DYSFUNCTION OF ENDOTHELIAL PROTEIN C ACTIVATION IN SEVERE MENINGOCOCCAL SEPSIS

SAUL N. FAUST, M.R.C.P., MICHAEL LEVIN, F.R.C.P., PH.D., ODILE B. HARRISON, B.SC., ROBERT D. GOLDIN, F.R.C.PATH., MARION S. LOCKHART, B.SC., SHEILA KONDAVEETI, PH.D., ZOLTAN LASZIK, M.D., CHARLES T. ESMON, PH.D., AND ROBERT S. HEYDERMAN, M.R.C.P., PH.D.

ABSTRACT

Background Impairment of the protein C anticoagulation pathway is critical to the thrombosis associated with sepsis and to the development of purpura fulminans in meningococcemia. We studied the expression of thrombomodulin and the endothelial protein C receptor in the dermal microvasculature of children with severe meningococcemia and purpuric or petechial lesions.

Methods We assessed the integrity of the endothelium and the expression of thrombomodulin and the endothelial protein C receptor in biopsy specimens of purpuric lesions from 21 children with meningococcal sepsis (median age, 41 months), as compared with control skin-biopsy specimens.

Results The expression of endothelial thrombomodulin and of the endothelial protein C receptor was lower in the patients with meningococcal sepsis than in the controls, both in vessels with thrombosis and in vessels without thrombosis. On electron microscopical examination, the endothelial cells were generally intact in both thrombosed and nonthrombosed vessels. Plasma thrombomodulin levels in the children with meningococcal sepsis (median, 6.4 ng per liter) were higher than those in the controls (median, 3.6 ng per liter; P=0.002). Plasma levels of protein C antigen, protein S antigen, and antithrombin antigen were lower than those in the controls. In two patients treated with unactivated protein C concentrate, activated protein C was undetectable at the time of admission, and plasma levels remained low.

Conclusions In severe meningococcal sepsis, protein C activation is impaired, a finding consistent with down-regulation of the endothelial thrombomodulin–endothelial protein C receptor pathway. (N Engl J Med 2001;345:408-16.)

NEISSERIA MENINGITIDIS is the leading infectious cause of death in children in developed countries and is a cause of disability resulting from extensive skin damage and loss of limbs.[1,2] Severe meningococcal sepsis is characterized by marked inflammatory-cell activation, disseminated intravascular coagulation, and vascular compromise.[3-5] As compared with other forms of septic shock, the coagulopathy and microvascular thrombosis that develop in this type of sepsis are particularly severe. Purpura fulminans occurs in 10 to 20 percent of cases[6,7] and in severe cases involves thrombosis of the large vessels with infarction of the digits and limbs.[6,8] This disorder results from complex dysregulation of normal hemostatic mechanisms.[6-8] Procoagulant pathways are activated,[9-12] and there is impairment of both the natural anticoagulant pathways[13-17] and the fibrinolytic system.[18-21] It remains unclear why purpura fulminans develops in some patients, whereas in others with equally severe septic shock there are no thrombotic complications.

Dysfunction of the protein C activation pathway appears to be critical to the development of thrombosis in purpura fulminans.[6,7,22] Protein C is a vitamin K–dependent glycoprotein that circulates in plasma as an inactive zymogen. Once activated, protein C requires protein S as a cofactor for its anticoagulant functions. Congenital and acquired deficiencies of protein C or of protein S may result in purpura fulminans.[22-24] Replacement therapy with protein C concentrate prevents purpura fulminans in children with a congenital deficiency of this glycoprotein, and infusions of activated protein C have been shown to moderate the development of coagulopathy and prevent death in animal models of gram-negative sepsis and in humans with severe sepsis.[25,26] In meningococcal disease, plasma levels of protein C and protein S are markedly reduced,[13,16,17,27] but dysfunction of the endothelial protein C activation pathway may also be involved.

Activation of protein C requires binding of the protein to two receptors on the endothelial surface: thrombomodulin and the endothelial protein C receptor.[22] The resulting complex acts as a molecular switch that limits the procoagulant activity of thrombin (Fig. 1); this complex also has a number of anti-inflammatory properties.[22] We postulated that disruption of the activated endothelial protein C complex is an early event in the development of the widespread thrombosis and disseminated intravascular coagulation associated with severe meningococcal disease. To test this hypothesis, we studied the expression of thrombomodulin and the endothelial protein C

From the Departments of Paediatrics (S.N.F., M.L., O.B.H., S.K.) and Pathology (R.D.G.), Imperial College School of Medicine at St. Mary's Hospital, London; the Oklahoma Medical Research Foundation, Oklahoma City (M.S.L., C.T.E.); the Departments of Pathology (Z.L., C.T.E.) and Biochemistry and Molecular Biology (C.T.E.), University of Oklahoma Health Sciences Center, Oklahoma City; the Howard Hughes Medical Institute, Oklahoma City (C.T.E.); and the Departments of Pathology and Microbiology, University of Bristol, Bristol, United Kingdom (R.S.H.). Address reprint requests to Dr. Levin at the Department of Paediatrics, Imperial College School of Medicine at St. Mary's Hospital, Norfolk Pl., London W2 1PG, United Kingdom, or at m.levin@ic.ac.uk.

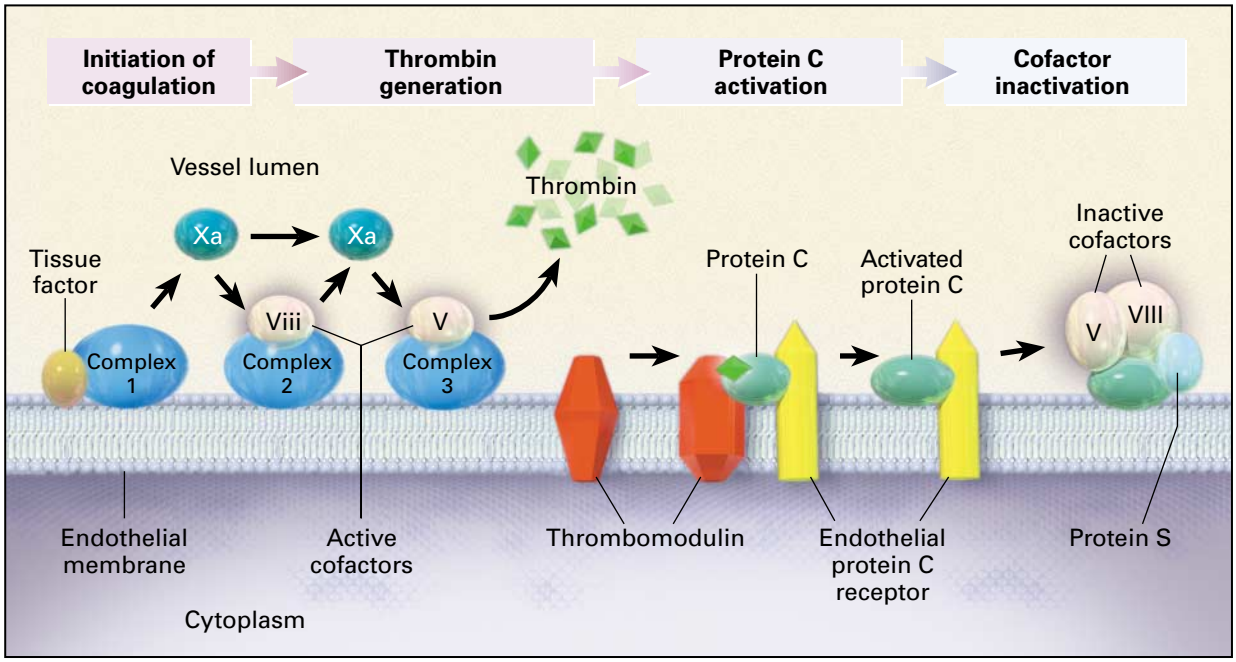

Figure 1. Endothelial Activation of Coagulation and the Protein C Pathway.

Coagulation is initiated by tissue factor and other coagulation-factor complexes on the surface of endothelial cells and monocytes. The activated factor X that is consequently generated requires activated cofactors V and VIII to produce thrombin, which in turn forms a complex with thrombomodulin. Protein C activation takes place by way of interaction between the thrombomodulin–thrombin complex and the endothelial protein C receptor. Activated protein C, together with its cofactor, protein S, inactivates factors V and VIII to provide negative feedback to the generation of thrombin. Complex 1 comprises tissue factor and coagulation factors VII, IX, and X; complex 2 comprises factors IX and X and cofactor VIII; and complex 3 comprises factor X, prothrombin, and cofactor V.

receptor on the dermal endothelium in skin-biopsy specimens from children with severe meningococcal disease and related these findings to events in the circulation.

METHODS

Patients and Specimens

Meningococcal disease was diagnosed according to clinical criteria and was confirmed microbiologically in children admitted to St. Mary's Hospital, London.[19,28] The study was approved by the St. Mary's local research-ethics committee and was conducted between January 1998 and November 2000. Informed consent was obtained from the parents of the hospitalized children for the collection of the clinical samples and from the healthy adults and the parents of the healthy children who served as controls.

Plasma samples were obtained from 83 children with meningococcal disease at recorded intervals after the first administration of parenteral antibiotics. The median age of the children was 37 months (range, 1 to 210), and the median Glasgow Meningococcal Septicemia Prognostic Score was 12 (range, 5 to 15) on a scale of 0 to 15 (higher scores denote more severe disease).[29] Two patients received treatment with unactivated protein C concentrate. The patients were categorized according to the clinical severity of their cutaneous disease, as follows: no scarring or only mild disease, severe scarring (no plastic surgery required), or purpura fulminans (requiring amputation or skin grafting or resulting in death due to severe disease). Control plasma samples were obtained from eight adults and eight healthy children before routine surgery.

Specimens of skin 3 mm in diameter were obtained by punch biopsy from the edge of purpuric or petechial lesions in 21 patients with severe meningococcal sepsis (a consecutive subgroup of the cohort of 83 children).[30] The median age of these 21 children was 41 months (range, 1 to 185). The median Glasgow Meningococcal Septicemia Prognostic Score was 11 of 15 (range, 6 to 15), and 9 of 15 patients had a Pediatric Risk of Mortality score above 50 percent.[31] All the biopsy specimens were taken within 24 hours after the administration of the first dose of parenteral antibiotics. In two additional patients, biopsy specimens were also obtained three months into recovery, during plastic-surgery procedures. Control skin-biopsy specimens were obtained from five children during routine surgery.

Immunohistochemical Studies

Formalin-fixed, paraffin-embedded sections of the skin-biopsy specimens were immunostained for thrombomodulin, endothelial protein C receptor, a neutrophil marker (neutrophil elastase), endothelial-cell markers (CD31 and CD34), and a monocyte marker (CD68) by the avidin–biotin–peroxidase method.[32] Sections 3 μm thick were incubated with monoclonal antibodies to detect expression of thrombomodulin (antibody, 7.8 μg per milliliter), endothelial protein C receptor (antibody, 0.2 mg per milliliter),[33] neutrophil elastase (M0752, Dako), CD31 (M0823, Dako), CD34 (M7165, Dako), and CD68 (M0876, Dako). Antigen retrieval by

microwave heat induction in citrate buffer (pH 6.0; HDS05, SD Supplies) was required for optimal staining with the anti-CD31, anti-CD34, and anti-CD68 antibodies.[34] Primary antibody binding was detected with an immunoperoxidase kit (Vectastain Elite ABC kit, Vector Laboratories).

In each section, the degree of thrombosis was assessed (severe [more than 66 percent of the vessels thrombosed], moderate [33 to 66 percent of the vessels thrombosed], or mild [less than 33 percent of the vessels thrombosed]), as was the degree of inflammation (severe, moderate, or mild). The intensity of immunostaining was graded semiquantitatively as strong, moderate, weak, or absent by comparison with the staining observed in control skin specimens, with strong staining equivalent to that in the controls. For each antigen, two to five sections, with 30 to 75 μm between sections, were studied to ensure that in all the biopsy specimens, both normal and abnormal tissue was obtained.

To avoid bias, all the sections were initially compared with the controls by a single investigator, who was unaware of the identity of the patients and the severity of their illness. For each antigen, 20 random sections were then assessed separately by two independent, blinded investigators to ensure that the interpretation of the staining was consistent and accurate. In addition, 15 biopsy specimens were examined by two independent histopathologists who had no knowledge of the nature of the study. Additional information is in Supplementary Appendix 1 (available with the full text of this article at http://www.nejm.org).

Electron Microscopy

For electron microscopy, skin-biopsy specimens were fixed in 4 percent glutaraldehyde and stained with osmium tetroxide. Final staining was with uranyl acetate and Reynold's lead citrate. Transmission electron microscopy (model 400, Philips) was carried out at magnifications of 1000 to 2800.

Assays of Plasma Antigens

Thrombomodulin antigen and thrombin–antithrombin complexes were detected with the use of a thrombomodulin enzyme-linked immunosorbent assay (ELISA) kit (Immubind [no. 837], American Diagnostica) and a TAT-complex ELISA kit (Enzygnost, Dade Behring). Endothelial protein C receptor, protein C, protein S, and antithrombin antigens were measured by ELISA.[35-38] Activated protein C in plasma was measured with the use of a modified enzyme-capture assay.[39] The lower limit of detection was 3 ng per milliliter. Details of this method are in Supplementary Appendix 2 (available with the full text of this article at http://www.nejm.org).

Statistical Analysis

Data are expressed as medians, means, and ranges. Comparisons between the patients and the controls were performed with use of paired t-tests with log-transformed data.

RESULTS

Histologic and Ultrastructural Findings

In all the biopsy specimens, the general tissue structure was well preserved; there was evidence of thrombosis and frequently of a perivascular, acute inflammatory-cell infiltrate of neutrophils and monocytes (Fig. 2). Thrombosis was classified as severe in 5 of the 21 patients, moderate in 9, mild in 4, and absent in 3. Inflammation was assessed as severe in 6 of these 21 patients, moderate in 12, and mild in 3. The intensity of staining for the endothelial-cell markers CD31 and CD34 in the biopsy specimens from the patients with meningococcal sepsis was generally equivalent to that observed in the control skin specimens, but discontinuous staining was occasionally observed in both thrombosed and nonthrombosed vessels. Transmission electron microscopy of skin-biopsy specimens from five children with purpuric lesions revealed that although some vessels (both those with and those without thrombosis) showed loss of endothelial cells with a characteristic lack of organelles, this finding was not widespread. The integrity of the endothelial cells was generally preserved in both thrombosed and nonthrombosed vessels (Fig. 3).

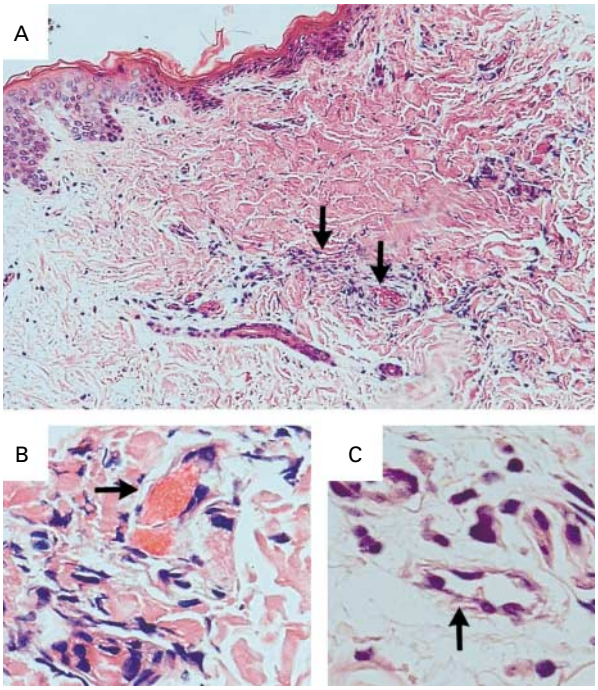

Figure 2. Skin-Biopsy Specimen from a Patient with Meningococcal Sepsis.

A biopsy specimen from a purpuric lesion shows areas containing thrombosed vessels (right-hand arrow in Panel A and arrow in Panel B) and a perivascular infiltrate (left-hand arrow in Panel A) (hematoxylin and eosin, ×100 [Panel A] and ×400 [Panel B]). Panel C shows inflammatory cells (arrow) around nonthrombosed vessels (hematoxylin and eosin, ×400). The cellular infiltrate consisted of both neutrophils (identified by neutrophil-elastase staining) and monocytes and macrophages (identified by CD68 staining).

Thrombomodulin and Endothelial Protein C Receptor Expression on Endothelium

The intensity of staining for endothelial thrombomodulin in the biopsy specimens from all the patients with meningococcal sepsis was less than that in the specimens from the controls (Fig. 4). Thrombomodulin staining was weak in 15 and moderate in 6 of the 21 specimens from the patients with menin-

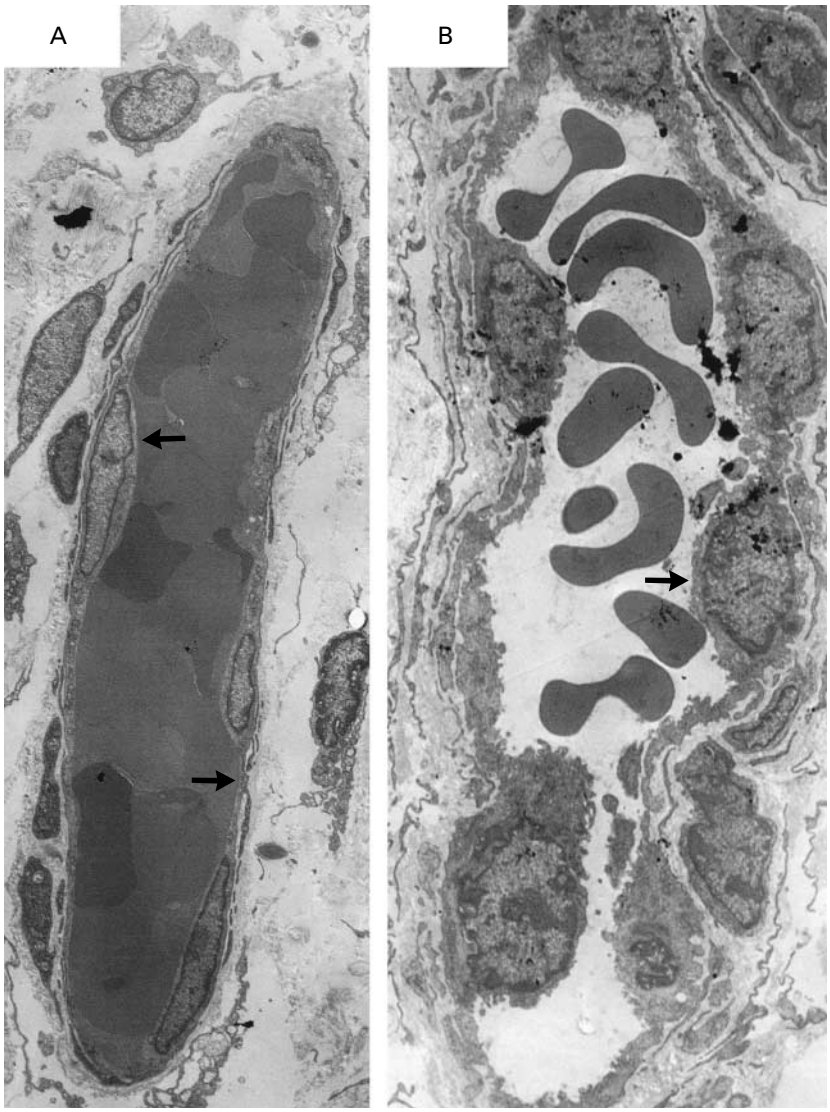

Figure 3. Transmission Electron Micrographs of Skin-Biopsy Specimens from Patients with Meningococcal Sepsis.

The endothelium is intact (arrows) in both a thrombosed vessel (Panel A, ×1300) and a nonthrombosed vessel (Panel B, ×2200). The black areas are artifacts that result from the processing of small and fragile specimens.

gococcal sepsis. The intensity of staining for endothelial protein C receptor was lower in 17 of these 21 specimens than in the control specimens: it was judged to be weak in 11 and moderate in 6, but staining was strong (i.e., equivalent to that of the controls) in the other 4 specimens (Fig. 5). Comparatively weak staining for thrombomodulin and endothelial protein C receptor was also observed in areas with little thrombosis. Occasionally, areas of weak thrombomodulin staining were identified where moderate or strong staining for endothelial protein C receptor was preserved in adjacent sections. In two patients with severe purpura fulminans, comparative examination of skin-biopsy specimens over time revealed the resolution of most of the initial changes, with some residual acute inflammatory infiltrate three months later (Fig. 6).

Plasma Levels of Thrombomodulin, Endothelial Protein C Receptor, Protein C, Protein S, and Antithrombin Antigens and Thrombin–Antithrombin Complexes

The plasma levels of thrombomodulin antigen were significantly higher in all the patients with meningococcal disease on day 1 after the initiation of treat-

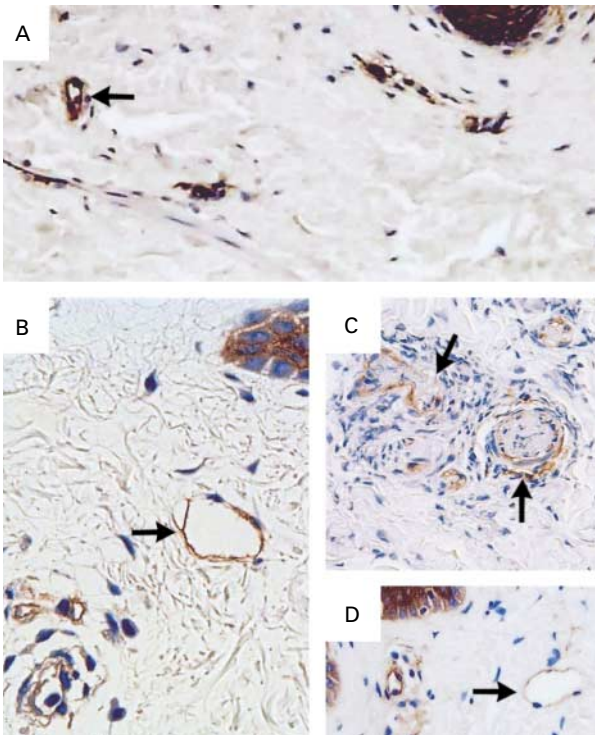

Figure 4. Skin-Biopsy Specimens Incubated with a Monoclonal Antibody against Thrombomodulin (Immunoperoxidase Stain).

Panel A (×200) shows normal skin with intense thrombomodulin staining on endothelial cells (arrow). Epidermal cells are known to stain nonspecifically with this antibody[33] (as seen in the area of dense staining [upper right]). Panels B (×200), C (×400), and D (×200) show skin specimens from patients with meningococcal sepsis. There is reduced thrombomodulin staining in nonthrombosed vessels (arrows in Panels B and D) and thrombosed vessels (arrows in Panel C).

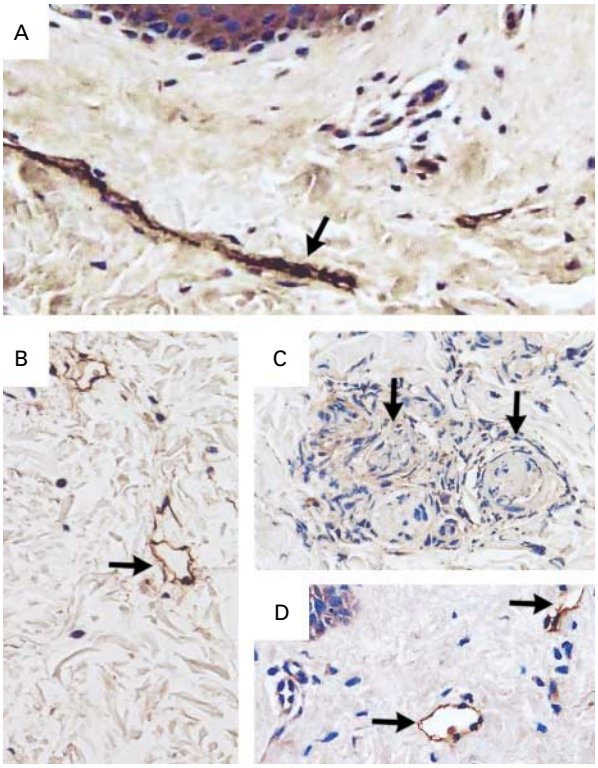

Figure 5. Skin-Biopsy Specimens Incubated with an Antibody against Endothelial Protein C Receptor (Immunoperoxidase Stain).

Panel A (×200) shows normal skin, with intense staining for endothelial protein C receptor on endothelial cells (arrow). Epidermal cells are known to stain nonspecifically with this antibody[33] (as seen in the area of weak staining [upper part of panel]). Panels B (×200), C (×400), and D (×200) show skin from children with meningococcal sepsis, in which the staining for endothelial protein C receptor is reduced, in both nonthrombosed vessels (arrows in Panels B and D) and thrombosed vessels (arrows in Panel C).

ment than in the controls ($P=0.002$). The levels had returned to normal six to eight weeks later. The levels of thrombomodulin antigen correlated with the severity of disease (Table 1). Plasma levels of endothelial protein C receptor antigen in the children with moderate disease were not significantly different from those in either the children or the adults who served as controls ($P=0.35$ and $P=0.30$, respectively). Plasma levels of protein C, protein S, and antithrombin antigens were also low on day 1 ($P<0.001$, $P=0.004$, and $P=0.01$, respectively, for the comparison with the levels in the controls). Activation of coagulation was confirmed by the detection of high levels of thrombin–antithrombin complexes on day 1.

Activated Protein C in Plasma

On day 1 after the initiation of treatment, 11 of 14 patients had undetectable plasma levels of activated protein C (lower limit of detection, 3 ng per milliliter). On days 2, 3, and 4, activated protein C was undetectable in all 14 of these patients. Two patients received unactivated protein C concentrate (50 IU per kilogram of body weight every eight hours) on days 1, 2, and 3. Although trough levels (measured immediately before an infusion) and peak levels (measured one hour after an infusion) of protein C antigen were within the normal range, activated protein C was not detected. To ensure that these observations were not due to the presence of a plasma inhibitor, in separate experiments plasma samples from patients with meningococcal sepsis were spiked with exogenous activated protein C before the assay. In these experiments, 80 to 90 percent of the expected function of the added activated protein C was recovered (data not shown).

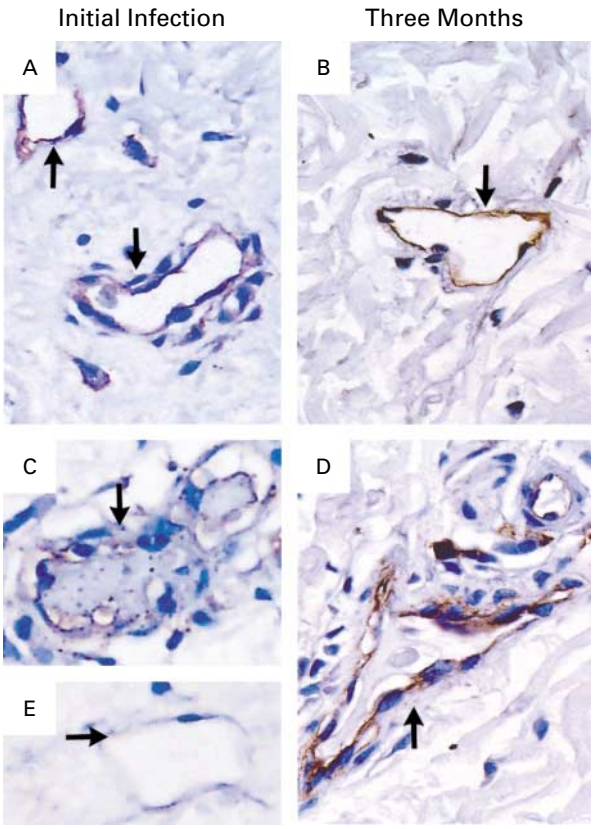

Figure 6. Immunostaining for Thrombomodulin in Skin-Biopsy Specimens from Patients with Meningococcal Sepsis during the Initial Infection and Three Months Later (Immunoperoxidase Stain, ×200).

Thrombomodulin immunostaining of skin-biopsy specimens from a patient with acute meningococcal sepsis during the initial infection (Panel A) and three months later (Panel B) is shown. The same sequence is shown for a second patient (Panels C and D). Panel E is another initial specimen from the second patient. The arrows in Panels A and E show unthrombosed vessels with reduced thrombomodulin staining, and the arrow in Panel C a thrombosed vessel with reduced thrombomodulin staining. Partial recovery of thrombomodulin expression (arrows in Panels B and D), together with some residual inflammatory infiltrate, is seen in both patients after three months.

DISCUSSION

The control of intravascular thrombosis depends on a carefully regulated balance of prothrombotic and antithrombotic mechanisms both on the vessel wall and in the circulation. Because of the difficulties of studying the vascular endothelium, most of what is known about changes in endothelial thromboregulatory pathways has been inferred from studies in vitro. We studied important regulators of the coagulation process in both the vascular endothelium and the plasma of children with acute meningococcal sepsis. The results show impairment of an endothelium-based anticoagulation pathway in vivo.

We found a marked reduction in the expression of thrombomodulin and endothelial protein C receptor on the endothelium of both thrombosed and nonthrombosed dermal vessels in children with early meningococcal disease. Ultrastructural studies showed that this reduction could not be explained simply by the loss of endothelial cells. The finding that plasma levels of activated protein C were low or undetectable in children with meningococcal sepsis, as well as the failure of activated protein C levels to rise after the administration of unactivated protein C concentrate (in two patients), suggests that the reduction in the endothelial expression of thrombomodulin and endothelial protein C receptor results in the impairment of protein C activation. Given the widespread generation of thrombin in meningococcal sepsis, we would have expected the plasma levels of activated protein C to increase markedly if the endothelial pathways for activation were intact. Alternative explanations are that the activated protein C is bound to cellular sites, that it has been inactivated, or that it has been cleared from the circulation. Whatever the mechanism, in meningococcal sepsis a functional impairment of the endothelial protein C activation pathway will exacerbate the existing state of hypercoagulability that is due to markedly reduced levels of protein C, protein S, and antithrombin.

Several processes during the acute inflammatory response have been implicated in the reductions in endothelial-cell thrombomodulin and endothelial protein C receptor. These include down-regulation of transcription of the genes encoding thrombomodulin and endothelial protein C receptor in response to cytokines and endotoxin[40,41] and enzymatic cleavage of the protein C activation complex.[42,43] As others have observed in studies of sepsis,[44,45] we found a marked elevation of plasma thrombomodulin levels in meningococcemia and a relation between plasma antigen levels and the severity of the disease. Given our finding of reduced thrombomodulin expression in dermal endothelium, there appears to be shedding of this molecule from the endothelial surface rather than failure of protein production.[46] This shedding may be mediated by an inflammatory process involving both meningococci in the dermis[47,48] and host inflammatory cells and mediators.[3,5,12,49] We have previously shown in vitro that meningococci adhering to the vascular endothelium act as a focus for the up-regulation of cell-adhesion molecules (intercellular adhesion molecule 1, vascular-cell adhesion molecule 1, and E-selectin [CD62E]) and for the attachment of neutrophils[50,51] and that endothelial-cell glycosaminoglycans are cleaved from the endothelial surface in response to activated neutrophils and other inflammatory stimuli.[50,52,53] Thrombomodulin is normally attached to a glycosaminoglycan (chondroitin sulfate)

TABLE 1. RESULTS OF ANALYSIS OF PLASMA ANTIGEN LEVELS IN CHILDREN WITH MENINGOCOCCAL SEPSIS ON DAY 1 AFTER THE INITIATION OF TREATMENT AND IN CONTROLS.*

ANALYTE	NO. OF CHILDREN	SEVERITY OF DISEASE	MEDIAN	RANGE	MEAN	P VALUE
				ng/liter		
Thrombomodulin antigen	76	Control	3.6	2.8–5.0	3.7	—
		Mild disease	4.7	2.9–15.5	5.7	0.05
		Moderate disease	6.4	4.3–20.8	7.5	0.002
		Severe disease	8.2	3.8–17.8	8.5	<0.001
		Any disease	6.4	2.9–20.8	7.1	0.002
		Convalescence	4.8	2.9–9.6	5.1	0.52
				ng/ml		
Endothelial protein C receptor antigen	65	Control	415	150–724	403	—
		Mild disease	238	118–514	260	0.99
		Moderate disease	314	99.9–412	276	0.35
		Severe disease	310	118–784	332	0.78
		Any disease	264	99.9–784	292	0.35
		Convalescence	451	244–1080	530	0.90
				IU/ml		
Protein C antigen	58	Control	0.72	0.63–1.14	0.79	—
		Mild disease	0.19	0.07–0.81	0.23	0.01
		Moderate disease	0.15	0.06–0.30	0.16	<0.001
		Severe disease	0.27	0.05–0.64	0.27	<0.001
		Any disease	0.19	0.05–0.81	0.24	<0.001
		Convalescence	0.75	0.51–1.30	0.79	0.28
				IU/ml		
Protein S antigen	61	Control	0.73	0.66–1.23	0.84	—
		Mild disease	0.43	0.16–0.89	0.45	0.03
		Moderate disease	0.38	0.15–0.69	0.41	0.004
		Severe disease	0.49	0.12–1.06	0.49	0.005
		Any disease	0.43	0.12–1.06	0.46	0.004
		Convalescence	0.68	0.49–1.16	0.75	0.03
				IU/ml		
Antithrombin antigen	74	Control	1.06	0.61–7.48	1.81	—
		Mild disease	0.40	0–0.90	0.40	0.01
		Moderate disease	0.32	0.01–0.64	0.31	0.01
		Severe disease	0.38	0–1.06	0.42	0.001
		Any disease	0.38	0–1.06	0.39	0.01
		Convalescence	1.17	0.59–1.88	1.21	0.61
				μg/ml		
Thrombin–antithrombin complexes	15	Control	5.3	0–24.3	6.6	—
		Mild disease	10.6	4.5–87.2	18.1	0.33
		Moderate disease	39.0	21.9–56.1	39.0	0.13
		Severe disease	76.7	57.0–150.0	83.9	0.001
		Any disease	38.4	4.5–150.0	47.1	0.19
		Convalescence	3.3	2.0–12.9	5.7	0.84

*P values are for the comparison with the children without meningococcal sepsis (controls). For each analyte, the number of samples varied according to the quantity of plasma available for analysis.

on the endothelial surface,[54] and disruption of the glycosaminoglycan component of thrombomodulin by these mediators may therefore lead to further dysfunction of this molecule. Why similar changes in the endothelial protein C receptor in plasma were not observed remains uncertain.[55] Soluble endothelial protein C receptor may be bound to activated leukocytes[56] or further degraded, reducing its immunoreactivity.

Severe meningococcal sepsis frequently presents a therapeutic challenge.[4] Current strategies involving the replacement of clotting factors, platelets, and fibrinogen may be insufficient to arrest the progression of widespread thrombosis.[15,25,57-61] An improvement in the outcome of children with meningococcal sepsis who were treated with unactivated protein C concentrates has been described in case reports and

one uncontrolled series.[57,62-64] The theoretical advantage of protein C is that it would be activated at the site of injury in response to the generation of thrombin and that the activation of the anticoagulant system would be proportional to the concentration of thrombin and cease when thrombin generation was controlled by the normal anticoagulant systems. However, this approach requires the vascular protein C activation complex to be intact. Our finding that the endothelial pathways required for protein C are impaired in severe meningococcal sepsis may have important therapeutic implications. For example, activated protein C does not require a functioning thrombomodulin–endothelial protein C receptor pathway, so it may be more effective than unactivated protein C concentrate in patients in whom the activation complex is compromised. A successful phase 3 clinical trial of activated protein C in adults has recently been reported.[26]

Endothelial events are important in controlling both coagulation and inflammation within the vascular compartment,[7,12] so studies of plasma events can provide only a partial picture of the processes involved. The strategy we used may be helpful in investigating the thrombotic and inflammatory processes in other acute disorders. Although observations made in dermal blood vessels may not fully reflect events in the systemic circulation, it is likely that for a generalized disease such as meningococcal sepsis, events in the cutaneous vasculature reflect those taking place elsewhere. The levels of protein C are low in many forms of sepsis,[65,66] and the endothelial dysfunction we observed may also occur in other severe bacterial infections. We speculate that the dysfunction of the endothelial protein C activation pathway that occurs in meningococcal sepsis is more profound than that in other forms of septic shock and that this difference may explain the common association of this disease with purpura fulminans.

Supported by a Clinical Training Fellowship from the Medical Research Council, United Kingdom (to Dr. Faust); by a project grant from the Meningitis Research Foundation (to Dr. Levin and Dr. Heyderman); and by a grant (P50 HL54804) from the Howard Hughes Medical Institute (to Dr. Esmon).

We are indebted to Naomi Esmon and Deborah Stearns-Kurosawa (Oklahoma Medical Research Foundation); Rodney Rivers, Suresh Ladva, Marjorie Walker, Alick Stephens, Funmi Daramola, and Jane Webb (Imperial College at St. Mary's Hospital); the Paediatric Intensive Care clinical team and Mary Thompson (St. Mary's Hospital); Simon J. Davidson (Imperial College at the Royal Brompton Hospital); Nigel Klein, John Harper, and Marion Malone (Great Ormond Street Hospital); and Edward Sheffield (University of Bristol).

REFERENCES

1. Achtman M. Global epidemiology of meningococcal disease. In: Cartwright K, ed. Meningococcal disease. Chichester, England: John Wiley, 1995:159-75.
2. Pollard AJ, Britto J, Nadel S, DeMunter C, Habibi P, Levin M. Emergency management of meningococcal disease. Arch Dis Child 1999;80:290-6.
3. Brandtzaeg P. Pathogenesis of meningococcal infections. In: Cartwright K, ed. Meningococcal disease. Chichester, England: John Wiley, 1995:71-114.
4. Duncan A. New therapies for severe meningococcal disease but better outcomes? Lancet 1997;350:1565-6.
5. de Kleijn ED, Hazelzet JA, Kornelisse RF, de Groot R. Pathophysiology of meningococcal sepsis in children. Eur J Pediatr 1998;157:869-80.
6. Eley B, Levin M. Purpura fulminans. In: Harper J, Oranje A, Prose N, eds. Textbook of pediatric dermatology. Vol. 2. Malden, Mass.: Blackwell Science, 2000:1574-87.
7. Faust SN, Heyderman RS, Levin M. Disseminated intravascular coagulation and purpura fulminans secondary to infection. Baillieres Best Pract Res Clin Haematol 2000;13:179-97.
8. Heyderman RS, Habibi P. Skin manifestations of meningococcal infection. In: Harper J, Oranje A, Prose N, eds. Textbook of pediatric dermatology. Vol. 1. Malden, Mass.: Blackwell Science, 2000:384-94.
9. Osterud B, Flaegstad T. Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. Thromb Haemost 1983;49:5-7.
10. Heyderman RS, Klein NJ, Daramola OA, et al. Induction of human endothelial tissue factor expression by Neisseria meningitidis: the influence of bacterial killing and adherence to the endothelium. Microb Pathog 1997;22:265-74.
11. Mavrommatis AC, Theodoridis T, Orfanidou A, Roussos C, Christopoulou-Kokkinou V, Zakynthinos S. Coagulation system and platelets are fully activated in uncomplicated sepsis. Crit Care Med 2000;28:451-7.
12. Levi M, de Jonge E, van der Poll T, ten Cate H. Disseminated intravascular coagulation. Thromb Haemost 1999;82:695-705.
13. 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;55:459-70.
14. Shimura M, Wada H, Wakita Y, et al. Plasma tissue factor and tissue factor pathway inhibitor levels in patients with disseminated intravascular coagulation. Am J Hematol 1996;52:165-70.
15. Fourrier F, Lestavel P, Chopin C, 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;16:121-4.
16. Hazelzet JA, Risseeuw-Appel IM, Kornelisse RF, et al. Age-related differences in outcome and severity of DIC in children with septic shock and purpura. Thromb Haemost 1996;76:932-8.
17. Sheth SB, Carvalho AC. Protein S and C alterations in acutely ill patients. Am J Hematol 1991;36:14-9.
18. 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;57:271-8.
19. Hermans PW, Hibberd ML, Booy R, et al. 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Lancet 1999;354:556-60.
20. Kornelisse RF, Hazelzet JA, Savelkoul HF, et al. The relationship between plasminogen activator inhibitor-1 and proinflammatory and counter-inflammatory mediators in children with meningococcal septic shock. J Infect Dis 1996;173:1148-56.
21. Paramo JA, Perez JL, Serrano M, Rocha E. Types 1 and 2 plasminogen activator inhibitor and tumor necrosis factor alpha in patients with sepsis. Thromb Haemost 1990;64:3-6.
22. Esmon CT, Ding W, Yasuhiro K, et al. The protein C pathway: new insights. Thromb Haemost 1997;78:70-4.
23. Dreyfus M, Magny JF, Bridey F, et al. Treatment of homozygous protein C deficiency and neonatal purpura fulminans with a purified protein C concentrate. N Engl J Med 1991;325:1565-8.
24. Levin M, Eley BS, Louis J, Cohen H, Young L, Heyderman RS. Postinfectious purpura fulminans caused by an autoantibody directed against protein S. J Pediatrics 1995;127:355-63.
25. 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;79:918-25.
26. Bernard GR, Vincent J-L, Laterre P-F, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001;344:699-708.
27. Powars D, Larsen R, Johnson J, et al. Epidemic meningococcemia and purpura fulminans with induced protein C deficiency. Clin Infect Dis 1993;17:254-61.
28. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Lancet 1999;353:1049-53.
29. Thomson AP, Sills JA, Hart CA. Validation of the Glasgow Meningococcal Septicemia Prognostic Score: a 10-year retrospective survey. Crit Care Med 1991;19:26-30.

30. van Deuren M, van Dijke BJ, Koopman RJ, et al. Rapid diagnosis of acute meningococcal infections by needle aspiration or biopsy of skin lesions. BMJ 1993;306:1229-32.
31. Pollack MM, Ruttimann UE, Getson PR. Pediatric Risk of Mortality (PRISM) score. Crit Care Med 1988;16:1110-6.
32. Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981;75:734-8.
33. Laszik Z, Mitro A, Taylor FB Jr, Ferrell G, Esmon CT. Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway. Circulation 1997;96: 3633-40.
34. Cattoretti G, Pileri S, Parravicini C, et al. Antigen unmasking on formalin-fixed, paraffin-embedded tissue sections. J Pathol 1993;171:83-98.
35. Kurosawa S, Stearns-Kurosawa DJ, Hidari N, Esmon CT. Identification of functional endothelial protein C receptor in human plasma. J Clin Invest 1997;100:411-8.
36. Deutz-Terlouw PP, Ballering L, van Wijngaarden A, Bertina RM. Two ELISA's for measurement of protein S, and their use in the laboratory diagnosis of protein S deficiency. Clin Chim Acta 1990;186:321-34.
37. Soria J, Soria C, Samama M, Nicolas G, Kisiel W. Severe protein C deficiency in congenital thrombotic disease — description of an immunoenzymological assay for protein C determination. Thromb Haemost 1985;53:293-6.
38. Edgar P, Jennings I, Harper P. Enzyme linked immunosorbent assay for measuring antithrombin III. J Clin Pathol 1989;42:985-7.
39. Gruber A, Griffin JH. Direct detection of activated protein C in blood from human subjects. Blood 1992;79:2340-8.
40. Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. J Biol Chem 1994;269:26486-91.
41. Moore KL, Andreoli SP, Esmon NL, Esmon CT, Bang NU. Endotoxin enhances tissue factor and suppresses thrombomodulin expression of human vascular endothelium in vitro. J Clin Invest 1987;79:124-30.
42. Furuno T, Mitsuyama T, Hidaka K, Tanaka T, Hara N. The role of neutrophil elastase in human pulmonary artery endothelial cell injury. Int Arch Allergy Immunol 1997;112:262-9.
43. Esmon CT, Xu J, Gu JM, et al. Endothelial protein C receptor. Thromb Haemost 1999;82:251-8.
44. Ikegami K, Suzuki Y, Yukioka T, Matsuda H, Shimazaki S. Endothelial cell injury, as quantified by the soluble thrombomodulin level, predicts sepsis/multiple organ dysfunction syndrome after blunt trauma. J Trauma 1998;44:789-94.
45. Iba T, Yagi Y, Kidokoro A, Fukunaga M, Fukunaga T. Increased plasma levels of soluble thrombomodulin in patients with sepsis and organ failure. Surg Today 1995;25:585-90.
46. Krafte-Jacobs B, Brilli R. Increased circulating thrombomodulin in children with septic shock. Crit Care Med 1998;26:933-8.
47. Harrison OB, Faust SN, Goldin RD, Levin M, Robertson BD, Heyderman RS. Meningococcal host cell interactions in the skin of children with purpura fulminans: variation in the expression of capsule and type IV pili. In: Abstracts of the 12th International Pathogenic Neisseria Conference, Galveston, Tex., Nov. 12–17, 2000:18. abstract.
48. 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;133:506-14.
49. Nadel S, Levin M, Habibi P. Treatment of meningococcal disease in childhood. In: Cartwright K, ed. Meningococcal disease. Chichester, England: John Wiley, 1995:207-43.
50. Klein NJ, Ison CA, Peakman M, et al. The influence of capsulation and lipooligosaccharide structure on neutrophil adhesion molecule expression and endothelial injury by Neisseria meningitidis. J Infect Dis 1996; 173:172-9.
51. Dixon GL, Heyderman RS, Kotovicz K, et al. Endothelial adhesion molecule expression and its inhibition by recombinant bactericidal/permeability-increasing protein are influenced by the capsulation and lipooligosaccharide structure of Neisseria meningitidis. Infect Immun 1999;67:5626-33.
52. Heyderman RS, Klein NJ, Shennan GI, Levin M. Reduction of the anticoagulant activity of glycosaminoglycans on the surface of the vascular endothelium by endotoxin and neutrophils: evaluation by an amidolytic assay. Thromb Res 1992;67:677-85.
53. Klein NJ, Shennan GI, Heyderman RS, Levin M. Alteration in glycosaminoglycan metabolism and surface charge on human umbilical vein endothelial cells induced by cytokines, endotoxin and neutrophils. J Cell Sci 1992;102:821-32.
54. Sadler JE. Thrombomodulin structure and function. Thromb Haemost 1997;78:392-5.
55. Kurosawa S, Stearns-Kurosawa DJ, Carson CW, D'Angelo A, Della Valle P, Esmon CT. Plasma levels of endothelial cell protein C receptor are elevated in patients with sepsis and systemic lupus erythematosus: lack of correlation with thrombomodulin suggests involvement of different pathological processes. Blood 1998;91:725-7.
56. Kurosawa S, Esmon CT, Stearns-Kurosawa DJ. The soluble endothelial protein C receptor binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18. J Immunol 2000;165:4697-703.
57. Smith OP, White B, Vaughan D, et al. Use of protein-C concentrate, heparin, and haemodiafiltration in meningococcus-induced purpura fulminans. Lancet 1997;350:1590-3.
58. Zenz W, Muntean W, Gallistl S, Zobel G, Grubbauer HM. Recombinant tissue plasminogen activator treatment in two infants with fulminant meningococcemia. Pediatrics 1995;96:44-8.
59. Creasey AA, Chang AC, Feigen L, Wun TC, Taylor FB Jr, Hinshaw LB. Tissue factor pathway inhibitor reduces mortality from Escherichia coli septic shock. J Clin Invest 1993;91:2850-6.
60. Fourrier F, Chopin C, Huart JJ, Runge I, Caron C, Goudemand J. Double-blind, placebo-controlled trial of antithrombin III concentrates in septic shock with disseminated intravascular coagulation. Chest 1993;104:882-8.
61. Taylor FB Jr, Chang A, Ruf W, et al. Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock 1991;33:127-34.
62. Rintala E, Seppala OP, Kotilainen P, Pettila V, Rasi V. Protein C in the treatment of coagulopathy in meningococcal disease. Crit Care Med 1998; 26:965-8.
63. de Jonge E, Levi M, Stoutenbeek CP, van Deventer SJ. Current drug treatment strategies for disseminated intravascular coagulation. Drugs 1998;55:767-77.
64. White B, Livingstone W, Murphy C, Hodgson A, Rafferty M, Smith OP. An open-label study of the role of adjuvant hemostatic support with protein C replacement therapy in purpura fulminans-associated meningococcemia. Blood 2000;96:3719-24.
65. Lorente JA, Garcia-Frade LJ, Landin L, et al. Time course of hemostatic abnormalities in sepsis and its relation to outcome. Chest 1993;103: 1536-42.
66. Hesselvik JF, Malm J, Dahlback B, Blomback M. Protein C, protein S and C4b-binding protein in severe infection and septic shock. Thromb Haemost 1991;65:126-9.