Fulminant Meningococcemia (Purpura Fulminans)

Internal Medicine Grand Rounds
Parkland Memorial Hospital
July 16, 1998

Robert S. Munford, M.D.

Robert S. Munford III, M.D., has no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

R. S. Munford, M.D., is a Professor in the Infectious Diseases Division. His clinical interests include sepsis, meningococcal and other invasive gram-negative bacterial diseases, and head and neck infections. His laboratory research goals are to learn how animals detoxify bacterial lipopolysaccharides (endotoxins) and to use genetic engineering to prevent and treat inflammatory diseases.

Many thanks to Dr. Dennis Burns for providing several pathology slides for this presentation and to Ms. Martha Rogers for excellent library assistance.

The following cases represent the two extremes of the spectrum of meningococcal disease (MD), as encountered during the epidemic in Sao Paulo, Brazil, in 1972 [1]:

1. According to his mother, this 16 year-old Sao Paulo high school student had complained of muscle soreness, headache and chilly sensations for two days before he became stuporous and had a grand mal seizure. When examined at Hospital Emilio Ribas he was arousable but disoriented, he had striking nuchal rigidity, there were a few small petechiae on his chest, and his oral temperature was 102.8°F. When a lumbar puncture was performed, the opening pressure was 300 mm H_20; the CSF contained 1,250 WBC/mm^3, the protein was 230 mg/dL, and the glucose was 13 mg/dL. The CSF and blood grew *Neisseria meningitidis*, serogroup C, resistant to sulfadiazine. He was treated with penicillin G, 4 MU q4h. His mental status returned to baseline within 3 days and he was discharged from the hospital in one week.

2. According to his commanding officer, this 18 year-old man was in excellent health when he arrived at recruit camp in a Sao Paulo suburb two weeks earlier. At line inspection on the morning of admission the young man was noted to have blood spots on his face. When questioned, he said that he had felt "sick" since he awoke that morning. He was immediately transported in a military truck to Hospital Emilio Ribas. On arrival he was stuporous, febrile (101.6°F), and hypotensive (80/50), with widespread purpuric lesions on his face, chest, and extremities. Blood was drawn for culture and intravenous penicillin G was begun; it was difficult to stop bleeding at the venepuncture sites. Large doses of glucocorticoids were given. He expired at 2 P.M. The blood cultures subsequently grew *Neisseria meningitidis*, serogroup C, resistant to sulfadiazine.

The first patient had meningococcal meningitis (MM), while the second case illustrates the clinical course of fulminant meningococcemia (FM). In the words of Herrick (1910), in FM there is

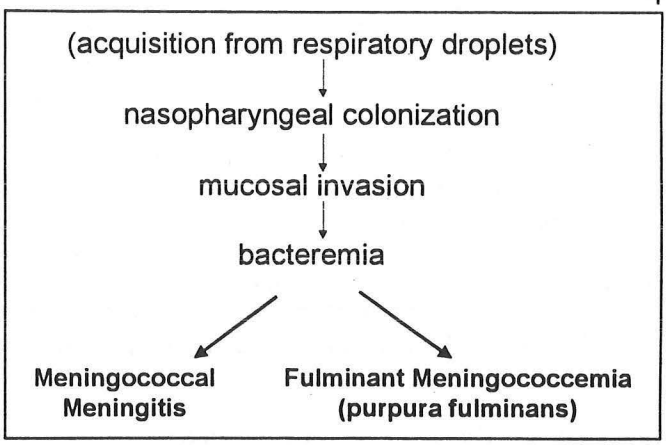
> "a sudden onset with chill, moderate or high fever, great prostration, restlessness with apathy, deepening into stupor or coma, which endures until death. The pulse is rapid, running, of small volume, weak and of low pressure. The color is a cyanotic pallor that is a mask of death. The rash begins as purple blotches on the trunk or elsewhere. These increase in size with incredible rapidity until coalescent areas may transform a considerable area of the body surface – even one-third—by ominous purple patches, continually extending until the fatal issue. In addition to the diffuse purpura, punctate petechial spots are thickly sprinkled over trunk, face, extremities and mucosa of the mouth and eyes and seem an independent lesion. No other infection so quickly slays."[2]

This Grand Rounds presentation will address the following questions:

1. When epidemic meningococcal disease (MD) occurs in a population, why do some individuals develop meningitis (MM) while others develop fulminant meningococcemia (FM) ("purpura fulminans")? (I shall assume that, during an epidemic, almost all individuals are infected by the same strain of *Neisseria meningitidis*.) 2. What is the nature of the coagulopathy? 3. What is the pathogenesis of septic shock and organ injury? 4. What pathophysiology-based therapies are being tested? 5. What do studies on patients with FM tell us about the host response to invasion by other bacteria?

General overview:

Neisseria meningitis are aerobic gram-negative diplococci. The species is classified into serogroups based on the antigenicity of the capsular polysaccharide. In recent years, serogroups B and C have caused most endemic MD in the U.S., and 5 distinct but closely related serogroup C strains have caused small outbreaks [3], including an epidemic in east Texas during the early 1990's.



Meningococci are transmitted from person to person via respiratory droplets. They colonize the nasopharynx and, in some individuals, invade through non-ciliated cells in the respiratory mucosa to enter the submucosa, from which they may make their way into the bloodstream [4]. Protection from meningococcal bacteremia is conferred by bactericidal antibody (IgG and IgM) and complement. Meningococci entering the blood may have several fates: (a) relatively slow multiplication in the blood, eventually seeding one or more organs in which host defenses (neutrophils, complement, etc.) are weaker than those in blood – the meninges, joints, pericardium, etc., or (b) more rapid multiplication in the blood, with invasion of vascular endothelium and induction of DIC and shock. There is also a chronic form of (recurrent) meningococcal bacteremia, and some individuals recover from meningococcemia without treatment [5].

Although meningococcal disease is principally a disease of childhood (peak attack rates occur in the 3 - 9 month age group), cases occur at all ages. During epidemics, attack rates increase in teenagers and young adults.

The two (extreme) forms of meningococcal disease

	Fulminant meningococcemia (FM)	*Meningococcal Meningitis (MM)*
Onset of symptoms to presentation for medical care	Short (often < 12 h)	Longer (days)
Presenting signs/symptoms	Prostration, petechiae/ purpura, fever	Headache, meningismus, fever
CSF culture	Often negative	Usually positive
CSF endotoxin, cytokines	Low	High
Blood endotoxin, cytokines	High	Low
Case-fatality rate	30 – 50%	3 – 10%
Fraction of total cases	~10%	~30%

Most patients with MD have meningococcemia and meningitis – there is a wide spectrum, with many overlapping features. This Table describes the extremes of the clinical spectrum.

Neisseria meningitidis: virulence mechanisms

Although some clones of *N. meningitidis* are more virulent (cause more severe disease) in humans than others, the basis for this difference is not understood.

One interesting and probably important phenotypic change may occur when meningococci are grown in medium containing cytidine monophospho-N-acetylneuraminic acid (CMP-NANA). Meningococci have an enzyme, sialyl transferase, that can transfer both endogenous (meningococcal) and exogenous (host) CMP-NANA to its LPS. Both endogenous and exogenous sialylation are associated with increased resistance to complement-mediated killing. It appears that

sialylation may mask an epitope, Lacto-N-neotetraose, that is a target in the complement-meningococcus interaction [6].

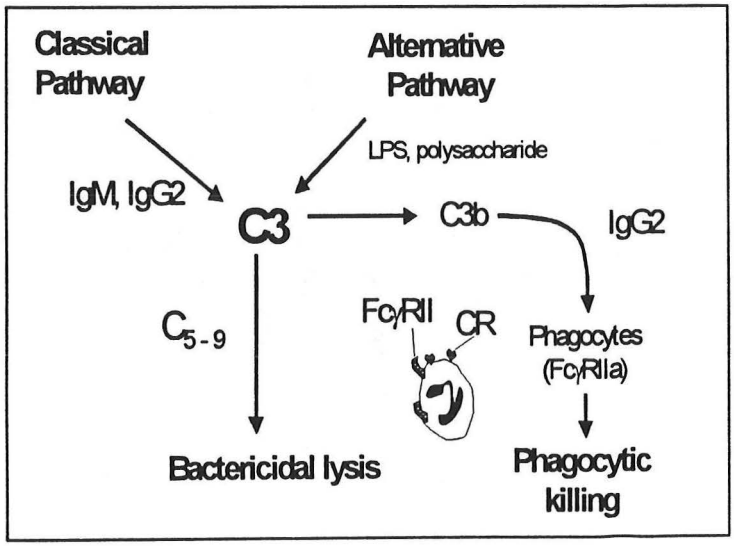
What host factors determine the clinical course of meningococcal disease?

It is a generally accepted notion that bacteremia results from the survival and multiplication of a single invading bacterium [7]. If meningococci double every 20 minutes *in vivo,* a blood concentration of 10^5 bacteria/ml would be reached within 10 hours after bloodstream invasion. (At this density, the organisms would be readily observable on the peripheral blood smear.) Little is known about the growth of meningococci in animal blood, however, since *N. meningitidis* is exclusively a human pathogen and there are no relevant animal models. Perhaps, like many other microbes, meningococci adapt to life in vivo by switching on or off cassettes of genes that favor their survival in blood, their ability to invade cells, etc. Perhaps some individuals provide a more favorable environment for sialylation of meningococcal LPS. Maybe this happens more readily in some individuals than in others, for unclear reasons. Most scientific attention has focused on the host factors that kill meningococci or limit their growth.

Bactericidal antibodies The results of a remarkable experiment support the conclusion that bactericidal antibodies prevent meningococcal bloodstream invasion. Army recruits surrendered blood specimens when they entered boot camp at Fort Dix, NJ, in 1966. They submitted nasopharyngeal cultures biweekly and cases of meningococcal disease were noted as they occurred. Bactericidal activity was measured by mixing serum with growing meningococci in the presence of an exogenous complement source. After incubation for 1 hour, the surviving meningococci were enumerated [8].

So 5 of 13 (38%) recruits who acquired nasopharyngeal infection with group C meningococci and did not have bactericidal antibody to the infecting strain became ill with meningococcal disease. There were no cases in other recruits. The bactericidal antibodies recognized capsular polysaccharide antigens.

Total recruits	492
Sera lacking bactericidal activity to a mixture of case strains	54
Group C meningococcal isolate from nasopharynx	24
No bactericidal activity to acquired group C strain	13
Cases of meningococcal disease	5

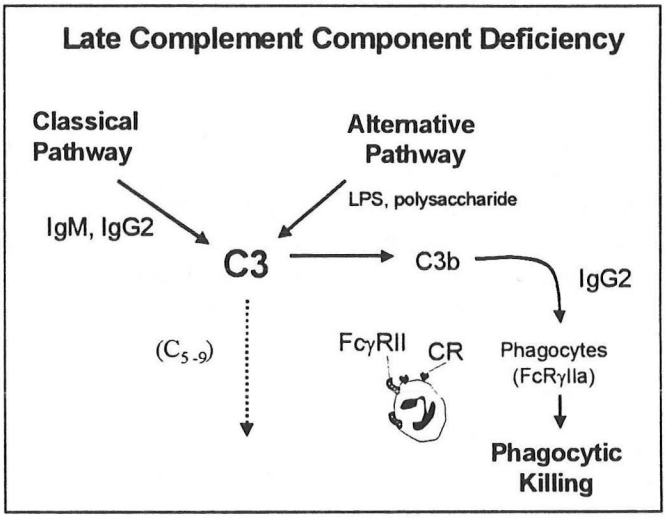


The major bactericidal antibodies are IgM and IgG antibodies that bind to the capsular polysaccharide. So immunity to meningococcal disease is serogroup-specific. Antibodies to other surface (subcapsular) antigens (proteins, LPS) may confer cross-serogroup protection. Colonization with non-pathogenic, unrelated yet antigenically cross-reactive bacteria is thought to account for the development of bactericidal antibodies to pathogenic meningococci. IgA antibodies to meningococcal surface antigens may block lysis by IgG and IgM [9]; although this

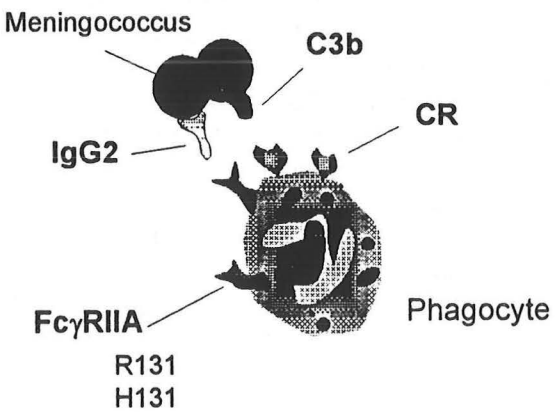
phenomenon was recognized almost 20 years ago, its significance remains uncertain.

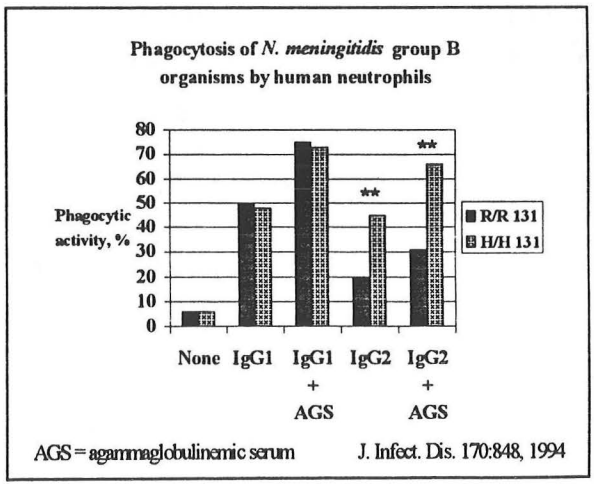
Complement is required for both opsonophagocytosis and bactericidal activity. Individuals deficient in one of the late complement components (C5 – C9) cannot assemble the "attack complex" needed to kill *Neisseria*. Interestingly, they typically develop *less severe* meningococcal disease (case-fatality rate ~ 3%), often with unusual serogroups [10,11], and at an older age, than complement-sufficient individuals [12]. Meningococcal disease may recur, sometimes on 4 or 5 occasions [13]. All of the terminal complement components exhibit autosomal recessive inheritance [14] and the prevalence of the different component deficiencies varies widely among ethnic groups [13].

Properdin-deficient males, in contrast, often develop overwhelming MD (the case-fatality rate approximates 75%)[15], as do female heterozygotes [14]. Interestingly, the age of onset is typically in the teens or twenties. There is a report of one girl who had homozygous deficiency of factor H and recurrent menngococcal disease [16]; while properdin is a positive regulator of the alternative pathway, factor H is a negative (control) factor, and its absence is evidently sufficient to cause spontaneous activation of the pathway and depletion of the other pathway components.



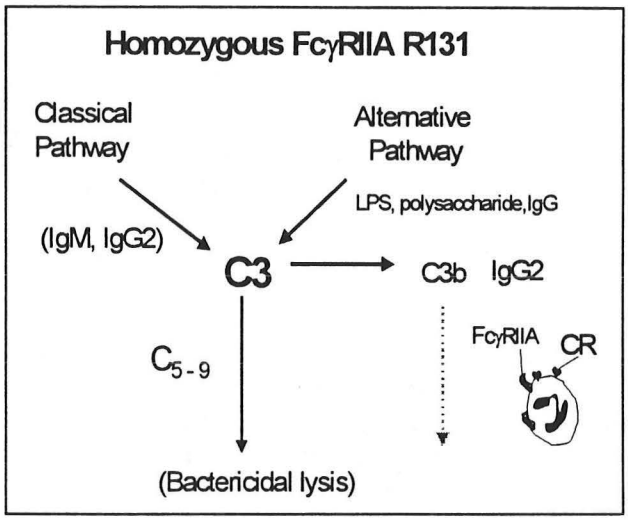
The susceptibility of LCCD patients to recurrent Neisserial infections argues strongly that bactericidal killing is necessary for effective immunity to Neisseria. So why is meningococcal disease in LCCD patients usually mild? Hypothesis: individuals with LCCD (a) have cross-reactive IgG antibodies to meningococcal antigens, and therefore they can (b) produce C3b by activating both the classical and alternative pathways, allowing (c) opsonization by both IgG and C3b. Particles that are opsonized by both IgG and C3b are more effectively phagocytosed than are particles opsonized only by C3b or C3bi. This hypothesis is supported by the observation that persons who have LCCD plus a defective Fc receptor that binds IgG poorly (the FcγRIIa R-131 allele) have more severe disease. More severe ('grave') disease occurred in 14 of 31 episodes in LCCD patients with R/R or R/H genotypes, compared with 1 of 18 episodes in children with the H/H genotype (odds ratio = 14) [17]. Individuals who have the R131 allele thus may be unable to use antimeningoccal (IgG2) antibodies to phagocytose meningococci because the critical Fc receptor on their neutrophils binds IgG2 poorly. Phagocytosis is evidently sufficient to restrain meningococcal growth in patients who have only LCCD.





Receptors for the constant region of IgG (FcγR) are important in the phagocytosis of IgG-opsonized microorganisms [18]. Neutrophils express two of the 3 classes of FcγR, FcγRII (CD32) and FcγRIII (CD16). The key polymorphism in CD32 involves a single amino acid residue, at position 131, that is critical for IgG binding. The allele that has arginine at this site (R-131) internalizes IgG2-coated particles less effectively than the allele that has histidine (H-131)[19]. IgG2 is the major antibody that binds polysaccharide antigens. Another study found a disproprtionately high rate of R/R-131 genotype among children who died with FM [20].

Assuming accurate surveillance data, the incidence of disease caused by *Haemophilus influenzae* type b and meningococci is roughly 10-fold lower in Japan than in the U.S. and many other countries. Approximately 50% of Japanese who develop meningococcal disease have LCCD, usually component 7 or 9 deficiency [21]. This prevalence of LCCD among MD patients is considerably higher than has been found in countries with higher MD incidence [13], suggesting that in a relatively immune population complement deficiency is a greater risk factor for developing MD [13]. The prevalence of the FcγRIIa H131 allele is said to be very high among Japanese (arout 85%)[18], but the connection between this observation and the low incidence of disease caused by encapsulated bacteria in Japan is uncertain.



Summary: Meningococcal disease seems to occur in three stages: colonization, invasion (bacteremia) and growth in vivo. Individuals who have bactericidal antimeningococcal antibody and normal complement are immune – they do not develop bacteremia. When bacteremia occurs, effective phagocytosis seems to play an important role in determining the subsequent course of MD. The evidence for this is obviously circumstantial: LCCD increases susceptibility to meningococcal disease, but C3b can be generated and meningococcal growth is probably checked by robust phagocytosis, so that the clinical course is usually mild. A deficiency of properdin, which stabilizes the alternative pathway convertase and allows continued activation of this pathway, is associated with more severe MD. Activation of the alternative pathway by Ig, LPS or capsular polysaccharide is thus important for producing and maintaining C3b; without C3b, neither bactericidal lysis nor phagocytosis can proceed effectively. A phagocytic defect (FcγRIIa R131 allele) that impairs the phagocytosis of IgG2-coated particles may favor more severe disease in otherwise normal individuals, again suggesting that phagocytic killing is important for restraining meningococcal growth; this allele also has been associated with a more severe clinical course in patients with LCCD.

It is especially important to immunize LCCD and properdin-deficient individuals with meningococcal vaccine [13].

*Is phagocyte **activation** required to control meningococcal growth?*

Is phagocyte *activation* also required to control meningococcal growth? This idea was recently suggested by a group of Dutch pediatricians [22]. They took blood from first degree relatives of children

who had had meningococcal disease. They added endotoxin to the blood and measured the levels of IL-10 and TNF-α in the blood after incubation for 18 hours. Their surprising result was that the blood from relatives of children who survived made more TNF than IL-10, while the blood of relatives of children who died made more IL-10 than TNF. The authors suggested that a primary IL-10 (i.e., antiinflammatory) response to endotoxin might prevent activation of neutrophils and monocyte-macrophages, impairing phagocytosis and intracellular killing, thereby allowing meningococci to grow in the absence of symptoms. The patient would become ill only when purpura and vascular collapse ensue.

This scenario has some problems, including the fact that it will be impossible to test prospectively. A spirited exchange of letters to the Lancet raised other issues. The analysis should be repeated in another population.

Pathogenesis of Meningococcal Meningitis

N. meningitidis has a striking tropism for the meninges. Infection of the CNS seems to begin in the choroid plexus or the ependyma that lines the cerebral ventricles. Meningococci adhere to cerebral capillary endothelial cells prior to entering the subarachnoid space. A vigorous local inflammatory response ensues, probably triggered by meningococcal endotoxin. Both bacterial growth and the inflammatory response occur within the CSF compartment, where levels of endotoxin, IL-6, TNF-α, IL-1ß, IL-1Ra, and IL-10 greatly exceed the concentrations found in plasma [23,24].

Patients with MM have usually been sick for 24 – 48 hours before they seek medical attention. Common presenting symptoms include nausea and vomiting, headache, neck stiffness, lethargy, and confusion. C-reactive protein levels are usually > 100 mg/L [25], indicating that an acute phase response has been underway for many hours.

Patients who develop MM may be those in whom meningococci do not grow rapidly in the blood. They may have a more vigorous initial inflammatory response to invading meningococci, or they may lack the (unknown) factors that allow *N. meningitidis* to fluorish *in vivo* (presumably this involves activation/repression of critical bacterial genes). In any case, circulating meningococci eventually "home" to the cerebral vessels, enter the SAS, and induce meningitis. (The CSF [like many other extravascular spaces – pericardial fluid, joint fluid] is relatively deficient in both antibody and complement – thus providing a safe harbor for bacterial growth.) With prompt antimicrobial chemotherapy the prognosis, perhaps surprisingly, is pretty good – at least it is much better than that of FM.

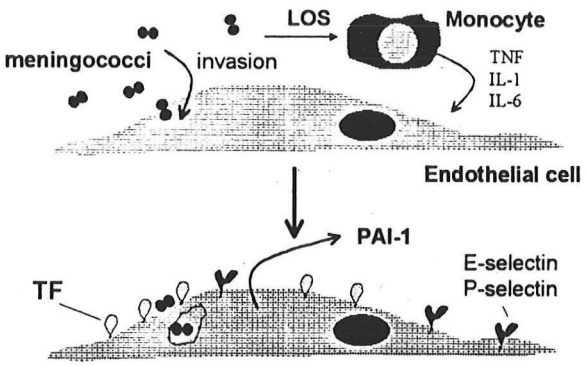
The pathophysiology of meningococcal purpura

The distinctive feature of FM: bacterial adhesion to, and invasion of, vascular endothelial cells.

Few other bacterial pathogens adhere to and invade vascular endothelial cells so well as meningococci. Studies from the pre-antibiotic era found that vascular endothelial cells in patients dying with FM were stuffed with organisms [26].

> "The sequence of events in the pathogenesis of the [skin] lesions is the localization of the meningococci in the endothelium followed by endothelial damage and inflammation of the vessel walls, with resultant necrosis and thrombosis. These changes, by permitting the extravasation of red cells, account for the hemorrhagic cutaneous lesions."[26]

Later studies [27] confirmed this finding but failed to note a correlation between the extent of the skin lesions and the severity of the coagulopathy. Others found **meningococci in the endothelium in skin lesions but not in internal organs,** even though the endothelium in both sites was badly necrotic [28]. The latter authors suggested that this might reflect the inability of meningococci to grow at high (core) temperatures and attributed the endothelial damage in these sites to circulating meningococcal endotoxin.



Activated endothelial cell harboring meningococci

A rough but plausible scenario: *N. meningitidis*, having entered the bloodstream and avoided bactericidal killing by Ab, C and phagocytes, (a) attach to, and may invade, vascular endothelial cells, particularly in cutaneous vessels, (b) multiply on or within the EC, (c) induce expression of EC surface leukocyte adhesion molecules, tissue factor, von Willebrand factor, PAI-1, etc. (d) are released when EC die and lyse. Meningococci that have multiplied within or on ECs release large amounts of endotoxin-containing membrane fragments [29,30], which contribute to the activation of complement (alternative > classical pathways)[31], stimulation of monocytes and endothelial cells at distant sites, etc.

(? Anti-inflammatory host response)
Meningococci attach to vascular endothelium
(preferentially in the skin?)
↓
Meningococci grow on and in endothelial cells,
releasing endotoxin
activating EC to express procoagulant
molecules, to attract neutrophils, etc.
↓
Endothelial cells lyse,
releasing meningococci and
meningococcal endotoxin
↓
Massive activation of monocytes and endothelial cells,
releasing both pro-and anti-inflammatory cytokines,
triggering DIC and hypotension.

Meningococcal pili and outer membrane proteins (particularly, the opacity proteins) are involved in EC invasion [32]., and EC toxicity can be related to the ability of meningococci to adhere to the EC surface [33]. Interestingly, a polysialic acid capsule or sialylated LPS seems to retard EC adherence and invasion [34-36]. Release of endotoxin onto the EC surface may induce tissue factor expression [35]. In fact, very little is known about the conditions that favor meningococcal growth *in vivo*, or about the changes in surface antigen expression and metabolic machinery that undoubtedly occur as meningococci adapt to the *in vivo* environment. Understanding these issues should be a major research goal.

The meningococcal cell wall has one dominant pro-inflammatory molecule, LPS (LOS), and the outer membrane that contains it is poorly tethered to the underlying peptidoglycan. Presumably this structural peculiarity accounts for the fact that meningococci, more than other gram-negative bacteria, shed lots of LPS-containing membrane blebs in vivo. Almost surely this LPS activates monocytes, neutrophils, and other cells, which release various mediators; these mediators can also activate the endothelium. So endothelial cells may be stimulated from within and without – in any case, they are clearly activated to produce several molecules that can be profoundly pro-coagulant and sticky for leukocytes. There is also evidence that monocyte-derived membrane vesicles ("microparticles") are highly pro-thrombotic, and that these are released from monocytes in response to LPS stimulation [37]. The concentrations of endotoxin detected in the blood of patients with FM are often 10- to 100-fold greater than those found in the blood of patients with sepsis due to other gram-negative bacteria.

At one time it was thought that meningococcal LOS is more potent than LOS from other gram-negative bacteria [38]. That possiblity was ruled out in a careful series of experiments performed by Alice Erwin when she was a UTSW Microbiology graduate student. She found that meningococcal LOS was no

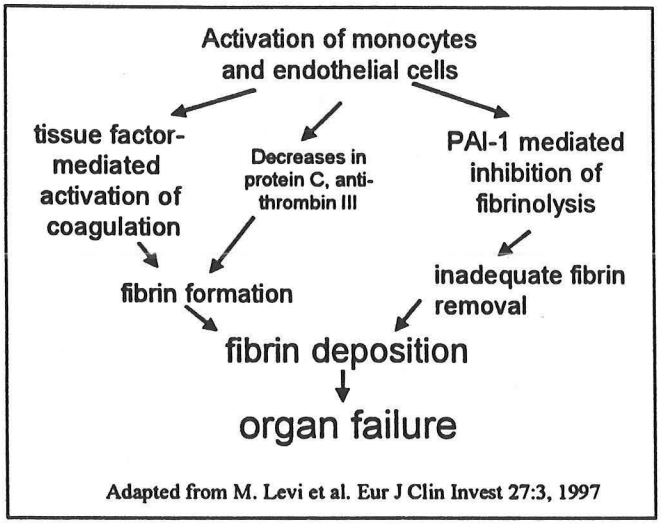
more potent at inducing the dermal Shwartzman reaction in rabbits than was *E. coli* LOS of similar size [39].

Summary: it's reasonable to suppose (although most of the evidence is circumstantial) that adhesion to and invasion of EC by meningococci is a critical event in the pathogenesis of FM, and that much pathology can be related to local (endothelial disruption, platelet-leukocyte-fibrin microthrombi, vasculitis, hemorrhage) and systemic (DIC, shock) consequences of this invasion.

Coagulopathy: the dominant determinant of outcome?

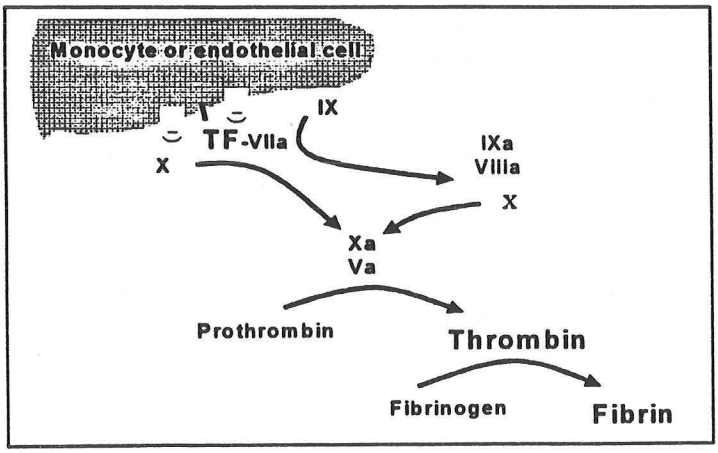
If one categorizes children with MD according to outcome (worst case = mortality or significant morbidity [e.g., amputation]), the best clinical predictor appears to be the presence or absence of coagulopathy (PTT > 50 sec, or fibrinogen < 140 mg/dL)[40]. There are numerous prognostic scoring systems for meningococcal disease, and virtually all incorporate some measure of coagulopathy as a heavily weighted variable [41-44]. The following discussion will assume that coagulopathy is a central feature of FM. It is possible, of course, that coagulopathy is an epiphenomenon, with other processes having more direct roles in generating the clinical picture, but this seems unlikely.

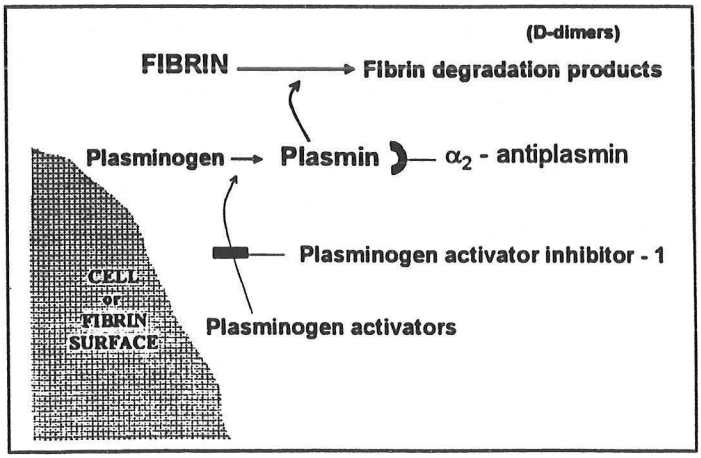
FM: a hypercoagulable state



When patients develop FM, the pro-coagulant, anti-fibrinolytic forces dominate in the peripheral blood [45]. Monocyte expression of tissue factor increases [46]. Fibrinopeptide A and thrombin-antithrombin (TAT) levels are high, reflecting active clotting; antithrombin and fibrinogen levels are low [30,47]. The contact (factors XII, XI, prekallikrein, high molecular weight kininogen) system is activated [48]. Striking deficiencies of antithrombin III and proteins C and S occur [49-52]; there may be a strong negative correlation between protein C activity and both the size of the skin lesions and mortality [53]. Plasminogen levels are decreased while plasmin-antiplasmin complexes and PAI-1 levels in the blood are very high [54]. PAI-1 levels correlate strongly with mortality risk [55 54,56].

Fibrin deposition (thus, platelet-leukocyte-fibrin microthrombi) is favored by (a) a *procoagulant* tendency, promoted by activation of tissue factor and deficiencies of proteins C, S, and anti-thrombin III, and (b) an *anti-fibrinolytic* tendency, favored by excessive PAI-1.





Neutrophils are stimulated by endotoxin to express cell-surface CD11b/CD18, which binds ICAM-1 on endothelium; at the same time, EC are activated to express other adhesion molecules (E-selectin, P-selectin, ICAM-1) that attract neutrophils and encourage their diapedesis through the endothelium. Neutrophils release reactive oxygen intermediates as well as potentially harmful enzymes (blood elastase levels have been very high in patients with severe sepsis [57]) that may contribute to EC damage and initiate hemorrhage into tissues. Although some pathologists have had difficulty finding platelets in the vascular lesions noted at autopsy, these cells are also involved in the formation of microthrombi; the thrombocytopenia of severe sepsis is poorly understood but its pathogenesis may be no more complicated than simple entrapment in thrombi.

Diagnosis of DIC in a patient with the clinical features of FM should not be difficult. Of the readily available tests, D-dimer formation is the most specific and sensitive [58]; the results of a semi-quantitative, rapid D-dimer assay correlated with both organ injury and mortality in a recent study of septic patients [59]. The platelet count is decreased, as is the fibrinogen level, and there are frequently schistocytes on the peripheral smear. Prolongation of the PT and/or PTT is usually evident but these tests lack sensitivity and specificity for DIC. For a detailed discussion of the laboratory diagnosis of DIC, see the recent review by Bick [58].

Coagulation and fibrinolysis may be regulated independently..

IL-6 seems to be the major pro-coagulant cytokine, while it has little effect on fibrinolysis in humans [60] or chimpanzees [61]. In contrast,TNF promotes fibrinolysis but has little direct effect on coagulation *in vivo* [62,63][though, at least in high doses, infused TNF-α may activate coagulation [64], perhaps by increasing IL-6 production]; it has been suggested that TNF antagonists may enhance the tendency toward thrombosis during sepsis [65]! Epinephrine inhibits coagulation and stimulates fibrinolysis [66]. IL-1 is not thought to be a critical early mediator of coagulopathy but high doses of IL-1Ra improved both coagulant and fibrinolytic parameters in humans with severe sepsis [67], and may have improved survival in those with DIC [68]. IL-10 inhibits coagulant responses and blocks the PAI-1 response to endotoxin in human volunteers [69]. IL-4 also inhibits TF surface expression in stimulated monocytes.

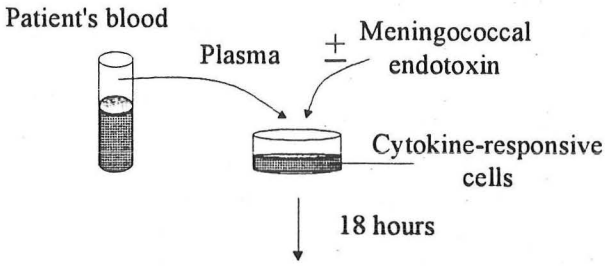
The pathophysiology of organ injury and shock

FM: endotoxin and host mediator levels in the blood.

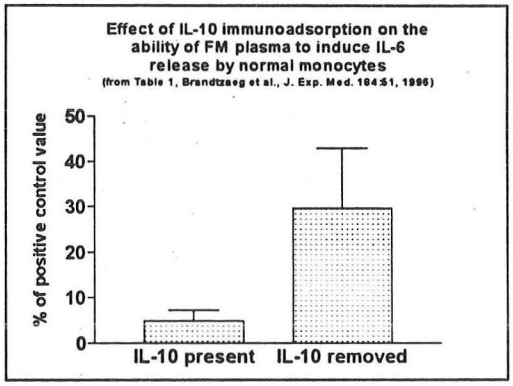
At the time they come for medical care, patients with FM typically have extremely high blood levels of meningococcal endotoxin and both pro-inflammatory (TNF-α, IL-1, interferon-γ, IL-6, IL-8) and anti-inflammatory (IL-1Ra, IL-1RsII, sTNF-RI, sTNF-RII, IL-10) cytokines.

Mediator	*Higher than in MM*	*Lower than in MM*	*References*
Bacterial products	Endotoxin (often very high levels)		29,30,70
Cytokines Pro-inflammatory	TNF-α, IL-1, IFN-γ, IL-6, IL-8		55,71-74
Anti-inflammatory	IL-1Ra, IL-1sRII, IL-10, sTNF-RI, sRNF-RII		24,55,72-77
Acute phase reactants		C-reactive protein	25
Clotting parameters Procoagulant	PAI-1, Fibrinopeptide A,		30,54,55,71
Anticoagulant/ pro-fibrinolysis	TPA, TFPI	Fibrinogen, protein S, protein C, anti-thrombin III	49,50,78

How can one make sense of all these values? To estimate the overall balance (pro- vs. anti-inflammatory) of the mediators in plasma, Brandtzaeg et al. [79] took plasma from patients acutely ill with FM and MM. They incubated the plasma with normal human monocytes and measured the procoagulant activity, TNF-α, and IL-6 released over an 18 hr incubation ex vivo. They found that (a) plasma from FM patients did not elicit release of these mediators from normal monocytes, even when a large dose of meningococcal endotoxin was added; (b) plasma from MM patients also was not pro-inflammatory, but it did not prevent endotoxin from inducing mediator release; (c) removing IL-10 from the FM plasma partially restored pro-inflammatory forces (Figure). Similar findings have been reported in studies of patients with severe sepsis caused by other bacteria [80].



These interesting results suggest that the dominant forces in the venous blood of patients with FM are *anti-*inflammatory, and that IL-10 is an important (but not the only) contributor to this balance.



Other studies have found that the blood monocytes of patients with FM (as well as other forms of septic shock) are hyporesponsive to endotoxin stimulation[81]. Again, IL-10 seems to play a major anti-inflammatory role, along with TGF-ß [82]. Interestingly, while LPS induces less TNF-α and IL-10 release from septic monocytes than from normal monocytes, LPS-induced IL-1Ra production is normal – again this would contribute to an anti-inflammatory milieu.

Summary: the blood concentrations of various mediator molecules suggest that full-blown *FM is an anti-inflammatory, pro-coagulant state in which circulating blood leukocytes are relatively unresponsive to stimulation by endotoxin and most other agonists.*

> *(Caveat: most of what we know about mediator interactions comes from studies performed on venous blood samples. Clotting occurs principally on surfaces, so the factors we measure in circulating plasma may represent what has not been "consumed" in local tissues. Similarly, inflammation can be compartmentalized in such a way that the blood levels of various mediators may not reflect the concentrations present in the tissues where the action really is.)*

How is it possible that in a lethally "pro-inflammatory" disease, the dominant mediators – both those that prime the patient to develop the disease and those that characterize its full-blown state—are anti-inflammatory? Some tentative answers:

(1) The major pathological process is microthrombosis. Often accompanied by vasculitis, thrombosis induces diffuse, severe organ dysfunction that leads to death. The major trigger for microthrombosis, at least during the early stage of the disease, may be meningococcal adhesion/infection of the endothelium and local elaboration of tissue factor, PAI-1, etc. The cytokines that circulate systemically may be less important.

(2) In patients with severe sepsis, the blood concentrations of over 30 molecules are elevated and the levels of others are decreased. Faced with this exceptionally complex array of abnormal signals, cells may become stunned—they revert to their intrinsic regulatory rhythms and ignore most external information. This represents a loss of normal physiological complexity. According to students of chaos theory [84], severe sepsis may be a state in which organs lose their ability to communicate normally with each other. Isolated from normal control mechanisms, they don't function normally. In keeping with this notion, regularization of the heart rate is an ominous prognostic factor in critically ill patients [85].

The pathogenesis of shock in FM

While there are several candidate mediators of septic shock, none has proven primacy. With regard to the relationship between coagulopathy and shock, numerous studies have suggested that (a) DIC follows activation of the "extrinsic" (TF-regulated) arm, (b) shock and DIC are not intimately linked [86,87], and (c) the "contact" arm of clotting plays at least a contributory role in the pathogenesis of shock [88]. This independence of DIC and shock suggests that therapies that prevent or reverse DIC may not prevent shock or other untoward manifestations of sepsis.

The Waterhouse-Friderichsen Syndrome (WFS): when DIC causes adrenal dysfunction.

No aspect of FM is better known to medical students than the WFS, the occurrence of collapse and death in association with massive adrenal hemorrhage. Old-timers can remember when adrenal hemorrhage was invoked to explain the occurrence of shock in patients with MD. We now recognize the WFS as one dramatic example of DIC-induced microthrombosis, hemorrhage, and tissue injury. Although it occurs in only a minority of patients with FM [89], others may have partial adrenal insufficiency and fail to mount the normal hypercortisolemic response to severe sepsis [90]. Partial or comlete adrenal insufficiency has also been found in patients with sepsis of other etiologies [91-93]. Diagnosing adrenal insufficiency by measurements of plasma basal or stimulated cortisol levels [92] is sometimes problematic[94]. Patients with FM in whom shock persists despite vigorous fluid resuscitation should receive supplemental glucocorticoid (hydrocortisone, 100 mg iv q8h).

Other genetic polymorphisms that may favor a fulminant course

The experiment by Westendorp et al. discussed above [22] suggests that the course of meningococcal disease may be determined by whether one has a pro-inflammatory or anti-inflammatory response to meningococcal endotoxin. If this hypothesis is substantiated, specific genetic polymorphisms that underly this phenomenon will doubtless be found.

Thus far, three defined polymorphisms have been associated with more severe meningococcal disease.

FcγRIIa (CD32) [20]. See above.

TNF promoter [95]. One polymorphism involves a single base substitution at –308 nt relative to the transcriptional start site of the TNF-α gene. The TNF2 allele is thought to be associated with higher constitutive and inducible levels of transcription than is the TNF1 allele, but this point has been challenged [96,97]. In one study of 98 children with MD, 10 of 33 with TNF2 died, compared with 8 of 65 with the TNF1 allele ($p = 0.03$, RR = 2.5, CI = 1.1 – 5.7)[95]. Although this polymorphism has also been associated with increased risk of cerebral malaria, its relevance to severe sepsis is disputed. Westendorp et al. did not find an association between the –308 allele and severe meningococcal disease in their study population, for example [22].

PAI-1 promoter. A polymorphism in the PAI-1 promoter has been associated with higher basal PAI-1 levels and higher inflammation-induced PAI-1 levels. It may occur with increased frequency in patients who have experienced myocardial infarction [98,99] (although this is controversial [100,101]) and unpublished reports have linked it to the severity of meningococcal disease.

Other genes. Other studies have found that the prevalence of deficiencies or mutations in clotting factors (proteins C or S, antithrombin-III, APC resistance [factor V Leiden][102]) is very low in patients with MD who develop severe purpura [103].

Meningococcal disease: therapy

In the U.S., meningococci remain susceptible to penicillin G. Nevertheless, a second-generation cephalosporin (cefotaxime, ceftriaxone) is favored for initial therapy, for its greater ease of administration and because other bacteria that may be penicillin-resistant (*S.pneumoniae*, *H. influenzae,* other gram-negative bacteria) can cause the same syndromes. In the ß-lactam-allergic patient, chloramphenicol (75 – 100 mg/kg q6h) is suitable. Patients with meningococcal meningitis should be treated for 7 days. While glucocorticoid therapy for adult meningitis is controversial, the efficacy of dexamethasone treatment for *H. influenzae* meningitis in children is now generally accepted and many experts would administer dexamethasone (8-12 mg q6h) to adults with bacterial meningitis, beginning if possible before antibiotic therapy is initiated. Patients with meningococcal disease should be hospitalized with respiratory isolation precautions for the first 24 hours.

Patients with FM typically experience severe shock (low SVR), diffuse leakage of fluid into extravascular spaces, and multiple organ dysfunction. Myocardial dysfunction may be prominent. Supportive therapy for FM (or, for that matter, any form of septic shock) has never been studied in randomized, placebo-controlled trials. Standard measures include vigorous fluid resuscitation (often requiring 10 – 12 liters over the first 24 hours), elective ventilation, pressors (epinephrine and/or dopamine), and, in patients with refractory shock, supplemental glucocorticoids (see above). Some authorities recommend early hemodialysis or hemofiltration. Fresh frozen plasma or cryoprecipitate is often given to patients who are actively bleeding, or who have severely deranged clotting parameters. For an excellent discussion of the management of children with severe meningococcal disease, see the review by B. Giroir and his colleagues in the UT Southwestern Department of Pediatrics [44].

Meningococcal disease: prevention

Meningococcal polysaccharide vaccines

A single injection of tetravalent meningococcal vaccine (serogroups A,C,W-135,Y) immunizes (protects) ~85 - 100% of immunocompetent adults. In addition to individuals with LCCD or properdin deficiency, persons who should receive the vaccine include those with sickle cell anemia, asplenia or splenectomy, military recruits, and individuals traveling to the African "meningitis belt" during the dry months (December to June) or to other areas with epidemic meningococcal disease. In general, the vaccine should be given only to persons $\geq$2 years of age [104].

Antimicrobial chemoprophylaxis

The attack rate for meningococcal disease in household contacts of cases is ~500-fold greater than that in the total population. It is recommended that close contacts of cases receive chemoprophylaxis with rifampin (adult dose: 600 mg po q12h x 4 doses) or ciprofloxacin (adult dose: 500 mg po once)[104,105]. A single injection of ceftriaxone (250 mg i.m.) is also effective. Close contacts include household members, day care center contacts, and anyone directly exposed to the patient's oral secretions. Casual contacts are not at increased risk. Chemoprophylaxis should be administered as soon as possible after the case is identified. Most secondary cases occur during the two weeks following the onset of the primary case.

Possible new therapies for FM:

In over a dozen phase III trials, anti-inflammatory drugs did not improve the survival of patients with severe sepsis or septic shock. Attention has thus turned to other kinds of interventions. Anti-endotoxin drugs are intended to neutralize the microbial trigger for the septic response, while it is hoped that anticoagulant drugs will prevent or reverse microthrombosis without promoting bleeding.

An unanticipated problem has made it harder to evaluate new drugs in patients with FM: conventional therapy has improved over the last decade, so that the case-fatality rate in experienced centers (such as those served by our Department of Pediatrics[44]) has fallen to around 15%. This welcome improvement has increased the numbers of patients that must be studied to measure significant differences between drug and placebo.

Anti-endotoxin drugs

The blood of patients with FM often contains extremely high concentrations of meningococcal endotoxin [70]. After therapy with penicillin is initiated, the endotoxin is cleared rather rapidly, so that within 12 – 24 hours there may be a 10- to 100-fold decrease in its concentration in the blood. There is no evidence that plasma endotoxin concentrations increase after antimicrobial chemotherapy-induced lysis of meningococcal cells [70]. Drugs that neutralize endotoxin will be beneficial only to the extent that endotoxin remains a trigger for pathologic changes as it is being cleared – a point that is still unsettled.

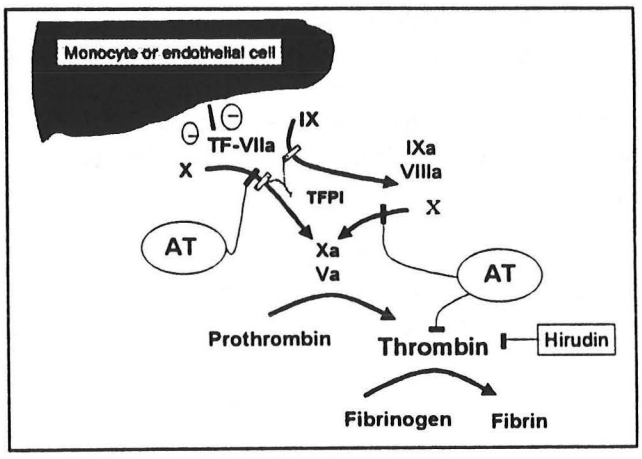
HA-1A. This antiendotoxin antibody was tested in a large phase III trial in which children with FM were randomized to receive HA-1A or placebo. The results showed a non-significant survival advantage among recipients of HA-1A. HA-1A is an autoantibody that binds to numerous non-LPS antigens [106] and is probably toxic to patients who do not have gram-negative sepsis [107]

BPI. Bactericidal-permeability increasing protein is a normal constituent of human neutrophils [108]. It has both bactericidal and endotoxin-neutralizing properties. In a phase II trial in children with FM who had received antibiotic treatment for no more than 8 hours, it appeared very promising [109]. Only 1 of 26 treated patients died, whereas predicted mortality, based on various clinical criteria, was > 30%. A phase III trial is in progress. The results of this trial should answer important questions about the role played by endotoxin during the treatment phase of FM.

E5531. This is the most potent endotoxin inhibitor yet described [110]. It blocks essentially all of the responses of animals and humans to endotoxin challenge. Phase II – III studies in humans are underway in septic patients.

Anticoagulant drugs.

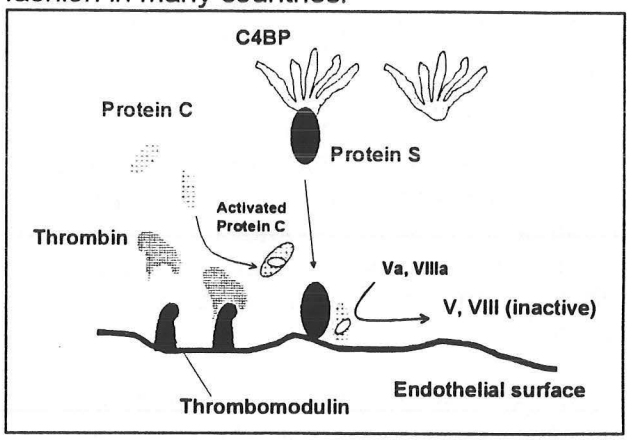
Here the goal is to prevent or reverse microthrombosis without promoting bleeding or other adverse events. As discussed above, the rationale for intervention is strong and the experience with anticoagulants, while very preliminary, is rather encouraging [111]. These drugs offer the theoretical advantage that their action does not require a cellular intermediary – their target molecules act in the plasma space – so the state of cellular activation should be relatively unimportant. In addition, the presence of potent anti-fibrinolytic forces in the blood would theoretically reduce the risk of bleeding after coagulation is inhibited.



Antithrombin. AT is a 58 kDa plasma glycoprotein (serpin) anticoagulant that inhibits factors Xa, thrombin, and many other serine proteases. Its activity is greatly enhanced by heparin. AT levels are typically reduced in patients with FM, and AT infusions have been used in several uncontrolled studies [51,111,112]. In one small randomized controlled trial conducted in patients with severe sepsis, a positive (beneficial) effect of AT III infusion was found [113]. Trials are underway in

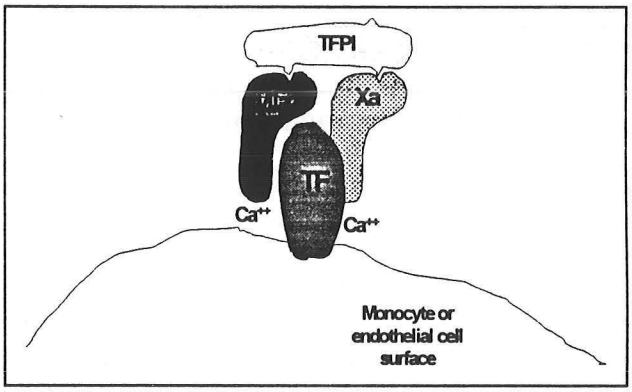
Europe [114] and in the U.S. Unfortunately, the anecdotal experience with antithrombin has been so positive that it is now being used in an uncontrolled fashion in many countries.

Antithrombin is thought to bind to a glycosaminoglycan, heparan sulfate, on endothelial surfaces. Heparan sulfate contains the pentasacharide sequence that binds AT and, by inducing a conformational change, profoundly increases its ability to interact with its target proteases. It is thought that this AT-heparan interaction plays a physiological role (producing an antithrombogenic surface) and that it can be anti-inflammatory by inducing the release of prostacyclin [115]. It is also possible that antithrombin is anti-inflammatory simply because it blocks the action of thrombin, a pro-inflammatory molecule [116].



Activated Protein C. Protein C is a plasma protein. It is activated by thrombin-thrombomodulin (TM) complexes, and in concert with protein S, it inactivates factors Va and VIIIa. Protein C levels are often greatly reduced in patients with FM [51,117,118], protein C administration can prevent coagulopathy and death in *E. coli*-infused baboons [119], and intravenous infusions of a protein C concentrate have seemed to benefit some patients [50,120]. A trial in children with FM is currently underway in the Netherlands.

TFPI. Tissue factor pathway inhibitor is a 32 – 43 kDa, lipoprotein-bound (LDL > HDL >> VLDL), Kunitz-type protease inhibitor that binds factors VIIa and Xa and blocks the ability of the TF-VIIa-Xa complex to initiate clotting [121,122]. Circulating levels of TFPI increase dramatically following heparin infusion. Levels actually increase during FM [30]. TFPI is made by many kinds of cells in culture, but in vivo the endothelium may be the most important source of the protein [122]. It is tethered to the endothelial cell membrane by a GPI anchor and may be located (along with urokinase) in caveolae [123].



The rationale for using TFPI therapeutically is two-fold: (a) given the massive up-regulation of TF on monocytes, endothelial cells, and other cell types, even supranormal levels of endogenous TFPI may be insufficient to prevent initiation of clotting, and (b) TFPI is an endotoxin-binding protein that can block cellular responses to LPS [124]. In experimental models of peritonitis in rabbits [125] and *E. coli* infection in baboons [126,127], TFPI was protective even when treatment was delayed by four hours. In baboons (which do not always mimic human responses to endotoxin or bacteria), TFPI infusion can prevent both coagulopathy and hypotension [127]. A phase II-III trial of TFPI is now underway in septic patients.

Antibodies to TF have also prevented DIC and death in animal models [128,129] without attenuating the cytokine response. An active site-inhibited factor VIIa (DEGR VIIa) attenuated both coagulant and cytokine responses to *E. coli* infusion in baboons [130], while a monoclonal Fab fragment against Factor VII/VIIa blocked the early endotoxin-induced coagulation changes in chimpanzees [131]. These results support the notion that assembly of TF-VIIa complexes is important in the hemostatic responses to bacteria. Inhibition of this pathway does not always block other endotoxin-induced responses, such as TNF-α production [126,128,130].

Hirudin The active anticoagulant in leech saliva, hirudin is a potent thrombin inhibitor. It binds thrombin in a 1:1 complex and inhibits not only the conversion of fibrinogen to fibrin, but also all other activities of thrombin [132]. Recombinant hirudins lack a sulfate at tyrosine 63 and are called desulfatohirudins. Addition of polyethylene glycol to hirudin prolongs its half life, permitting once-a-day dosing. Several hirudin analogs are under development [132].

Hirudin and its analogs have been tested in several clinical studies in non-septic patients, usually in comparison with heparin (and often in combination with tPA or streptokinase), and they have generally performed well. Hirudin has also prevented fibrin deposition in animal models of septic shock [133,134].

In addition to its uncertain efficacy in septic patients, hirudin has been associated with increased incidence of intracranial hemorrhage in some studies and the recombinant protein is expensive [132]. Its place in the anticoagulant armamentarium is unclear.

Heparin Although several case series failed to show a survival benefit from heparin therapy in patients with septic shock or FM [135], there are reports that children with FM who receive heparin have fewer necrotic digits and less cutaneous necrosis than children who do not (summarized in [136]). One reason that heparin may be ineffective: AT III levels are typically low, and heparin further promotes the inactivation of AT III by neutrophil elastase, which is released into the blood during severe sepsis [137]. On the other hand, plasma levels of TFPI typically increase following the infusion of heparin.

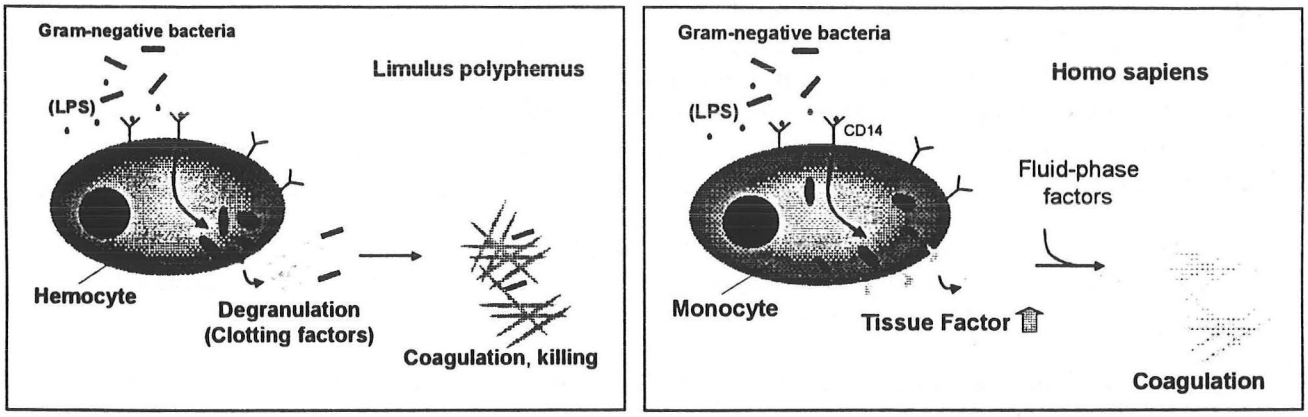
Tissue plasminogen activator. Like streptokinase, tPA has been used in a limited number of children with FM [138]. Its use has generally been limited to patients who have ischemic limbs.

Overview: FM and the septic response to invading microbes

FM is one of the most dramatic syndromes in infectious diseases. "No other infection so quickly slays."[2] It is also the most extreme, overwhelming syndrome of severe sepsis and septic shock. Unlike most other patients who develop severe sepsis, individuals who experience FM are generally fit prior to the onset of the illness. They thus have provided an opportunity to study the overwhelming host response to bacterial invasion against a normal (and young) physiological background. While it can be argued that other forms of severe sepsis are sufficiently different from FM to preclude useful generalizations, I think several points may be made:

1. *Anti-inflammatory "priming" may predispose patients to severe sepsis.* Patients who undergo major surgery or sustain major trauma may experience a state of "immune suppression" in which anti-inflammatory cytokines dominate in the blood. This state seems to predispose to subsequent infection and severe sepsis. If it is true that some individuals react to microbial invasion with a primary anti-inflammatory response, identifying these individuals could lead to major improvements in the prevention and treatment of patients who are admitted to ICUs, where sepsis remains the leading cause of death.

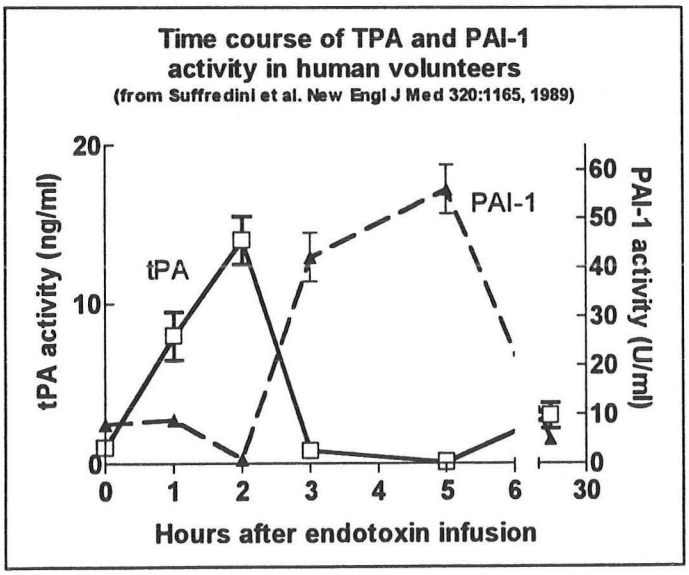
2. *There is a close, highly conserved relationship between inflammation and coagulation.* Coagulation is a component of the innate host defense toward invading microbes. This reponse is hard-wired and ready-to-go; it also includes the alternative complement system and the phagocytes. In horseshoe crabs, for example, endotoxin-induced clotting is a major mechanism of protection from invading gram-negative bacteria [139]. The Limulus amoebocyte (hemocyte) releases a bouquet of clotting factors that, activated by endotoxin, trap invading bacteria. This response is conserved in higher animals, with important changes: the monocyte reacts to endotoxin by expressing tissue factor, which initiates clotting by engaging extracellular clotting factors on the monocyte surface.



Modern recognition of the relationship between inflammation and clotting is generally credited to Colvin and Dvorak, who pointed out that fibrin deposition is involved in the delayed hypersensitivity response[140]. A practical point: anticoagulation with coumadin diminishes the size of delayed hypersensitivity skin test reactions (induration) [141]. Coagulopathy thus might account for a state of "pseudoanergy" in some patients.

Conversely, coagulation (e.g., venous thrombosis) induces inflammation; thrombin is probably the most pro-inflammatory trigger for this reaction [142]. Antithrombins are antiinflammatory as well as anticoagulant.

It is now highly likely that *most, if not all, forms of systemic inflammation involve activation of coagulation, fibrinolysis, and anti-fibrinolysis.* In fact, much has been learned about the pathogenesis of inflammation-induced coagulopathy by studying the responses of human volunteers to intravenous infusions of low doses (4 ng/kg) of endotoxin. Following endotoxin infusion, volunteers typically experience chills, fever, and myalgias for a few hours. The blood pressure may decrease transiently. In general, the infusion is well-tolerated, and there have been no known deaths.



Coagulation reponses to endotoxin in human volunteers [143]. Circulating thrombin-antithrombin complexes and prothrombin fragments (F_{1+2}) begin to increase between 90 - 120 min after an intravenous endotoxin infusion, after the peak of TNFα and roughly coinciding with the peak of IL-6. Factor V coagulant activity decreases, probably due to cleavage of V by activated protein C [144]. There is also evidence for activation of the intrinsic (contact) pathway [145], which is thought to contribute to hypotension, but not so much to coagulopathy [86,146]. Abnormalities in the blood concentrations of the natural anticoagulants are not so evident in the mild coagulopathy induced by endotoxin infusion.

Fibrinolytic responses to endotoxin in human volunteers [143,147] (see Figure). Endotoxin also triggers

endothelial cells to release tPA and PAI-1. Notably, a rapid increase in tPA activity peaks at 2 hr after the endotoxin infusion, and this pro-fibrinolytic stage is followed by an increase in PAI-1 that counteracts it, producing *net anti-fibrinolysis*.

3. *Regardless of the triggering microbe, EC injury and microthrombosis may be the fundamental pathological processes that lead to septic organ injury, shock, and death.*

4. *The important reactions in clotting and fibrinolysis occur on cell surfaces.* Key initiators and regulators of coagulation (TF), anti-coagulation (TM), and fibrinolysis (plasminogen receptors) are cell-surface proteins. The catalytic efficiency of clotting enzymes (kcat/Km) can increase by several thousand-fold when factor complexes form on negatively charged surfaces [148]. Similarly, plasminogen is more readily activated after it binds to cellular receptors; the catalytic efficiency of bound plasmin exceeds that of free plasmin and bound plasmin is protected from inactivation by fluid-phase inhibitors.

The total concentrations of clotting enzyme inhibitors (antithrombin, α_2-macroglobulin, α_1-antitrypsin) in blood far exceed the clotting enzyme concentrations that could be achieved [148]. Having coagulation enzyme complexes form on membrane surfaces favors high local enzyme concentrations and allows clotting to occur more efficiently. Similar concepts apply to fibrinolysis. Both systems are evidently designed to localize clotting to specific vascular beds and to avoid propagation of clots in the fluid phase.

- inflammatory and clotting events that occur in tissues and are concentrated on cell surfaces may not be accurately measured by studying the composition of the peripheral blood. This would apply to all forms of severe sepsis.

- meningococci may be so profoundly provocative to humans because they adhere to and invade the vascular endothelium, directly eliciting pro-coagulant responses. In other forms of sepsis, endothelial damage may result from activation by circulating endotoxin, cytokines, etc., and be less severe than in FM. Other microbes that invade endothelial cells (such as rickettsiae) do not release endotoxin – perhaps this accounts for the lower incidence of profound coagulopathy seen with these pathogens.

5. *There is a genetic component to susceptibility to severe sepsis and/or septic shock.* Identifying polymorphisms that contribute to a "high risk" profile may allow physicians to know which patients are most likely to develop severe sepsis. Forward-looking academic ICU physicians are banking DNA from their patients, so that this DNA can be analyzed for such polymorphisms (markers)--those discussed here as well as others certain to be identified in the future. At some point there should be enough information from such studies to allow useful prognostication. We should try to find ways (patterns of polymorphisms) that will identify those patients who, if infected, will develop severe sepsis. Then we can target our efforts toward preventing infection, and/or early recognition of infection/ inflammation. Since sepsis often progresses through recognizable stages [149], it may be possible to prevent severe sepsis in many individuals.

6. *Systemic inflammation is regulated by mediators that act on cells.* By and large, the pro- and anti-inflammatory mediators act by stimulating or inhibiting target cells. If the cells are already overwhelmed by abnormal signal information, the impact of circulating cytokines (or their inhibitors) may be greatly reduced. In contrast, coagulation and fibrinolysis occur in the extracellular milieu, where they are (may be) susceptible to modulation by circulating antagonists, whether or not the involved cells are responsive to cytokines or other stimuli. This may offer a therapeutic opportunity.

1. de Morais JS, Munford RS, Risi JB, Antezana E, Feldman RA. Epidemic disease due to serogroup C Neisseria meningitidis in Sao Paulo, Brazil. J.Infect.Dis. 1974; 129:568-571.

2. Herrick WW. Extrameningeal meningococcus infections. Arch.Intern.Med. 1919; 23:409-418.

3. Jackson LA, Schuchal A, Reeves MW, Wenger JD. Serogroup C meningococcal outbreaks in the United States. JAMA 1995; 273:383-389.

4. Stephens DS, Farley M. Pathogenic events during infection of the human nasopharynx with *Neisseria meningitidis* and *Haemophilus influenzae*. Rev Infect Dis 1991; 13:22-33.

5. Sullivan DT, LaScolea LJ. Neisseria meningitidis bacteremia in children: quantitation of bacteremia and spontaneous clinical recovery without antibiotic therapy. Pediatrics 1987; 80:63-67.

6. Odegaard TJ, Kaltashov IA, Cotter RJ, Steeghs L, Van der Ley P, Khan S, et al. Shortened hydroxyacyl chains on lipid A of *Escherichia coli* cells expressing a foreign UDP-*N*-acetylglucosmaine *O*-acyltransferase. J.Biol.Chem. 1997; 272:19688-19696.

7. Rubin LG. Bacterial colonization and infection resulting from multiplication of a single organism. Rev.Infect.Dis. 1987; 9:488-493.

8. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus I. The role of humoral antibodies. J.Exp.Med. 1969; 129:1307-1326.

9. Jarvis GA, Griffiss JM. Human IgA1 blockade of IgG-initiated lysis of *Neisseria meningitidis* is a function of antigen-binding fragment binding to the polysaccharide capsule. J.Immunol. 1991; 147:1962-1967.

10. Fijen CAP, Kuijper EJ, Hannema AJ, Sjöholm AG, Van Putten JPM. Complement deficiencies in patients over ten years old with meningococcal disease due to uncommon serogroups. Lancet 1989; 2:585-588.

11. Fijen CAP, Kuijper EJ, Tjia HG, Daha MR, Dankert J. Complement deficiency predisposes for meningitis due to nongroupable meningococci and *Neisseria*-related bacteria. Clin.Infect.Dis. 1994; 18:780-784.

12. Ross SC, Densen P. Complement deficiency states and infection: Epidemiology, pathogenesis and consequences of Neisserial and other infections in an immune deficiency. Medicine 1984; 63:243-273.

13. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. Clin.Microbiol.Rev. 1991; 4:359-395.

14. Frank MM. Complement in disease: inherited and acquired complement deficiencies. In: Frank MM, Austen KF, Claman HN, Unanue ER, editors. *Samter's* Immunologic Diseases. Fifth ed. Boston: Little, Brown, 1995:489-500.

15. Densen P, Weiler JM, Griffiss JM, Hoffman LG. Familial properdin deficiency and fatal meningococcemia. New Eng.J.Med. 1987; 316:922-926.

16. Nielsen HE, Christensen KC, Koch C, Thomsen BS, Heegaard NHH, Tranum-Jensen J. Hereditary, complete deficiency of complement factor H associated with recurrent meningococcal disease. Scand.J.Immunol. 1989; 30:711-718.

17. Platonov AE, Kuijper EJ, Vershinina IV, Shipulin GA, Westerdaal N, Fijen CA, et al. Meningococcal disease and polymorphism of FcgammaRIIa (CD32) in late complement component-deficient individuals. Clin.Exp.Immunol. 1998; 111:97-101.

18. Van de Winkel JGJ, Capel PJA. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. Immunol.Today 1995; 14:215-221.

19. Warmerdam PAM, Van de Winkel JG, Vlug A, Westerdaal NAC, Capel PJA. A single amino acid in the second Ig-like domain of the human Fcγ receptor II is critical for human IgG2 binding. J.Immunol. 1991; 147:1338-1343.

20. Bredius RGM, Derkx BHF, Fijen CAP, De Wit TPM, De Haas M, Weening RS, et al. Fcgamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. J.Infect.Dis. 1994; 170:848-853.

21. Nagata M, Hara T, Aoki T, Mizuno Y, Akeda H, Inaba S, et al. Inherited deficiency of ninth component of complement: an increased risk of meningococcal meningitis. J.Pediatr. 1989; 114:260-264.

22. Westendorp RGJ, Langermans JAM, Huizinga TWJ, Elouali AH, Verweij CL, Boomsma DI, et al. Genetic influence on cytokine production and fatal meningococcal disease. Lancet 1997; 349:170-173.

23. Brandtzaeg P, Ovstebo R, Kierulf P. Compartmentalization of lipopolysaccharide production correlates with clinical presentation in meningococcal disease. J.Infect.Dis. 1992; 166:650-652.

24. Van Deuren M, Van der Ven-Jongekrijg J, Vannier E, Van Dalen R, Pesman G, Bartelink AKM, et al. The pattern of interleukin-1 β (IL-1β) and its modulating agents IL-1 receptor antagonist and IL-1 soluble receptor type II in acute meningococcal infections. Blood 1997; 90:1101-1108.

25. Marzouk O, Bestwick K, Thomson APJ, Sills JA, Hart CA. Variation in serum C-reactive protein across the clinical spectrum of meningococcal disease. Acta Paediatr. 1993; 82:729-733.

26. Hill WR, Kinney TD. The cutaneous lesions in acute meningococcemia: a clinical and pathologic study. J.Amer.Med.Assoc. 1947; 134:513-518.

27. Sotto MN, Langer B, Hoshino-Shimizu S, de Brito T. Pathogenesis of cutaneous lesions in acute meningococcemia in humans: light, immunofluorescent, and electron microscopic studies of skin biopsy specimens. J.Infect.Dis. 1976; 133:506-514.

28. Evans RW, Glick B, Kimball F, Lobell M. Fatal intravascular consumption coagulopathy in meningococcal sepsis. Am.J.Med. 1969; 46:910-918.

29. Brandtzaeg P, Bryn K, Kierulf P, Ovstebo R, Namork E, Aase B, et al. Meningococcal endotoxin in lethal septic shock plasma studied by gas chromatography, mass-spectrometry, ultracentrifugation, and electron microscopy. J.Clin.Invest. 1992; 89:816-823.

30. Brandtzaeg P, Sandset PM, Joo GB, Ovstebo R, Abildgaard U, Kierulf P. The quantitative association of plasma endotoxin, antithrombin, protein C, extrinsic pathway inhibitor and fibrinopeptide A in systemic meningococcal disease. Thromb.Res. 1989; 55:459-470.

31. Brandtzaeg P, Hogasen K, Kierulf P, Mollnes TE. The excessive complement activation in fulminant meningococcal septicemia is predominantly caused by alternative pathway activation. J.Infect.Dis. 1996; 173:647-655.

32. Virji M, Makepeace K, Peak IRA, Ferguson DJP, Moxon ER. Pathogenic mechanisms of *Neisseria meningitidis*. Ann.NY Acad.Sci. 1996; 797:273-276.

33. Dunn KLR, Virji M, Moxon ER. Investigations into the molecular basis of meningococcal toxicity for human endothelial and epithelial cells: the synergistic effect of LPS and pili. Microb.Pathog. 1998; 18:81-96. Abstract.

34. Read RC, Zimmerli S, Broaddus VC, Sanan DA, Stephens DS, Ernst JD. The (α2--8)-linked polysialic acid capsule of group B *Neisseria meningitidis* modifies multiple steps during interaction with human macrophages. Infect.Immun. 1996; 64:3210-3217.

35. Heyderman RS, Klein NJ, Daramola OA, Hammerschmidt S, Frosch M, Robertson BD, et al. Induction of human endothelial tissue factor expression by *Neisseria meningitidis*: the influence of bacterial killing and adherence to the endothelium. Microbial Pathogenesis 1997; 22:265-274.

36. Klein NJ, Ison CA, Peakman M, Levin M, Hammerschmidt S, Frosch M, et al. The influences of capsulation and lipooligosaccharide structure on neutrophil adhesion molecule expression and endothelial injury by *Neisseria meningitidis*. J.Infect.Dis. 1996; 173:172-179.

37. Satta N, Freyssinet JM, Toti F. The significance of human monocyte thrombomodulin during membrane vesiculation and after stimulation by lipopolysaccharide. Br.J.Haematol. 1997; 96:534-542.

38. Davis CE, Arnold K. Role of meningococcal endotoxin in meningococcal purpura. J.Exp.Med. 1974; 140:159-171.

39. Erwin AL. Studies of bacterial lipopolysaccharides and of a leukocyte lipopolysaccharide-deacylating enzyme, acyloxyacyl hydrolase. Doctoral dissertation. Dallas, TX: University of Texas Southwestern Medical Center at Dallas, 1990.

40. McManus ML, Churchwell KB. Coagulopathy as a predictor of outcome in meningococcal sepsis and the systemic inflammatory response syndrome with purpura. Crit Care Med 1993; 21:706-711.

41. Barquet N, Domingo P, Caylà JA, González J, Rodrigo C, Fernández-Viladrich P, et al. Prognostic factors in meningococcal disease - Development of a bedside predictive model and scoring system. JAMA 1997; 278:491-496.

42. Kornelisse RF, Hazelzet JA, Hop WCJ, Spanjaard L, Suur MH, Van der Voort E, et al. Meningococcal septic shock in children: Clinical and laboratory features, outcome, and development of a prognostic score. Clin.Infect.Dis. 1997; 25:640-646.

43. Malley R, Huskins WC, Kuppermann N. Multivariable predictive models for adverse outcome of invasive meningococcal disease in children. J.Pediatr. 1996; 129:702-710.

44. Kirsch EA, Barton RP, Kitchen L, Giroir BP. Pathophysiology, treatment and outcome of meningococcemia: a review and recent experience. Pediatr.Infect.Dis.J. 1996; 15:967-979.

45. Vervloet MG, Thijs LG, Hack CE. Derangements of coagulation and fibrinolysis in critically ill patients with sepsis and septic shock. Seminars in Thrombosis and Hemostasis 1998; 24:33-44.

46. Osterud B, Flaegstad T. Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. Thromb.Haemostas.(Stuttgart) 1983; 49:5-7.

47. Lorente JA, García-Frade LJ, Landín L, De Pablo R, Torrado C, Renes E, et al. Time course of hemostatic abnormalities in sepsis and its relation to outcome. Chest 1993; 103:1536-1542.

48. Wuillemin WA, Fijnvandraat K, Derkx BHF, Peters M, Vreede W, Ten Cate H, et al. Activation of the intrinsic pathway of coagulation in children with meningococcal septic shock. Thromb.Haemost. 1995; 74:1436-1441.

49. Powars DR, Rogers ZR, Patch MJ, McGehee WG, Francis RB. Purpura fulminans in meningococcemia: association with acquired deficiencies of proteins C and S. New Eng.J.Med. 1987; 317:571-572.

50. Smith OP, White B, Vaughan D, Rafferty M, Claffey L, Lyons B, et al. Use of protein-C concentrate, heparin, and haemodiafiltration in meningococcus-induced purpura fulminans. Lancet 1997; 350:1590-1593.

51. Fourrier F, Lestavel P, Chopin C, Marey A, Goudemand J, Rime A, et al. Meningococcemia and purpura fulminans in adults: acute deficiencies of proteins C and S and early treatment with antithrombin III concentrates. Intensive Care Med. 1990; 16:121-124.

52. Fourrier F, Chopin C, Goudemand J, Hendrycx S, Caron C, Rime A, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation; Compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest 1992; 101:816-823.

53. Fijnvandraat K, Derkx B, Peters M, Bijlmer R, Sturk A, Prins MH, et al. Coagulation activation and tissue necrosis in meningococcal septic shock: Severely reduced protein C levels predict a high mortality. Thromb.Haemost. 1995; 73:15-20.

54. Brandtzaeg P, Joo GB, Brusletto B, Kierulf P. Plasminogen activator inhibitor 1 and 2, alpha-2-antiplasmin, plasminogen, and endotoxin levels in systemic meningococcal disease. Thromb.Res. 1990; 57:271-278.

55. Kornelisse RF, Hazelzet JA, Savelkoul HFJ, Hop WCJ, Suur MH, Borsboom ANJ, et al. The relationship between plasminogen activator inhibitor- 1 and proinflammatory and counterinflammatory mediators in children with meningococcal septic shock. J.Infect.Dis. 1996; 173:1148-1156.

56. Pralong G, Calandra T, Glauser M-P, Schellekens J, Verhoef J, Bachmann F, et al. Plasminogen activator inhibitor 1: A new prognostic marker in septic shock. Thromb.Haemost. 1989; 61:459-462.

57. Philippe J, Dooijewaard G, Offner F, Turion P, Baele G, Leroux-Roels G. Granulocyte elastase, tumor necrosis factor-α and urokinase levels as prognostic markers in severe infection. Thromb.Haemost. 1992; 68:19-23.

58. Bick RL. Disseminated intravascular coagulation: pathophysiological mechanisms and manifestations. Seminars in Thrombosis and Hemostasis 1998; 24:3-18.

59. Kollef MH, Eisenberg PR, Shannon W. A rapid assay for the detection of circulating D-dimer is associated with clinical outcomes among critically ill patients. Crit.Care Med. 1998; 26:1054-1060.

60. Stouthard JML, Levi M, Hack CE, Veenhof CHN, Romijn HA, Sauerwein HP, et al. Interleukin-6 stimulates coagulation, not fibrinolysis. Thromb.Haemost. 1996; 76:738-742.

61. van der Poll T, Levi M, Hack CE, Ten Cate H, van Deventer SJH, Eerenberg AJM, et al. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J.Exp.Med. 1994; 179:1253-1259.

62. 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.

63. van der Poll T, Coyle SM, Levi M, Jansen PM, Dentener M, Barbosa K, et al. Effect of a recombinant dimeric tumor necrosis factor receptor on inflammatory responses to intravenous endotoxin in normal humans. Blood 1997; 89:3727-3734.

64. Bauer KA, Ten Cate H, Barzegar S, Spriggs DR, Sherman ML, Rosenberg RD. Tumor necrosis factor infusions have a procoagulant effect on the hemostatic mechanisms of humans. Blood 1989; 74:165-172.

65. van der Poll T, Levi M, van Deventer SJH, Ten Cate H, Haagmans BL, Biemond BJ, et al. Differential effects of anti-tumor necrosis factor monoclonal antibodies on systemic inflammatory responses in experimental endotoxemia in chimpanzees. Blood 1994; 83:446-451.

66. 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.

67. Boermeester MA, Van Leeuwen PAM, Coyle SM, Wolbink GJ, Hack CE, Lowry SF. Interleukin-1 blockade attenuates mediator release and dysregulation of the hemostatic mechanism during human sepsis. Arch.Surg. 1995; 130:739-748.

68. Fisher CJ, Jr., Dhainaut J-FA, Opal SM, Pribble JP, Balk RA, Slotman GJ, 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. JAMA 1994; 271:1836-1843.

69. Pajkrt D, van der Poll T, Levi M, Cutler DL, Affrime MB, Van den Ende A, et al. Interleukin-10 inhibits activation of coagulation and fibrinolysis during endotoxemia. Blood 1997; 89:2701-2705.

70. Brandtzaeg P, Kierulf P, Gaustad P, Skulberg A, Bruun JN, Halvorsen S, et al. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. J.Infect.Dis. 1989; 159:195-204.

71. Girardin E, Grau GE, Dayer J-M, Roux-Lombard P, The J5 Study Group, Lambert P-H. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. New Eng.J.Med. 1988; 319:397-400.

72. Van Deuren M, Van der Ven-Jongekrijg J, Bartelink AKM, Van Dalen R, Sauerwein RW, Van der Meer JWM. Correlation between proinflammatory cytokines and antiinflammatory mediators and the severity of disease in meningococcal infections. J.Infect.Dis. 1995; 172:433-439.

73. Van Deuren M, Van der Ven-Jongekrijg J, Demacker PNM, Bartelink AKM, Van Dalen R, Sauerwein RW, et al. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. J.Infect.Dis. 1994; 169:157-161.

74. Lehmann AK, Halstensen A, Sornes S, Rokke O, Waage A. High levels of interleukin 10 in serum are associated with fatality in meningococcal disease. Infect.Immun. 1995; 63:2109-2112.

75. Girardin E, Roux-Lombard P, Grau GE, Suter P, Gallati H, The J5 Study Group, et al. Imbalance between tumour necrosis factor-alpha and soluble TNF receptor concentrations in severe meningococcaemia. Immunology 1992; 76:20-23.

76. Villers D, Dao T, Nguyen J-M, Bironneau E, Godard A, Moreau M, et al. Increased plasma levels of human interleukin for DA1.a cells/leukemia inhibitory factor in sepsis correlate with shock and poor prognosis. J.Infect.Dis. 1995; 171:232-236.

77. Van Deuren M, Frieling JT, Van der Ven-Jongekrijg J, Neeleman C, Russel FG, Van Lier HJ, et al. Plasma patterns of tumor necrosis factor-α (TNF) and TNF soluble receptors during acute meningococcal infections and the effect of plasma exchange. Clin.Infect.Dis. 1998; 26:918-923.

78. Giraud T, Dhainaut J-F, Schremmer B, Regnier B, Desjars P, Loirat P, et al. Adult overwhelming meningococcal purpura: A study of 35 cases, 1977-1989. Arch.Intern.Med. 1991; 151:310-316.

79. 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.

80. Döcke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, et al. Monocyte deactivation in septic patients: Restoration by IFN-gamma treatment. Nature Med. 1997; 3:678-681.

81. Van Deuren M, Netea MG, Hijmans A, Demacker PN, Neeleman C, Sauerwein RW, et al. Posttranscriptional down-regulation of tumor necrosis factor-α and interleukin-1β production in acute meningococcal infections. Journal of Infectious Diseases 1998; 177:1401-1405.

82. Randow F, Syrbe U, Meisel C, Krausch D, Zuckermann H, Platzer C, et al. Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor β. J.Exp.Med. 1995; 181:1887-1892.

83. Williams MA, White SA, Miller JJ, Toner C, Withington S, Newland AC, et al. Granulocyte-macrophage colony-stimulating factor induces activation and restores respiratory burst activity in monocytes from septic patients. Journal of Infectious Diseases 1998; 177:107-115.

84. Godin PJ, Buchman TG. Uncoupling of biological oscillators: a complementary hypothesis concerning the pathogenesis of multiple organ dysfunction syndrome. Crit.Care Med. 1996; 24:1107-1116.

85. Yien H-W, Hseu S-S, Lee L-C, Kuo TBJ, Lee T-Y, Chan SHH. Spectral analysis of systemic arterial pressure and heart rate signals as a prognostic tool for the prediction of patient outcome in the intensive care unit. Crit.Care Med. 1997; 25:258-266.

86. Esmon CT, Taylor FB, Jr., Snow TR. Inflammation and coagulation: linked processes potentially regulated through a common pathway mediated by protein C. Thromb.Haemostas.(Stuttgart) 1991; 66:160-165.

87. Warr TA, Rao VM, Rapaport SI. Disseminated intravascular coagulation in rabbits induced by administration of endotoxin or tissue factor: effect of anti-tissue factor antibodies and measurement of plasma extrinsic pathway inhibitor activity. Blood 1990; 75:1481-1489.

88. Colman RW. Disseminated intravascular coagulation due to sepsis. Semin.Hematol. 1994; 31 Suppl. 1:10-17.

89. Ferguson JH, Chapman OD. Fulminating meningococcic infections and the so-called Waterhouse-Friderichsen Syndrome. Am.J.Pathol. 1948; 24:763-796.

90. Wajchenberg B, Leme CE, Tambascia M, Boulos M, Okada H, Cesar FP, et al. The adrenal response to exogenous adrenocorticotrophin in patients with infections due to *Neisseria meningitidis*. J.Infect.Dis. 1978; 138:387-391.

91. Rothwell PM, Udwadia ZF, Lawler PG. Cortisol response to corticotropin and survival in septic shock. Lancet 1991; 337:582-583.

92. 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.

93. Lamberts SWJ, Bruining HA, de Jong FH. Corticosteroid therapy in severe illness. New Eng.J.Med. 1997; 337:1285-1292.

94. Matot I, Sprung CL. Corticosteroids in septic shock: resurrection of the last rites? Crit.Care Med. 1998; 26:627-630.

95. 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.

96. Stuber F, Udalova IA, Book M, Drutskaya LN, Kuprash DV, Turetskaya RL, et al. -308 Tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. J.Inflammation 1996; 46:42-50.

97. Brinkman BMN, Zuijdgeest, Kaijzel EL, Breedveld FC, Verweij CL. Relevance of the tumor necrosis factor alpha (TNFα) -308 promoter polymorphism in TNFα gene regulation. J.Inflammation 1996; 46:32-41.

98. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. J.Biol.Chem. 1993; 268:10739-10745.

99. Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-acrtivator inhibitor 1 gene is associated with myocardial infarction. Proc.Natl.Acad.Sci.USA 1995; 92:1851-1855.

100. Ossei-Gerning N, Mansfield MW, Strickland MH, Wilson IJ, Grant PJ. Plasminogen activator inhibitor-1 promoter 4G/5G genotype and plasma levels in relation to a history of myocardial infarction in patients characterized by coronary angiography. Arterioscler.Thromb.Vasc.Biol. 1997; 17:33-37.

101. Ye S, Green FR, Scarabin PY, Nicaud V, Bara L, Dawson SJ, et al. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 activity but not with risk of myocardial infarction in the ECTIM study. Etude CasTemoins de l'nfarctus du Mycocarde. Thromb.Haemost. 1995; 74:837-841.

102. Sandra L.Hofmann. Resistance to activated protein C: the most common inherited defect underlying the prothrombotic state. Medical Grand Rounds, Parkland Memorial Hospital . 9-28-1995. (GENERIC)
Ref Type: Unpublished Work

103. Westendorp RGJ, Reitsma PH, Bertina RM. Inherited prethrombotic disorders and infectious purpura. Thromb.Haemostas.(Stuttgart) 1996; 75:899-901.

104. U.S.Department of Health and Human Services.Public Health Service.Centers for Disease Control and Prevention. Control and Prevention of Meningococcal Disease. MMWR 1997; 46:1-21.

105. Cuevas LE, Kazembe P, Mughogho GK, Tillotson GS, Hart CA. Eradication of nasopharyngeal carriage of *Neisseria meningitidis* in children and adults in rural Africa: A comparison of ciprofloxacin and rifampicin. J.Infect.Dis. 1995; 171:728-731.

106. Bhat NM, Bieber MM, Chapman CJ, Stevenson FK, Teng NNH. Human antilipid A monoclonal antibodies bind to human B cells and the i antigen on cord red blood cells. J.Immunol. 1993; 151:5011-5021.

107. McCloskey RV, Straube RC, Sanders C, Smith SM, Smith CR, CHESS Trial Study Group. Treatment of septic shock with human monoclonal antibody HA-1A: A randomized, double-blind, placebo-controlled trial. Ann.Intern.Med. 1994; 121:1-5.

108. Elsbach P, Weiss J. Role of the bactericidal/permeability-increasing protein in host defence. Curr.Opin.Immunol. 1998; 10:45-49.

109. Giroir BP, Quint PA, Barton P, Kirsch EA, Kitchen L, Goldstein B, et al. Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeability-increasing protein in children with severe meningococcal sepsis. Lancet 1997; 350:1439-1443.

110. Christ WJ, Asano O, Robidoux ALC, Perez M, Wang Y, Dubuc GR, et al. E5531, a pure endotoxin antagonist of high potency. Science 1995; 268:80-83.

111. Fourrier, F., Jourdain, M., Tournois, A., Caron, C., Goudemand, J., and Chopin, C. Coagulation inhibitor substitution during sepsis. Intensive Care Medicine 21, S264-S268. 1998. (GENERIC)
Ref Type: Generic

112. Vinazzer H. Therapeutic use of antithrombin III in shock and disseminated intravascular coagulation. Seminars in Thrombosis and Hemostasis 1989; 15:347-352.

113. Fourrier F, Chopin C, Huart J-J, Runge I, Caron C, Goudemand J. Double-blind, placebo-controlled trial of antithrombin III concentrates in septic shock with disseminated intravascular coagulation. Chest 1993; 104:882-888.

114. Eisele B, Lamy M. Clinical experience with antithrombin III concentrates in critically ill patients with sepsis and multiple organ failure. Seminars in Thrombosis and Hemostasis 1998; 24:71-80.

115. Mammen EF. Antithrombin: its physiological importance and role in DIC. Seminars in Thrombosis and Hemostasis 1998; 24:19-25.

116. Johnson K, Aarden L, Choi Y, DeGroot E, Creasey A. The proinflammatory cytokine response to coagulation and endotoxin in whole blood. Blood 1996; 87:5051-5060.

117. Leclerc F, Hazelzet JA, Jude B, Hofhuis W, Hue V, Martinot A, et al. Protein C and S deficiency in severe infectious purpura of children: a collaborative study of 40 cases. Intensive Care Med. 1992; 18:202-205.

118. Alcaraz A, Espana F, Sanchez-Cuenca J, Zuazu I, Estelles AaJ, Vicente V. Activation of the protein C pathway in acute sepsis. Thromb.Res. 1995; 79:83-93.

119. Taylor FB, Jr., Chang A, Esmon CT, D'Angelo A, Vigano-D'Angelo S, Blick KE. Protein C prevents the coagulopathic and lethal effects of *Escherichia coli* infusion in the baboon. J.Clin.Invest. 1987; 79:918-925.

120. Rivard GE, David M, Farrell C, Schwarz HP. Treatment of purpura fulminans in meningococcemia with protein C concentrate. J.Pediatr. 1995; 126:646-652.

121. Frenkel, E. P. Tissue factor and factor VII. Medical Grand Rounds, Parkland Memorial Hospital . 1-6-94. (GENERIC)
Ref Type: Unpublished Work

122. Broze GJ. The role of tissue factor pathway inhibitor in a revised coagulation cascade. Semin.Hematol. 1992; 29:159-169.

123. Lupu C, Goodwin CA, Westmuckett AD, Emeis JJ, Scully MF, Kakkar VV, et al. Tissue factor pathway inhibitor in endothelial cells colocalizes with glycolipid microdomains/caveolae - Regulatory mechanism(s) of the anticoagulant properties of the endothelium. Arterioscler.Thromb.Vasc.Biol. 1997; 17:2964-2974.

124. Park CT, Creasey AA, Wright SD. Tissue factor pathway inhibitor blocks cellular effects of endotoxin by binding to endotoxin and interfering with transfer to CD14. Blood 1997; 89:4268-4274.

125. Camerota AJ, Creasey AA, Patla V, Larkin VA, Fink MP. Delayed treatment with recombinant tissue factor pathway inhibitor improves survival in rabbits with gram-negative peritonitis. J.Infect.Dis. 1998; 177:668-676.

126. Creasey AA, Chang ACK, Feigen L, Wün T-C, Taylor FB, Jr., Hinshaw LB. Tissue factor pathway inhibitor reduces mortality from *Escherichia coli* septic shock. J.Clin.Invest. 1993; 91:2850-2860.

127. Carr C, Bild GS, Chang ACK, Peer GT, Palmier MO, Frazier RB, et al. Recombinant *E. coli*-derived tissue factor pathway inhibitor reduces coagulopathic and lethal effects in the baboon gram-negative model of septic shock. Circ.Shock 1995; 44:126-137.

128. Dackiw APB, McGilvray ID, Woodside M, Nathens AB, Marshall JC, Rotstein OD. Prevention of endotoxin-induced mortality by antitissue factor immunization. Arch.Surg. 1996; 131:1273-1278.

129. Taylor FB, Jr., Chang A, Ruf W, Morrisey JH, Hinshaw L, Catlett R, et al. Lethal *E. coli* septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ.Shock 1991; 33:127-134.

130. Taylor FB, Jr., Chang AC, Peer G, Li A, Ezban M, Hedner U. Active site inhibited factor VIIa (DEGR VIIa) attenuates the coagulant and interleukin-6 and -8, but not tumor necrosis factor, responses of the baboon to LD_{100} *Escherichia coli*. Blood 1998; 91:1609-1615.

131. Biemond BJ, Levi M, Ten Cate H, Soule HR, Morris LD, Foster DL, et al. Complete inhibition of endotoxin-induced coagulation activation in chimpanzees with a monoclonal Fab fragment against factor VII/VIIa. Thromb.Haemost. 1995; 73:223-230.

132. Pineo GF, Hull RD. Hirudin and hirudin analogues as new anticoagulant agents. Curr.Opin.Hematol. 1995; 2:380-385.

133. Siebeck M, Spannagl M, Bichler J, Hoffmann H, Gippner-Steppert C, Jochum M. Delayed treatment with desulfato-hirudin prevents fibrin formation in lipopolysaccharide-induced shock. Thromb.Res. 1997; 88:473-480.

134. Hermida J, Montes R, Páramo JA, Rocha E. Endotoxin-induced disseminated intravascular coagulation in rabbits: Effect of recombinant hirudin on hemostatic parameters, fibrin deposits, and mortality. J.Lab.Clin.Med. 1998; 131:77-83.

135. Corrigan JJ, Jordan CM. Heparin therapy in septicemia with disseminated intravascular coagulation. New Eng.J.Med. 1998; 283:778-782.

136. Kuppermann N, Inkelis SH, Saladino R. The role of heparin in the prevention of extremity and digit necrosis in meningococcal purpura fulminans. Pediatr.Infect.Dis.J. 1994; 13:867-873.

137. Jordan RE, Kilpatrick J, Nelson RM. Heparin promotes the inactivation of antithrombin by neutrophil elastase. Science 1987; 237:777-779.

138. Zenz W, Muntean W, Gallistl S, Zobel G, Grubbauer HM. Recombinant tissue plasminogen activator treatment in two infants with fulminant meningococcemia. Pediatrics 1995; 96:144-148.

139. Muta T, Iwanaga S. The role of hemolymph coagulation in innate immunity. Curr.Opin.Immunol. 1996; 8:41-47.

140. Colvin RB, Dvorak H. Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. J.Exp.Med. 1973; 138:686-698.

141. Edwards RL, Rickles FR. Delayed hypersensitivity in man: effects of systemic anticoagulation. Science 1978; 200:541-543.

142. Johnson K, Choi Y, DeGroot E, Samuels I, Creasey A, Aarden L. Potential mechanisms for a proinflammatory vascular cytokine response to coagulation activation. J.Immunol. 1998; 160:5130-5135.

143. van Deventer SJH, Büller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. Experimental endotoxemia in humans: Analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood 1990; 76:2520-2526.

144. Wyshock EG, Suffredini AF, Parrillo JE, Colman RW. Cofactors V and VIII after endotoxin administration to human volunteers. Thromb.Res. 1995; 80:377-389.

145. DeLa Cadena RA, Suffredini AF, Page JD, Pixley RA, Kaufman N, Parrillo JE, et al. Activation of the kallikrein-kinin system after endotoxin administration to normal human volunteers. Blood 1993; 81:3313-3317.

146. 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.

147. Suffredini AF, Harpel PC, Parrillo JE. Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. N.Engl.J.Med. 1989; 320:1165-1172.

148. Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. Blood 1990; 76:1-16.

149. 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.