Effect of Glycoprotein IIb/IIIa Antagonist Abciximab on Monocyte-Platelet Aggregates and Tissue Factor Expression

Sabine Steiner, Daniela Seidinger, Kurt Huber, Christoph Kaun, Erich Minar, Christoph W. Kopp

Objective—Activated platelets rapidly adhere to monocytes and upregulate the expression of tissue factor (TF), the major trigger of the coagulation cascade. In this study, we examined the effect of abciximab, a nonselective glycoprotein IIb/IIIa-receptor antagonist, on monocyte TF expression in thrombin receptor activator–stimulated whole blood in vitro.

Methods and Results—Abciximab (50 μg/mL) reduced the mass of platelets attached to monocytes, measured by the mean fluorescence intensity (MFI) of CD42b on CD14+ cells, 1 (CD42b, 471±197 versus 1073±217 MFI, mean±SD, P<0.05), 5, and 10 minutes after thrombin receptor activator stimulation of whole blood to the same extent as anti-P-selectin (50 μg/mL; 288±177 MFI, P<0.05) when determined by flow cytometry. In parallel, the expression of the platelet activation marker P-selectin colocalized with CD14+ monocytes was reduced up to 25% by abciximab at the same time points. Expression of monocyte TF antigen (CD14+/TF+, 39.9±8.7% versus 66.3±19.9%, P<0.05), chromogenic TF-activity (TF, 8.4±1.9 versus 13.2±2.8 U, arbitrary units, P<0.05), and TF mRNA was suppressed in the presence of abciximab as a consequence of reduced platelet mass attached to monocytes.

Conclusions—Our data suggest that heterotypic monocyte-platelet aggregates are a target for abciximab, which suppresses monocyte TF because of a reduction of monocyte-platelet cross talk. (Arterioscler Thromb Vasc Biol. 2003;23:1697-1702.)

Key Words: tissue factor ■ glycoprotein IIb/IIIa inhibition ■ abciximab ■ monocyte-platelet aggregates ■ flow cytometry

Activated platelets expressing P-selectin (CD62P) rapidly adhere to human blood leukocytes by interacting with leukocyte P-selectin glycoprotein (GP) ligand-1 (PSGL-1).1 As a consequence of platelet-leukocyte cross talk, tissue factor (TF), the major initiator of blood coagulation,2 was reported to be induced.3 In thrombin receptor activator (TRA)-stimulated whole blood, induction of monocyte TF was shown to depend predominantly on P-selectin/PSGL-1 counterligation and to a minor degree on the interaction of leukocyte CD40 with the platelet activation marker CD40 ligand (CD40L).5 Abciximab, a nonselective GP IIb/IIIa-receptor antagonist, inhibits platelet aggregation6 and reduces ischemic complications after percutaneous coronary interventions.7–10 In addition, abciximab was shown to suppress leukocyte Mac-1 expression in patients with acute myocardial infarction by reducing the amount of platelets attached to leukocytes.11 These data suggest that GP IIb/IIIa inhibitors may regulate adhesive processes of leukocytes in acute coronary syndromes by targeting monocyte-platelet aggregate (MPA) formation.

In this study, we show that abciximab suppresses monocyte TF in TRA-stimulated whole blood by reducing the mass of platelets attached to monocytes. We used whole blood flow cytometry, immunohistochemistry, chromogenic activity assay, and reverse transcriptase–polymerase chain reaction (RT-PCR) to demonstrate this effect of the GP IIb/IIIa-antagonist abciximab on TRA-dependent TF induction and compared it to the extent of monocyte TF suppression achieved by P-selectin. Because TF pathway inhibitor (TFPI) regulates TF activity,12 we additionally studied monocyte TFPI expression in response to TRA-induced monocyte-platelet cross talk.

Methods

Blood Collection and Cell Preparation

Blood was drawn from the antecubital vein with loosened tourniquet to minimize platelet activation from healthy volunteers who had not taken aspirin or other nonsteroidal antiinflammatory drugs within the past 14 days. After discarding the first 2 mL, blood was collected into syringes prefilled with recombinant desulfatohirudin (Refludan, Hoechst Marion Russel; final concentration, 900 anti-IIa U/mL) for flow cytometry. For isolation of peripheral blood mononuclear cells (PBMCs) and platelets, citrate (Vacutainer tubes containing 1/10th volume of 3.8% trisodium citrate; Becton Dickinson) was used as anticoagulant. PBMCs for coculture experiments were isolated by density gradient centrifugation on Ficoll (Pharmacia Biotech) and resuspended in RPMI 1640 (Gibco) after 3 washes. Platelet-rich plasma (PRP) was prepared by centrifugation at 200g for 10 minutes at 4°C, and platelet count was adjusted to 2×10^10/L.

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TABLE 1. Characterization of Monocyte-Platelet Interactions by Flow Cytometry

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<th>Platelet Marker</th>
<th>MPAs, %</th>
<th>Monocyte Platelet Load, MFI</th>
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<tbody>
<tr>
<td>GP Ibα</td>
<td>CD14+/CD42b</td>
<td>CD42bMFI</td>
</tr>
<tr>
<td>P-selectin</td>
<td>CD14+/CD62P</td>
<td>CD62PMFI</td>
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<tr>
<td>CD40 ligand</td>
<td>CD14+/CD40L</td>
<td>CD40LMFI</td>
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with autologous platelet poor plasma (PPP; centrifugation at 2000g for 10 minutes at 4°C). All samples were processed immediately.

Flow Cytometry

For whole blood flow cytometry, aliquots of hirudin anticoagulated blood (0.5 mL) were placed into sterile polystyrene tubes (Becton Dickinson) and preincubated for 10 minutes with either c7E3 Fab (0 to 50 μg/mL; abcmixim, Centocor) or anti-CD62P (0 to 50 μg/mL; Instrumentation Laboratories) or anti-CD11b (50 μg/mL; Calbiochem) or control antibody (mouse IgG1, 50 μg/mL; clone, 2T8–2F5; Instrumentation Laboratories). After addition of TRA (final concentration, 12.5 μmol/L; Calbiochem) for platelet activation, whole blood was additionally incubated for up to 1 hour at 37°C in 5% CO2 in air.

One hundred microliters of whole blood was stained with saturating concentrations of the following fluorochrome-conjugated monoclonal antibodies (mAb): FITC-labeled mAb for TF (American Diagnostica), PE-Cy5-labeled mAb for monocyte CD14 (endotoxin receptor), PE-labeled mAb for the platelet activation marker CD62P (P-selectin), PE-labeled mAb for CD40L (CD40 ligand; all Ab from Instrumentation Laboratories), APC-labeled mAb for the constitutive platelet marker CD42b (GP Ibα of von Willebrand factor receptor complex; Becton Dickinson) and corresponding isotype controls. After 10 minutes of preincubation with antibodies in the dark at room temperature, the samples were fixed and erythrolysed with Optilyse B (Instrumentation Laboratories). For analysis of TFPI, cells were incubated with biotinylated (BiotinTag micro Biotinylation Kit B; Instrumentation Laboratories) and corresponding isotype controls. After 10 minutes at room temperature served as secondary antibody. TFPI expression was additionally studied in PBMC platelet coculture (2 × 10^5 /μL) whole blood was additionally incubated for up to 1 hour at 37°C in 5% CO2 in air and were seeded on plastic Petri dishes. Monocyte TF mRNA expression was determined by semiquantitative RT-PCR, amplifying a 282-bp fragment specific for human TF using 5'-CTA CTT TGG TTT CCT GGG ACA AGT GA'−3' as forward primer and 5'-CAG TGC AAT ATA GCA TTT GCA GTA GC'−3' as reverse primer in 30 cycles at 55°C annealing and 72°C extension temperature. Amplification of cloned TF cDNA served as positive control. A 266-bp fragment specific for the housekeeping gene GAPDH was amplified using the same cycle conditions with 5'-ACA GTC CAT GCC ATC ACT GCA GCC'−3' as forward primer and 5'-GGCTGTT TCT ACC ACC TTC TTG-3' as reverse primer. The intensity of electrophoresed TF PCR bands corrected for GAPDH was compared using NIH image. Gene Amp PCR System 2400 (Perkin Elmer Applied Biosystems) was used as a cycler.

TFPI mRNA Expression in PBMC-Platelet Coculture

Induction of TFPI mRNA was detected in PBMC-platelet coculture (cell ratio, 1:20) after 2 hours of TRA stimulation. RNA prepared

the mean channel fluorescence intensity (MFI) of constitutive and platelet activation markers was determined, gating on CD14+ events. Whereas CD42b MFI characterizes the mass of platelets attached to monocytes, CD62P MFI is a measure of the mean epitope density of P-selectin molecules on the platelet surface, which provides a parameter of platelet activation within the MPA.13

TF Activity in PBMC Platelet Coculture

TF activity of PBMC platelet coculture was measured by a chromogenic assay. Platelet suspension was preincubated with either control antibody (50 μg/mL), abciximab (50 μg/mL), or anti-CD62P (50 μg/mL) before activation by TRA (12.5 μmol/L) and coculture with 1 × 10^5 PBMCs at a ratio of 1:20 for 5 minutes. PPP served as a control for PRP. After centrifugation at 200g for 10 minutes at 4°C, samples were resuspended in assay buffer (pH 7.4; 10 mMol/L) containing HEPES, 137 mMol/L NaCl, 5 mMol/L KCl, 0.75 mMol/L Na,HPO4, 11 mMol/L glucose, 2.5 mMol/L CaCl2, and 0.5% fatty acid–free BSA. Assay buffer was supplemented with hirudin (900 μg/mL) to inhibit factor X cleavage and platelet activation by thrombin. After 30 minutes, incubation of the samples with purified coagulation factor VIIa (7 mMol/L; Calbiochem) and factor X (300 mMol/L; Calbiochem) at 37°C, amidolytic activity of generated factor Xa on Spectrozyme-Xa (125 μmol/L) was recorded using a kinetic plate reader (Dias, Dynatech). Factor Xa activity was compared with a standard curve generated with relipidated TF/VIIa complex and factor X and was converted to arbitrary units of TF activity. TF-dependent Xa generation was shown by omitting factor VIIa in these incubations.

Immunocytochemistry and Confocal Laser Microscopy

PBMC and platelet isolation was performed as described above. PRP (2 × 10^5 /μL) was preincubated with abciximab (50 μg/mL) or control antibody (mouse IgG1, 50 μg/mL; clone, 2T8–2F5; Instrumentation Laboratories) for 10 minutes at room temperature before coculture of platelets (2 × 10^5) with PBMCs (1 × 10^5) at a ratio of 1:20. PPP served as control for PRP. Cocultures were stimulated with TRA (12.5 μmol/L) for 5 minutes at 37°C in 5% CO2 in air and were seeded on adhesion slides (Bio-Rad) and fixed with cold acetone for 10 minutes at 4°C. Unspecific binding was blocked with PBS–BSA–NaN3/10% AB serum for 15 minutes at room temperature. For detection by confocal laser microscopy (Zeiss, Axiover), samples were incubated with anti-TF polyclonal Ab (rabbit anti-human IgG, American Diagnostica) or control antibody (IgG; Sigma) overnight at 4°C. TRITC-labeled, goat anti-rabbit IgG antibody (Sigma) incubated for 45 minutes at room temperature served as secondary antibody.

Monocyte TF mRNA

Hirudin anticoagulated whole blood was stimulated with TRA (12.5 μmol/L) for 45 minutes with or without abciximab preincubation at 37°C and 5% CO2 in air. LPS stimulation (100 ng/mL) served as positive control. Monocyte isolation from 100 mL whole blood was performed by immunomagnetic separation followed by mRNA extraction (Monocyte mRNA Isolation Kit, Dynal). TF mRNA expression was determined by semiquantitative RT-PCR, amplifying a 282-bp fragment specific for human TF using 5'-CTA CTT TGT CTC TCA AGC ATG GA-3' as forward primer and 5'-CAG TGC AAT ATA GCA TTT GCA GTA GC-3' as reverse primer in 30 cycles at 55°C annealing and 72°C extension temperature. Amplification of cloned TF CDNA served as positive control. A 266-bp fragment specific for the housekeeping gene GAPDH was amplified under the same cycle conditions with 5'-ACA GTC CAT GCC ATC ACT GCA GCC-3' as forward primer and 5'-GGCTGTT TCT ACC ACC TTC TTG-3' as reverse primer. The intensity of electrophoresed TF PCR bands corrected for GAPDH was compared using NIH image. Gene Amp PCR System 2400 (Perkin Elmer Applied Biosystems) was used as a cycler.

TFPI mRNA Expression in PBMC-Platelet Coculture

Induction of TFPI mRNA was detected in PBMC-platelet coculture (cell ratio, 1:20) after 2 hours of TRA stimulation. RNA prepared
from LPS (100 ng/mL) stimulated PBMCs or from HUVECs served as positive control. Real-time PCR was performed using LightCycler-RNA Master SYBR Green I (Roche) according to the manufacturer’s instructions. TFPI forward primer (5’-ACA AGA GAT GCA AAC AGG-3’), TFPI reverse primer (5’-GGC ATC CAC CAT ACT TGA A-3’), GAPDH forward primer (5’-ACA GTC CAT GCC ATC ACT GCC-3’), and GAPDH reverse primer (5’-GCC TGC TTC ACC ACC TTC TTG-3’) were designed using the LightCycler Probe Design Software Version 1.0 and the Primer3 Software (http://www.genome.wi.mit.edu). The amplification conditions consisted of an initial incubation at 61°C for 20 minutes, followed by incubation at 95°C for 30 seconds, 50 cycles of 95°C for 1 second, the respective annealing temperature for 10 seconds and 72°C for 10 seconds, a melting step from 45°C to 95°C increasing 0.1°C per second, and a final cooling to 40°C. Data were analyzed using LightCycler Software Version 3.5 (Roche).

Endotoxin Contamination

No endotoxin contamination of cell suspensions and buffers was detected. (E-toxate, Sigma).

Statistical Analysis

Values are given as mean±SD. Data were analyzed by Student’s t test or ANOVA followed by Tukey’s post hoc test. Correlation was calculated according to Pearson to assess correlations between TF expression and MPA. Significance was defined as P<0.05. All analyses were made with a SPSS software package 9.0 for Windows.

Results

Abciximab Reduces Platelet Mass and Tissue Factor of MPAs

Monocyte TF expression correlated with the percentage of MPA formation (CD14+/CD42b%; r=0.96; P<0.05) and with the mass of platelets attached to monocytes as measured by the MFI of the constitutive platelet marker CD42b (r=0.97, P<0.05) and a platelet activation marker CD62P (r=0.98, P<0.05) 1 minute after TRA stimulation.

In dose-response studies, a significant inhibition of monocyte TF expression as well as monocyte platelet load measured by CD42b MFI and CD62P MFI was observed at abciximab concentrations of 10 to 50 μg/mL (Figure 1; see the online data supplement available at http://atvb.ahajournals.org). Because 50 μg/mL abciximab resulted in optimal inhibition of monocyte TF expression, this concentration was used in all additional experiments (Figure 1). Preincubation of whole blood with abciximab or anti–P-selectin suppressed TRA-induced TF expression to a similar extent (Figure 1C) 1, 5, and 10 minutes after stimulation and was paralleled by a significant reduction of monocyte platelet load (CD42b MFI; Figure 1B). No change in MPA formation and TF expression was observed in the presence of a control antibody. Anti–P-selectin blocked MPA formation (CD14+/CD42b%) in time course up to 64% immediately after TRA stimulation (Figure 1A). In contrast, abciximab increased MPA formation (CD14+/CD42b%) 1 minute after TRA stimulation compared with control antibody (Figure 1A) at low CD42b MFI (Figure 1B), indicating dispersed single platelets attached to CD14+ cells. TRA stimulation of whole blood induced activated MPA formation (CD14+/CD62P+) to nearly 100% in the presence of both control antibody and abciximab (data not shown). In contrast, activated monocyte platelet load (CD62P MFI on CD14+ cells) was reduced by up to 25% in the presence of abciximab during the same time course of

Figure 1. MPA formation (CD14+/CD42b%; A), the constitutive platelet load (CD42b MFI; B), and the activated platelet load (CD62P MFI; D) on monocytes as well as the percentage of TF+ monocytes (CD14+/TF+; C) were determined in the presence of abciximab (dotted line), anti-P-selectin (broken line), or control antibody (solid line) using whole blood flow cytometry in time course. Data are given as mean±SD. *P<0.05.
TRA stimulation, although this was not statistically significant because of high standard deviations (P=0.1; Figure 1D).

Activated MPA formation characterized by the percentage of (CD14+/CD40L+), double-positive events was induced significantly faster in TRA-stimulated samples in the presence of abciximab compared with control antibody (Table 2). TRA-induced platelet activation in whole blood as measured by CD40L immunofluorescence intensity (CD40L MFI) on CD14+ cells resulted in a modest 2.1-fold increase, which was not inhibited by abciximab preincubation (Table 2). In contrast, blocking anti–P-selectin antibody significantly suppressed both the percentage (CD14+/CD40L+ %) and immunofluorescence intensity (CD40L MFI) of CD40L on monocytes immediately after TRA stimulation and in time course (Table 2). Inhibition of Mac-1 by anti-CD11b antibody had no influence on MPA formation or TF expression compared with TRA stimulation only (data not shown).

### Tissue Factor Pathway Inhibitor Expression in MPAs

Monocyte TFPI expression of unstimulated (CD14+/TFPI+, 2±0.2%) or TRA-stimulated (CD14+/TFPI+, 1.9±0.2%) whole blood was hardly above background, and no change was observed in the presence of abciximab or anti–P-selectin in time course up to 1 hour. Twenty-four-hour coculture of PBMC and TRA-stimulated platelet-rich plasma did not increase TFPI surface expression compared with unstimulated samples (Table 3) and was not affected by preincubation with abciximab or anti–P-selectin. LPS stimulation for 24 hours resulted in an approximately 1.8-fold increase of TFPI surface expression (P=0.07).

### Absciximab Blocks TF Activity

After showing that abciximab suppressed TF antigen expression in TRA-stimulated whole blood, we next examined the effect of abciximab on TF activity in PBMC-platelet coculture by chromogenic assay. TF-dependent Xa generation did not differ between isolated PBMCs and PBMCs in coculture with unstimulated platelets (arbitrary units [U], 4.8±1.6 versus 5.8±0.8 U, P=0.33; Figure 2). TRA stimulation of platelets for 5 minutes significantly increased Xa generation of PBMC-platelet coculture (13±2.8 U, P<0.001) and was dependent on TF because no chromogenic activity was detected in the absence of coagulation factor VIIa. Preincubation of platelets with anti–P-selectin or abciximab significantly blocked TF activity of stimulated PBMC-platelet coculture (anti–P-selectin, 9.0±1.4 U, P<0.05; abciximab, 8.4±1.9 U, P<0.05; Figure 2).

### TF Immunocytochemistry

In unstimulated PBMC-platelet coculture, no heterotypic aggregate formation was observed with little monocyte TF expression (Figure 3A). TRA stimulation triggered MPA formation and induced TF expression (Figures 3B and 3D), which was clearly suppressed in the presence of abciximab (Figure 3C).

### TF mRNA Expression in TRA-Stimulated Whole Blood

Induction of monocyte TF mRNA in TRA-stimulated whole blood was observed after 45 minutes and was suppressed by approximately 80% in the presence of abciximab in 2 independent experiments (Figure IIA; see the online data supplement available at http://atvb.ahajournals.org). LPS stimulation of whole blood and TF cDNA served as positive control. No TF mRNA was detected in monocytes isolated from unstimulated whole blood of healthy volunteers, confirming the lack of monocyte activation by the isolation procedure or the incubation period.

### TFPI mRNA Expression in PBMC-Platelet Coculture After TRA Stimulation

The level of TFPI mRNA expression in 24-hour PBMC-platelet coculture was unaffected by stimulation with TRA and the presence of blocking antibodies against GP IIb/IIIa-receptor or P-selectin (Figure IIB; see the online data supplement available at http://atvb.ahajournals.org). LPS stimulation of PBMC-platelet coculture resulted in a significant, approximately 5-fold increase in TFPI mRNA expression (P<0.05). For comparison, the level of TFPI mRNA in cultured HUVECs used as positive control was 190-fold higher than in PBMC-platelet coculture (data not shown).

### Discussion

MPA formation was recognized as an early marker of acute coronary syndrome and as a more sensitive parameter for platelet activation than platelet surface P-selectin expression. Rapid induction of monocyte TF mediated by platelet-monocyte cross talk triggered the development of a procoagulant state as a consequence of heterotypic aggregate formation in vitro. Modulation of platelet-leukocyte interactions by GP IIb/IIIa inhibition was previously shown to

<table>
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<tr>
<th>CD14+/TFPI+, %</th>
<th>Unstimulated</th>
<th>TRA+IgG1</th>
<th>TRA+GPIb</th>
<th>TRA+Anti–P-Selectin</th>
<th>LPS</th>
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<tr>
<td></td>
<td>4.3±1.2</td>
<td>3.7±2.5</td>
<td>4.7±2</td>
<td>3.7±0.7</td>
<td>8.3±0.9</td>
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regulate leukocyte-adhesive properties by decreasing the expression of monocyte Mac-1 (CD11b/CD18) primarily because of a reduction of the platelet mass attached to monocytes.11

In this study, we demonstrated suppression of monocyte TF expression by abciximab in TRA-activated whole blood at early time points. This was attributable to a significant decrease in platelet mass attached to monocytes (≈56%, Figure 1B) and reduced activation (≈25%, Figure 1D) of platelets incorporated into these heterotypic aggregates as measured by the expression of P-selectin. These data suggest reduced platelet-monocyte interaction via P-selectin/PSGL-1 in the presence of abciximab.

Rapid induction of monocyte TF expression in TRA-stimulated whole blood was shown to depend largely on P-selectin/PSGL-1 interaction and to a minor extent on CD40L/CD40 cross talk.3 Therefore, we compared the effect of abciximab to the effect of blocking anti–P-selectin antibody on MPA formation and TF expression. Although abciximab reduced the mass of platelets attached to monocytes, the percentage of MPA was not diminished. In contrast, anti–P-selectin suppressed both the percentage of MPA formed and the platelet mass attached to monocytes. Despite this different effect on MPA formation, the extent of monocyte TF suppression by abciximab and anti–P-selectin at early time points was comparable, supporting platelet mass as the critical determinant of monocyte TF induction.

In contrast to the platelet activation marker P-selectin, only a minor increase of CD40 ligand expression was observed on MPA after TRA stimulation of whole blood, which did not change significantly with abciximab preincubation (Table 2). This is in line with recent data showing stabilization of CD40L on heterotypic aggregates by abciximab.16 Furthermore, abciximab was shown to interfere with firm adhesion of platelets to monocytes because of its cross reactivity with Mac-1.17 However, this interaction does not seem to be crucial for platelet-mediated monocyte TF induction, because we did not find inhibition of TF expression in TRA-stimulated whole blood in the presence of anti-CD11b (anti-Mac-1).

Immunocytochemistry confirmed our observation obtained by flow cytometry showing a decrease of monocyte-platelet coaggregation and TF expression by preincubation of PBMC-platelet coculture with abciximab. In accordance with a previous report, monocytes of unstimulated coculture showed faint TF staining by confocal fluorescence microscopy.18 TRA stimulation of PBMC-platelet coculture triggered the formation of large MPAs with intense TF expression on both cell types, which was suppressed by abciximab.

In parallel to TF antigen, TF activity was significantly reduced in TRA-stimulated PBMC-platelet coculture by preincubation with abciximab, indicating regulation of TF-dependent procoagulant activity by GP IIb/IIIa inhibition. In addition to early expression of surface TF in MPA, TRA-dependent induction of monocyte TF mRNA in whole blood was reduced in the presence of abciximab, suggesting decreased TF synthesis by GP IIb/IIIa blockade.

No relevant expression of TFPI was detected on MPA in TRA-stimulated whole blood by flow cytometry, although a previous report showed release of TFPI from platelets on thrombin activation.19 We additionally studied TFPI expression in 24-hour PBMC-platelet coculture by flow cytometry and RT-PCR, because monocyte TF-induction was shown to be antagonized by delayed induction (24 to 48 hours) of monocyte TFPI.12 In contrast to LPS, TRA-stimulated monocyte-platelet cross talk did not increase monocyte TFPI mRNA after 24 hours, and preincubation with abciximab or anti–P-selectin did not change TFPI mRNA expression significantly.

In conclusion, our data provide in vitro evidence for a potential regulation of monocyte TF by abciximab interfering with monocyte-platelet cross talk. Abciximab administered as adjunct antithrombotic therapy in percutaneous coronary intervention improved clinical outcome,7–10 in particular in

Figure 2. TF activity of PBMC cocultured with PPP, PRP, and TRA-stimulated PRP for 15 minutes at 37°C was determined by chromogenic assay in the presence of factors VIIa and X. TRA-stimulated samples were preincubated with control antibody (IgG1), abciximab, or anti–P-selectin for 10 minutes at room temperature to test inhibition of TF activity. In selected experiments, factor VIIa was omitted (−VIIa) to confirm TF-dependent activity. Data are given as mean ± SD. *p < 0.05; **p < 0.01.

Figure 3. Expression of TF in untreated (A), TRA-stimulated (B and D), and abciximab-blocked, TRA-stimulated PBMC-platelet coculture (C) was assessed by immunocytochemistry and visualized by confocal laser fluorescence microscopy. Scale bar indicates distance at ×800 (12.5 μm) and ×1800 (25 μm) magnification.

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patients with acute coronary syndrome (ACS). Because MPA formation is an early marker of ACS and TF-dependent procoagulant activity was found induced 48 hours after percutaneous revascularization in patients with acute myocardial infarction, it is of interest to what extent adjunct antiplatelet therapy with GP IIb/IIIa antagonists may affect platelet-induced TF expression in vivo. The clinical relevance of the high abciximab concentration used in this study was supported by a recent clinical trial showing 50% reduction of major adverse cardiac events in patients with ACS undergoing percutaneous coronary intervention by intracoronary application of abciximab compared with intravenous application. In emergency angioplasty, high local doses of abciximab may facilitate the dissolution of thrombi at the culprit lesion and suppress monocyte-platelet cross talk with reduced TF activity.

Acknowledgments

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Figure Legends to online Figures

**Figure I.** In dose-response studies, the percentage of TF$^+$ monocytes (CD14$^+$/TF$^+$%; A) was determined in the presence of increasing concentrations of abciximab (0-50µg/mL) and anti-P-selectin (0-50µg/mL) in whole blood after 5 minutes stimulation with TRA (12.5µg/mL). TF-expression was significantly suppressed by 10µg/mL antibody concentration with optimal inhibition at 50µg/mL. Monocyte platelet load measured by CD42b MFI and CD62b MFI significantly decreased with preincubation of 10-50µg/mL abciximab (B). Data are given as mean±SD. *P<0.05

**Figure II.** (A) Monocyte TF and GAPDH expression was determined by RT-PCR in unstimulated (1) and TRA-stimulated whole blood in the presence of abciximab (2) or control antibody (3). LPS stimulated whole blood (4) and dilute TF phage cDNA (5) served as positive control. (B) TFPI mRNA expression of unstimulated and TRA-stimulated PBMC-platelet coculture was measured by Real-Time PCR in the presence of either control antibody, abciximab or anti-P-selectin. LPS-stimulation of PBMC-platelet coculture for 24 hours served as positive control. *P<0.05
Figure II

A

282bp  
266bp

TRAP
Abciximab
Control antibody

-  
+  
+  
LPS  
TFcDNA

TF
GAPDH

B

TFPI mRNA (x-fold control)

PPP  
PRP  
PRP+TRA  
+IgG1  
+Abciximab  
+anti-P-selectin  
LPS

*
Figure I

A

TF-positive monocytes (%)

Concentration of antibody (µg/mL)

0 0.5 5 10 25 50

abciximab
anti-P-selectin

B

Mean fluorescence intensity

Concentration of abciximab (µg/mL)

0 0.5 5 10 25 50

CD42b MFI
CD62P MFI

* * *