Interventional Cardiology

Monocyte-Derived Tissue Factor Contributes to Stent Thrombosis in an In Vitro System

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OBJECTIVES	This study evaluated the role of circulating tissue factor (TF) in mediating thrombus formation on stents in an in vitro model of stent perfusion.
BACKGROUND	The traditional view of coagulation has recently been challenged by the demonstration that TF is present in circulating blood. The potential contribution of this intravascular pool of TF to thrombus formation on stents is not known.
METHODS	Coronary stents were placed in parallel silicone tubes connected to a roller pump that was set to pump blood at a flow rate of 10 ml/min. Stents were then exposed to heparinized blood from healthy volunteers for 120 min.
RESULTS	The presence of the stent in the circuit caused a significant increase in monocyte TF expression, but only monocytes with attached platelets stained positive for TF. Thrombi formed on stents and the thrombi stained positive for TF. Pretreatment of blood with a monoclonal antibody against TF (cH36) caused a 56% reduction in ^{125}I-fibrin(ogen) deposition on stents compared with controls (p = 0.002). Monocyte depletion of blood reduced ^{125}I-fibrin(ogen) deposition by 45% (p = 0.01) and TF staining in the thrombus by 83% (p = 0.01). Pretreatment of blood with a monoclonal antibody against P-selectin reduced ^{125}I-fibrin(ogen) deposition by 24% (p = 0.04). Perfusion of stents with leukocyte-reduced platelet-rich plasma (PRP) produced small thrombi and treatment of PRP with cH36 reduced ^{125}I-fibrin(ogen) deposition by 43% (p = 0.01).
CONCLUSIONS	Circulating TF plays a pivotal role in thrombus formation on stents. Monocytes appear to be the main, but not only, source of TF depositing in the thrombus. (J Am Coll Cardiol 2004; 44:1570–7) © 2004 by the American College of Cardiology Foundation

Tissue factor (TF) is a transmembrane glycoprotein that triggers the extrinsic pathway of the coagulation cascade. Tissue factor binds to factor VII/VIIa and the resulting complex activates factor IX and X, with the subsequent generation of thrombin and deposition of fibrin (1,2).

The classic view of coagulation implies that upon disruption of the vessel wall, TF sequestered in the adventitia is exposed to the flowing blood, with the consequent activation of coagulation and thrombus formation (3). This view of coagulation has been challenged by the discovery that TF is also present in circulating blood in association with small lipid vesicles that can bind to platelet aggregates that form on collagen-coated surfaces in vitro (4). The precise source of these vesicles has not, however, been identified.

Coronary stents have improved the outcome of patients undergoing percutaneous coronary interventions (5). Nevertheless, around 1% of patients may experience subacute thrombosis of the stent, and as many as 37% may have an increase in creatine phosphokinase muscle brain isoenzyme release after stent placement (6).

A better understanding of the thrombotic mechanisms triggered by stents may provide new therapeutic approaches to prevent clinical events following coronary interventions.

In this study we found that circulating TF plays a crucial role in mediating thrombus formation on stents in an in vitro model of stent perfusion. Moreover, monocytes were identified as a main source of the TF that contributed to stent thrombus formation.

METHODS

Blood sample collection and preparation of monocyte-reduced blood. Blood samples (30 ml) were collected in a syringe containing 10 IU of unfractionated heparin (Epsoclar; Biologici Italia, Milano, Italy) from healthy volunteers who were not taking medication and who were on average 30 years of age (range 23 to 35 years). Activated clotting time was measured with a HEMOCRON 401 (Cremascoli, Iris, Milano, Italy) and adjusted to 180 to 230 s by adding additional heparin (range 2.5 to 5 IU).

To prepare platelet-rich plasma (PRP), blood was centrifuged at 90 g × 10 min at 22°C and the platelet count adjusted to 200,000/μl. To reduce the leukocyte content of the PRP, it was passed through a filter of precision-woven

Abbreviations and Acronyms

IOD = integrated optical density
Mab = monoclonal antibody
MRB = monocyte-reduced blood
PPP = platelet-poor plasma
PRP = platelet-rich plasma
TF = tissue factor

nylon filaments with 5 μ pores (CellMicroSieves, Biodesign Inc., Carmel, New York). This process virtually abolished leukocyte contamination, as judged by flow cytometry. Platelet-poor plasma (PPP) was prepared by further centrifugation of the remaining blood at 2,000 g $\times$ 10 min.

In some experiments blood was reacted with superparamagnetic polystyrene beads (Dynabeads M-450 CD 14; Dynal Biotech, Oslo, Norway) coated with a primary monoclonal antibody (Mab) specifically for the CD14 monocyte membrane marker by diluting 2.5 ml of blood with an equal volume of buffer (phosphate-buffered saline) (137 mM NaCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4) and then adding 50 μl beads/ml of blood. The blood and beads were placed on a mixer (Dynal Bitoech) at 4°C for 60 min and then the beads and attached cells and/or other material were separated using a magnetic particle concentrator (Dynal Biotech) for 5 min. The remaining blood was analyzed for monocyte depletion by flow cytometry.

Depending on the experiment, 5 ml of whole blood, monocyte-reduced blood (MRB), PRP, or PPP was placed into polystyrene tubes and incubated for 10 min with 25 μg/ml of ^{125}I-fibrin(ogen) (Amersham Biosciences, Milano, Italy), and with saline, 200 μg/ml of cH36, a chimeric Mab against TF (kindly supplied by Sunol Molecular Corporation, Miramar, Florida), or 60 μg/ml of anti-P-selectin antibody (9E1, R & D Systems, Minneapolis, Minnesota). In preliminary experiments, cH36 (250 μg/μl) was incubated with recombinant TF (Hemoliance, Instrumentation Laboratory, Milano, Italy) and then the TF was used in a prothrombin assay. Compared with the 12-s control, incubation with cH36 resulted in prolongation of the prothrombin time to 82 s. After taking 5 μl of the sample to measure the ^{125}I-specific activity, blood or plasma from each tube was transferred to the corresponding silicone tubing of the flow system.

This protocol was approved by the local ethics committee and informed consent was obtained from all the blood donors.

Flow system and experimental design. Twenty-three-millimeter long Tetra Multilink stents (kindly supplied by Guidant, Temecula, California) were inserted and then deployed in silicone tubing (3 mm inner diameter) (Gilson Italia, Milano, Italy) by inflating the balloon at 14 atm for 20 s. The silicone tubing was then connected to a roller pump (Minipuls 3; Gilson, Villers, France), which was set to pump blood at a flow rate of 10 ml/min, with a theoretically calculated shear rate of $\approx$64 s^{-1}. The circuit was then closed using a silicone connector and the perfusion performed for 120 min. The temperature was kept stable at 37°C by a water bath.

The study consisted of three different sets of experiments. The first set (group 1) consisted of 10 experiments; in each case, four silicone tubing circuits were perfused in parallel: circuit A contained saline-treated blood with no stent and served as a control, whereas circuits B, C, and D all contained stents and the blood was pretreated with saline, cH36, or 9E1, respectively.

The second set (group 2) consisted of six experiments; in each case four perfusions were performed in parallel: circuit A was the control as before, whereas in the remaining three perfusion stents were exposed to whole blood (circuit B), MRB (circuit C), or MRB pretreated with cH36 (circuit D).

The third set (group 3) consisted of six experiments; in each case five perfusions were performed in parallel: circuit A contained saline-treated PRP with no stent and served as a control, whereas in the remaining four perfusion stents were exposed to PPP (circuit B), PPP + cH36 (circuit C), PRP (circuit D), and PRP + cH36 (circuit E).

Radioactivity measurements. At the end of the perfusion the stents were recovered from the tubing and their ^{125}I-radioactivity measured using a gamma spectrometer (Canberra Industries, Meriden, Connecticut). In some experiments, in addition to thrombi on the stent, thrombi were found free in the tubing; these thrombi were added to the stent thrombi before measuring the radioactivity.

Flow cytometry. Mab to CD45 (phycoerythrin [PE]-labeled), CD14 (PE, fluorescein isothiocyanate [FITC], and allophycocyanin-labeled), and CD42b (PE-labeled), as well as isotype controls, were from Pharmingen (San Diego, California). Anti-TF (FITC-labeled) Mab was from American Diagnostica (Greenwich, Connecticut). Samples were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, California) equipped with CELLQuest analysis software (Becton Dickinson). In studies involving more than one labeled antibody, compensation was performed using Calibrite beads and FACSComp software (Becton Dickinson).

Monocyte TF expression was determined in the first set of experiments at baseline and at the end of the perfusion as previously described by Lindmark et al. (7). In brief, monocytes were identified by gating on CD14-positive cells, and TF positivity was defined as a fluorescence intensity greater than a threshold set at the upper 2% value using the isotype control. In two cases, triple staining was performed using an anti-CD14 (APC) Mab, an anti-TF (FITC) Mab, and an anti-CD42b (PE) Mab, which enabled an assessment of the correlation between platelet-monocyte aggregates and monocyte TF expression. Monocytes were identified by both their characteristic scatter properties and CD14 positivity, and thereafter subjected to two-color analysis using TF-FITC and CD42b-PE.

Table 1. ^{125}I-fibrin(ogen) Deposition (Mean Values ± SEM) on Stents After 120 Min Perfusion in the Three Different Sets of Experiments

First set of experiments (10 cases)			
Perfusion 1A	Perfusion 1B	Perfusion 1C	Perfusion 1D
No stent	Stent/saline	Stent/cH36	Stent/9E1
	20,554 ± 3,445 ng	9,007 ± 3,044 ng*	15,562 ± 3,237 ng†
Second set of experiments (6 cases)			
Perfusion 2A	Perfusion 2B	Perfusion 2C	Perfusion 2D
No stent	Stent/WB	Stent/MRB	Stent/MRB +cH36
	7,233 ± 1,546 ng	3,987 ± 1,825 ng‡	3,789 ± 1,637 ng§

Third set of experiments (6 cases)				
Perfusion 3A	Perfusion 3B	Perfusion 3C	Perfusion 3D	Perfusion 3E
No stent/fPRP	Stent/PPP	Stent/PPP + cH36	Stent/fPRP	Stent/fPRP + cH36
	129 ± 7 ng	134 ± 8 ng	1,339 ± 223 ng‖	766 ± 100 ng¶

*p = 0.002 and †p = 0.04 versus perfusion 1B; ‡p = 0.01 and §p = 0.01 versus perfusion 2B; ‖p < 0.001 versus perfusion 3B; ¶p = 0.01 versus perfusion 3D.
cH36 = monoclonal antibody against tissue factor; MRB = monocyte-reduced blood; 9E1 = monoclonal antibody against P-selectin; PPP = platelet-poor plasma; fPRP = filtered platelet-rich plasma; WB = whole blood.

To assess monocyte depletion achieved with the anti-CD14 beads, leukocytes were identified by their characteristic scatter properties and thereafter subjected to two-color analysis with CD45-PE and CD14-FITC.

Histology and immunohistochemistry. Thrombi formed on stents were scraped off the stent surface, fixed in 10% formalin, and processed for paraffin embedding. Sections (2 μ) were cut and stained with hematoxylin and eosin. For immunohistochemistry, sections were deparaffinized, processed for antigen retrieval as described by Pileri et al. (8), and incubated for 30 min with an anti-TF Mab (American Diagnostica). Thereafter the slides were incubated with rabbit antimouse immunoglobulins (Dako, Carpinteria, California) for 20 min, followed by 20 min with the APAAP complex (Dako), which is a complex of mouse alkaline phosphatase and antialkaline phosphatase Mab. The color was then developed for 20 min with fucsin, after which the slides were counterstained with hematoxylin.

Image cytometry. TF staining was quantified by image cytometry using a CCD monochromatic camera (Sony XC77CE, Kangawa-Ken, Japan) connected to a light microscope (Leitz GmbH, Wetzlar, Germany). Briefly, in each specimen the three most reactive areas of 0.5 mm^2 were selected with a 25× objective. Two images were collected under monochromatic light: the first at 530 nm, where TF staining had the highest absorbance, and the second at 630 nm, where nuclei stained most intensely. Nuclei were then eliminated from the image using an image processing procedure, allowing for the measurement of TF staining at 530 nm. For each specimen, results were expressed as integrated optical density (IOD) units/mm^2.

Statistics. Data are presented as mean values ± SEM. A Smirnov-Kolmogorov test was used to test for normality. Because data were normally distributed, comparisons among the effects that different treatments (control, cH36, and 9E1 in the first set of experiments) or conditions (whole blood, MRB, and MRB plus cH36 in the second set of experiments, etc.) had on the same individuals were performed using one-way analysis of variance for repeated measures and Bonferroni's test for all pairwise multiple comparisons, as well as paired t test as appropriate. A p value <0.05 was considered statistically significant.

RESULTS

Hematologic Parameters

Hematologic parameters (hemoglobin, hematocrit, and both leukocyte and platelet counts) were within the normal ranges in all the donors. The mean activated clotting times, measured at the beginning of the experiments, were 216 ± 10 s in the first set of experiments, 211 ± 3 s in the second set of experiments, and 207 ± 4 s in the third set of experiments. Adjusted platelet count in the third set of experiments was 206,700 ± 3,900/μl.

Group 1

Thrombus formation on stents. In group 1, in the absence of a stent (circuit A), no visible thrombus formed in the tubing. In the stent samples treated with saline (circuit B), visible thrombus covered the stent surface, and in four of 10 experiments free thrombus was also observed in the tubing. When blood was pretreated with anti-TF antibody (circuit C) or anti-P-selectin (circuit D) antibody, the visible thrombus formed was reduced relative to the saline control (circuit B).

^{125}I-Fibrin(ogen) deposition was 20,544 ± 3,445 ng in circuit B (saline), 9,007 ± 3,044 ng in circuit C (anti-TF), and 15,562 ± 3,237 ng in circuit D (anti-P-selectin) (p = 0.002; for multiple comparisons see Table 1). Figure 1 shows thrombus formation on stents exposed to blood pretreated with either saline or anti-TF.

Histology and immunohistochemistry. The thrombi formed on stents perfused with blood pretreated with saline (circuit B) showed irregular areas of erythrocytes trapped in a fibrin network. Large and irregular platelet aggregates were scattered throughout the thrombi. Numerous granulocytes and monocytes were deposited as irregular clusters within the thrombi. Tissue factor staining was scattered throughout the thrombi, in close contact with the platelet aggregates and intermingled with fibrin strands (Fig. 2). In

Figure 1. Thrombus formation after 120 min perfusion on stent surface exposed to blood pretreated with either saline (control) or with a monoclonal antibody against tissue factor (cH36). Note the striking reduction of thrombus formation on the stent in which blood was pretreated with cH36.

addition, monocytes consistently stained strongly and diffusely positive for TF, whereas some neutrophils gave weak and irregular TF staining.

Monocyte TF expression and platelet-monocyte aggregates. In perfusion A (no stent), monocyte TF expression did not significantly change throughout the perfusion (3.3 ± 1% at baseline vs. 3.6 ± 1% at 120 min; p = ns). When saline-treated blood was perfused for 120 min in tubing containing a stent (circuit B), however, monocyte TF expression increased significantly (3.5 ± 1% vs. 17.9 ± 5%; p = 0.02) (Figs. 3A and 3B). In perfusions in which blood
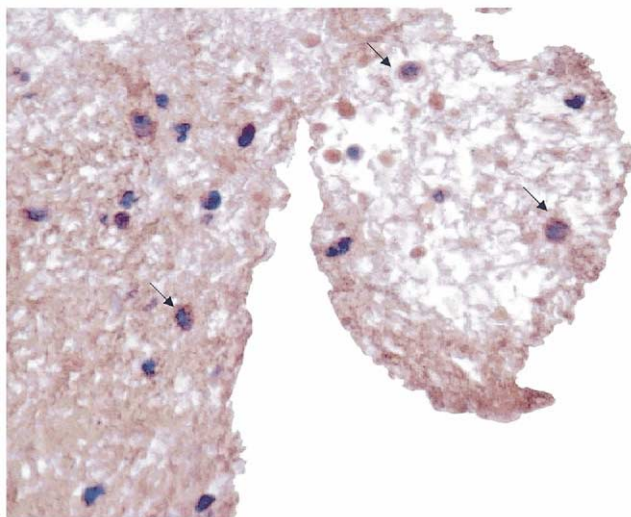

Figure 2. Histologic assessment of thrombi formed on a stent surface showing intense tissue factor staining **(red)**. Monocytes **(arrows)** found in the thrombus displayed strong tissue factor staining, while granulocytes displayed weak and irregular staining. Original magnification 400×.

was pretreated with 9E1 (4 cases), at the end of the perfusion there was a 46% reduction in monocyte TF expression compared with perfusion B (9.7 ± 2% vs. 17.9 ± 5%, respectively; p = 0.3). When monocytes were divided into those without and those with attached platelets, virtually all of the monocytes that stained positive for TF also stained positive for the platelet marker CD42b (Figs. 3C and 3D). The percentage of monocytes that had platelets attached increased in both experiments where this was analyzed (pre-perfusion values 10% and 15%, post-perfusion values 42% and 53%).

Group 2

Thrombus formation on stents perfused with MRB. Pretreatment of blood with anti-CD14 beads reduced the monocyte count by 93 ± 1% as judged by flow cytometry (Figs. 4A and 4B). Stents perfused with MRB showed a significant reduction in thrombus formation as compared with whole blood. Adding an anti-TF Mab to MRB produced only a small, nonsignificant reduction in ^{125}I-fibrin(ogen).

Thus, ^{125}I-fibrin(ogen) deposition on stents perfused with whole blood, MRB, and MRB + anti-TF were 7,233 ± 1,546 ng, 3,987 ± 1,825 ng, and 3,789 ± 1,637 ng, respectively (p = 0.006; for multiple comparisons see Table 1).

To determine whether the reduction of thrombus formation with MRB was accompanied by a decrease in TF expression in thrombi, we analyzed the thrombi immunohistochemically. As shown in Figure 5, thrombi formed in perfusions with MRB had less TF staining than thrombi formed in perfusions with control whole blood (16,620 ± 1,126 IOD/mm^2 vs. 2,942 ± 518 IOD/mm^2, respectively, p = 0.01). In addition, thrombi formed in perfusions with MRB showed markedly reduced numbers of monocytes. Granulocytes, which gave weak and irregular TF staining, were more prominent in thrombi from MRB than in thrombi from control whole blood.

Group 1

Thrombus formation on stents perfused with PPP or PRP. When stents were exposed to PPP (circuit B), only a small amount of thrombus deposited on the stents (^{125}I-fibrin[ogen] deposition 129 ± 7 ng) and cH36 did not affect thrombus deposition (134 ± 8 ng) (p = ns). With filtered PRP, thrombus formation was much greater than with PPP (^{125}I-fibrin(ogen) deposition 1,340 ± 223 ng; p < 0.001), but much less than with whole blood. Pretreatment of PRP with cH36 significantly reduced ^{125}I-fibrin(ogen) deposition (766 ± 100 ng; p = 0.01).

DISCUSSION

Our studies provide new data on the contributions of TF and monocytes to stent thrombosis. Utilizing a closed circuit, we demonstrated that perfusion of whole, heparin-
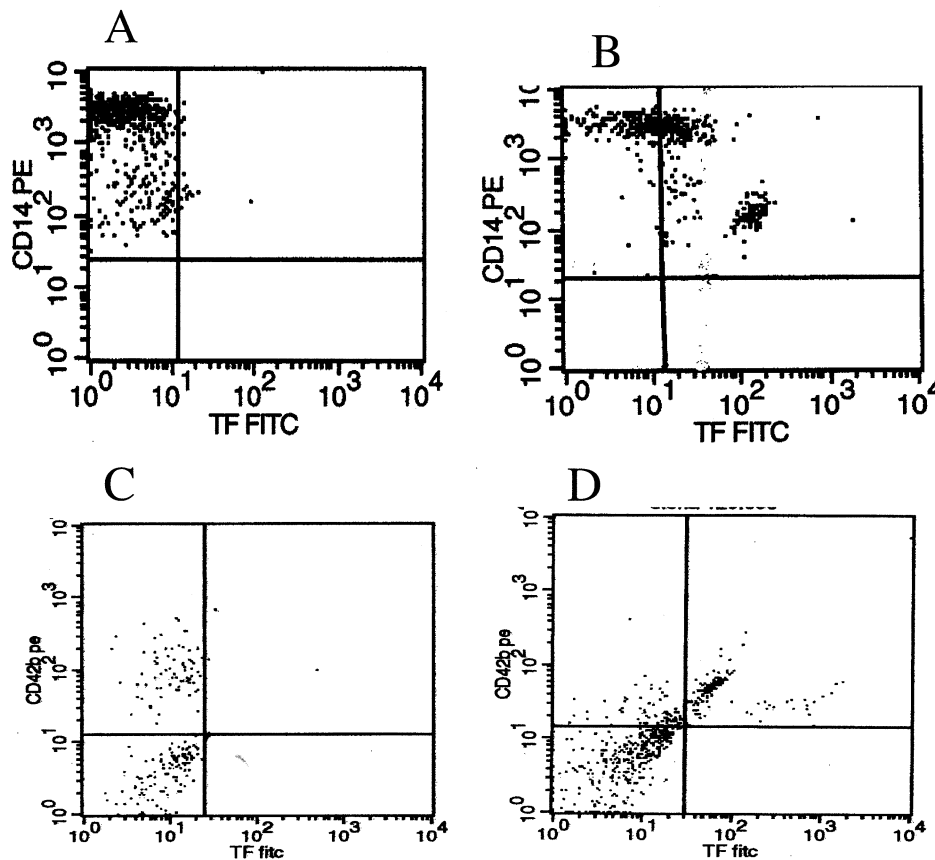
Figure 3. Tissue factor (TF) expression on monocyte membranes at baseline **(A)** and at the end of the perfusion **(B)**. In two experiments monocytes were subjected to two-color analysis using TF-FITC as the abscissa and CD42b-PE as the ordinate: at baseline **(C)** there are some platelet-monocyte aggregates that do not display TF staining. At the end of the perfusion **(D)** only monocytes with attached platelets displayed TF staining.

anticoagulated blood from normal controls for 120 min at $64\ s^{-1}$ consistently resulted in thrombus formation when a stent was deployed in the circuit, but not when there was no stent. The perfusion conditions were designed to simulate the diameter and blood flow of a typical coronary artery after stent placement. In our first series of experiments we observed that an antibody to TF decreased the amount of fibrin(ogen) deposited in the thrombus by 56%. In the second series of experiments we observed that nearly depleting the blood of monocytes with an antibody to the monocyte-specific antigen CD14 produced a 45% decrease in fibrin(ogen) deposition. Adding an antibody to TF to the blood nearly depleted of monocytes caused a further small reduction in fibrin(ogen) deposition that was not statistically significant. These data suggest that TF contributes to thrombus formation and that monocytes are a major, but perhaps not the sole, source of TF. To assess the potential role of other sources of TF, in the third series of experiments we used PRP that was nearly devoid of leukocytes. Small thrombi formed on stents and an antibody to TF reduced the thrombus by 43%, indicating that TF derived from platelets (9) and/or circulating microvesicles probably also contribute to stent thrombosis. Further support for the importance of monocytes in TF deposition came from the direct observation of decreased TF staining of thrombi

formed by blood nearly depleted of monocytes. We further found that after the perfusion more monocytes stained positive for the platelet-specific antigen CD42b and more monocytes expressed TF. Moreover, the increased expression of TF was confined to monocytes that stained positive for the platelet-specific antigen. This raises the possibility that platelet activation results in the attachment of platelets to monocytes which, in turn, induces monocyte expression of TF. This hypothesis is consistent with studies conducted by others that indicate that platelet-monocyte aggregates increase after percutaneous coronary interventions (10,11) and that both P-selectin (7,12,13) and CD40 ligand (7), molecules expressed on the surface of activated but not unactivated platelets, can stimulate monocyte synthesis of TF. Support for a role for P-selectin in stent-associated thrombus formation comes from our additional studies demonstrating that an antibody to P-selectin decreased TF expression on monocyte membranes and fibrin(ogen) deposition in stent thrombi. These data, in turn, are consistent with prior studies by Palabrica et al. (14), who demonstrated decreased fibrin deposition in a Dacron graft implanted within an arteriovenous shunt in nonhuman primates when the animals were treated with an antibody to P-selectin. Such antibodies may operate by decreasing platelet-monocyte interactions (and the subsequent enhancement of
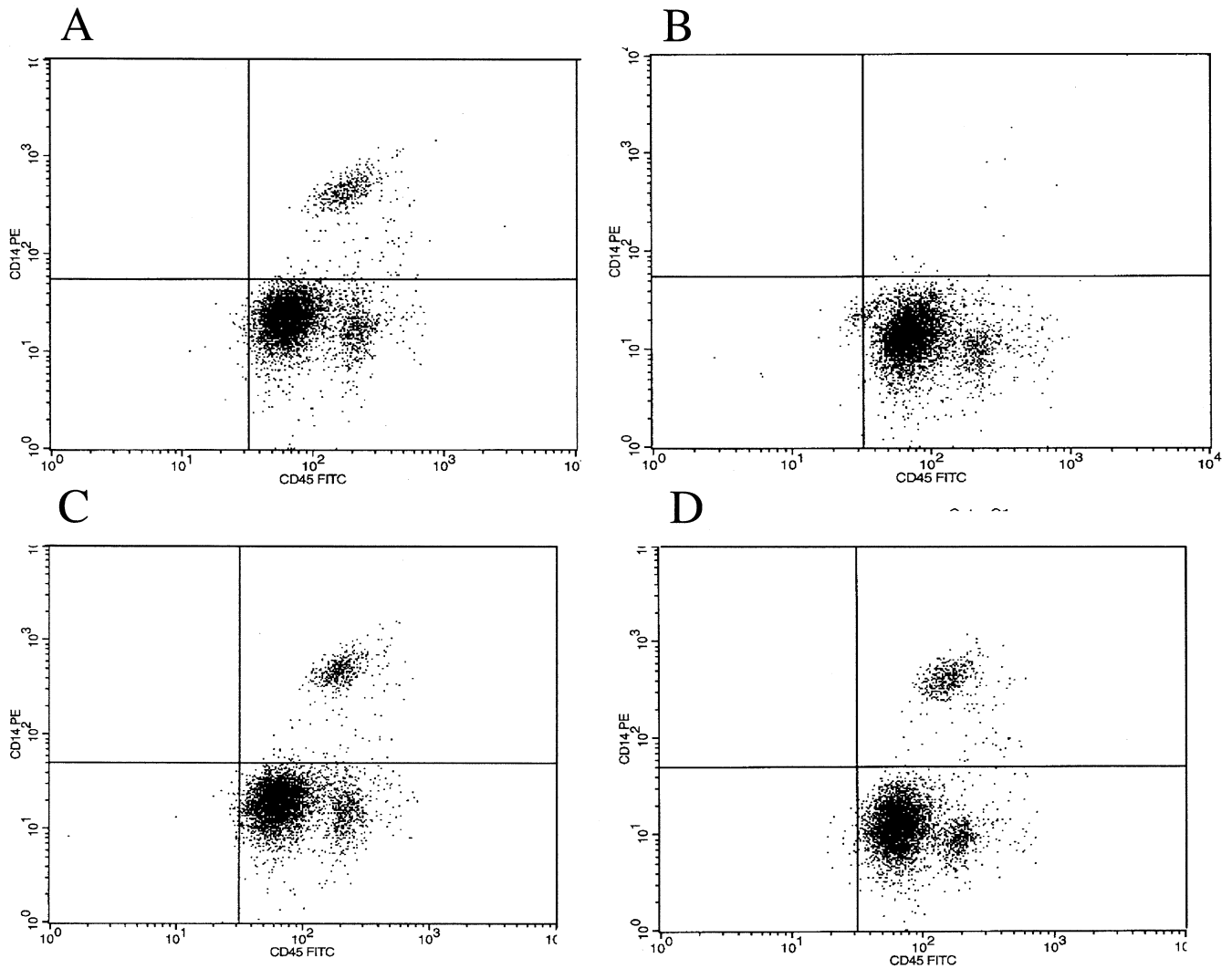
Figure 4. Whole blood before **(A)** and after **(B)** anti-CD14 bead addition. Note in **B** the dramatic reduction of monocyte count. In controls that were subjected to all of the procedures used to deplete monocytes except for adding the anti-CD14-coupled beads, no difference was noticed between the baseline sample **(C)** and the sample after the procedures **(D)**.

TF expression) and/or by decreasing the ability of leukocyte-derived microparticles containing both P-selectin glycoprotein ligand-1 and TF to attach to activated platelets expressing P-selectin at the site of injury (15,16). Because the effects of the antibody were observed very soon after administration, the latter mechanism is likely to have contributed, but it remains possible that the sustained effect was due to the former mechanism. Nevertheless, the anti-P selectin Mab caused a partial reduction on monocyte TF expression, and its effect on thrombus formation was much less profound than the anti-TF Mab. This finding may be the consequence of the existence of multiple pathways of monocyte TF activation, which may be still operative even in the presence of P-selectin blockade. Finally, our observations on the association of platelet-leukocyte aggregates and monocyte TF expression may explain why the presence of circulating platelet-leukocyte aggregates after percutaneous coronary interventions in humans correlated with recur-

rent ischemic events in an intriguing pilot study conducted by Mickelson et al. (17).

Our current studies complement our previous studies of stent insertion in nonhuman primates, where we demonstrated that platelet adhesion and aggregate formation was accompanied by recruitment of monocytes and neutrophils (18). The platelet thrombus stained positive for TF antigen, the neutrophils gave variably weak staining for TF, and the monocytes stained strongly positive for TF. We also observed leukocytes adjacent to stent struts, an observation consistent with recent evidence of monocyte adhesion to stent metal (19). In our previous study we could not, however, determine the source of TF that we observed in the thrombi. Our new data reinforce the recent evidence that bloodborne TF can contribute to thrombus formation and extend those observations to thrombi formed on stents (4,20,21). Because there was no blood vessel in our system, the TF could not come from the blood vessel wall and thus
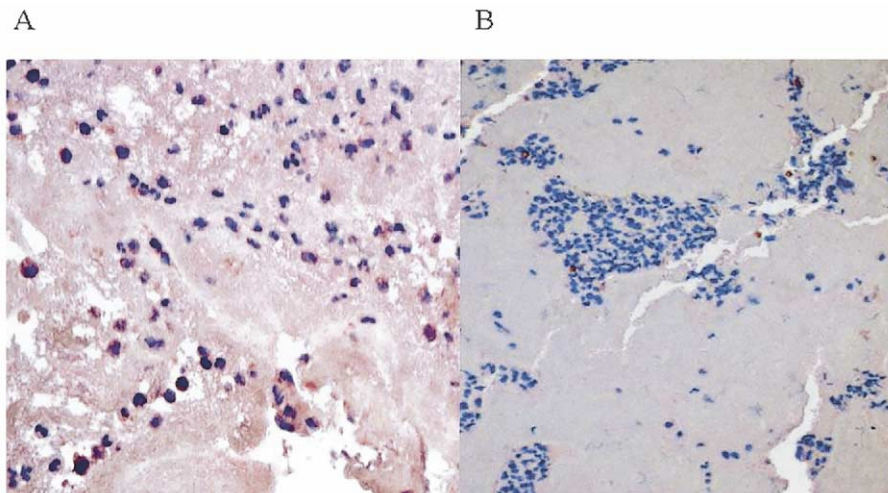
Figure 5. Immunohistochemistry of thrombi formed on stents perfused with whole blood **(A)** or monocyte-depleted blood **(B)**. In **B** a strong reduction in tissue factor staining **(red)** was observed compared with **A**. Original magnification 200×.

must be derived from the blood. Moreover, our data indicate that not only is TF deposited, but it plays an important role in thrombus formation because an antibody to TF reduced thrombus formation.

Among the proposed sources of blood TF are microparticles derived from monocytes or neutrophils (15,16,20,22) or an alternatively spliced form of TF (21). Although we cannot exclude the possibility that our antibody to CD14 also depleted CD14-positive microparticles containing TF, we and others (23) have not identified such microparticles in normal individuals, and although others have identified small numbers of such microparticles in normals (24,25), there is uncertainty about the activity of TF in such microparticles (24). Platelets and platelet-derived microparticles have also been proposed as sources of blood TF (9,24,25). Because we observed some TF in thrombi despite nearly complete elimination of monocytes, we tried to assess the contribution of platelets to blood TF in the near absence of leukocytes. We observed that pretreating PRP with cH36 reduced thrombus formation, thus suggesting TF is also important in PRP-supported thrombus formation. We could not, however, differentiate between whether platelets are themselves a source of TF, as suggested by Camera et al. (9), or rather serve as a nidus upon which microvesicle-containing TF deposits after platelets form thrombi, as shown by Rauch et al. (15). It is important, however, to differentiate between TF antigen and activity. Thus, data from Diamant et al. (24) raise doubts about the functional activity of TF in microparticles isolated from plasma, and it has been postulated that interactions of platelets with leukocytes or leukocyte-derived microparticles may be necessary to "de-encrypt" TF (26–28).

One limitation of our study is that we used blood from normal controls rather than patients with cardiovascular disease. In preliminary experiments in patients with myocardial infarction who were taking aspirin, we observed

similar or greater thrombus formation than with the controls in this study. A separate study of patients is planned.

In conclusion, our data support an important role of circulating TF in stent thrombosis. Monocytes appear to be an important source of TF and it is possible that platelet activation and platelet-monocyte interactions may enhance TF expression. Thus, the elements in the reverberating circuit among platelets, monocytes, and TF may be potential therapeutic targets, with specific inhibition of TF particularly appealing.

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