

Identification of Active Tissue Factor in Human Coronary Atheroma

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Background Recent observations suggest that thrombosis in vivo is initiated via the tissue factor (TF) pathway. The TF activity of human coronary atheroma has not been reported.

Methods and Results Directional coronary atherectomy (DCA) specimens from 63 lesions were analyzed with the use of a quantitative TF-specific activity assay. The median content of TF was 10 ng/g plaque (95% CI, 6 to 13 ng/g; range, 0 to 47 ng/g). After homogenization of the specimens, TF activity was detected in 28 of 31 lesions (90%). With a polyclonal anti-human TF antibody, the use of immunohistochemistry detected TF antigen in 43 of 50 lesions (86%); TF antigen was expressed in cellular and acellular areas of the plaque. Histologically defined thrombus was present in 19 of the 43 lesions with detectable TF antigen and in none of the 7 lesions without detectable TF antigen (19 of 43 versus 0 of 7; $P < .02$).

TF antigen was undetectable with immunohistochemistry in 4 of 13 restenotic lesions (31%) and in 3 of 37 de novo lesions (8%) ($P < .05$).

Conclusions TF contributes to the procoagulant activity of most atherosclerotic lesions treated with DCA. The association of immunohistochemically detectable TF with plaque thrombus suggests that TF plays a role in coronary thrombosis. Diminished TF expression in restenotic lesions may in part account for the lower complication rate that has been associated with DCA of restenotic versus de novo lesions. Inhibition of TF may represent a therapeutic goal for the prevention of thrombotic complications associated with percutaneous coronary interventions. (*Circulation*. 1996;94:1226-1232.)

Key Words • angioplasty • coronary disease • immunohistochemistry • thrombosis • thrombus

Thrombosis after the disruption of an atherosclerotic plaque plays a central role in the pathogenesis of acute coronary syndromes such as unstable angina and myocardial infarction.¹ When the contents of a disrupted plaque come into contact with circulating blood, thrombosis may be initiated. TF is a membrane-bound procoagulant molecule that acts as an essential cofactor with factor VII/VIIa to form a complex that cleaves factors IX and X, thereby activating the coagulation cascade.² A number of recent observations in humans and in nonhuman primates suggest that activation of the coagulation cascade in vivo may be initiated via the TF pathway.³⁻⁵ Patients with factor VII deficiency demonstrate depressed levels of factor IX activation, indicating that basal factor IXa generation in vivo results mainly from the activity of TF.³ The infusion of recombinant factor VIIa into patients with hereditary factor VII deficiency results in thrombin generation, as indicated by increases in plasma F1+2 levels. In contrast, the infusion of factor IX or VIII (intrinsic pathway) into patients who are deficient in these factors does not result in thrombin generation.⁴ These observations suggest that TF plays a predominant role in the basal activity of the coagulation cascade in vivo.

The expression of TF mRNA and antigen within the normal vessel wall and in the atherosclerotic plaque has been characterized by in situ hybridization and immunohistochemistry, respectively.⁶⁻⁸ With the use of these techniques, TF is detectable in the adventitia but not in the media or endothelium of normal arteries. Balloon dilatation of rat aorta rapidly induces the expression of TF mRNA and activity in the media.⁹ In atherosclerotic carotid endarterectomy specimens, TF mRNA and antigen are detectable in macrophages, in mesenchymal intimal cells, and in the extracellular matrix.⁶ In comparison with patients with stable angina, patients with unstable coronary syndromes demonstrate higher levels of TF antigen in their coronary atherectomy specimens.⁸

Studies in which TF antigen has been identified in atherosclerotic plaque have not demonstrated functional procoagulant activity. In addition, TF antigen has often been detected in a small number of cells and, in some studies, in a minority of samples.⁶⁻⁸ To determine the extent to which TF activity is present in atherosclerotic coronary arteries, we examined DCA specimens from a series of lesions ($n=63$) with a functional TF-specific colorimetric activity assay. TF procoagulant activity was detected in 84% of the atherectomy specimens. Specimens from 50 (79%) of these lesions were also studied immunohistochemically; 86% were positive for TF antigen. Thus, TF contributes to the procoagulant activity of the majority of obstructive atherosclerotic lesions found in human coronary arteries.

Methods

Patient Population

Protocols for all studies were approved by the institutional review board, and specimens were harvested from patients who provided informed consent. All coronary atherectomy specimens

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Selected Abbreviations and Acronyms

DCA	= directional coronary atherectomy
IPA-pNA	= D-isoleucine-L-proline-L-arginine- <i>p</i> -nitroanilide
mOD	= milli-optical density units
pNA	= <i>p</i> -nitroanilide
PTCA	= percutaneous transluminal coronary angioplasty
sTF	= soluble TF
TF	= tissue factor

were derived from patients treated in the cardiac catheterization laboratory of the Mount Sinai Hospital, New York, NY. A patient with a stable coronary syndrome was defined as a patient with stable or crescendo exertional angina or an asymptomatic patient with a positive exercise test. A patient with an unstable coronary syndrome was defined as a patient with angina pectoris at rest or after myocardial infarction or an asymptomatic patient with a positive exercise test after myocardial infarction (<1 month). A restenotic lesion was defined as a lesion that had been treated percutaneously within the preceding 12 months. DCA was performed with the use of standard techniques.¹⁰ All patients received aspirin and intravenous heparin before DCA. In all cases, the activated clotting time was maintained at >300 seconds.

Specimens were collected from a group of 63 patients who were treated with DCA between February 17, 1993, and January 27, 1994. Only one atherectomized lesion per patient was studied. The total number of percutaneous coronary interventions performed during this period was 792 (the study population accounted for 8%), 176 of which were DCA (the study population accounted for 36% of the DCA interventions). Specimens from all 63 lesions were studied with a functional TF-specific colorimetric assay; immunohistochemical studies were performed on 50 of these 63 lesions (79%). The activity assays and immunohistochemical stains were performed on separate portions of the plaque specimen, which was randomly divided at the time of the procedure. Immunohistochemistry was not performed in 13 cases due to an insufficient quantity of tissue.

TF Activity Assay

To quantify TF activity, plaques were rinsed with saline and then incubated at 37°C in 500 μ L of a reaction mixture consisting of factor X (150 nmol/L), factor VIIa (10 nmol/L), and CaCl_2 (5 mmol/L) in HEPES/BSA buffer (0.14 mol/L NaCl, 0.01 mol/L HEPES, 1 mg/mL BSA, pH adjusted to 7.5). Under these conditions, factor Xa is generated at a rate proportional to the amount of bioavailable plaque TF (Fig 1). After various periods of incubation, 50- μ L aliquots were removed from the reaction mixture and placed in a 96-well microtiter plate (Dynatech) preloaded with 125 μ L of EDTA (50 mmol/L) to arrest further generation of factor Xa. To quantify the amount of Xa, 25 μ L of the factor Xa-sensitive chromogenic substrate IPA-pNA (10 mmol/L) was then added to each well, and the reaction product, pNA, was measured over 10 minutes at 35°C with the use of a kinetic ELISA plate reader (Thermomax, Molecular Devices). The slope of the change in absorbance at 405 nm was used as the measure of factor Xa concentration. Results are expressed mOD over time and are normalized to 1 mg of plaque. Control wells were filled with aliquots of the reaction mixture and no DCA specimen. These wells consistently yielded readings of <25 mOD/min, which was therefore defined as the threshold for TF detection.

To assess the bioavailability of TF, activity assays were also performed on homogenized specimens. Samples were homogenized in 500 μ L of HEPES/BSA buffer with a 6-mm-diameter circular blade at 50 000 rpm for 20 seconds with a high-speed homogenizer (Virtis). A 50- μ L aliquot of the homogenized specimen was then added to the reaction mixture, and the activity assays were performed as described above.

Tissue Factor : Factor VIIa

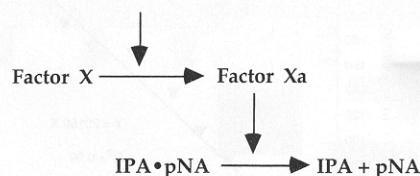


FIG 1. Principle of the chromogenic TF-specific procoagulant activity assay. TF binds factor VIIa to form a complex that converts factor X to its active form, Xa. The amount of factor Xa generated is proportional to the TF activity of the sample and determines the rate of pNA release from the Xa-sensitive substrate IPA-pNA. pNA generation results in a color change due to increased light absorption at 405 nm.

Assay Specificity

The ability of the assay to measure procoagulant activity specifically due to TF was assessed with the use of a monoclonal anti-human TF antibody.¹¹ Twelve sets of specimens were homogenized and then divided into equal portions that were incubated either with anti-TF antibody (10 μ g/mL) or with vehicle (HEPES/BSA buffer) alone at 4°C overnight. Samples with and without antibody were then assayed for TF activity. Specimens pretreated with antibody demonstrated a mean $\pm$ SD reduction of $80 \pm 17\%$ in procoagulant activity in comparison with specimens harvested from the same lesion that were not pretreated with antibody (Fig 2). The lack of total (ie, 100%) inhibition of plaque TF activity may be due to the presence of TF within areas of the plaque that are not fully accessible to the antibody.

Generation of a Standard Curve for TF Quantification

The colorimetric assay used to analyze the DCA specimens expresses TF activity in relative units of mOD/min. To convert these results into recombinant human TF equivalent units, a standard activity curve relating TF concentration (nmol/L) to factor Xa activity (mOD/min) was generated (Fig 3). Recombinant human TF in octyl β -glucopyranoside was provided by Dr Robert Kelley (Genentech, San Francisco, Calif). TF was reconstituted in phospholipid vesicles (30% phosphatidylserine, 70% phosphatidylcholine) in a protein-to-lipid molar ratio of 1:10 000.¹² Readings were taken after incubation of the reaction mixture for 3 hours with solutions containing varying concentrations of recombinant human TF in HEPES/BSA buffer. These readings were then converted to ng/g plaque by assuming a molecular weight of 40 000 for TF.¹³ A standard curve was generated for a 3-hour incubation period because the TF activity of individual DCA specimens was discernible at this time point (see "Results").

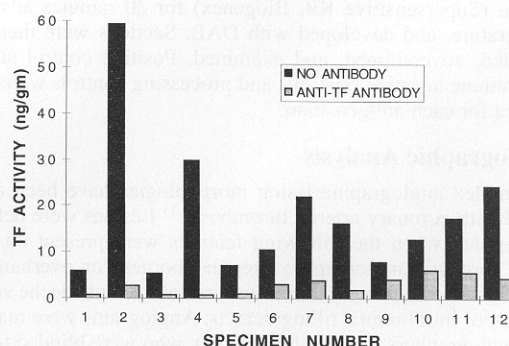


FIG 2. TF activity measurements in homogenized coronary atherectomy specimens preincubated in vitro with or without an anti-human TF antibody. Each set of specimens ($n=12$) is derived from a single lesion. Procoagulant activity is markedly diminished in the portion of the specimen pretreated with anti-TF antibody.

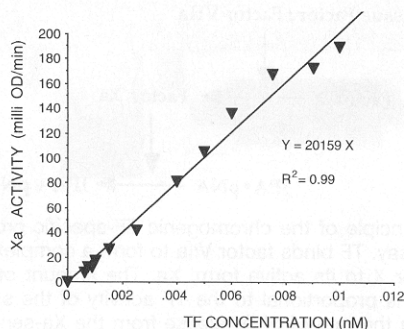


FIG 3. Standard TF activity curve relating the concentration of recombinant human TF to factor Xa activity. After a 3-hour incubation of recombinant human TF in a reaction mixture containing factors VIIa and X, the Xa-sensitive substrate IPA-pNA was added, and the reaction product was read over 10 minutes with the use of optical densitometry at 405 nm.

Antibody Preparation for Immunohistochemistry

Polyclonal anti-human TF antibody was raised in rabbits to the extracellular domain of recombinant sTF, residues 1 to 218. An IgG fraction was prepared from the crude antiserum by adsorption to and elution from a Protein-A Sepharose column (Pharmacia). Immunopurification was achieved through binding of sTF to an Affigel-10 column (Bio-Rad) and then adsorption of the IgG fraction to the column. After exhaustive washing of the buffer, specific anti-sTF antibody was eluted with 4 mol/L guanidinium hydrochloride. The antibody was dialyzed into Tris buffer before use.

Histological and Immunohistochemical Analyses

After division of samples, specimens were immediately fixed in 10% buffered formalin and routinely processed for paraffin embedding. The formalin-fixed, paraffin-embedded tissue blocks were serially sectioned at 5 μ m onto lysine-coated slides and stored at room temperature until use. Before staining, slides were heated at 57°C for 2 hours, rinsed in xylene for 10 minutes, rehydrated in successive rinses of graded ethanols, and placed in buffer. Slides were routinely stained with hematoxylin and eosin and by a combined Masson elastic technique. Immunohistochemical staining was performed on tissue sections to localize TF antigen and for identification of macrophages and smooth muscle cells with the use of 0.5 μ g/mL anti-sTF antibody, 7.6 μ g/mL anti-CD68 (KP-1 [M814] DAKO), and 0.1 μ g/mL anti-smooth muscle actin (1A4 [M851] DAKO), respectively. Rehydrated sections were blocked with normal goat serum and 3% H₂O₂ in water, washed in PBS, and incubated with the appropriate primary antibody for 0.5 to 2 hours at 37°C. Sections were washed in PBS, reacted with horseradish peroxidase-conjugated streptavidin (Supersensitive Kit, Biogenex) for 20 minutes at room temperature, and developed with DAB. Sections were then dehydrated, coverslipped, and examined. Positive control slides, nonimmune negative controls, and processing controls were performed for each antigen stain.

Angiographic Analysis

Complex angiographic lesion morphologies have been associated with coronary arterial thrombosis.¹⁴ Lesions were defined as complex when the following features were present on pre-DCA coronary angiography: irregular borders or overhanging edges, ulceration (clearly delineated area of dye outside the vessel lumen), or intraluminal filling defects. Angiograms were read by two angiographers (two of the authors), who were blinded to the histological and immunohistochemical data.

Statistical Analysis

In the population of DCA specimens examined, the level of TF-specific procoagulant activity was found to be nonnormally

distributed; therefore, nonparametric statistics were used. Paired data were analyzed with a Wilcoxon signed rank test. Descriptive statistics include mean and SD or median and 95% CI. The TF activity levels for a group of specimens were deemed significantly different from a reference group when the median was outside the 95% CI of the reference group and $P < .05$.¹⁵ With the Mann-Whitney U test, specimens associated with histological thrombus versus those with no thrombus were compared in terms of their TF content as assessed with immunohistochemistry and activity assays. To compare the frequency of undetectable TF staining in restenotic versus de novo lesions or in stable versus unstable coronary syndromes, the χ^2 test was used.

Results

Expression of TF Activity in Human Coronary Atheroma

Time Course of Factor Xa Generation

To quantify TF activity in coronary atheroma, factor Xa generation was measured over 300 minutes using the chromogenic activity assay (see "Methods"). Representative examples are shown in Fig 4. The rise in factor Xa levels was linear for both nonhomogenized ($n=7$) and homogenized ($n=6$) tissue preparations ($r=.94 \pm .05$ for nonhomogenized plaques; $r=.91 \pm .09$ for homogenized plaques). These data demonstrate that TF activity, indicated by the slope of the factor Xa versus time curve, varies among individual plaques. These variations were discernible after 180 minutes for both homogenized and nonhomogenized specimens, and therefore subsequent measurements of factor Xa were made after incubation of specimens in the reaction mixture for 3 hours.

Variations in Plaque TF Activity

TF activity measurements were performed on 32 nonhomogenized (mean $\pm$ SD weight, 2.4 ± 1.4 mg) and 31 homogenized (2.5 ± 1.6 mg) specimens derived from 63 patients (1 specimen per patient). With 25 mOD/min as the threshold for TF detection (see "Methods"), TF activity was detected in 25 of 32 nonhomogenized (78%) and 28 of 31 homogenized (90%) specimens, yielding an overall detection rate of 53 of 63 (84%).

The TF content for each specimen was calculated using a standard activity curve (Fig 3) and expressed in ng of

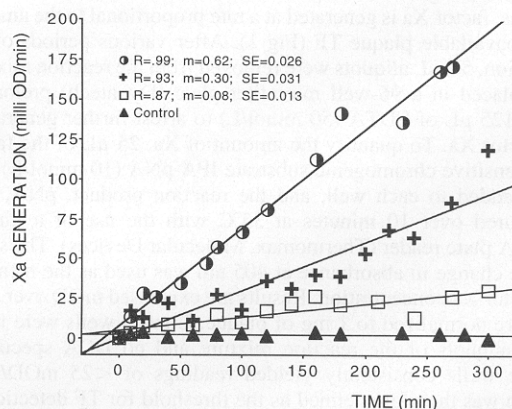


FIG 4. The rate of factor Xa generation by three representative nonhomogenized DCA specimens using a colorimetric assay read at 405 nm and expressed in mOD. Each regression line is drawn through points derived from a single specimen. TF activity is reflected in the slope (m), for which the SEM (SE) is given. Control wells were filled with aliquots of reaction mixture to which no DCA specimen had been added.

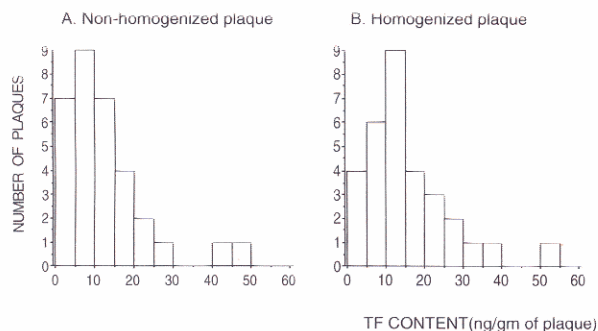


FIG 5. Frequency distribution of TF in nonhomogenized (A) and homogenized (B) human coronary atherectomy specimens, with the use of an interval width of 5 ng/g.

TF/g of plaque. A wide variation in TF content was found in both nonhomogenized and homogenized specimens (nonhomogenized plaque: median, 10 ng/g; 95% CI, 6 to 13 ng/g; range, 0 to 47 ng/g; homogenized plaque: median, 13 ng/g; 95% CI, 9 to 16 ng/g; range, 0 to 54 ng/g). The frequency distribution of TF in both nonhomogenized and homogenized samples of human coronary atheroma was skewed (Fig 5).

In 16 patients, the DCA specimens were harvested from restenotic lesions that had been previously treated with balloon angioplasty (n=14) or DCA (n=2). Restenotic lesions (n=16) demonstrated similar levels of TF activity to de novo lesions (n=47) (restenotic: median TF content, 10 ng/g; 95% CI, 5 to 19 ng/g; de novo: median, 12 ng/g; 95% CI, 10 to 15 ng/g; $P=NS$).

DCA specimens harvested from lesions with a pre-DCA complex angiographic morphology (n=33) were associated with similar levels of TF compared with specimens collected from lesions without a complex morphology (n=30) (complex lesions: median TF content, 11 ng/g; 95% CI, 8 to 16 ng/g; noncomplex lesions: median, 12 ng/g; 95% CI, 9 to 15 ng/g; $P=NS$). The levels of TF activity were also similar in patients with stable (n=22) versus unstable (n=41) coronary syndromes (stable syndromes: median TF content, 12 ng/g; 95% CI, 5 to 19 ng/g; unstable syndromes: median, 12 ng/g; 95% CI, 9 to 15 ng/g; $P=NS$). The levels of TF activity in plaques with or without a complex angiographic morphology were compared after pooling of the results derived from homogenized and nonhomogenized specimens. Analysis of these specimen preparations separately did not alter the conclusions.

Bioavailability of Plaque TF

TF expressed on a plaque surface that is in direct contact with circulating blood in vivo or with the reaction mixture in vitro is more likely to be available for interaction with factor VII/VIIa than is TF that is embedded in the deeper layers. To compare the bioavailability of surface and total plaque TF, procoagulant activity was assayed in homogenized and nonhomogenized portions of plaque harvested from the same lesion (see "Methods"). Assays were performed simultaneously on eight sets of specimens with a 3-hour incubation period in the reaction mixture. In each set, homogenized specimens demonstrated a higher level of TF activity than did nonhomogenized specimens (Fig 6). The median TF activity of the homogenates (median, 15 ng/g; 95% CI, 10 to 73 ng/g) was approximately two-fold higher than that associated with the nonhomogenates (median, 8 ng/g; 95% CI, 7 to 28 ng/g; $P=.01$). Although

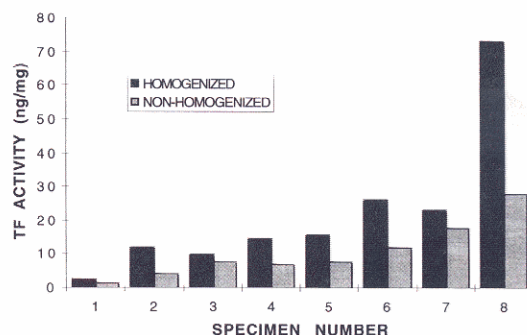


FIG 6. TF activity levels in homogenized and nonhomogenized portions of atherectomy specimens. Each set of specimens (n=8) was harvested from a single lesion. TF-specific procoagulant activity is detectable at consistently higher levels in homogenized specimens than in nonhomogenized specimens.

homogenization resulted in a significant increase in the detection of TF, the TF activity levels of homogenized and nonhomogenized specimens were highly correlated ($r=.94$; $P<.001$).

Expression of TF Antigen in Human Coronary Atheroma

Of the 63 lesions examined with the TF activity assay, sufficient tissue from 50 lesions was available for both

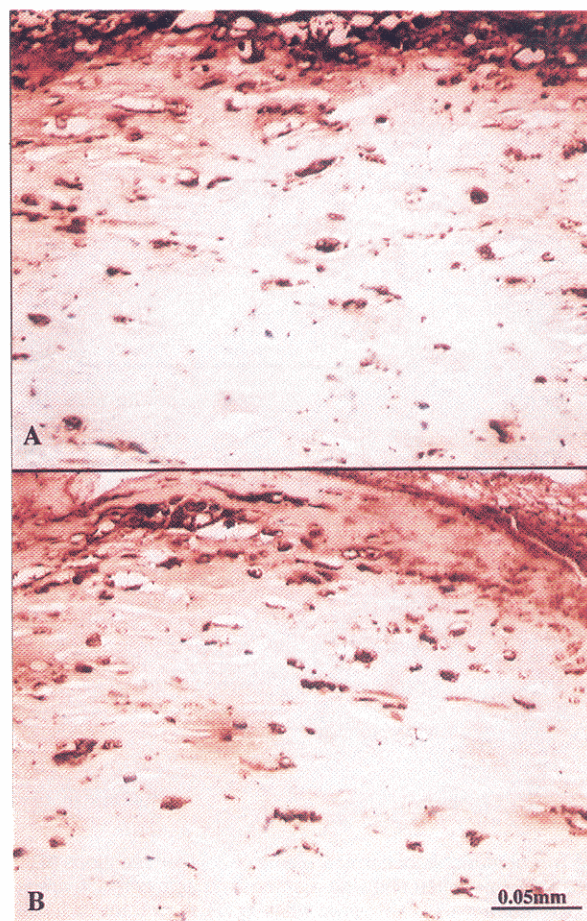


FIG 7. Immunohistochemical stains of adjacent sections of atherectomy specimens showing a macrophage-rich area (A) and TF antigen (B). Most of the TF antigen appears intracellularly. (Peroxidase-DAB with hematoxylin counterstain; A: KP-1; B: anti-sTF.)

histological and immunohistochemical study. TF antigen was detected in 43 of 50 lesions (86%) examined. TF antigen was expressed focally in both cellular (Figs 7 and 8) and acellular (Figs 8 and 9) areas. Intracellular staining was detected in 32 of the 50 lesions (64%) examined, and extracellular staining was detected in 39 (78%). The cells expressing TF antigen appeared to be of monocyte/macrophage and vascular smooth muscle cell origin (Figs 7 and 8). Extracellular staining was also seen and was particularly intense in cholesterol crystal-rich areas of the plaque (Fig 9). No extracellular staining was seen in the absence of anti-sTF antibodies, after staining with anti-sTF antibody preabsorbed with a 10-fold excess of TF protein, and after staining with irrelevant antibodies, such as anti-smooth muscle cell actin antibody. Of the 7 samples that did not display TF antigenicity, 5 had TF activity. Therefore, of the 50 samples subjected to both procedures, TF was detectable in 48 (96%) through the use of at least one of the two methods. In this regard, it should be noted that TF activity and antigenicity were measured on different portions of the plaque specimen.

Histological sections were analyzed by a pathologist (one of the authors), who was blinded to the results of

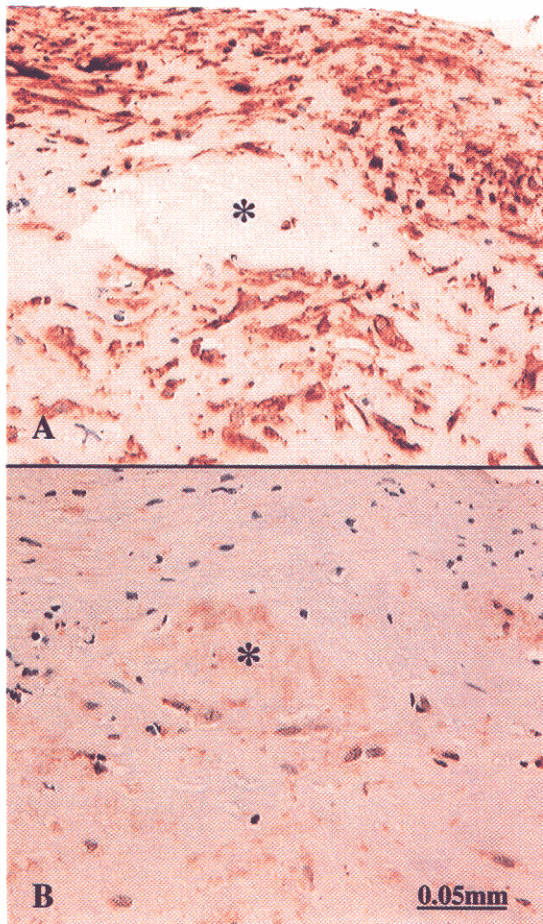


FIG 8. Immunohistochemical stains of adjacent sections of atherectomy specimen showing a smooth muscle cell-rich area (A) and TF antigen (B). Compared with Fig 7B, the intracellular staining for TF is less intense but is positive in many of the smooth muscle cells. Note that the acellular area seen in the center of A (*) corresponds to an area of extracellular staining for TF in B (*). (Peroxidase-DAB with hematoxylin counterstain; A: anti-smooth muscle cell α -actin; B: anti-sTF.)

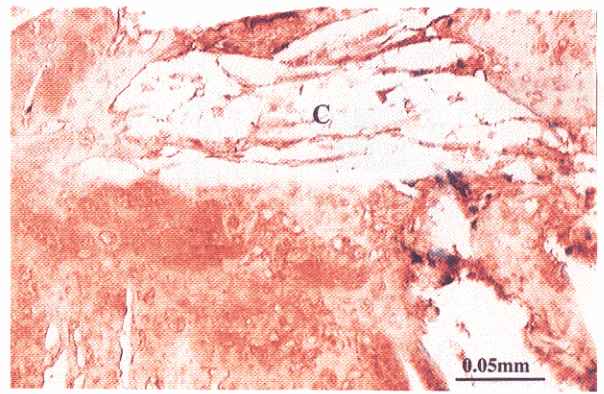


FIG 9. Immunohistochemical stain of cholesterol crystal-rich area (C) typical of the core of an atherosclerotic plaque. There is diffuse staining for TF antigen in the extracellular debris surrounding the crystals. (Peroxidase-DAB with hematoxylin counterstain; anti-sTF.)

immunohistochemistry for the presence or absence of thrombus. Histological thrombus was detected in specimens from 19 of the 50 lesions (38%) examined (Fig 10). Thrombus was present in 19 of the 43 lesions (44%) with detectable TF antigen and in 0 of the 7 lesions (0%) without detectable TF antigen (19 of 43 versus 0 of 7; $P < .02$). The thrombus seen in association with the DCA specimens was organized, suggesting that these thrombi were not formed within the catheter as the specimens were withdrawn.

Thirteen of the 50 lesions studied with histology and immunohistochemistry were restenosis lesions that had undergone intervention within the preceding 12 months. A complete lack of immunostaining for TF was more common in restenotic than in de novo lesions. TF antigen was undetectable in 4 of the 13 restenotic lesions (31%). In the 37 de novo lesions, TF antigen was undetectable in only 3 (8%; $P < .05$).

Twenty-nine of the 50 immunohistochemically stained samples were harvested from lesions with a complex pre-DCA angiographic morphology. Twenty-five of these 29 lesions (86%) had detectable TF antigen; the 21 lesions without a complex angiographic morphology also had an 86% (18 of 21) detection rate of TF antigen ($P = \text{NS}$). Similar detection rates of TF antigen were found in patients with stable (15 of 18, or 83%) versus unstable coronary syndromes (29 of 32, or 91%; $P = \text{NS}$).

Discussion

Intravascular thrombosis plays an important role in the pathogenesis of acute coronary syndromes¹ and in abrupt closure and restenosis after PTCA and DCA.^{16,17} Spontaneous and angioplasty-induced fractures¹⁸ of atherosclerotic plaques may trigger thrombosis by exposing circulating blood to procoagulant plaque elements previously sequestered within the vessel wall. Plaque constituents that have been hypothesized to participate in the thrombogenicity of atheroma include collagen, fatty acids, phospholipids, and TF.^{6,19}

Previous studies have detected TF antigen in atherosclerotic plaque.^{6,8} This is the first study to our knowledge that demonstrates that this TF is active and thus may contribute to the thrombogenicity of human coronary atheroma. With the use of a quantitative colorimetric assay, TF

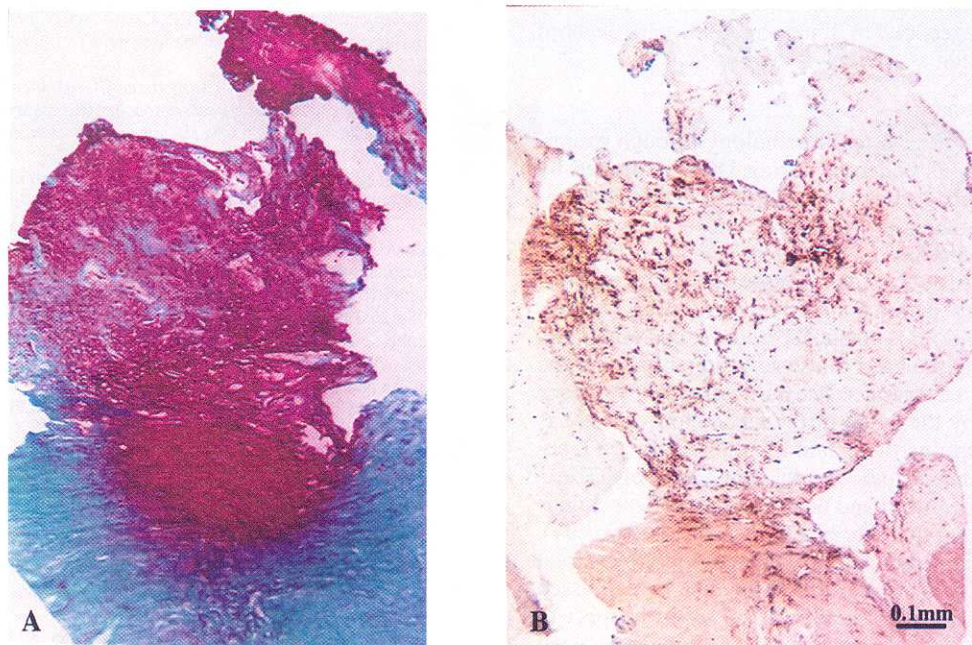


FIG 10. Micrographs of adjacent sections from an atherectomy specimen demonstrating a large area of organizing thrombus (dense red staining of fibrin in A) containing both cellular and extracellular TF antigen. (A: combined Masson's elastic stain; B: peroxidase-DAB for anti-tSF.)

activity in DCA specimens was found to be ubiquitous but highly variable in amount. Using plasma markers of coagulation, a number of investigators have demonstrated marked variability in the degree to which clotting is activated in patients with clinical syndromes associated with coronary plaque rupture.²⁰⁻²² Plasma levels of prothrombin fragment 1+2, a marker of thrombin generation,²³ and fibrinopeptide A, a marker of thrombin activity,²⁴ have been measured in coronary arterial blood before and after balloon angioplasty.²⁵ Changes in the plasma levels of these peptides after balloon-mediated plaque disruption demonstrated activation of the coagulation cascade leading to intracoronary thrombin generation in $\approx 20\%$ of patients treated with PTCA, despite adequate doses of heparin. Variations in the level of TF activity may in part account for the variable incidence of thrombosis and thrombin generation occurring after iatrogenic or spontaneous plaque disruption. Disruption of atherosclerotic plaque has been hypothesized to trigger the initiation of coronary thrombosis.²⁶ The approximately twofold increase in TF activity seen after plaque homogenization (Fig 6) is consistent with the hypothesis that plaque disruption exposes previously sequestered sites for factor VII/VIIa binding.

Previous immunohistochemical studies on human atherosclerotic tissue have yielded variable detection rates for TF antigen. Wilcox et al⁶ found TF antigen in 100% of carotid endarterectomy specimens examined ($n=16$). TF protein was detected in both cellular and acellular areas of the plaque,⁶ which is consistent with the findings of the present study. Using immunohistochemical staining of DCA specimens, Annex et al⁸ detected TF antigen in 6% of restenotic lesions ($n=18$) and in 33% of de novo lesions ($n=43$). In the report by Annex et al, patients with unstable coronary syndromes demonstrated an increased rate of detection of TF on immunohistochemistry compared with patients with stable coronary syndromes. In the present study, the clinical presentation (unstable versus stable an-

gina) and angiographic morphology (complex versus non-complex) did not distinguish patients with respect to plaque TF expression. The lack of a differential expression in clinical and angiographic subsets and the higher immunohistochemical TF detection rate (86%) in our series of DCA specimens may relate to differences in the sensitivity of the anti-human TF antibody, to differences in the patient populations, and to differences in tissue processing (frozen sections of paraformaldehyde-fixed specimens were used in the study by Annex et al and paraffin sections of formalin-fixed specimens were used in our study).

The above factors may also account for the apparently more diffuse immunohistochemical localization of TF antigen (Figs 7 through 9) compared with those previously reported.^{6,8} In the present study, some specimens with predominantly extracellular staining on immunohistochemistry demonstrated high levels of TF procoagulant activity. The finding of abundant and active TF in the extracellular matrix (Fig 9) raises the possibility that TF is released from cells in a functional and stable state. Indeed, the loss of TF from the plasma membrane has been previously reported and may be due to its shedding from the cell surface, perhaps in vesicles.²⁷ Alternatively, active TF may be released during cell death. The finding of TF in the lipid-rich core is also consistent with studies performed *in vitro* using a perfusion chamber to measure the thrombogenicity of different components of the atherosclerotic plaque.¹⁹ These authors found that the lipid-rich core was the most thrombogenic.

The present study supports the observation by Annex et al⁸ that de novo lesions have a higher incidence of TF antigen expression than do restenotic lesions. The lower levels of TF expression in restenotic lesions may contribute to the lower postatherectomy complication rate that has been reported for these lesions.²⁸ Despite these observations, TF may play a role in the development of restenosis after PTCA. In a rabbit carotid injury model, anti-TF antibodies have been shown to block cyclic flow variations that are due to platelet-rich

thrombi.²⁹ In humans treated with PTCA, antiplatelet therapy has been recently reported to diminish significantly the need for revascularization at 6-month follow-up.³⁰

Study Limitations

The investigation of arterial pathology through examination of specimens collected during DCA is limited by an inherent sampling error. Percutaneous atherectomy removes only fragments of the plaque under indirect visualization, and therefore the extracted specimens may not be representative of the total plaque or of the residual plaque remaining in the patient's arterial wall. In this study, the correlation of plaque TF activity and antigenicity was limited by the need to use separate portions of the plaque to perform the assays. The particular plaque surface that is exposed by the blade of the atherectomy device is random. To determine the extent to which surface TF activity reflects total plaque activity, the levels of TF expression in homogenized and nonhomogenized specimens were compared and found to be highly correlated ($r=.94$). This suggests that plaque surface TF activity reflects total plaque TF activity and that TF activity may be expressed rather diffusely in atherosclerotic tissue. With respect to bioavailability, it should be noted that in nonhomogenized samples, at least one side of the specimen was not in contact with circulating blood (ie, the side toward the arterial wall). Thus, even in the case of nonhomogenized samples, the measurement of TF bioavailability in vitro may not fully reflect bioavailability in vivo.

Varying levels of TF activity are expressed in atherosclerotic specimens obtained during DCA. Although the extent to which the surface procoagulant activity of these specimens reflects the activity of the plaque remaining within the coronary artery is unknown, the results of this study suggest that DCA is likely to create a surface that exposes active TF to circulating blood. Inhibition of TF may therefore represent a therapeutic goal for the prevention of thrombotic complications associated with PTCA and DCA.

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