Activated Platelets but Not Endothelial Cells Participate in the Initiation of the Consolidation Phase of Blood Coagulation*

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To address the question of whether initiation of the consolidation phase of coagulation occurs on platelets or on endothelium, we have examined the interaction of coagulation factor XI with human umbilical vein endothelial cells (HUVEC) and with platelets. In microtiter wells factor XI binds to more sites in the absence of HUVEC (1.8 × 10¹⁸ sites/well, KD = 2.6 nM) than in their presence (1.3 × 10¹⁸ sites/well, KD = 12 nM) when high molecular weight kininogen (HK) and zinc are present. Binding was volume-dependent and abrogated by HUVEC or Chinese hamster ovary cells and was a function of nonspecific binding of HK to the artificial plastic surface. Factor XI did not bind to HUVEC or to HEK293 cell monolayers anchored to microcarrier beads. Activation of HUVEC resulted in von Willebrand’s factor secretion, but factor XI binding was not observed. Only activated platelets supported factor XI binding in the presence of nonspecific binding of HK to the artificial plastic surface. Factor XI did not bind to HUVEC or to HEK293 cell monolayers anchored to microcarrier beads. Activation of HUVEC resulted in von Willebrand’s factor secretion, but factor XI binding was not observed. Only activated platelets supported factor XI binding in the presence of HK and zinc (KD = 8 nM, B max = 1319 sites/cell). Activation of factor XI was observed in plasma in the presence of platelets activated by the thrombin receptor activation peptide but not with activated HUVEC. These results support the concept that activated platelets, but not endothelial cells, expose a procoagulant surface for binding and activating factor XI, thereby initiating the consolidation phase of coagulation.

It is well known that plasma proteins and blood and vascular cells play a pivotal role in hemostasis. However, under normal physiological conditions blood is fluid, and yet a clot can form locally at the site of vascular injury. Fluidity of blood is maintained by the anti-thrombotic nature of the vascular endothelium, and thrombus formation is mediated in part by the activation of platelets. Contrary to the anticoagulant paradigm of endothelial function, it has been reported that quiescent endothelial cells can bind coagulation factor XI (FXI) and promote its activation and expression of its enzymatic activity in vitro (1, 2). The present study examines the role of both human platelets and human umbilical vein endothelial cells (HUVEC) in the binding and activation of FXI, which participates in the initiation of the intrinsic or consolidation pathway of blood coagulation.

FXI is a 160-kDa homodimeric protein found in plasma at a concentration of ~30 nM complexed with high molecular weight kininogen (HK) (3, 4). FXIIa, thrombin, and FXIa have been shown to activate FXI to FXIIa in the presence of an artificial negatively charged surface (3, 5, 6). Two potentially physiologically relevant cellular surfaces that might promote blood coagulation within the vasculature are the platelet and the endothelial cell. FXI binds to activated platelets with high affinity (KD = 10 nM, B max = 1,500 sites/platelet) in the presence of HK (45 nM) and zinc ions (25 μM) (7) or in the presence of protrombin (1 μM) and calcium ions (2 mM) (8), but this only occurs after the platelet has been pretreated with an agonist such as thrombin (7, 8). Activated platelets also promote the activation of platelet-bound FXI by thrombin, FXIIa, and FXIa with thrombin demonstrated to be the preferred activator (9, 10). Thus, the platelet exists in the blood stream in a dormant form that exposes a surface for assembly of coagulation complexes only after appropriate stimulation.

The negative charge associated with the endothelium comes in the form of constitutively expressed heparan sulfate and chondroitin sulfate glycosaminoglycans (11). Thus, the endothelium is constantly exposing a negative charge to plasma, yet coagulation does not ubiquitously occur. This anti-thrombotic property of the endothelium has been attributed to the potentiation of inhibitors of plasma serine proteases by glycosaminoglycans (12). It has been reported that FXI binds to HUVEC with a high affinity (KD = 4.5–6.9 nM, B max = 2.7–13 × 10⁶ sites/cell) requiring HK and zinc ions (1, 2). HUVEC-bound FXI was activated to FXIIa by FXIIa, and HUVEC-bound FXIa was able to generate FIXa (1). This suggests that an otherwise phenotypically anticoagulant cell type is capable of generating procoagulant activity.

We have examined the binding of FXI to endothelial cells and have found a discrepancy between the experimental results reported previously and our data. Using several assays with various controls, and under several assay conditions, we have shown that FXI does not associate with HUVEC but does bind specifically to high affinity receptors on activated platelets. Although we were able to reproduce the experimental results previously published (1, 2), we have shown that they represent an artifact of the binding assay utilized (13–15). Our studies of endothelial cell and platelet interactions with FXI suggest that the activated platelet, not the endothelium, is the preferred surface for FXI binding and consequent activation by thrombin.

EXPERIMENTAL PROCEDURES

Proteins—FXI was purchased from Hematologic Technologies, Inc. (Essex Junction, VT). HK and thrombin were purchased from Enzyme...
Generally, the purity was 0.6–1.0 by gel filtration through a 1-cc Sephadex G-50 column. 125I-FXI was purified, if not already, by polyethylene glycol precipitation and dialysis against 0.5% bovine serum albumin (BSA) in Hepes-buffered saline. 125I-FXI was determined by the absorbance at 405 nm and corrected for free 125I.

Modified Eagle’s medium (12) was used to determine percent radioactivity bound (Equation 1) and specific radioactivity (Equation 2).

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\text{SRA} = (P + S) / (\text{mol protein added}) = \text{cpm/mol}
\]

Measurement of Specific Radioactivity—Radiolabeled protein (1 µl) was added to 99 µl of 0.5% bovine serum albumin (BSA) in Hepes-buffered saline and 100 µl of 40% trichloroacetic acid. This was vortexed and incubated in an ice bath for 5 min. The solution was centrifuged at 14,000 × g for 5 min, and half of the supernatant was removed and placed in a separate vial. Both vials, 100 µl of the supernatant (S) and 100 µl of the pellet (P), were measured for γ-emission in a Wallace 1470 automatic gamma counter. The following equations were used to determine percent radioactivity bound (Equation 1) and specific radioactivity (Equation 2; SRA).

\[
\%\text{bound} = \left( \frac{P}{P + S} \right) \times 100
\]

Equilibrium Binding Experiments Performed in the Microtiter Wells—Binding studies were performed as described previously (1) except that HUVEC or CHO cells were grown to >90% confluence in 96-well fibronectin-coated plates instead of 48-well plates. 125I-FXI in incubation buffer was added to cells at varying concentrations for 1 h at 37 °C. In some experiments 100 nm HK, 1 µm protamine, or 8 µM forced HK peptide was added. In other experiments, 1% non-fat dry milk, 1% polyethylene glycol, or 1% Irish cream was substituted for BSA. Also fibronectin-coated 96-well plates were substituted with 96-well non-coated Primera plates (BD PharMingen) or fibronectin-coated filter-bottom plates in which the filter of individual wells could be removed and measured for radioactivity.

Equilibrium Binding Experiments Using Cells in Suspension—To eliminate the effect of FXI binding to an artificial surface, HUVEC were placed into suspension non-enzymatically or cultured in suspension on microcarrier beads, and binding was measured using a procedure similar to that described previously for platelets (19). HUVEC were grown to confluence on non-coated plates and detached using 5 mM EDTA in phosphate-buffered saline for 5 min at 37 °C. Cells (10^7) were tritium-labeled, washed, placed in a 15-ml conical flask, centrifuged for 5 min at 1,500 × g, and resuspended in 5 ml of incubation buffer. In other cases, HUVEC or HEK293 cells grown to confluence on microcarrier beads were washed and resuspended in incubation buffer to a density of 10^6 cells/ml. silicone oil (15 µl, DC500:DC200, 4:1) was centrifuged to the bottom of a 200-µl microcentrifuge tube with a narrow bore tip (Sarstedt, Inc., Princeton, NJ). The cell suspension (washed platelets, HUVEC or HEK293 cells) or microcarrier bead suspension (HUVEC or HEK293 cells) was added to the radiolabeled protein mixture (50 µl, 15°C) and incubated at 37 °C for 1 h with shaking by hand every 15 min. The supernatant (free protein) was measured for γ-emission.

RESULTS

Equilibrium Binding Studies Performed in the Microtiter Wells Assay—In order to determine the affinity and stoichiometry of FXI binding to the HUVEC surface, cells were grown to confluence in microcarrier wells, washed, and then incubated with 125I-labeled FXI for 1 h at 37 °C under the conditions reported previously (1). These experiments were conducted in the presence of zinc ions (100 µM) and in the presence or absence of HK (100 nm, Fig. 1). In the absence of HK no evidence of saturable binding was detected at a concentration as high as 100 nm added FXI, i.e. 3X the physiological concentration. The small amount of detectable binding was not significantly different from the baseline binding. No evidence was detected of nonsaturable binding of FXI to HUVEC.
displaceable by an excess of non-labeled FXI. Non-saturable binding also occurred in the absence of cells.

In the presence of HK, saturable binding was achieved by 100 nM added 125I-FXI both in the presence and absence of HUVEC (Fig. 1). The calculated $K_D$ values are 2.6 ± 0.7 and 12.1 ± 4.4 nM in the absence and presence of HUVEC, respectively. The calculated number of binding sites was $1.8 \times 10^{10} ± 0.1$ molecules/well in the absence of cells and 1.3 $\times 10^{10} ± 0.2$ molecules/well (or $1.3 \times 10^6$ molecules/cell) in the presence of cells. The difference in the number of binding sites (27.8%) is equivalent to the surface area of the microtiter well base (28.3 mm$^2$) that the monolayer of cells occupies, i.e. 27.3% of the total surface area (103.7 mm$^2$) covered by the incubation volume added (100 μl). This result suggests that the FXI binding detected was a property of the plastic surface of the microtiter wells and that binding was abrogated by the presence of the cells.

Because the vertical sides of the microtiter wells themselves appeared to be playing a significant role in the amount of FXI bound, the number of binding sites detected may be influenced by volume rather than protein concentration. The number of binding sites increased by varying the volume within the range of 50–200 μl in the presence of zinc, 30 nM FXI, and 100 nM HK (Fig. 2). A volume-dependent increase in the number of binding sites was detected when HUVEC, CHO cells, or empty wells were incubated with the reaction mixture (Fig. 2) but not in the absence of HK (data not shown). The significant amount of nonspecific background binding determined in the presence of HK may explain both the similarities and the discrepancies between our results and those described previously.

To ensure that our inability to detect FXI binding to HUVEC was a consequence of the absence of specific FXI binding sites on HUVEC and not the result of a deficiency in the binding assay, cell-coated microcarrier beads were replaced with HUVEC-coated beads.2 A titration with HK in the presence of zinc ions did not promote FXI binding to HUVEC or HEK293 cells at any concentration (data not shown). These results indicate that neither HUVEC nor HEK293 cells immobilized on microcarrier beads participate in FXI binding reactions. Also, HUVEC that had been dissociated from tissue culture dishes non-enzymatically did not support FXI binding in suspension (data not shown).

To optimize the microtiter well assay, several experimental conditions were examined in order to block the binding of FXI/HK to the microtiter well in the absence of HUVEC. BSA was replaced with gelatin, Irish cream, polyethylene glycol, or non-fat dry milk. None of these blocking agents prevented the binding of FXI/HK to the microtiter wells; in fact BSA appeared to be the best blocking agent (data not shown). Furthermore, the microtiter plates themselves were replaced with Primaria brand microtiter plates, which allow for the growth of endothelial cells without precoating with a matrix protein. Binding of FXI in the presence of HK was still detected when these plates were used, suggesting that matrix coatings were not playing a role. Other cofactors known to mediate the binding of FXI to platelets (8, 9) were then substituted for HK; however, neither prothrombin nor an HK-derived peptide consisting of the 31-amino acid residue region responsible for the HK/FXI interaction was able to mediate FXI binding to the HUVEC surface in microtiter wells (data not shown).

**FXI Binding Studies with Cells on Microcarrier Beads—** HUVEC were grown to confluence on Cytodex-3 collagen-coated microcarrier beads, and binding assays were conducted on cells in suspension similarly to the platelet binding experiments (20). Microcarrier beads allow for a monolayer of anchorage-dependent cells to be manipulated in suspension. HUVEC-coated beads did not release vWF until pretreated with agonists (data not shown). Accordingly, HUVEC-coated beads either untreated or pretreated with thrombin receptor activation peptide (TRAP) were assayed in suspension (Fig. 3A). Only trace amounts of non-saturable, nonspecific, and non-displaceable FXI binding were observed in either case. Also HEK293 cells were grown to confluence on the beads and used in the suspension assay. No detectable FXI binding was observed at any concentration (data not shown). HUVEC-coated beads were observed, and the results with HEK293 cells were similar to those with HUVEC grown on beads (Fig. 3A). On the other hand, saturable HK binding was detected to HUVEC-coated microcarrier beads but not to HEK293 cell-coated beads.2 A titration with HK in the presence of zinc ions did not promote FXI binding to HUVEC or HEK293 cells at any concentration (data not shown). These results indicate that neither HUVEC nor HEK293 cells immobilized on microcarrier beads participate in FXI binding reactions. Also, HUVEC that had been dissociated from tissue culture dishes non-enzymatically did not support FXI binding in suspension (data not shown).

To ensure that our inability to detect FXI binding to HUVEC was a consequence of the absence of specific FXI binding sites on HUVEC and not the result of a deficiency in the binding assay, cell-coated microcarrier beads were replaced with platelets. The binding of 125I-FXI to platelets (Fig. 3B) was specific, saturable, and reversible ($K_D = 8$ nM, $B_{max} = 1319$ sites/platelet), which is similar to previously published work (7, 21). The difference in the ordinate between Fig. 3, A (fmol/10$^5$ cells) and B (pmol/10$^8$ platelets) should be noted.

**Rate of FXIa Formation in Plasmas—** A critical reaction in the initiation of the intrinsic or consolidation pathway of blood coagulation is the activation of FXI to FXIa. It has been shown that both activated platelets (9) and the HUVEC surface (1) facilitate the activation of FXI in purified systems. To investigate the role of platelets, HUVEC, HEK293 cells, and kaolin in the FXI-dependent initiation of blood coagulation in a plasma-based assay, we utilized the chromogenic substrate S-2366 to detect generation of enzymatic activity in the wells of microtiter plates with normal or coagulation factor-deficient plasma in

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2 T. R. Baird and P. N. Walsh, unpublished results.
the absence or presence of various protease inhibitors. This chromogenic substrate, S-2366, is cleaved by FXIa ($K_m = 250 \mu M$) and thrombin ($K_m = 150 \mu M$) but not by other activated coagulation factors such as FXIIa, kallikrein, FIXa, FXa, or FVIIa. Therefore, the generation of FXIa and thrombin was monitored as the change in absorbance at 405 nm over a period of 1 h, and the results are presented in Fig. 4 as mOD/min. Neither incubation with HUVEC (4.2 mOD/min) nor HEK293 cells (3.4 mOD/min) resulted in the generation of significant amounts of amidolytic activity in normal plasma as measured by S-2366 hydrolysis. TRAP-activated platelets (43 mOD/min) but not unactivated platelets (8.2 mOD/min, Fig. 4A, inset) resulted in the generation of significant amounts of amidolytic activity in normal plasma as measured by S-2366 hydrolysis. TRAP-activated platelets (43 mOD/min) but not unactivated platelets (8.2 mOD/min, Fig. 4A, inset) resulted in the generation of amidolytic activity, which was inhibited completely by PNII (3.3 mOD/min, a potent FXIa inhibitor) and partially by hirudin (16.3 mOD/min, a thrombin inhibitor) but not by corn trypsin inhibitor (CTI, 38 mOD/min, a FXIIa inhibitor). Kaolin, a contact pathway activating surface, also supported an increase in the rate of S-2366 hydrolysis (56 mOD/min), which was inhibited by PNII (38 mOD/min) and CTI (0.73 mOD/min) but not by hirudin (41 mOD/min). Rates were reduced to <1 mOD/min in the presence of Glu-Gly-Arg-chloromethylketone, a nonspecific serine protease inhibitor, in all cases.

To detect the generation of amidolytic activity caused by the generation of enzymes other than FXIa, clotting factor-deficient plasmas were used in the presence of TRAP-activated platelets (Fig. 4B). Amidolytic activity was reduced from 43 mOD/min in normal plasma (Fig. 4A) to 15 mOD/min in FXI-deficient plasma (Fig. 4B) and was completely inhibited by hirudin (3.7 mOD/min). It is possible that this residual platelet-dependent amidolytic activity in FXI-deficient plasma is a consequence of platelet FXI (23) because this activity is also inhibited by PNII (Fig. 4B). FXII- and HK-deficient plasmas showed no reduction in the rate of hydrolysis unless PNII or hirudin were present, consistent with the conclusion that thrombin can hydrolyze S-2366 and preferentially activates FXI on the platelet surface (8, 9). The results of the experiments carried out in HK-deficient plasma, which were similar to those with normal plasma, suggest that the use of microtiter wells is valid in this assay.

**DISCUSSION**

The results presented in this paper demonstrate that FXI interacts with specific, saturable, high affinity binding sites exposed on TRAP-activated platelets where it is activated by thrombin to initiate the consolidation phase of blood coagulation. FXI does not bind to either resting HUVEC or HUVEC activated with TRAP to release vWF even in the presence of HK and zinc ions when coated on microcarrier beads, contrary to previously published results performed in microtiter wells (1, 2). Using a microtiter well assay, we were able to reproduce the results published previously (1, 2) for FXI binding in the presence of HK and zinc ions, i.e. saturable, specific, reversible high affinity ($K_D = 12 \text{ nM}$) binding of FXI to microtiter wells containing HUVEC ($1.3 \times 10^{10}$ sites/well = $1.3 \pm 0.2 \times 10^6$ sites/cell). However, we found a higher number of FXI binding
sites in the absence of cells (1.8 ± 0.1 × 10¹⁰ sites/well) with high affinity binding (KD = 2.4 nM, Fig. 1). This suggests that saturable, specific, high affinity binding of FXI observed in the presence of HK is a property of the artificial surface within the microtiter wells. In the absence of HK there was no detectable binding, similar to earlier reports (1).

We recognize that the properties of cellular membranes can be vastly affected by minor manipulations, which can significantly differ between laboratories, thereby limiting comparisons between our results and those established previously. However, the large number of high affinity binding sites reported previously for FXI on the unactivated HUVEC surface (KD = 4.5–6.9 nM, Bmax = 2.7–13 × 10⁶ sites/cell) (1, 2) suggests that the entire pool of plasma FXI should be bound under normal physiological conditions as determined by the following calculations. There are ~7.23–9.03 × 10¹⁶ molecules of FXI physiologically present in circulation (30 nM × 4–5 liters × 6.023 × 10²³ molecules/mol). Since there are ~1–6 × 10¹³ endothelial cells per adult vascular system (24), there would be 2.7–78 × 10¹⁹ high affinity endothelial sites for FXI per adult, i.e. 373–8,637 more receptors than FXI molecules. Thus, FXI always would be associated with the endothelium, and none would be found free in plasma.

Furthermore, a single endothelial cell could not accommodate 2.7–13 million molecules of FXI. The surface area of the HUVEC luminal membrane is ~100 µm², assuming a flat rectangular shape. If it is assumed that 2.7 × 10⁶ FXI molecules occupy 100% of the cellular surface, 37 nm² per binding site (100 µm²/2.7 × 10⁶ sites) would be available, each with a radius of 3.4 nm ((37 nm²/π)⁻¹/2). Thus, the diameter of the widest dimension of FXI when bound to its receptor would be 6.8 nm. The same calculation can be performed assuming 13 million sites/cell, providing a binding contact of 7.7 nm² with a cylindrical maximum diameter of 3.2 nm. Therefore, the diameter of the FXI molecule interacting with the entire exposed endothelial cell surface would have to be ~3.2–6.8 nm, assuming 100% occupancy and precluding cellular interaction with any other molecules. According to crystallographic data, the smallest dimension of the FVIIa (50 kDa) unit cell is 10 nm, FXa (58.8 kDa) is 5.5 nm, and FIXa (56 kDa) is 9 nm (25–27). The size of the FVIIa/tissue factor complex (90 kDa) is 4–5 nm in diameter, and the smallest dimension of the unit cell is 7.11 nm (28). These calculations indicate that the FXI molecule is too small to interact with the endothelial cell surface in such a way that it would fill the available area. Therefore, it is unreasonable for a single endothelial cell to bind as many as 13 million molecules of FXI.

To eliminate the high level of background binding detected in the presence of HK, we attempted to block the microtiter wells using a variety of potential blocking agents and microtiter plates themselves. The 27% decrease in the number of FXI binding sites detected when HUVEC were present compared with that detected in their absence is nearly identical to the 28% of a 96-well microtiter well surface area, including the sides, that is covered by HUVEC when incubated in 100 µl of buffer. Furthermore, we report about half as many sites per HUVEC as was reported earlier (1) using smaller microtiter wells with approximately half the surface area. This strongly suggests that cells abrogate the number of binding sites by coating the base of the microtiter well. To test the hypothesis that the microtiter well surface was the cause of the high background binding, we determined the effect of incubation volume on FXI binding, which was predicted to be volume-dependent because an increase in volume would increase the available surface area of the microtiter well without changing the number of cells. As the volume of the reaction mixture was varied while maintaining the FXI (30 nM) and HK (100 nM) concentrations we found that the number of FXI binding sites detected per well increased in direct proportion to the incubation volume regardless of whether HUVEC, CHO cells, or empty wells were studied. The high number of detected binding sites is related not to the presence of cells but to the artificial surface contained within the microtiter wells, and it is a property of HK (13, 14), which binds nonspecifically to plastic surfaces.

The inability to detect specific binding sites in the microtiter well assay was suggestive but not conclusive proof of the absence of FXI receptors on HUVEC. Therefore, to reexamine the binding of FXI to HUVEC in comparison to platelets, HUVEC were grown to confluence on microcarrier beads and assayed in suspension. This method eliminates interference by artificial surfaces because only radioactive proteins bound to the cellular pellet are measured. Binding studies performed with HUVEC in suspension directly may have induced anoikis, or cell death caused by lack of surface contacts. However, neither quiescent nor TRAP-pretreated HUVEC-coated microcarrier beads bound FXI in the presence or absence of HK. The amount of FXI detected in the pellets of quiescent or activated HUVEC was no different when substituted with HEK293 cell-coated beads. The amount of FXI bound was negligible, in contrast to the 2.7–13 × 10⁶ sites/cell previously reported (1, 2). When TRAP-activated platelets were used instead of HUVEC, specific and saturable FXI binding was detected in the presence of HK (45 µM) and zinc ions (25 µM). Platelets did not bind FXI in the absence of HK, zinc ions, or TRAP stimulation.

The examination of the functional consequences of FXI binding to cellular surfaces supports the conclusion that activated platelets but not endothelial cells participate in the initiation of the consolidation phase of blood coagulation. Our studies were carried out in plasma, providing the advantage of incorporating all blood proteins in one reaction. To elucidate candidate FXI activators and cofactors as well as enzymes that may hydrolyze S-2366, studies were carried out in the presence or absence of various specific inhibitors, coagulation proteins, and cells. As predicted from the binding experiments, neither HUVEC nor HEK293 cells facilitate the activation of FXI. Activated platelets and kaolin support the activation of FXI via separate and distinct mechanisms as shown by the inhibition profile. The ability of hirudin, a specific thrombin inhibitor, to inhibit the rate of substrate hydrolysis in the presence of activated platelets but not kaolin suggests that thrombin is a major activator of FXI on platelets but not on artificial surfaces. However, the incomplete inhibition of amidolytic activity by hirudin on platelets suggests that FXI activation proceeds via another mechanism, possibly FXIIa. Kaolin supports the activation of FXI through FXIIa as seen by the complete inhibition of amidolytic activity in the presence of CTI but not hirudin. These conclusions are confirmed by the factor-deficient plasma studies. FXI-deficient plasma had no effect on the rate of hydrolysis in the presence of activated platelets, but in the presence of hirudin a condition in which FXIIa and thrombin are absent or inactive resulted in a 9-fold reduction in rate. On the other hand, the rate of hydrolysis on kaolin in FXII-deficient plasma was reduced by 4.5-fold and was reduced 18-fold by the combined absence of FXII and inactivation of thrombin by hirudin.

The present studies demonstrate conclusively that activated platelets express saturable and specific high affinity receptors for FXI that are functionally important in promoting the initiation of the consolidation or intrinsic pathway of blood coagulation. In contrast, neither resting nor thrombin-activated HUVEC have any capacity to interact specifically with FXI or to promote blood coagulation reactions involved in the initiation of the intrinsic pathway. These observations are consistent
with the concept that the primary phenotype of resting endothelium is anticoagulant and non-thrombogenic, whereas the major function of activated (but not resting) platelets is to participate in the initiation of the consolidation pathway of blood coagulation, leading to the local explosive generation of thrombin at sites of vascular injury.

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