelimomab in hyperinflammatory response during severe sepsis: The RAMSES Study. *Crit.Care Med.*, 2001. 29: 765-9.
76. Ma, P., D. Chen, J. Pan, and B. Du, Genomic polymorphism within interleukin-1 family cytokines influences the outcome of septic patients. *Crit.Care Med.*, 2002. 30: 1046-50.
77. Fisher, C.J., Jr., *et al.*, Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA*, 1994. 271: 1836-43.
78. Skerrett, S.J., T.R. Martin, E.Y. Chi, J.J. Peschon, K.M. Mohler, and C.B. Wilson, Role of the type 1 TNF receptor in lung inflammation after inhalation of endotoxin or Pseudomonas aeruginosa. *Am. J. Physiol.*, 1999. 276: L715-27.
79. Wang, H., *et al.*, HMG-1 as a late mediator of endotoxin lethality in mice. *Science*, 1999. 285: 248-51.
80. Puglielli, M.T., *et al.*, Reversal of virus-induced systemic shock and respiratory failure by blockade of the lymphotoxin pathway. *Nature Med.*, 1999. 5: 1370-4.
81. De Gregorio, E., P.T. Spellman, G.M. Rubin, and B. Lemaitre, Genome-wide analysis of the Drosophila immune response by using oligonucleotide microarrays. *Proc. Natl. Acad. Sci. USA*, 2001. 98: 12590-5.
82. Chinnaiyan, A.M., *et al.*, Molecular signatures of sepsis: multiorgan gene expression profiles of systemic inflammation. *Am.J.Pathol.*, 2001. 159: 1199-209.
83. Munford, R.S. and K.J. Tracey, Is severe sepsis a neuroendocrine disease? *Molec. Med.*, 2002. In Press.
84. De Backer, D., J. Creteur, J.C. Preiser, M.J. Dubois, and J.L. Vincent, Microvascular blood flow is altered in patients with sepsis. *Am. J. Respir. Crit. Care Med.*, 2002. 166: 98-104.
85. Landry, D.W., *et al.*, Vasopressin deficiency contributes to the vasodilation of septic shock. *Circulation*, 1997. 95: 1122-5.
86. Holmes, C.L., B.M. Patel, J.A. Russell, and K.R. Walley, Physiology of vasopressin relevant to management of septic shock. *Chest*, 2001. 120: 989-1002.