Coronary no-reflow is caused by shedding of active tissue factor from dissected atherosclerotic plaque

Diana Bonderman, Alexander Teml, Johannes Jakowitsch, Christopher Adlbrecht, Mariann Gyöngyosi, Wolfgang Sperker, Harald Lass, Wilhelm Mosgoeller, Dietmar H. Glogar, Peter Probst, Gerald Maurer, Yale Nemerson, and Irene M. Lang

Introduction

The no-reflow (NR) phenomenon is defined as profound reduction in antegrade coronary flow in the absence of epicardial vessel obstruction. Despite a 0.6% to 2% incidence during percutaneous coronary interventions and a 15% mortality, the mechanisms underlying coronary NR are unclear. A common denominator of various disorders leading to NR appears to be microvascular damage.

One candidate protein to cause coronary NR is tissue factor (TF), a 47-kd transmembrane glycoprotein and a member of the cytokine-receptor superfamily. TF initiates blood coagulation by binding activated coagulation factor VII (VIIa) with a dissociation constant of around 10 pM. The TF-VIIa complex proteolytically activates factors IX and X, triggering the coagulation system. The activity of TF is lipid dependent and is enhanced by a factor of 2000 to 3000 in the presence of phosphatidylserine/phosphatidylcholine, allowing almost immediate thrombus formation. Because TF-containing microparticles with procoagulant activity are abundant in atherosclerotic plaques, spontaneous or mechanical disruption of a coronary atherosclerotic plaque by angioplasty and/or stenting could lead to release of active TF into the coronary blood.

First, we demonstrate that ex vivo plaque disruption by scraping leads to release of membrane-bound active TF. Secondly, we show that in vivo plaque disruption by angioplasty and/or stenting causes shedding of active TF into the coronary blood. Third, we show that TF antigen levels during coronary NR are elevated compared with the levels that are measured in the same patients after restoration of flow. Finally, we demonstrate that intracoronary injection of atherosclerotic material or purified relipidated TF was performed in a porcine model. TF induced NR in the model, thus strengthening the concept that TF is causal, not just a bystander to atherosclerotic plaque material. The data suggest that active TF is released from dissected coronary atherosclerotic plaque and is one of the factors causing the NR phenomenon. Thus, blood-borne TF in the coronary circulation is a major determinant of flow.

Patients, materials, and methods

Harvest of carotid thromboendarterectomy specimens

Specimens were harvested in the operating room, and 1 cm² of the intimal tissue flap was immediately scraped under sterile conditions and resuspended in cold Hepes-buffered saline (HBS) (10 mM Hepes, 0.1% bovine serum albumin [BSA], 5 mM CaCl₂). Large particles were removed by centrifugation (1300 g). Microvesicles and microparticles were recovered by ultracentrifugation (100,000 g).

Patient characteristics

Patients with acute coronary syndromes were admitted to the catheter laboratory with symptoms of longer than 30 minutes and shorter than 4 hours duration and with negative plasma creatine phosphokinase values. In addition, patients experiencing NR (Table 2) were treated with 100 to 500 μg intracoronary verapamil.
The PercuSurge GuardWire (Traatek, Miami, FL) protection device was used in 6 patients to prevent embolization of material into the capillary bed (Table 1). In these patients, the location of the target stenosis in venous grafts or in native vessel segments without side branches allowed its use. The system served the study in that it prevented losses of vessel wall-derived particles of any size into the distal vascular bed. The distal over-the-wire occlusion balloon was inflated shortly before the intervention and was held inflated during the intervention with the use of the hypotube as angioplasty guidewire. After removal of the intervention catheter, an aspiration catheter was advanced into the coronary artery and 40 mL blood was drawn from the coronary bed with the inflated distal balloon still in place (Figure 1A) and with a syringe used to serve as a collection chamber. Then the distal balloon was deflated, and coronary flow was restored. The aspirated material was passed over a small filter unit (Falcon, 40-μm nylon) (Becton Dickinson, San Jose, CA). Material trapped in the filter was resuspended in 1 mL HBS, and stored at −70°C before TF measurement. For control, blood from healthy individuals was passed over a filter, and rinsing buffers were assayed in parallel.

In patients experiencing NR, blood was drawn through a no-sidehole guiding catheter from the target coronary artery (Figure 1B) after discarding 5-mL or 8-mL aspirates, respectively (with the use of a 6 or 7 French guiding catheter).

Flow was classified according to the Thrombolysis In Myocardial Infarction (TIMI) study.1 Flow was defined as TIMI 0 when no antegrade flow was present; as TIMI 1 when penetration of contrast was present without perfusion, ie, without opacification of the entire coronary bed; as TIMI 2 when the rate of entry of contrast material into the distal vessel or its rate of clearance from the distal bed was perceptibly slower than its flow into or clearance from comparable areas not perfused by the target vessel; and as TIMI 3 when coronary flow was normal, ie when the rate of entry of contrast material into the distal vessel or its rate of clearance from the distal bed was as rapid as flow into or clearance from a normal uninvolved coronary vessel.

Coronary blood samples from subjects undergoing coronary angiography for other indications, eg, valvular heart disease, or for a preoperative risk stratification and showing normal epicardial coronary arteries were collected as controls.

Sodium citrate (at a final concentration of 0.129 M) was added to blood samples, and platelet-poor plasma (PPP) was prepared by centrifugation (1300g, 4°C, 10 minutes). To expose membrane-associated TF,14 PPP samples were also subjected to 4 freeze-thaw cycles prior to antigen measurements. All human studies were approved by the Ethics Committee of the University of Vienna, Austria.

**Differential centrifugation experiments**

Rinsing buffers from carotid scrapings were centrifuged at 23,000g (15 minutes, 20°C) (Universal 30RF centrifuge) (Hettich, Tuttingen, Germany) and at 250,000g (60 minutes, 20°C) (Beckman L-80 ultracentrifuge SW 50.1-Rotor, Beckman Coulter, Fullerton, CA), corresponding to a particle size of 0.5 to 2 μm and 10 to 15 nm, respectively. TF activity measurements were performed in the starting materials, 23,000g and 250,000g pellets, and their respective supernatants.

**Reagents**

Human recombinant factor VIIa was a kind gift from Novo-Nordisk (Copenhagen, Denmark) and human recombinant TF (TF1-243) was a gift from Robert Kelley (Genentech, San Francisco, CA), to Yale Nemerson. The phospholipids used for relipidation of TF consisted of 40% 1,2-dioleoyl-sn-glycero-3-phosphatidylserine and 60% 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL). Factor X was purified from human plasma.14 Spectrozyme-Xa was from American Diagnostica (Greenwich, CT).

**Preparation of TF-phospholipid**

Human recombinant TF was incorporated into phospholipid vesicles at a phospholipid to protein molar ratio of 44 000:1 as previously described.15 Human TF has been shown to be a good initiator of clotting in pig plasma.16

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**Figure 1. Schematic drawing of coronary blood sampling.** The right coronary artery (RCA) is shown. Arrows within the circles point to the exact site of blood drawing. Stasis of the blood column is indicated by the use of gray. (A) PercuSurge distal protection device. Blood is aspirated from the tip of the export catheter, while the catheter is slowly withdrawn proximally. The harvested blood is passed over a filter. TF activity of the material on the filter is measured. (B) NR in the course of percutaneous transluminal coronary angioplasty (PTCA). Blood is aspirated from the static blood column at the tip of the guiding catheter, which is deeply intubated into the proximal vessel segment.
TF activity assay

TF activity was measured by adding the sample to a solution containing 1 nM factor VIIa, 150 nM factor X, and 5 nM CaCl₂. At intervals, samples were transferred to a microtiter plate in which each well contained 100 µL EDTA buffer (50 mM bicine, pH 8.5, 20 mM EDTA, 1 mg/mL BSA), which terminates production of factor Xa. Spectrozyme Xa (25 µL, 0.5 nM final concentration) (American Diagnostica) was added, and optical density was measured at 405 nm for 30 minutes by means of a kinetic enzyme-linked immunosorbent assay (ELISA) plate reader (BTK Microquant, Software KC4) (Biotech, Winooski, VT) at 35°C. A standard curve was generated with the use of relipidated recombinant human TF.

TF antigen assay

Imubind Tissue Factor ELISA Kit No. 845 (American Diagnostica) was used according to the manufacturer’s instructions.

Immunoelectron microscopy

The PercuSurge specimens were fixed in 3% freshly depolymerized paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4) for 1 hour at 4°C, rinsed in PBS, and dehydrated in increasing concentrations of ethanol (30%, 50%, 70%, 95%, and 100%, 30 to 60 minutes each). The resin infiltration was achieved by increasing concentrations of HM20 resin (Lowicryl, Chemische Werke Lowi, Waldkraiburg, Germany) at −35°C. The resin was cured under UV light for 48 hours at 35°C and polymerized. For immunostaining, the resin was cured under UV light for 48 hours at room temperature. For immunostaining, 100-nm thin sections were mounted on grids and incubated in PBS with 3% BSA and 0.05% Tween-20, pH 7.4 (PBS-ST) to block unspecific binding sites. The primary antibody was monoclonal anti-TF (1:40, 2.5 hours at 4°C, rinsed in PBS, and dehydrated in increasing concentrations of ethanol (30%, 50%, 70%, 95%, and 100%, 30 to 60 minutes each). The resin infiltration was achieved by increasing concentrations of HM20 resin (Lowicryl, Chemische Werke Lowi, Waldkraiburg, Germany) at −35°C. The resin was cured under UV light for 48 hours at room temperature. For immunostaining, 100-nm thin sections were mounted on grids and incubated in PBS with 3% BSA and 0.05% Tween-20, pH 7.4 (PBS-ST) to block unspecific binding sites. The primary antibody was monoclonal anti-TF (1:40, 2.5 hours at room temperature) in PBS-ST, followed by 3 rinses in PBS and incubation with protein A–14-nm colloidal gold for 1 hour. The sections were rinsed in PBS and distilled water 3 times each and contrasted in aqueous 2% uranylacetate for 8 minutes and lead acetate for 2.5 minutes before examination in a Zeiss transmission electron microscope (Zeiss, Munich-Hallbergmoos, Germany).

Animal protocol

Ten domestic pigs (33 ± 6 kg) of both genders fed on a standard natural diet were studied. The animals were anesthetized with intravenous ketamine (30 mg/kg), acepromazin (12 mg/kg), thiopental (5 mg/kg), and rocuron (0.025 mg/kg) and a continuous infusion of fentanyl (0.08 mg/kg). Following endotracheal intubation, the pigs were mechanically ventilated with a mixture of 20% pure oxygen and 80% room air. Arteriotomy of the carotid artery and insertion of a 7F sheath were performed under sterile conditions. Electrocardiogram, arterial blood pressure, and temperature were recorded throughout the procedure. After administration of 2000 IU heparin, the RCA was cannulated, and a 0.014-inch Doppler ultrasound guide wire (FloWire) (Cardiometrics, Mountain View, CA) was introduced to the midpart of the artery. The average peak flow velocity (APV) was assessed at baseline and during maximal hyperemia after intracoronary bolus injection of 18 µg adenosine (centimeters per second, peak APV), and the coronary flow reserve was calculated as a ratio of peak APV and baseline APV. We injected 1 mL carotid scraping material containing 33 pg/mL TF activity or 250 µL of 39 nmol relipidated human TF, respectively, as a bolus through a deeply intubated 7F Judkins standard RCA guiding catheter. Angiograms were performed before and during the first 30 minutes after injection. The pigs were killed by saturated potassium chloride. The protocol was approved by the Animal Subjects Committee of the University of Vienna.

Immunohistochemical analysis

Immediately after the pigs were killed, multiple transmural biopsies from the RCA-dependent left ventricular posterior wall and from the anterior wall myocardium were obtained. Tissues and PercuSurge material were fixed in 7.5% buffered formalin and were embedded in paraffin, and serial 3-µm sections were stained as described by means of mouse monoclonal antibodies against fibrin (1:50) (Biodesign International, Saco, ME) and TF (1:100, human TF). In addition, for immunostaining of PercuSurge samples, anti-CD15 (1:25) (DAKO, Glostrup, Denmark) and anti-CD61 (1:25) (DAKO) mouse monoclonal antibodies were used.

Computer-assisted quantitative histological evaluation

Following immunohistochemistry, sections from all specimens were scanned in their full size by means of the AxioCam color digital camera (Zeiss). Computer-based planimetry of the area of microvascular thrombosis was performed by means of the AxioVision 2.05 software package (Zeiss). Fibrin stains and modified trichrome stains served to identify thrombus. Two observers who were blinded to the origin of the specimens independently analyzed 4 different areas of each section, and 4 sections per specimen.

Statistical analysis

Results are expressed as means ± SD. Comparisons were made with the Mann-Whitney U test and the paired t test. P < .05 was considered significant.

Results

Mechanical plaque disruption leads to shedding of membrane particles with procoagulant activity

To simulate intervention-related mechanical plaque damage ex vivo, 6 carotid thromboendoarterectomy specimens were scraped, and TF activity was determined in the rinsing buffers. Mean procoagulant activity corresponded to 33.03 ± 13.00 pg/mL authentic TF per square centimeter scraped carotid intimal flap area. The TF activity in fractions obtained after differential centrifugation of carotid atherosclerotic plaque scrapings were as follows:

Table 1. Characteristics and tissue factor values from patients undergoing percutaneous coronary revascularization by means of the distal protection device PercuSurge

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Culprit vessel</th>
<th>Anginal status</th>
<th>Initial TIMI flow grade</th>
<th>TF activity a</th>
<th>TF antigen, NR/R</th>
<th>Time interval, min b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47/m</td>
<td>Degenerated RCA vein graft</td>
<td>Acute MI</td>
<td>2. extensive thrombus</td>
<td>198.91</td>
<td>64.9/63.3</td>
<td>100/140</td>
</tr>
<tr>
<td>2</td>
<td>58/m</td>
<td>Large RCA</td>
<td>Unstable angina</td>
<td>1</td>
<td>94.34</td>
<td>113.3/81.8</td>
<td>120/200</td>
</tr>
<tr>
<td>3</td>
<td>44/m</td>
<td>Large RCA</td>
<td>Acute MI</td>
<td>2</td>
<td>110.15</td>
<td>139.2/27.1</td>
<td>100/140</td>
</tr>
<tr>
<td>4</td>
<td>76/f</td>
<td>RCA vein graft</td>
<td>Stable angina</td>
<td>3</td>
<td>12.89</td>
<td>38.4/19.8</td>
<td>5/45</td>
</tr>
<tr>
<td>5</td>
<td>61/f</td>
<td>Degenerated RCA vein graft</td>
<td>Acute MI</td>
<td>0</td>
<td>74.89</td>
<td>126.6/80.6</td>
<td>90/140</td>
</tr>
<tr>
<td>6</td>
<td>78/m</td>
<td>Degenerated LAD vein graft</td>
<td>Unstable angina</td>
<td>0-1</td>
<td>60.50</td>
<td>115.3/39.9</td>
<td>90/200</td>
</tr>
</tbody>
</table>

TIMI indicates Thrombolysis in Myocardial Infarction study; TF, tissue factor; NR, no-reflow; RCA, right coronary artery; MI, myocardial infarction; LAD, left anterior descending coronary artery.

a TF activity was measured in the rinsing buffer of the PercuSerge Filter.

b Time interval from the onset of chest pain to the first blood harvest/interval from the onset of chest pain to the end of the percutaneous intervention and second blood draw.
180.16 ± 95.55 for the 23 000 g pellet; 5.63 ± 2.32 for the corresponding supernatant; 98.23 ± 34.60 for the 250 000 g pellet; and 0 for the corresponding supernatant. Thus, complete removal of active TF from the rinsing buffers was accomplished by ultracentrifugation at 250 000 g, indicating that TF activity was membrane associated. P = .03 for the differences between the starting material and the respective pellets. Part of the plaque material was used for injection into porcine coronary arteries.

**Coronary angioplasty and stenting are associated with shedding of procoagulant activity from the plaque into the coronary blood**

To confirm that intervention-related disruption of a coronary atherosclerotic plaque in vivo leads to release of active TF into the coronary blood, blood samples were harvested during angioplasty with a distal protection device, PercuSurge, in 6 patients (Table 1). Fine particulate material was trapped in the filter and was recovered in rinsing buffer. By light microscopy, the filter material contained mainly leukocytes, platelets, and vast amounts of amorphous material with cholesterol crystals. Immunohistochemically, TF colocalized with leukocytes, platelets, and fibrin (Figure 2). Manual cell counts showed that only approximately 2% of CD15+ cells demonstrated immunoreactivity with anti-TF antibody. By immunoelectron microscopy, TF was identified within platelets (insert, Figure 2C) and found attached to fibrin (insert, Figure 2D).

The rinsing solutions contained procoagulant activity corresponding to 91.10 ± 62.16 pg per milliliter of authentic TF (Table 1). In addition, plasma TF was measured in the blood column proximal to the PercuSurge occlusion balloon (Figure 1A) and after release of the occlusion balloon with reflow (R) in the distal part of the vessel (Table 1). TF antigen was significantly lower under conditions of R (99.62 ± 39.16 pg/mL versus 48.78 ± 23.80 pg/mL, P = .028).

**Table 2. Characteristics of patients experiencing acute no-reflow in the course of percutaneous coronary interventions**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Culprit vessel</th>
<th>Circumstance of NR</th>
<th>Initial TIMI flow grade</th>
<th>TF antigen, pg/mL NR/R</th>
<th>Increase of TF antigen after freeze thawing of NR plasma, pg/mL</th>
<th>Timing (min)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64/f</td>
<td>proximal LAD</td>
<td>dissection after PTCA</td>
<td>3</td>
<td>180.95/119.51</td>
<td>72.24</td>
<td>5/50</td>
</tr>
<tr>
<td>2</td>
<td>50/m</td>
<td>high-grade mid RCA</td>
<td>unstable angina</td>
<td>1</td>
<td>124.45/79.32</td>
<td>24.05</td>
<td>120/200</td>
</tr>
<tr>
<td>3</td>
<td>47/m</td>
<td>proximal LAD</td>
<td>directional atherectomy</td>
<td>3</td>
<td>119.55/50.73</td>
<td>13.76</td>
<td>35/60</td>
</tr>
<tr>
<td>4</td>
<td>52/m</td>
<td>proximal RCA</td>
<td>unstable angina</td>
<td>2</td>
<td>38.50/17.65</td>
<td>10.29</td>
<td>15/70</td>
</tr>
<tr>
<td>5</td>
<td>76/f</td>
<td>proximal CX</td>
<td>CX thrombosis during LAD PTCA</td>
<td>0</td>
<td>269.45/90.46</td>
<td>194.90</td>
<td>30/100</td>
</tr>
<tr>
<td>6</td>
<td>52/m</td>
<td>proximal RCA</td>
<td>unstable angina</td>
<td>0</td>
<td>144.23/75.82</td>
<td>55.81</td>
<td>150/200</td>
</tr>
<tr>
<td>7</td>
<td>71/m</td>
<td>proximal LAD</td>
<td>distal dissection after stenting</td>
<td>2</td>
<td>88.76/79.42</td>
<td>19.80</td>
<td>20/60</td>
</tr>
<tr>
<td>8</td>
<td>60/m</td>
<td>mid RCA</td>
<td>thrombus dislodged by guide wire</td>
<td>2</td>
<td>158.08/*</td>
<td>29.97</td>
<td>120/180</td>
</tr>
<tr>
<td>9</td>
<td>86/f</td>
<td>proximal LAD</td>
<td>unstable angina</td>
<td>0</td>
<td>269.45/*</td>
<td>89.50</td>
<td>120/180</td>
</tr>
<tr>
<td>10</td>
<td>60/f</td>
<td>proximal LAD</td>
<td>unstable angina</td>
<td>1</td>
<td>203.44/*</td>
<td>10.31</td>
<td>160/250</td>
</tr>
<tr>
<td>11</td>
<td>53/m</td>
<td>mid LAD</td>
<td>dissection after PTCA</td>
<td>2</td>
<td>63.48/*</td>
<td>15.16</td>
<td>15/45</td>
</tr>
<tr>
<td>12</td>
<td>78/m</td>
<td>CX vein graft</td>
<td>acute MI</td>
<td>1</td>
<td>595.25/*</td>
<td>18.55</td>
<td>180/220</td>
</tr>
<tr>
<td>13</td>
<td>44/m</td>
<td>proximal LAD</td>
<td>acute MI</td>
<td>0</td>
<td>269.75/*</td>
<td>134.50</td>
<td>120/260</td>
</tr>
</tbody>
</table>

Initial TIMI flow grade refers to the first angiogram of the target vessel. PTCA indicates percutaneous transluminal coronary angioplasty; CX, circumflex coronary artery.

*Other abbreviations are explained in Table 1.
†Time interval from the onset of chest pain/NR to the first blood harvest/interval from the onset of chest pain/NR to the end of the percutaneous intervention and second blood draw. In patients with impaired flow at the beginning of the procedure, the onset of chest pain was used for the calculation of the time intervals because the TF–up-regulating process could have started prior to angiographic NR.
embolic obstruction was observed with surrounding thrombus with fibrin thrombi (Figure 4C,D). In plaque-injected animals, identified in the microvasculature distal to the RCA that was filled after the injection. NR/slow-flow spontaneously resolved in 5 pigs in 9 pigs, with a marked decrease of baseline flow immediately after the injection. NR/slow-flow spontaneously resolved in 5 pigs in 9 pigs, with a marked decrease of baseline flow immediately after the injection. NR/slow-flow spontaneously resolved in 5 pigs in 9 pigs, with a marked decrease of baseline flow immediately after the injection. NR/slow-flow spontaneously resolved in 5 pigs in 9 pigs, with a marked decrease of baseline flow immediately after the injection. NR/slow-flow spontaneously resolved in 5 pigs in 9 pigs, with a marked decrease of baseline flow immediately after the injection. NR/slow-flow spontaneously resolved in 5 pigs in 9 pigs, with a marked decrease of baseline flow immediately after the injection.

Histologically, positive TF staining (Figure 4A,B) was observed in all animals, with 9 of 10 demonstrating TIMI flow of 2 or less (Table 3). The most extensive area of microvascular fibrin thrombi was observed in animals with TIMI 0 flow. We speculate that NR occurs when the capacity of the regional coronary microvascular bed is exhausted; i.e., when all vessels in a given domain are obstructed. Such obstruction may occur spontaneously, through shedding of particulate material and TF from ruptured plaque during acute coronary syndromes, or iatrogenically, from PTCA and stent implantation. However, other factors that are as yet unknown may exacerbate the flow disturbances.

Comparison of the coagulation system to the pathogenesis of coronary NR is a novel observation. Recent experimental work of Giesens et al has lent support to the concept that blood-borne TF is a powerful thrombogenic species. In the present work, we captured active TF in a distal protection device from patients undergoing coronary revascularization. Other studies found that 20- to 60-nm vesicles were recovered by scraping pig aorta, demonstrating that the vessel wall itself may be a source of circulating TF. TF captured in the PercuSurge filter colocalized with leukocytes (Figure 3). Because circulating white blood cells upregulate TF only 24 to 48 hours after the percutaneous intervention, these TF-positive cells most likely originate from ruptured plaque, which is in line with previous findings. In addition, TF was found associated with platelets and fibrin (Figure 2C,D), which is in accord with recently published data. TF uptake by platelets and binding to fibrin may account for our observation that after restoration of flow, TF antigen returns to normal in plasma.

We found an increase of TF antigen after freeze thawing of PPP from NR (Figure 3), indicating some TF is in a protected state. It is known that TF incorporates into vesicles in such a way that half the molecules are facing outward and are therefore available to initiate coagulation while the other half are sequestered within the vesicle. Freeze thawing has been shown to reorient TF in such a way that half the sequestered molecules become available for coagulation. We have no information regarding the symmetry of the particles released following angioplasty, but the increase in immunologically detectable TF after freeze thawing suggests that some of the TF is sequestered. These molecules may, however, become available during thrombogenesis. There is currently no information regarding this hypothesis although it is being investigated in our laboratories.

It is known that TF antigen is elevated and that TF-positive microparticles have been captured on annexin V–coated plates from the blood of patients with unstable coronary artery disease.

However, Mallat et al investigated systemic venous blood
samples up to 8 days following an acute ischemic episode. It is therefore possible that secondary immunological or inflammatory effects resulted in TF release from other sites. In contrast, the present study focuses on a single atherosclerotic target lesion or vessel as the culprit for the clinical syndrome. Former work has emphasized the roles of microvascular spasm and inflammation in the development of NR. The lack of a significant inflammatory response in our animal experiments is due to the short observation period. TF initiates the coagulation cascade within seconds, resulting in thrombin generation and fibrin deposition. Thrombin is able to induce vasospasm by activation of protease-activated receptors. In fact, inhibition of thrombin with hirudin was shown to limit infarct size in a rabbit coronary ligation model.

Microvascular patency and consequently myocardial perfusion are predictors of mortality after thrombolytic therapy and after

Table 3. Histological analysis of the area of microvascular thrombosis in vessels of myocardium supplied by the RCA and TIMI flow grades after the injection

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Area of microvascular thrombosis, %</th>
<th>TIMI flow grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM1</td>
<td>7.4</td>
<td>0</td>
</tr>
<tr>
<td>AM2</td>
<td>2.8</td>
<td>1</td>
</tr>
<tr>
<td>AM3</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>AM4</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>TF1</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>TF2</td>
<td>5.7</td>
<td>2</td>
</tr>
<tr>
<td>TF3</td>
<td>3.1</td>
<td>3</td>
</tr>
<tr>
<td>TF4</td>
<td>4.1</td>
<td>2</td>
</tr>
<tr>
<td>TF5</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>TF6</td>
<td>1.6</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are expressed in percent of the area of the total field that was counted. AM indicates atherosclerotic plaque-injected animals; TF, TF-injected animals.
stent implantation in acute myocardial infarction.\textsuperscript{32} The observation that administration of antibody directed against glycoprotein IIb/IIIa had no effect on peri-interventional slow-flow in a meta-analysis of the EPIC and EPILOG studies\textsuperscript{33} lends support to the concept that small tears liberating the lipid core of atherosclerotic plaque allow active TF to enter the coronary bed and cause coronary flow deceleration, culminating in NR. In this regard, it is noteworthy that unstable plaques contain more TF activity than stable plaques.\textsuperscript{12,13} In the present study, 11 of 13 patients experiencing NR (Table 2) were unstable prior to the occurrence of NR. Active TF shed from dissected atherosclerotic plaque is associated with microvascular thrombosis and macrovascular slow-flow/NR. The concept that TF is a major player in the pathogenesis of NR is also supported by previous studies in a baboon model of middle cerebral artery occlusion, where NR was reversed with the administration of a TF antibody.\textsuperscript{34} In more recent experimental work, recombinant human, active site-blocked factor VIIa reduced infarct size and NR in rabbits.\textsuperscript{35,36} These observations support the view that clinical studies should be undertaken to investigate whether anti-TF agents are useful in the treatment of peri-interventional slow-flow and NR.

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The authors are grateful to Dr Marin Guentsch for expert technical advice. The study would not have been possible without the support of the interventional coronary catheter laboratory staff and animal laboratory personnel of the University of Vienna.

\section*{References}

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