Blood Coagulation and Fibrinolysis, 7, 459–464

Transmission of a procoagulant signal from tissue factor-bearing cells to platelets

D. M. Monroe, M. Hoffman and H. R. Roberts

(Received 2 January 1996; accepted in revised form 19 April 1996)

The goal of the current study was to examine the mechanism by which factor VIIa/tissue factor (TF) activity leads to platelet activation as the first step in initiation of coagulation. Adherent, endotoxin-treated monocytes were used as a cellular source of TF. The processes that led to platelet activation were rapid, since incubation of coagulation factors and platelets with TF for as little as 15 s initiated platelet activation. Further, direct contact between the TF source and platelets was not required since incubation of plasma levels of coagulation zymogens and inhibitors with TF generated the initiating signal for platelet activation. We hypothesized that thrombin generation on the cells that contained TF was the initiating signal for platelet activation. To test this hypothesis, factor VIIa, inhibitors, and different combinations of coagulation zymogens were incubated with TF-bearing cells. The supernatants were then transferred to a suspension of unactivated platelets with plasma concentrations of zymogen factors and inhibitors. Platelet activation was much more efficient when all the elements of the IIase complex (factors II, V and X) were preincubated with factor VIIa/TF than when only factor X was incubated with factor VIIa/TF. Finally, TF was incorporated into lipid vesicles containing phosphatidyl choline either with or without phosphatidyl serine. Vesicles without phosphatidyl sérine have no IIase activity. Platelets were incubated with TF, coagulation zymogens and inhibitors. Platelet activation only occurred when the lipid vesicles could support IIase activity. We conclude that sufficient thrombin generation occurs on the TF-bearing cell (or TF-bearing vesicle) in the absence of platelets, to provide the procoagulant signal that leads to platelet activation. The activated platelet surface then provides sites for TF-activated factor IXa to recruit factor Xa to bind and assemble into functional Xase and IIase complexes.

Key words: Blood coagulation, tissue factor, thromboplastin, platelets.

Introduction

Tissue factor (TF) is the main initiator of coagulation. TF is expressed as an integral membrane protein on stromal cells and the antiluminal side of endothelial cells, but not on platelets.[1] However, activated platelets provide the primary surface on which coagulation occurs. Therefore, contact of TF with plasma leads to production of a procoagulant signal that must be transmitted to platelets, resulting in platelet activation and subsequent assembly of procoagulant complexes on the platelet surface. The factor VIIa/TF complex activates both factor IX and X,[2] however, neither factor IXa nor Xa directly activates platelets. Several groups have suggested that exposure of TF to plasma components leads to the initial generation of small amounts of thrombin that initiates platelet activation,[3–5] and promotes platelet accumulation at sites of TF expression.[6] TF activity is responsible for platelet activation in some models of thrombosis, since anti-TF antibodies[6] or active site-inhibited factor VIIa[7] block platelet activation and deposition.

We have shown that addition of exogenous factor Xa to unactivated platelets and zymogen coagulation factors leads to platelet activation.[8] However, many TF-bearing cells can also assemble prothrombinase (IIase) complexes,[9] and might produce the small amounts of thrombin that serve as an initial procoagulant signal. Understanding the precise mechanism by which the activation of factors IX and X by VIIa/TF leads to platelet activation and accumulation could have profound implications for prevention of thrombosis.

The current studies were designed to test the hypothesis that TF-induced platelet activation is initiated by a

D. M. Monroe and H. R. Roberts are with the Center for Thrombosis and Hemostasis, University of North Carolina, Chapel Hill, North Carolina, USA. M. Hoffman is with the Department of Pathology, Duke University and Department of Veterans Affairs Medical Center, Durham, North Carolina, USA. Address correspondence to Dougald M. Monroe, CB# 7035, 932 FLOB, University of North Carolina, Chapel Hill, NC 27599–7035. Tel: 919–966–3311; Fax: 919–966–7639.

small amount of thrombin generated specifically on the surface of the TF-expressing cell. If true, this hypothesis predicts that the ability of a TF-bearing cell (or surface) to initiate platelet activation will be profoundly affected by its ability to also assemble prothrombinase complexes.

Materials and methods

Materials

Factor IX,[10] prothrombin,[11] and antithrombin III[12] were purified as previously described. Factor X was purchased from Enzyme Research Labs (South Bend, IN). Factor V and tissue factor apoprotein were purchased from Calbiochem (San Diego, CA). Factor VIII (Profilate) was purchased from Alpha Therapeutics (Los Angeles, CA) and further purified by gel filtration. Factor VIIa and full length recombinant tissue factor pathway inhibitor were the generous gift of Dr Ulla Hedner (NOVO Nordisk A/S, Gentofte, Denmark). Lipids were purchased from Avanti Polar Lipids (Birmingham, AL).

Components of the assay system

The cell-based experimental system used in these studies has been previously described.[8,13] Zymogen factors IX, X, and prothrombin were incubated overnight with inhibitors antithrombin III and tissue factor pathway inhibitor to remove traces of active factors in the zymogen proteins. Factors V and VIII were added immediately before the start of the experiment. The concentrated proteins (20% of the final volume) were added to unactivated platelets and the TF source to give plasma levels of zymogens, inhibitors, platelets, and a final calcium level of 3 mM. Small amounts of recombinant human factor VIIa (10 ng/ml) were added to initiate the procoagulant reactions. Platelet activation was monitored by removing 10 μl aliquots of the solution into 50 μl of 2% paraformaldehyde at timed intervals. The expression of CD-62 (P-selectin) was assayed on the fixed platelet samples by direct immunofluorescence staining and flow cytometry as described previously.[8]

Lipid vesicles

Bovine brain phosphatidylcholine (PC) and phosphatidylserine (PS) were supplied by the manufacturer as a chloroform solution in sealed ampoules. The starting lipid solutions were either pure PC or PC with PS added (30% on a molar basis). Chloroform was removed from the lipids in an argon stream. Lipids were taken up in cyclohexane, frozen in dry ice/ethanol, and lyophilized overnight. Dried lipids were taken up in 20 mM HEPES (pH 7.4), 150 mM NaCl to a concentration of 10 mM. The lipid suspensions were recycled through an 0.2 micron filter ten times to produce large unilamellar vesicles.[14,15] TF was incorporated into vesicles by adding apo-tissue factor in CHAPS detergent (as provided by the manufacturer) to pre-formed lipid vesicles, then dialyzing overnight against 20 mM HEPES (pH 7.4), 150 mM NaCl. This technique has been shown to lead to essentially quantitative incorporation of apo-TF into lipid vesicles, with the TF in the proper orientation.[16]

Factor Xa generation on lipid vesicles

Factor VIIa (1 nM) was incubated with TF that had been incorporated into lipid as described above. In pure PC vesicles, 50 pM TF in 500 nM lipid was used, in vesicles with 30% PS, 30 pM TF in 300 nM lipid was used. The incubation buffer was 20 mM HEPES (pH 7.4), 150 mM NaCl, 1 mg/ml albumin, 5 mM $CaCl_2$. The reaction was initiated by adding factor X (180 nM) and Spectrozyme FXa (0.5 mM). Factor Xa generation was detected by measuring cleavage of Spectrozyme FXa for 40 min at 30 s intervals. Reactions were run in which TF was omitted to measure background cleavage of factor X and Spectrozyme FXa. The rate of factor Xa generation was determined from the second order term of a fit to a polynomial. Under these conditions, TF incorporated into either PS:PC vesicles or PC vesicles supported similar rates of activation of factor X (33 pM Xa/min/nM TF *vs* 20 pM Xa/min/nM TF respectively). Thus, 50 pM TF in PC only vesicles supported the same rate of factor Xa activation as 30 pM TF in PS:PC vesicles.

Prothrombinase assay on lipid vesicles

Factor Xa (0.25 nM) and factor Va (10 nM) were incubated with lipid (25 mM) for 5 min in 20 mM HEPES (pH 7.4), 150 mM NaCl, 1 mg/ml albumin, 5 mM $CaCl_2$. The lipid was the same TF containing vesicles used to measure factor Xa generation as described above. The reaction was initiated by adding prothrombin (200 nM) and Chromozyme Th (0.5 mM). Thrombin generation was detected by measuring cleavage of Chromozyme Th for 30 min at 15 s intervals. Reactions were run in which factor Va and prothrombin were omitted to measure background cleavage of Chromozyme. The rate of thrombin generation was determined from the second order term of a fit to a polynomial as described previously.[17] Under these conditions, the rate of thrombin activation on PS:PC vesicles was 1000 times that seen on PC only vesicles (8.6 nM IIa/min *vs* 0.008 nM IIa/min respectively).

Statistical methods

Comparison of each treatment mean to every other treatment mean was carried out using Duncan's multiple-range test.

Results

Platelet activation is the end result of an initiation event

Small amounts of factor VIIa and plasma levels of zymogen factors and inhibitors were added to platelets and TF-bearing monocytes. At timed intervals, aliquots of the platelet/protein solution were removed to fresh microtiter wells that did not contain monocytes. Platelet activation was assessed at timed intervals after the transfer. Assays for TF activity (FVIIa dependent FX activation) showed that TF activity was not transferred with the platelets. As shown in Figure 1, platelets which had not been exposed to the TF source did not activate during the course of the experiment. However, as little as 15 s of exposure to the TF-bearing monocytes initiated events that led to subsequent platelet activation. Increased exposure to the TF-bearing cells led to an increased rate of platelet activation.

Platelet activation does not require contact between platelets and a TF source

Zymogen coagulation factors, inhibitors and factor VIIa were added to TF-bearing monocytes in the presence or absence of platelets. After 1 min incubation with monocytes, the platelet/protein solution was transferred to a fresh microtiter well or the protein solution was transferred to unactivated platelets. Aliquots were removed at timed intervals to assay for platelet activation. As shown in Figure 2, direct contact between platelets and the TF-bearing cells was not required to initiate platelet activation. Thus, coincubation of the TF-bearing cells with the coagulation proteins rapidly led to generation of a soluble factor that initiated platelet activation. If thrombin is the signal for platelet generation, we expect that the signal for platelet activation will be generated only when all of the components for prothrombinase assembly are incubated with the TF-bearing monocytes. Factor VIIa (10 ng/ml) and plasma levels of the indicated zymogen factors and inhibitors were incubated with 2000 monocytes (15 monocytes/μl) as a source for TF for 1 min. The supernatants were then transferred to platelets with additional proteins to give plasma concentrations of all of the proteins. The time to 50% platelet activation was determined from the activation curves. In Figure 3, the bar for each protein combination shows the rate of platelet activation compared to the rate of activation when all of the proteins were incubated with the TF source and factor VIIa (0 rate of platelet activation means that platelets were less than 50% activated after 10 min). Platelet activation was most rapid when prothrombin, factor V, and factor X (as well as factor VIIa) were present in the monocyte incubation mixture. All other combinations of proteins gave significantly slower rates of platelet activation ($P < 0.05$ Duncan's Multiple Range Test).

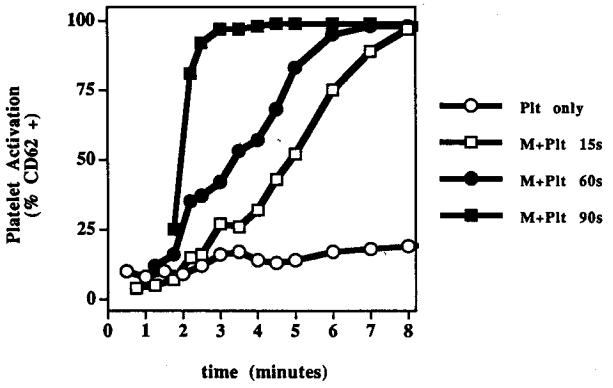

Figure 1. Only brief action of TF is required to initiate platelet activation. Unactivated platelets, coagulation proteins (factors II, V, VIII, IX, X and VIIa), inhibitors (ATIII and TFPI), and calcium were incubated with adherent, TF-bearing monocytes as described in Materials and methods. After co-incubation for 0 s (transferred immediately, open circles), 15 s (open squares), 60 s (closed circles) or 90 s (closed squares), the supernatants containing platelets and proteins were removed from the TF source and transferred to a fresh microtiter well. Samples were fixed at timed intervals for assay of platelet activation (measured as CD62 expression). The results shown are representative of five experiments performed on different days.

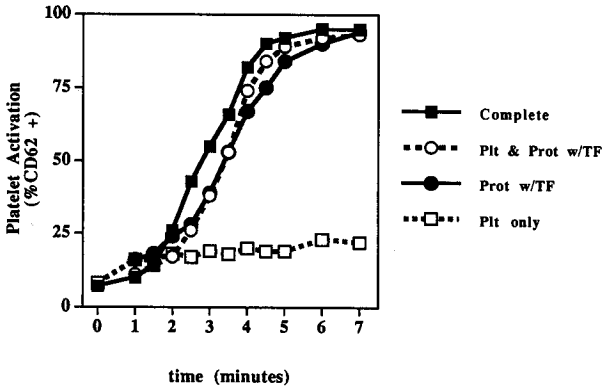

Figure 2. Physical contact is not required to transmit the activation signal from the TF-bearing cells to platelets. TF-bearing monocytes were incubated for 60 s with unactivated platelets, procoagulant proteins and inhibitors (open circles); or with only the procoagulant proteins and inhibitors (closed circles). After 60 s, the platelets and proteins were transferred to a clean microtiter well, or the proteins were transferred to a clean microtiter well containing a suspension of unactivated platelets as described in Materials and methods. The concentration of proteins was the same for each treatment. Platelet activation in these wells was compared to platelet activation in wells in which platelets, TF-bearing monocytes and proteins were incubated together for the entire experiment (closed squares); and wells in which unactivated platelets and proteins were incubated without a TF source for the entire experiment (open squares). The results shown are representative of five experiments performed, and show that preincubation of the TF source with the entire complement of proteins was as effective in initiating platelet activation as was preincubation of both platelets and proteins with the TF source.

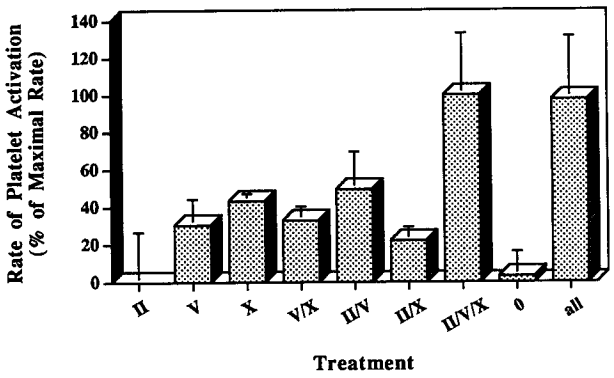

Figure 3. Initiation of platelet activation by protein solutions preincubated with a TF source. TF-bearing monocytes were incubated for 1 min with factor VIIa and the indicated coagulation proteins. The protein solutions were then transferred to a suspension of unactivated platelets, procoagulant proteins and inhibitors so that the final concentrations were about plasma level, and were the same in each final well. Samples were fixed at 1 min intervals, to be assayed for platelet activation. The time required for 50% of the platelets to become activated was determined for each treatment. The results of five separate experiments were averaged. The average value for the maximal rate of platelet activation was 4.1 min to half activation. This value was designated as 100%. Preincubation of TF-bearing monocytes (and factor VIIa) with factors II, V and X gave a subsequent rate of platelet activation that was not different from that obtained when all the coagulation proteins were preincubated with the TF source. The rates of platelet activation for 'All' proteins and 'II/V/X' were significantly greater than for any other treatment group ($P < 0.05$, Duncan's multiple range test).

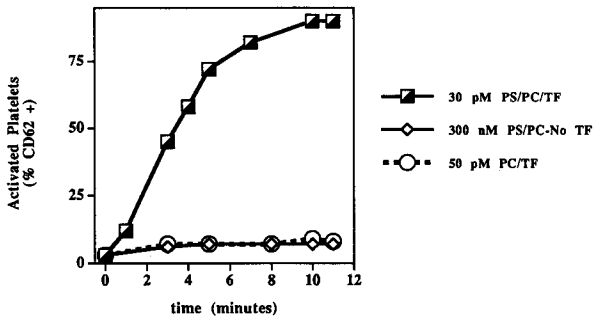

Figure 4. Initiation of platelet activation by apo-TF reconstituted into phospholipid vesicles. TF was relipidated into vesicles composed of PS and PC, or PC alone as described in Materials and methods. Unactivated platelets, procoagulant proteins (including factor VIIa) and inhibitors were incubated with 30 pM TF relipidated into PS/PC (divided squares), 50 pM TF in PC vesicles (open circles), or 300 nM PS/PC vesicles with no TF (open diamonds). The concentrations of relipidated TF used gave equal levels of TF activity, measured as FVIIa-dependent activation of FX. Just as in the experiments using the cellular source of TF, samples were removed at timed intervals for assay of platelet activation. The data shown is from one of two experiments which gave identical results: TF incorporated into PS/PC vesicles rapidly initiated platelet activation, while TF in PC only vesicles did not initiate platelet activation.

Mononuclear phagocytes can synthesize some coagulation factors, such as factor VII[18] and factor V or a factor V-like molecule.[19] The platelet activation seen when TF-bearing monocytes were incubated with some, but not all, of the components of the prothrombinase complex could occur because the monocytes supply small amounts of the missing components. To eliminate this confounding factor, we next conducted platelet activation experiments using recombinant apo-TF incorporated into synthetic phospholipid vesicles instead of a cellular source of TF.

Lipids that support IIase activity can act to initiate platelet activation

Previous studies have shown that while factor VIIa/TF and factor Xa/factor Va are both more active on a phospholipid surface, the two complexes differ in their requirements for lipid. The activity of the factor Xa/factor Va is enhanced by three orders of magnitude in the presence of PS[20,21] while the activity of the factor VIIa/TF complex is only enhanced by 2 to 20-fold in the presence of PS.[16,22–24] We incorporated apo-TF into vesicles containing either PC alone or PC with 30% PS. The ratio of lipid to TF was 10 000:1 on a molar basis. Vesicles were assayed for IIase activity and factor VIIa-dependent factor Xa generation. TF-containing vesicles composed of only PC had nearly as much Xa generating ability as PS:PC vesicles, but had virtually no IIase activity. When assayed as described in methods, PS:PC vesicles supported a rate of thrombin generation by factor Xa/Va that was 1000 times that seen on PC only vesicles.

Factor VIIa and plasma concentrations of zymogen factors and inhibitors were then incubated with platelets, factor VIIa, and TF that had been incorporated into lipid vesicles containing either PC or PS:PC. No TF-bearing monocytes were present in these experiments, so the phospholipid vesicles provided the only source of TF. Concentrations of TF/PC (50 pM TF, 500 nM lipid) and TF/PS:PC (30 pM TF, 300 nM lipid) were selected to give identical rates of factor Xa generation. Reactions that contained TF in PS:PC vesicles initiated platelet activation after 2 min (Figure 4). In contrast, reaction mixtures that contained TF in PC-only vesicles showed no platelet activation. If factor VIIa or TF was omitted from the reaction mixtures, no platelet activation occurred. Thus, despite having identical factor Xa-generating abilities, a TF-bearing surface that can also assemble prothrombinase (FXa/Va) complexes is much more effective at initiating platelet activation under these conditions.

Discussion

We have shown that thrombin generated on TF-bearing cells (monocytes) can play an important role in TF-mediated initiation of coagulation in a model system. We can reliably measure the nanomolar concentrations of thrombin generated by the platelet surface prothrombinase following platelet activation.[13] However, considerably lower levels of thrombin are required to initiate platelet activation. Addition of exogenous thrombin to a concentration of about 0.1 nM leads to platelet activation with a time course similar to that seen in the presence of TF-bearing monocytes (data not shown). Thus, initiation of platelet activation by a TF-bearing cell is most efficient when the cell can also support generation of small amounts of thrombin.

The TF/VIIa pathway is accepted as being the major mechanism by which coagulation is initiated *in vivo*. However, our data demonstrate that TF expression is not the only feature that determines whether a cell surface initiates coagulation. We demonstrated that the ability of a TF-bearing surface to initiate coagulation was highly dependent on the ability of that surface to also support prothrombinase assembly. We found that vesicles with the same TF activity, but different phospholipid composition, differed in their ability to initiate platelet activation. Platelet activation was efficiently initiated only when the TF-bearing vesicles also contained PS. The PS-containing vesicles could support assembly of the FXa/Va complex and, therefore, generate thrombin on the same membrane surface on which the FXa was activated.

We have also shown that monocytes were most effective in initiating platelet activation when the prothrombinase complex, as well as the TF/VIIa complex, could be assembled on their surfaces. Our conclusions are also supported by our previous work which showed that LPS-treated monocytes and fibroblasts with the same TF activity, differed in their abilities to induce platelet activation. This difference was due, as least in part, to the greater ability of monocytes to generate thrombin when supplied with the components of the prothrombinase complex (factors X, V and prothrombin).[25]

Mechanisms in addition to TF expression may play important roles both in initiating hemostatic coagulation and thrombosis. We think our findings on the ability of a TF-bearing cell to support prothrombinase activity have application to several pathologic conditions, including thrombosis in the setting of malignancy, inflammation and possibly atherosclerosis. Many workers have demonstrated TF expression on malignant cells (as reviewed in Ref. 26). It has also been demonstrated that the level of TF antigen expression does not necessarily correlate with TF activity.[27] Two cells or tissues with the same level of TF antigen, may have different levels of TF-dependent FX activation. Therefore, it has been suggested that features of the membrane surface, such as PS content, play a role in modulating TF activity.[26,28] Also, it has been noted that malignant cells differ greatly in their overall procoagulant activity as measured in clotting assays. This difference has been attributed to differences in TF activity, because procoagulant activity can be largely inhibited by anti-TF antibodies.[29] However, even though the tumor procoagulant activity is TF-dependent, the degree of tumor thrombogenicity does not necessarily correlate with either the level of TF antigen or activity.[26,30] Since malignant cells have also been shown to support prothrombinase activity,[31] we speculate that prothrombinase activity may also be an important determinant of overall tumor procoagulant activity, and thrombogenicity *in vivo*.

It has been hypothesized that induction of TF expression on monocytes may play a role in inducing thrombosis in malignancy,[26] as well as in inflammation. Inflammation is well known to be a risk factor for local thrombosis. The mechanism of thrombosis is felt to be related to the expression of TF on endothelial cells or monocytes induced by proinflammatory cytokines or bacterial endotoxin. Since we have shown that the ability to support prothrombinase activity can dramatically affect the ability of a TF-bearing cell to initiate coagulation, the level of prothrombinase activity expressed by monocytes may play an important role in the development of thrombosis during infection and inflammation. Robinson *et al.*[32] have shown that monocyte prothrombinase activity can be regulated by bacterial endotoxin. Therefore, in some settings, upregulation of prothrombinase activity may be as important as upregulation of TF activity in predisposing to thrombosis.

Acknowledgements—The authors would like to acknowledge the excellent technical assistance of Ms T. S. Wortham. This work was supported in part by Grant R01 HL48320 from the National Institutes of Health, and by the United States Department of Veterans Affairs.

References

1. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol* 1989; **134:** 1087–1097.
2. Østerud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proc Natl Acad Sci USA* 1977; **74:** 5260–5264.

3. Beguin S, Lindhout T, Hemker HC. The effect of trace amounts of tissue factor on thrombin generation in platelet rich plasma, its inhibition by heparin. *Thromb Haemost* 1989; **61:** 25–29.
4. Orvim U, Roald HE, Stephens RW, Roos N, Sakariassen KS. Tissue factor-induced coagulation triggers platelet thrombus formation as efficiently as fibrillar collagen at arterial blood flow conditions. *ArterioscIerThromb* 1994; **14:** 1976–1983.
5. Grignani G, Jamieson G. Tissue factor-dependent activation of platelets by cells and microvesicles of SK-OS-10 human osteogenic sarcoma cell line. *Invasion Metastasis* 1987; **7:** 172–182.
6. Barstad R, Hamers M, Kierulf P, Westvik A, Sakariassen K. Procoagulant human monocytes mediate tissue factor/factor VIIa-dependent platelet-thrombus formation when exposed to flowing nonanticoagulated human blood. *Arteriosclerosis Thromb Vascular Biol* 1995; **15:** 11–16.
7. Kirchhofer D, Tschopp T, Baumgartner H. Active site-blocked factor VIIa and IXa differentially inhibit fibrin formation in a human *ex vivo* thrombosis model. *Arteriosclerosis Thromb Vascular Biol* 1995; **15:** 1098–1106.
8. Hoffman M, Monroe DM, Oliver JA, Roberts HR. Factors IXa and Xa play distinct roles in tissue factor-dependent initiation of coagulation. *Blood* 1995; **86:** 1794–1801.
9. Tracy PB. Regulation of thrombin generation at cell surfaces. *Semin Thromb Hemost* 1988; **14:** 227–233.
10. McCord DM, Monroe DM, Smith KJ, Roberts HR. Characterization of the functional defect in factor IX Alabama. *J Biol Chem* 1990; **265:** 10250–10254.
11. Church FC, Whinna HC. Rapid sulfopropyl-disk chromatographic purification of bovine and human thrombin. *Anal Biochem* 1986; **157:** 77–83.
12. Church FC, Pratt CW, Treanor RE, Whinna HC. Antithrombin action of phosvitin and other phosphate-containing polyanions is mediated by heparin cofactor II. *Febs Lett* 1988; **237:** 26–30.
13. Monroe DM, Roberts HR, Hoffman M. Platelet procoagulant complex assembly in a tissue factor-initiated system. *Br J Haematol* 1994; **88:** 364–371.
14. Mayer LD, Hope MJ, Cullis PR. Vesicles of variable sizes produced by a rapid extrusion procedure. *Biochem Biophys Acta* 1986; **858:** 161–168.
15. Hope MJ, Bally MB, Webb G, Cullis PR. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochem Biophys Acta* 1985; **812:** 55–65.
16. Krishnaswamy S. The interaction of human factor VIIa with tissue factor. *J Biol Chem* 1992; **267:** 23696–23706.
17. Griffith MJ, Breitkreutz L, Trapp H, *et al.* Characterization of the clotting activities of structurally different forms of activated factor IX. *J Clin Invest* 1985; **75:** 4–10.
18. McGee MP, Wallin R, Devlin R, Rothberger H. Identification of mRNA coding for factor VII protein in human alveolar macrophages-coagulant expression may be limited due to deficient postribosomal processing. *Thromb Haemost* 1989; **61:** 170–174.
19. Altieri DC, Edgington TS. Sequential receptor cascade for coagulation proteins on monocytes. Constitutive biosynthesis and functional prothrombinase activity of a membrane form of factor V/Va. *J Biol Chem* 1989; **264:** 2969–2972.
20. Connor J, Bucana C, Fidler IJ, Schroit AJ. Differentiation-dependent expression of phosphatidylserine in mammalian plasma membranes: quantitative assessment of outer-leaflet lipid by prothrombinase complex formation. *Proc Natl Acad Sci USA* 1989; **86:** 3184–3188.
21. 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.
22. Bom VJ, Bertina RM. The contributions of Ca^{2+}, phospholipids and tissue-factor apoprotein to the activation of human blood-coagulation factor X by activated factor VII. *Biochem J* 1990; **265:** 327–336.
23. Rao LV, Tait JF, Hoang AD. Binding of annexin V to a human ovarian carcinoma cell line (OC-2008). Contrasting effects on cell surface factor VIIa/tissue factor activity and prothrombinase activity. *Thromb Res* 1992; **67:** 517–531.
24. Wildgoose P, Jorgensen T, Komiyama Y, *et al.* The role of phospholipids and the factor VII Gla-domain in the interaction of factor VII with tissue factor. *Thromb Haemost* 1992; **67:** 679–685.
25. Hoffman M, Monroe DM, Roberts HR. Cellular interactions in hemostasis. *Haemostasis* 1996; **26:** 12–16.
26. Rao LV. Tissue factor as a tumor procoagulant. *Cancer Metastasis Rev* 1992; **11:** 249–266.
27. Walsh JD, Geczy CL. Discordant expression of tissue factor antigen and procoagulant activity on human monocytes activated with LPS and low dose cycloheximide. *Thromb Haemost* 1991; **66:** 552–558.
28. Rao LV, Robinson T, Hoang AD. Factor VIIa/tissue factor-catalyzed activation of factors IX and X on a cell surface and in suspension: a kinetic study. *Thromb Haemost* 1992; **67:** 654–659.
29. Hu T, Bach RR, Horton R, Konigsberg WH, Todd MB. Procoagulant activity in cancer cells is dependent on tissue factor expression. *Oncol Res* 1994; **6:** 321–327.
30. Tanaka M, Yamanishi H. The expression of tissue factor antigen and activity on the surface of leukomic cells. *Leukemia Res* 1993; **17:** 103–111.
31. Sakai T, Noguchi M, Kisiel W. Tumor cells augment the factor Xa-catalyzed conversion of prothrombin to thrombin. *Haemostasis* 1990; **20:** 125–135.
32. Robinson RA, Worfolk L, Tracy PB. Endotoxin enhances the expression of monocyte prothrombinase activity. *Blood* 1992; **79:** 406–416.