Cellular origin and procoagulant properties of microparticles in meningococcal sepsis

Rienk Nieuwland, René J. Berckmans, Sarah McGregor, Anita N. Böing, Fred P. H. Th. M. Romijn, Rudi G. J. Westendorp, C. Erik Hack, and Augueste Sturk

Patients with meningococcal sepsis generally suffer from disseminated intravascular coagulation (DIC). The aim of this study was to address whether these patients have elevated numbers of circulating microparticles that contribute to the development of DIC. Plasma samples from 5 survivors, 2 nonsurvivors, and 5 healthy volunteers were analyzed for the presence of microparticles by flow cytometry. Ongoing coagulation activation in vivo was quantified by enzyme-linked immunosorbent assay of plasma prothrombin fragment F_{1+2}, and procoagulant properties of microparticles in vitro were estimated by thrombin-generation assay. On admission, all patients had increased numbers of microparticles originating from platelets or granulocytes when compared with controls ($P = .004$ and $P = .008$, respectively). Patients had elevated levels of F_{1+2} ($P = .004$), and their microparticles supported thrombin generation more strongly in vitro ($P = .003$) than those of controls. Plasma from the patient with the most fulminant disease course and severe DIC contained microparticles that expressed both CD14 and tissue factor, and these microparticles demonstrated extreme thrombin generation in vitro. We conclude that patients with meningococcal sepsis have elevated numbers of circulating microparticles that are procoagulant. These findings may suggest a novel therapeutic approach to combat clinical conditions with excessive coagulation activation. (Blood. 2000;95:930-935)

© 2000 by The American Society of Hematology

Introduction

Meningococcal sepsis is a life-threatening disease that occurs most frequently during childhood and is characterized by excessive activation of many cells and cascades, which results in disseminated intravascular coagulation (DIC) and shock.[1,2] Although it is clear that the cascade of inflammatory and clotting reactions is triggered by the meningococcal bacteria, and particularly by their release of endotoxin, the precise mechanisms underlying these reactions are not well understood. For example, the mechanism underlying the development of DIC, a typical threatening feature of meningococcal sepsis, is unknown.

Clotting requires the presence of phospholipid cofactors that serve as a surface to assemble the various complexes to activate the clotting factors. In vitro studies have shown that activated platelets, and in particular microparticles generated from them, contain a large number of binding sites for activated factor IX (IXa),[3] factor Va,[4] and factor VIII[5] and support both factor Xa activity[6,7] and prothrombinase activity.[4,7] Increased numbers of platelet-derived microparticles are present in the circulation of patients who have an increased risk for thromboembolic complications, such as patients undergoing cardiac surgery[8] or plasmapheresis[9] and in patients suffering from diabetes,[10] heparin-induced thrombocytopenia,[11] myocardial infarction,[12] uremia,[13] idiopathic thrombocytopenic purpura,[14] or thrombotic thrombocytopenic purpura.[15] Functional studies of these microparticles were not performed. Other studies about the presence of microparticles of nonplatelet origin in the circulation have not been reported thus far. We have shown recently that elevated levels of platelet- and erythrocyte-derived microparticles are present in wound blood collected directly from the pericardial cavity in patients undergoing cardiac surgery.[16] These in vivo–generated microparticles strongly bind annexin V, a protein known for its interaction with negatively charged phospholipids such as phosphatidylserine, one of the essential lipid cofactors for clotting. Upon addition to normal plasma, these microparticles supported the generation of thrombin by a tissue factor–factor VII-mediated pathway. Hence, microparticles may be involved in activation of the systemic coagulation in vivo.

In the present study, we investigated the presence, cellular source, and function of circulating microparticles in patients suffering from meningococcal sepsis. Plasma samples of these patients were analyzed for the presence of microparticles (flow cytometry) and their procoagulant activity (thrombin generation assay) and were compared with the number and properties of microparticles in plasma from 5 healthy volunteers. Our results show that patients with meningococcal sepsis have elevated numbers of circulating microparticles derived from various blood cells and that these microparticles support clotting. We suggest that extreme DIC is strongly linked to circulating tissue factor–expressing microparticles in this disease and possibly also in other clinical conditions with excessive coagulation activation.

From the Departments of Clinical Chemistry, General Internal Medicine, and Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; Department of Physiology, University of Edinburgh, Edinburgh, United Kingdom; University Hospital Vrije Universiteit, Amsterdam; and CLB, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands.

Submitted April 6, 1999; accepted September 24, 1999.

Reprints: Rienk Nieuwland, Department of Clinical Chemistry, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands; e-mail: rnieuwland@ckcl.azl.nl.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2000 by The American Society of Hematology

Materials and methods

Reagents and assays

Reptilase was obtained from Boehringer Mannheim (Mannheim, Germany), thrombin chromogenic substrate S2238 from Chromogenix AB (Mölndal, Sweden), and normal mouse serum and fluorescein isothiocyanate (FITC)-labeled anti-CD4 (anti-CD4–FITC) from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB, Amsterdam, The Netherlands). Anti–glycophorin A–FITC and anti-CD61–FITC were obtained from Dakopatts (Glostrup, Denmark). Mouse IgG_1-FITC, IgG_{2a}-FITC, IgG_1-phycoerythrin (PE; all used as controls), anti-CD14–PE, anti-CD8–FITC, and anti-CD20–FITC monoclonal antibody (mAb) were from Becton Dickinson (San Jose, CA). Annexin V–FITC was from Nexins Research B.V. (Hoeven, The Netherlands), anti–factor VII and anti–factor XII were from CLB, and anti–E-selectin–FITC (anti-CD62E–FITC) was from Serotec Ltd (Kidlington, UK). Anti-CD14–FITC was from Biosource (Camarillo, CA), anti-CD66b–FITC and IgG_{2b}-PE (control mAb) were from Immuno Quality Products (Groningen, The Netherlands), and anti–tissue factor–FITC and polyclonal rabbit anti-human tissue factor were from American Diagnostics (Greenwich, CT). Annexin V–PE was from PharMingen (San Jose, CA). F_{1+2} was determined by enzyme-linked immunosorbent assay (Enzygnost F_{1+2} micro) as described by the manufacturer (Behring Diagnostics GmbH, Marburg, Germany).

Clinical studies

All patients included in the study had (1) a positive blood culture for *Neisseria meningitidis*; (2) signs and symptoms of septic shock; (3) a disease duration of less than 24 hours at study entry; and (4) a characteristic rash (macular, petechial, purpuric, or ecchymotic). Seven patients (age range, 1-29 years; male-female ratio, 2:5) were included. Patients had been included in an open, prospective study on the effects of leukaplasmapheresis in patients with meningococcal septic shock, and patient samples were collected between 1989 and 1993.[17] Leukaplasmapheresis, an effective treatment that improves survival and reduces the chance of complications,[18] was applied after admission and was repeated 4, 10, 16, 24, and 36 hours after initial treatment. The protocol was approved by the local hospital ethical committee. Informed consent for the blood collection was obtained from the patients or their relatives, as well as the attending physician.

Collection of blood samples

EDTA-anticoagulated blood was collected at admission and before each leukaplasmapheresis procedure. Cells were removed by centrifugation for 15 minutes at 1550*g* at room temperature. Plasma samples were stored in aliquots at −70°C until use. All plasma samples from a single patient were tested in the same experiment to avoid day-to-day variation of the flow cytometer between samples from 1 patient. Healthy volunteer samples, collected in the same period and stored identically, were used for comparison.

Flow cytometric analysis

For flow cytometry, 250 μL of plasma was centrifuged for 15 minutes at 17 500*g* and 20°C to obtain microparticle pellets. Subsequently, 225 μL of supernatant was removed, 225 μL of apopbuffer (10 mmol/L HEPES, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, and 136 mmol/L NaCl; pH 7.4) was added, and the microparticles were recentrifuged. Finally, 225 μL of supernatant was removed and the pellets were resuspended with 75 μL of apopbuffer. From this suspension, 5-μL aliquots were diluted with 35 μL of apopbuffer containing 2.5 mmol/L $CaCl_2$ and 5 μL of microparticle-free normal mouse serum (1:5000, v/v, final concentration) and were incubated for 15 minutes at room temperature. Subsequently, to identify the total microparticle population and their cellular origin, we added 5 μL PE-labeled annexin V and 5 μL FITC-labeled mAb, respectively, and incubated the samples for 15 minutes in the dark. To identify monocyte-derived, tissue factor–expressing microparticles, we used anti-CD14–PE and anti–tissue factor–FITC. The following (final) concentrations were used: anti-CD4–FITC (0.5 μg/mL), anti-CD8–FITC (25 ng/mL), anti-CD20–FITC (0.5 μg/mL), IgG_1-FITC (0.5 μg/mL), IgG_{2a}-FITC (0.5 μg/mL), IgG_1-PE (0.5 μg/mL), anti-CD14–PE (0.25 μg/mL), anti-CD14–FITC (0.5 μg/mL), anti-CD61–FITC (1 μg/mL), anti-CD62E–FITC (1 μg/mL), anti–glycophorin A–FITC (0.25 μg/mL), anti-CD66b–FITC (0.25 μg/mL), IgG_{2b}-PE (0.5 μg/mL), anti–tissue factor–FITC (1 μg/mL), and annexin V–PE (40 pg/mL). The incubation with mAb and annexin V was terminated by the addition of 200 μL of apopbuffer containing 2.5 mmol/L $CaCl_2$, followed by recentrifugation. After removal of 200 μL of supernatant, another 300 μL of apopbuffer containing 2.5 mmol/L $CaCl_2$ was added and the pellets were resuspended. Samples were analyzed in a FACScan flow cytometer with CellQuest software (Becton Dickinson, San Jose, CA). Both forward scatter and sideward scatter were set at logarithmic gain. Microparticles were identified on forward scatter, sideward scatter, binding of annexin V, and binding of a cell-specific mAb. Annexin V measurements were corrected for autofluorescence, and binding of cell-specific mAbs was corrected with identical concentrations of control IgG antibodies.[16] The number of microparticles per liter of plasma was calculated as: Number/L = N × [100/5] × [355/150] × [10^6/250].

Thrombin generation by microparticles

The thrombin generation assay as described by Kessels et al[19] was used to assess the in vitro thrombin-generating capacity of microparticles. To prepare normal plasma, we collected citrate-anticoagulated blood from 40 healthy volunteers who had not taken any medication during the previous 10 days. Plasma was prepared by centrifugation for 15 minutes at 1550*g* at room temperature. The plasma samples were pooled and treated with reptilase (40 μL per 2 mL plasma) for 10 minutes at 37°C and then for 10 minutes on melting ice. Subsequently, fibrin and microparticles were removed by centrifugation for 1 hour at 17 500*g* (20°C), and plasma was stored in 1-mL aliquots at −70°C until use. Microparticles were prepared as described in flow cytometric analyses. At t = 0, thrombin generation was started by the addition of 30 μL $CaCl_2$ (17 mmol/L) to 120 μL of the prewarmed (37°C) normal plasma, to which 20 μL of buffer A (50 mmol/L Tris-HCl, 100 mmol/L NaCl; pH 7.35) and 10 μL of the washed microparticle suspension had been added. At fixed intervals after t = 0, 3 μL portions were removed from this mixture and added to prewarmed (37°C) buffer A containing 4 mmol/L of the chromogenic substrate S2238 and 20 mmol/L EDTA (to block further thrombin generation). After 180 seconds, the conversion of S2238 was stopped by the addition of 90 μL citric acid (1.0 mol/L), and the generated p-Nitroaniline was determined on a spectrophotometer at λ = 405 nm. For inhibition experiments, mAbs (anti–tissue factor, anti–factor VII, or anti–factor XII; 07-1.0 mg/mL) were added to both plasma (20 μL) and microparticles (10 μL), which were preincubated separately for 30 minutes at room temperature before the microparticles were added in the thrombin generation assay.

Statistical methods

Data were analyzed with SPSS for Windows, release 8. Differences were considered statistically significant at $P < .05$. For direct comparison of the number of microparticles in blood samples, the Wilcoxon matched-pairs signed-rank test was used.

Results

Number and cellular origin of circulating microparticles in patients with meningococcal sepsis

Microparticles were isolated from plasma samples, labeled, and analyzed by flow cytometry as described in "Materials and Methods." Figure 1 shows a representative picture of microparticles stained with annexin V, which binds to negatively charged phospholipids and can be used to stain microparticles,[16] and anti-CD66b, which labels granulocytes. To correctly identify

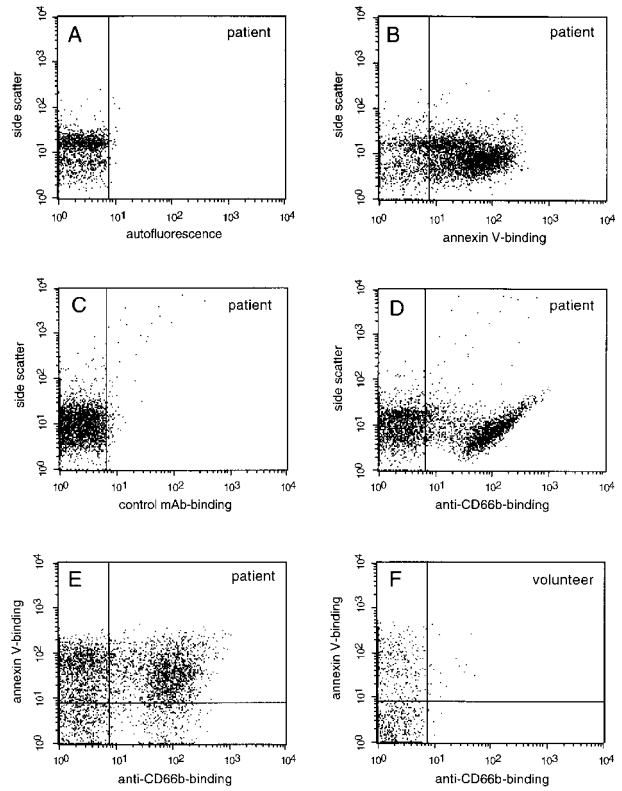

Figure 1. Representative fluorescence-activated cell sorter (FACS) dot plots of granulocyte-derived microparticles in plasma from a patient who survived meningococcal sepsis. Microparticles were isolated from the plasma of a (surviving) patient on admission, labeled, and analyzed by flow cytometry as described in "Materials and Methods." (A) Unlabeled microparticles (autofluorescence); (B) labeled with annexin V–PE; (C) labeled with IgG_1-FITC (control mAb); (D) labeled with anti-CD66b–FITC; and (E) double staining with annexin V–PE and anti-CD66b–FITC. (F) Representative dot plot of microparticles isolated from healthy volunteer plasma, double stained with annexin V–PE and anti-CD66b–FITC, for comparison.

annexin V–positive microparticles, we determined a threshold in a microparticle sample that was prepared without any additions to correct for intrinsic autofluorescence (Figure 1A). This threshold is also depicted in panels B, E, and F. To identify microparticles that bound cell-specific mAbs, we also incubated microparticles with identical concentrations of control antibodies to set a threshold. This threshold is shown in Figure 1C and is also used in panels D, E, and F. Labeling with anti-CD66b revealed that part of the microparticles originated from granulocytes (Figure 1D) and that the microparticles also bound annexin V (Figure 1E, upper right). Figure 1F shows the virtual absence of granulocyte-derived microparticles in a representative dot plot of microparticles from a healthy volunteer.

Table 1 summarizes the numbers of circulating microparticles, identified by staining with annexin V–PE and anti-CD mAb-FITC, in patients at study entry (n = 7) and healthy volunteers (n = 5). For these experiments, a panel of mAbs was used directed against T_H cells (CD4), T_S cells (CD8), monocytes (CD14), B cells (CD20), platelets (CD61), endothelial cells (CD62E), granulocytes (CD66b), and erythrocytes (glycophorin A). Compared with healthy volunteers, the patients had significantly increased numbers of circulating platelet (CD61)-derived and granulocyte (CD66b)-derived microparticles at study entry. Monocyte (CD14)-, B cell (CD20)-, and endothelial cell (CD62E)-derived microparticles were also increased, although the difference was not statistically significant. Notably, the nonsurviving patient A, who died on admission after a disease course of less than 18 hours, had the highest plasma levels of microparticles derived from T_H cells, monocytes, B cells, endothelial cells, platelets, granulocytes, and erythrocytes. The other nonsurvivor showed no marked differences in microparticle numbers when compared with patients who survived meningococcal sepsis.

Table 1. Number of circulating microparticles on admission in patients with meningococcal sepsis and healthy volunteers

Marker	Patients (n = 7)	Volunteers (n = 5)	N_x/N_t*	P†
CD4 (T_H cells)‡	6.1 (0.0-65)§	19.3 (2.65-47.9)	1/7	.432
CD8 (T_S cells)	ND¶	ND	ND	ND
CD14 (monocytes)	15.3 (0.0-735)	7.7 (0.0-16.1)	3/7	.315
CD20 (B cells)	10.0 (0.0-89)	0.0 (0.0-6.6)	5/7	.202
CD61 (platelets)	597 (101-1692)	41 (16-80)	7/7	.004
CD62E (endothelial cells)	61 (8.0-244)	18 (8.0-40)	5/7	.318
CD66b (granulocytes)	234 (29-294)	1.3 (0.0-5.5)	7/7	.008
Glycophorin A (erythrocytes)	72 (42-191)	51 (22-95)	1/7	.993

*N_x indicates the number of patient plasma samples that contained more microparticles (for the indicated CD marker) than the highest number found in volunteers; N_t indicates the total number of patient samples tested.

†Patients versus volunteers (Mann-Whitney test).

‡Microparticles were isolated, labeled, and analyzed, and their numbers were calculated as described in "Materials and Methods."

§Data are expressed as median (range) and represent number $\times 10^6$/L plasma.

¶Not detectable.

Time course of circulating microparticles

The time course of microparticle numbers in the patients is given in Figure 2. In all 5 survivors, the number of granulocyte-derived

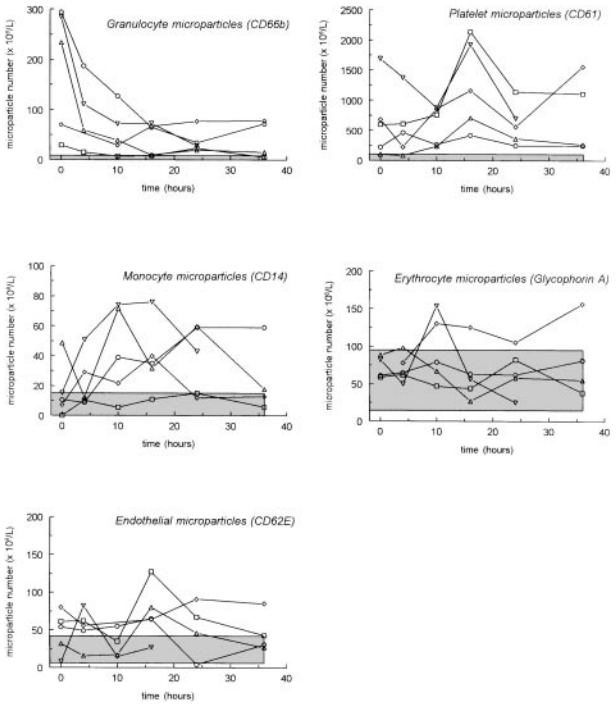

Figure 2. Time course of circulating microparticles in patients who survived meningococcal sepsis. Microparticles were assessed and analyzed by flow cytometry as described in "Materials and Methods." Figure shows the number of microparticles double stained with annexin V–PE and the indicated FITC-labeled mAb. The shaded area represents the range found in the 5 healthy volunteers. Note that the range of the y-axis varies and that most of the microparticles were of platelet origin.

microparticles decreased during the first 10 hours after admission (Figure 2, upper left). During the first 10 hours, the number of monocyte-derived microparticles (CD14; Figure 2, middle left) increased slightly in 4 of the survivors, although this increase was not significant ($P = .117$ at 24 hours and $P = .462$ at 36 hours). In contrast, microparticles originating from platelets, erythrocytes, or endothelial cells (Figure 2; upper right, lower right, and lower left, respectively) showed no apparent changes during the observation period.

Relation of circulating microparticles to coagulation in vivo and in vitro

To investigate whether the microparticles detected in the patients promoted coagulation, we first measured the activation of the coagulation system in vivo by assessing the concentration of the prothrombin fragment F_{1+2} in plasma during the course of the disease, which reflects the concentration of thrombin formed in vivo.[20] Table 2 shows that the concentration of F_{1+2} on admission was higher in the patients than in volunteers ($P = .004$). This difference was still present at 24 hours ($P = .016$), but not thereafter. The concentration of F_{1+2} in survivors decreased slowly after admission, becoming significantly lower than baseline values at 36 hours ($P = .016$), indicating progressively less activation of coagulation in vivo. To substantiate a link between coagulation activation and microparticles, we studied the thrombin-generating capacity of isolated microparticles from patients and healthy volunteers in vitro. Virtually all microparticle preparations of the patients generated more thrombin than those of healthy controls (data are summarized in Table 2).

Microparticles released from monocytes in vitro express tissue factor,[21,22] so we investigated whether such particles also circulate in vivo. Nonsurvivor A, who suffered from severe DIC as evidenced by a low platelet number (7×10^9/L), a prolonged prothrombin time (> 60 seconds; normal range, < 14.5 seconds), a decreased fibrinogen level (< 0.1 g/L; normal range, 1.7-3.7 g/L), the presence of fibrin degradation products, and a prolonged activated partial thromboplastin time (> 120 seconds; normal range, < 36 seconds), had extremely high numbers of monocyte-derived microparticles (CD14 positive). These microparticles double stained for tissue factor (Figure 3A). In contrast, microparticles of nonsurvivor B, with a less fulminant disease course (platelet number 258×10^9/L, prothrombin time 17.1 seconds, fibrinogen 2.5 g/L, presence of fibrinogen degradation products, and activated partial thromboplastin time of 42.9 seconds), hardly stained for CD14 or tissue factor (Figure 3B).

Table 2. Thrombin generation in plasma of patients with meningococcal sepsis in vivo and by microparticles in vitro

	In Vivo			In Vitro		
Time (h)*	F_{1+2} (nmol/L)	N_x/N_t†	P‡	TGT (OD 405 nm)§	N_x/N_t¶	P‡
0	5.5 (4.1-10)#	7/7	.004	0.105 (0.088-0.149)#	7/7	.003
4	4.1 (3.4-20)	5/5	.009	0.080 (0.070-0.132)	5/5	.009
10	5.5 (2.1-8.6)	4/5	.026	0.084 (0.068-0.106)	5/5	.009
16	3.8 (2.2-12.7)	4/5	.016	0.100 (0.090-0.116)	5/5	.009
24	4.2 (2.4-4.9)	4/5	.016	0.070 (0.055-0.079)	3/5	.117
36	3.2 (1.4-3.8)	4/5	.140	0.078 (0.067-0.142)	4/4	.014
Volunteers	2.1 (1.1-2.8)	—	—	0.057 (0.044-0.063)	—	—

*Time after admission (t = 0).

†N_x indicates the number of patient plasma samples that contained a higher concentration of F_{1+2} than the highest concentration found in healthy volunteers; N_t indicates the total number of patient samples tested.

‡Patients versus volunteers (Mann-Whitney test).

§TGT indicates thrombin generation test.

¶N_x indicates the number of patient microparticle fractions that generated more thrombin than the highest of the volunteer samples tested.

#Median (range).

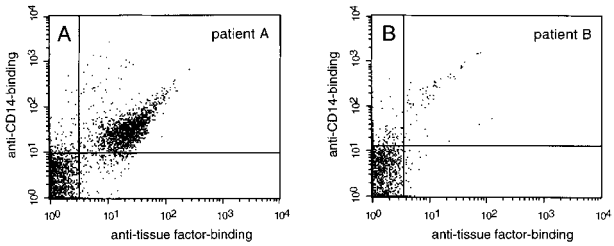

Figure 3. Identification of tissue factor on circulating microparticles of the nonsurviving patients. Microparticles of nonsurviving patient A (A) and patient B (B) were stained with CD14-PE and anti–tissue factor–FITC and analyzed by flow cytometry.

As indicated in Figure 4A, the addition of microparticles from nonsurvivor A to normal plasma resulted in a strong generation of thrombin, whereas those of nonsurvivor B induced only modest thrombin generation. The generation of thrombin in normal plasma by the microparticles from nonsurvivor A was extremely delayed when both microparticles and the normal plasma were preincubated with mAbs against tissue factor or factor VII (Figure 4B), indicating that the microparticle-associated tissue factor is active and stimulates the extrinsic pathway of the coagulation system. In contrast, preincubation with OT-2, a mAb that inhibits factor XIIa and blocks kaolin-induced thrombin generation in normal plasma (data not shown), had no effect.

Discussion

This study shows that patients with meningococcal sepsis have elevated numbers of microparticles originating from various cell populations in their circulation. These microparticles evoked a stronger generation of thrombin in normal plasma than those from volunteers, suggesting that the elevated numbers of microparticles in the circulation or their cellular origin may be related to the increased activation of the coagulation system in vivo. It could be argued that the increased procoagulant activity of the patients' microparticle fractions is mainly due to the increased number of microparticles and not to their cellular origin or properties. Because we wished to estimate the total procoagulant activity of the fractions, the microparticles were not diluted to a standard concentration in the thrombin generation assay. In addition, dilution cannot be done easily because the cellular composition of microparticles varied among patients, by the course of the disease, and most likely also by the type of disease. We also demonstrated tissue factor on microparticles, especially in a patient with an extremely fulminant course of DIC, which was functional in the thrombin generation assay as established with specific activity-blocking antibodies to tissue factor, factor VII, and factor XII.

Tissue factor is a transmembrane protein, the extracellular domain of which functions as a receptor for factor VII.[23] Binding of factor VII to tissue factor is a first step in a series of events in which soluble coagulation proteins become assembled on a phospholipid surface. Evidence that tissue factor is important for coagulation and inflammation in vivo comes from a number of animal studies. Infusion of recombinant activated factor VII into normal chimpanzees raised the plasma levels of activation peptides of factor IX, factor X, and prothrombin. This was blocked by the administration of an anti–tissue factor mAb.[24] Infusion of endotoxin reduced the

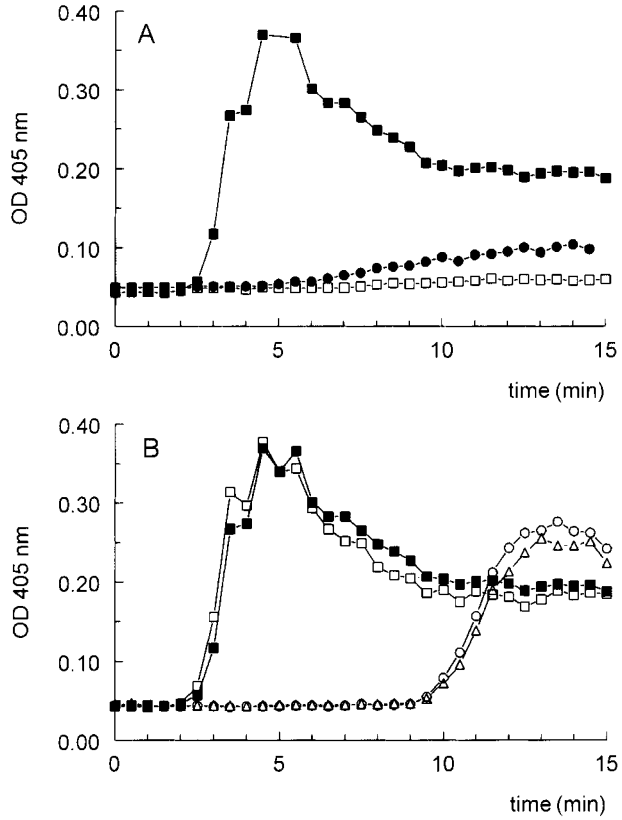

Figure 4. Thrombin generation by microparticles of the nonsurviving patients. After reconstitution of microparticle-free, defibrinated normal plasma with washed microparticles, thrombin generation was assessed as described in "Materials and Methods." (A) Microparticles of nonsurvivor A are indicated as ■, and those of nonsurvivor B as ●. For comparison, thrombin generation is also shown for a representative healthy volunteer (□). (B) Microparticles of nonsurvivor A in the absence of mAbs (■) or after preincubation with anti–tissue factor (○), anti–factor VII (△), or anti–factor XII (□).

number of platelets in rabbits and decreased the concentrations of fibrinogen, antithrombin, and factor VIII, whereas it prolonged the activated partial thromboplastin time. These changes were counteracted by tissue factor pathway inhibitor.[25] Infusion of *Escherichia coli* into baboons caused sepsis with severe DIC, which could be prevented by concurrent infusion of tissue factor pathway inhibitor.[26,27] Although these studies clearly indicate the importance of tissue factor for the development of DIC and sepsis, the location of functionally active tissue factor expression is not well known. Our results suggest that tissue factor exposed by microparticles, particularly those released by monocytes, may be important in this respect. However, another component required for the assemblage of the coagulation factor complexes on the phospholipid surface is phosphatidylserine, which is not exposed on normal cells but on, for example, activated platelets. In the present study, microparticles stained positive for annexin V, indicating the presence of phosphatidylserine on their surfaces.[28] Presumably, this also explains in part the thrombin-generating capacity of circulating microparticles. The relative contributions of phosphatidylserine and tissue factor to coagulation activation remain to be established. Possibly, the presence of tissue factor may enhance the coagulation activation associated with phosphatidylserine.

Monocytes are the only cells found in peripheral blood currently known to be capable of expressing tissue factor.[23] Isolated monocytes stimulated by endotoxin express tissue factor.[29] Under flow conditions, endotoxin-stimulated monocytes stimulate fibrin deposition and thrombus formation. Anti–tissue factor mAb inhibits both of these processes.[30] Monocytes have been shown to express tissue factor in patients suffering from invasive tumors, leukemia, sepsis, myocardial infarction, and diabetes, and in patients requiring extracorporeal circulation.[31] In addition to expressing tissue factor, monocytes can release tissue factor–exposing microvesicles in vitro upon stimulation with endotoxin.[21,22] Mallat et al[32] recently reported the presence of membrane vesicles of monocytic and lymphocytic origin that retained tissue factor activity in atherosclerotic plaques. Our present results extend these findings and demonstrate, for the first time, that procoagulant microparticles of monocyte, granulocyte, and endothelial cell origin can be detected in the circulation. It is interesting that of the 7 patients studied, only the patient with severe DIC had an extremely elevated (7-fold increase) number of endothelial cell–derived microparticles (244×10^6/L) compared with controls. Although we cannot exclude the possibility that these microparticles also express tissue factor, 85% of the tissue factor–positive microparticles were CD14 positive. We therefore presume that the contribution of tissue factor to the overall procoagulant activity is especially due to the increased number of monocyte-derived microparticles.

The samples analyzed in the present study had been collected between 1989 and 1993. This may raise concerns about the validity of the present findings (i.e., the microparticle profile). Therefore, microparticles were also isolated immediately after blood collection from 2 patients with sepsis and multiple organ failure and 2 healthy controls. Both patients clearly showed granulocyte-derived microparticles, which were absent or present at low numbers in the controls. The numbers of monocyte- and endothelial cell–derived microparticles were not increased in these patients compared with the controls (data not shown). On the basis of these preliminary data, we cannot make definitive conclusions about the presence or absence of either monocyte- or endothelial cell–derived microparticles in other diseases, but we hypothesize that such microparticles may be especially prevalent in patients with severe DIC. Combes et al[33] recently showed the presence of endothelial cell–derived microparticles in blood from healthy individuals and their increased presence in blood obtained from patients with lupus anticoagulant. Thus, the presence of endothelial cell–derived microparticles is evidently not unique for patients with meningococcal sepsis. Combes et al,[33] however, did not report the presence of tissue factor on these microparticles.

All patients studied had elevated numbers of microparticles. It is tempting to speculate that interference with the release of microparticles may be a target for therapeutic intervention. Recently, an mAb against the glycoprotein IIb-IIIa complex on platelets, which inhibits platelet–platelet interaction or aggregation, has been used successfully in patients undergoing stent implantation.[34,35] In vitro, this mAb prevents the release of microparticles from platelets.[36] Our findings imply that this mAb may inhibit clotting in vivo as well. Indeed, infusion of this mAb into baboons with lethal *E. coli* sepsis prevented fibrin deposition and renal insufficiency.[37] Thus, therapeutic interference with microparticle release in general, and possibly of platelets in particular, seems a realistic option.

In conclusion, elevated levels of microparticles were observed in patients with meningococcal sepsis. These microparticles enhanced coagulation by providing a suitable phospholipid surface and, at least in part, by exposing tissue factor. We suggest that such microparticles are involved in the pathogenesis of DIC during meningococcal sepsis and may constitute a novel target for therapeutic intervention in this disease, and possibly also in other clinical conditions with enhanced coagulation activation.

References

1. Bidstrup BP. Disseminated intravascular coagulation: pathophysiological mechanisms and manifestations. Semin Thromb Hemost. 1998;24:3.
2. Verzin F, Thin LN, Hack CE. Derangements of coagulation and fibrinolysis in critically ill patients with sepsis and septic shock. Semin Thromb Hemost. 1998;24:33.
3. Hoffman M, Monroe DM, Roberts HR. Coagulation factor IXa binding to activated platelets and platelet-derived microparticles: a flow cytometric study. Thromb Haemost. 1992;68:74.
4. Sims PJ, Faioni EM, Wiedmer T, Shattil SJ. Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. J Biol Chem. 1988;263:18,205.
5. Gilbert GE, Sims PJ, Wiedmer T, Furie B, Furie BC, Shattil SJ. Platelet-derived microparticles express high affinity receptors for factor VIII. J Biol Chem. 1991;266:17,261.
6. Holme PA, Brosstad F, Solum NO. Platelet-derived microvesicles and activated platelets express factor Xa activity. Blood Coagul Fibrinolysis. 1995;6:302.
7. Sims PJ, Wiedmer T, Esmon CT, Weiss HJ, Shattil SJ. Assembly of the platelet prothrombinase complex is linked to vesiculation of the platelet plasma membrane. Studies in Scott syndrome: an isolated defect in platelet procoagulant activity. J Biol Chem. 1989;264:17,049.
8. Abrams CS, Ellison N, Budzynski AZ, Shattil SJ. Direct detection of activated platelets and platelet-derived microparticles in humans. Blood. 1990;75:128.
9. Wun T, Paglieroni T, Holland P. Prolonged circulation of activated platelets following plasmapheresis. J Clin Apheresis. 1994;91:10.
10. Monura S, Komiyama Y, Miyake T, et al. Amyloid-β protein precursor-rich platelet microparticles in thrombotic diseases. Thromb Haemost. 1994;72:519.
11. Warkentin ET, Hayward CPM, Boshkov LK, et al. Sera from patients with heparin-induced thrombocytopenia generate platelet-derived microparticles with procoagulant activity: an explanation for the thrombotic complications of heparin-induced thrombocytopenia. Blood. 1994;84:3691.
12. Lee YJ, Horstman LL, Janania J, Reyes Y, Kelley RE, Ahn YS. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. Thromb Res. 1996;72:295.
13. Nomura S, Shouzu A, Nishikawa M, Kokawa T, Yasunaga K. Significance of platelet-derived microparticles in uremia. Nephron. 1993;63:485.
14. Jy W, Horsman LL, Arce M, Ahn YS. Platelet microparticles in ITP. J Lab Clin Med. 1992;119:334.
15. Kelton JG, Moore JC, Warkentin TE, Hayward CP. Isolation and characterization of cysteine proteinase in thrombotic thrombocytopenic purpura. Br J Haematol. 1996;93:421.
16. Nieuwland R, Berckmans RJ, Rotteveel-Eijkman RC, et al. Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. Circulation. 1997;96:3534.
17. Westendorp RGJ, Brand A, Haanen J, et al. Leukaplasmapheresis in meningococcal septic shock. Am J Med. 1992;92:577.
18. Bjorvatn B, Bjertnaes L, Fadnes HO, et al. Meningococcal septicaemia treated with combined plasmapheresis and leucopheresis or with blood exchange. BMJ. 1984;288:439.
19. Kessels H, Beguin S, Andree H, Hemker HC. Measurement of thrombin generation in whole blood: the effect of heparin and aspirin. Thromb Haemost. 1994;72:78.
20. Mann KG, Krishnaswamy S, Lawson JH. Surface-dependent hemostasis. Semin Hematol. 1992;29:213.
21. Satta N, Toti F, Feugeas O, et al. Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharides. J Immunol. 1994;153:3245.
22. Bona R, Lee E, Rickles F. Tissue factor apoprotein: intracellular transport and expression in shed membrane vesicles. Thromb Res. 1987;48:487.
23. Nemerson Y. Tissue factor and hemostasis. Blood. 1988;71:1.
24. ten Cate H, Bauer KA, Levi M, et al. The activation of factor X and prothrombin by recombinant factor VIIa in vivo is mediated by tissue factor. J Clin Invest. 1993;92:1207.
25. Bregengard C, Nordfang O, Wildgoose P, Diness V, Hedner U. Effect of two-domain TFPI on endotoxin-induced disseminated intravascular coagulation in rabbits. Thromb Haemost. 1993;69:887.
26. Drake TA, Cheng J, Chang A, Taylor FB Jr. Expression of tissue factor, thrombomodulin, and E-selectin in baboons with lethal Escherichia coli sepsis. Am J Pathol. 1993;142:1458.
27. Park CT, Creaseay 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.
28. Meers P, Mealy T. Calcium-dependent annexin V binding to phospholipids: stoichiometry, specificity, and the role of negative charge. Biochemistry. 1993;32:11,711.
29. van der Logt CPE, Dirven RJ, Reitsma PH, Bertina RM. Expression of tissue factor and tissue factor pathway inhibitor in monocytes in response to bacterial lipopolysaccharide and phorbolester. Blood Coagul Fibrinolysis. 1994;5:211.
30. Barstad RM, Hamers MJAG, Kierulf P, Westvik AB, Sakariassen KS. Procoagulant human monocytes mediate tissue factor/factor VIIa-dependent platelet-thrombus formation when exposed to flowing nonanticoagulated human blood. Arterioscler Thromb Vasc Res. 1995;15:11.
31. Francis JL, Carvalho M, Francis DA. The clinical value of tissue factor assays. Blood Coagul Fibrinolysis. 1995;6:S37.
32. Mallat Z, Hugel B, Ohan J, Leseche G, Freyssinet JM, Tedgui A. Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques. Circulation. 1999;99:348.
33. Combes V, Simon AC, Grau GE, et al. In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. J Clin Invest. 1999;104:93.
34. EPIC Investigators. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. N Engl J Med. 1994;330:956.
35. Coller BS. GPIIb/IIIa antagonists: pathophysiologic and therapeutic insights from studies of c7E3 Fab. Thromb Haemost. 1997;78:730.
36. Gemmell CH, Sefton MV, Yeo EL. Platelet-derived microparticle formation involves glycoprotein IIb-IIIa. Inhibition by RGDS and a Glanzmann's thrombasthenia defect. J Biol Chem. 1993;268:14,586.
37. Taylor FB, Coller BS, Chang ACK, et al. 7E3 F9ab2, a monoclonal antibody to the platelet GPIIb/IIIa receptor, protects against microangiopathic hemolytic anemia and microvascular thrombotic renal failure in baboons treated with C4b binding protein and a sublethal infusion of Escherichia coli. Blood. 1997;89:4078.