Platelet-Leukocyte Cross Talk in Whole Blood

Nailin Li, Hu Hu, Malin Lindqvist, Eva Wikström-Jonsson, Alison H. Goodall, Paul Hjemdahl

Abstract—Thrombosis and inflammation involve complex platelet-leukocyte interaction, the details of which are not fully elucidated. Therefore, we investigated cross talk between platelets and leukocytes in whole blood, under the following physiological conditions: at 37°C, with normal calcium concentrations, and with shear force. Platelet P-selectin and leukocyte CD11b expression were used to monitor platelet and leukocyte activation, respectively, and platelet-leukocyte aggregation (PLA) was analyzed. The leukocyte-specific agonist N-formyl-methionyl-leucyl-phenylalanine (10^-6 mol/L) increased P-selectin-positive platelets from 2.5±0.1% to 5.1±0.6% (P<0.05). The increase was inhibited by either the platelet-activating factor (PAF) antagonist SR27417, the superoxide anion scavenger superoxide dismutase, the 5-lipoxygenase inhibitor Ziletum, or the 5-lipoxygenase–activating protein inhibitor MK-886, suggesting the involvement of PAF, superoxide anion, and 5-lipoxygenase products in leukocyte-induced platelet activation. The platelet-specific agonist collagen (1 μg/mL) increased leukocyte CD11b expression from 2.94±0.52 to 3.81±0.58 (P<0.05); this was not inhibited by the thromboxane A2 receptor antagonist ICI 192.605 or the PAF antagonist SR27417. Platelet P-selectin expression induced by N-formyl-methionyl-leucyl-phenylalanine and leukocyte CD11b expression induced by collagen could be suppressed by glycoprotein IIb/IIIa blockade or P-selectin blockade. This study documents platelet-leukocyte cross talk under conditions that mimic a physiological state and suggests that this involves multiple mediators and mechanisms. Furthermore, new evidence of integrin and selectin involvement in intracellular and intercellular signaling during platelet-leukocyte cross talk is provided. (Arterioscler Thromb Vasc Biol. 2000;20:2702-2708.)

Key Words: platelets ■ leukocytes ■ platelet-leukocyte aggregates ■ platelet-leukocyte cross talk ■ whole blood

Thrombosis and inflammation are closely related pathophysiological processes with multicellular activation involving platelets, leukocytes, and endothelial cells. Evidence is accumulating that there are complex interactions, or “cross talk,” between these cells. Thus, the functional states of the cells involved may be influenced by a variety of stimulatory or inhibitory mediators, and the end result will depend on the balance between these influences.

On activation, leukocytes respond with degranulation, increased respiratory bursts, chemotaxis, and phagocytosis. All of these processes may be influenced by platelets. For instance, platelet-derived adenosine diphosphate and platelet-derived growth factor (PDGF) may induce leukocyte degranulation. Adherent platelets, platelet-derived microparticles, and platelet-released substances, such as PDGF, platelet factor 4, and thromboxane A2 (TXA2), may enhance leukocyte rolling and adhesion to the vessel wall. Platelets bound to neutrophils and platelet-released adenine nucleotides may promote superoxide anion (O2⁻) generation by neutrophils. Furthermore, platelet factor 4 and PDGF are chemotactic and may enhance phagocytosis by neutrophils and monocytes. By contrast, intact platelets may inhibit neutrophil O2⁻ generation and cytotoxicity, whereas leukocyte chemotaxis, adhesion, and O2⁻ generation may be inhibited by platelet-released NO and soluble P-selectin. Thus, platelets and platelet-released products may influence leukocyte function in a complex manner.

Similarly, platelet activation may be influenced by leukocytes. Leukocytes per se and leukocyte-released O2⁻ may enhance platelet adhesion. Furthermore, leukocyte-released substances, such as O2⁻, platelet-activating factor (PAF), elastase, and cathepsin G, may induce platelet aggregation and secretion. Conversely, unstimulated or weakly activated leukocytes may also attenuate platelet aggregation via leukocyte-released NO and/or ADPase. Neutrophil-derived elastase may bring about proteolysis of the GP Iba subunit, which contains the von Willebrand factor binding site, and thus influence platelet adhesion.

Transcellular metabolism also contributes to the cross talk between platelets and leukocytes. Thus, neutrophils can use platelet-released arachidonic acid to synthesize metabolites, such as leukotriene (LT)B4, that are not produced by platelets alone because they lack 5-lipoxygenase. Conversely, platelets can use neutrophil-derived precursors to synthesize LTC4 and lipoxin A4 and thus greatly enhance their production. Cell-cell adhesion via selectins and integrins may...
also promote transcellular eicosanoid biosynthesis.\textsuperscript{13,14} Furthermore, platelets and leukocytes can produce PAF, but coinubcation of activated platelets and activated neutrophils further enhances PAF-acether generation.\textsuperscript{15}

As visualized previously,\textsuperscript{16} platelets and leukocytes may form platelet-leukocyte aggregates or conjugates (PLAs), mainly via platelet-expressed P-selectin and its receptors P-selectin glycoprotein ligand-1 (PSGL-1) and CD11b, as well as via fibrinogen bridging between glycoprotein (GP) Ib/IIa and CD11b/CD18. The heterotypic conjugation may facilitate platelet-leukocyte interaction. For example, platelet-monocyte conjugation may enhance thrombin generation, and conjugated platelets may facilitate leukocyte rolling, adhesion, and migration in vivo\textsuperscript{17} and in vitro.\textsuperscript{18}

Previous studies have mostly been performed on isolated cells\textsuperscript{19,20} and have thus neglected the possibly important influences of red blood cells and plasma components on these interactions. Studies in whole blood\textsuperscript{21} were conducted in the presence of citrate, ie, with subphysiological calcium concentrations, which may alter platelet responses. Thus, previous results may not have reflected the true physiological state.

Therefore, we investigated platelet-leukocyte cross talk under conditions designed to mimic physiological conditions, ie, in whole blood, at 37°C, with physiological calcium concentrations, and with stirring to induce a low shear force, which is likely to mimic the venous shear stress. We used the leukocyte-specific agonist N-formyl-methionyl-leucyl-phenylalanine (fMLP) and the platelet-specific agonist collagen to induce leukocyte and platelet activation, respectively. We used a panel of antagonists to investigate possible mediators involved in the cross talk and monoclonal antibodies (MAbs) that block platelet-leukocyte aggregation to elucidate the impact of heterotypic conjugation. Platelet-leukocyte cross talk was monitored by studies of platelet P-selectin expression and leukocyte CD11b expression by use of whole blood flow cytometry and methodology involving minimal artifacts.

\section*{Methods}

\section*{Subjects}

Twenty-four healthy subjects (11 men and 13 women, aged 23 to 55 years) gave informed consent to participate in the present study, which was approved by the ethics committee of the Karolinska Institute.

\section*{Reagents}

The platelet agonist used was equine collagen (Nycemed Arzneimittel GmbH). The leukocyte-specific agonist fMLP was from Sigma Chemical Co. The PAF antagonist SR27417 was a gift from Dr J.-M. Herbert (Sanofi Recherche, Toulouse, France); the TXA\textsubscript{2} analogue U-46619 and the TXA\textsubscript{2} receptor antagonist ICI 192,605 were from BIOMOL Research Laboratories Inc. The 5-lipoxygenase inhibitor Zileuton was from Abbott Laboratories; the 5-lipoxygenase–activating protein (FLAP) inhibitor MK-886, LTB\textsubscript{4}, LTC\textsubscript{4}, and LTD\textsubscript{4} were gifts from Drs N. Hogg (Imperial Cancer Research Fund, London, UK) and M. Robinson (Celltech Ltd, London, UK), respectively. MAb MOPC21 (a gift from Dr Robinson) was used as nonspecific IgG control. HEPES and other chemicals were from Sigma.

Fluorescent antibodies for flow cytometric analysis were used at optimal concentrations, as determined by titration. Platelets were identified with an FITC-conjugated anti-CD42a (GPIIIa) MAb Beb1 (Becton Dickinson). Leukocytes were identified with an R-phcoerythrin (RPE)-conjugated panleukocyte, CD45 MAb T29/33 (Dakopatts AB). Platelet P-selectin expression was determined by an RPE-conjugated anti–P-selectin MAb AC1.2 (Becton Dickinson), and leukocyte CD11b expression was determined by an FITC-conjugated MAb, BEAR 1 (Immunotech). FITC- and RPE-conjugated isotypic MAb DAK-G01 were used as negative controls.

\section*{Blood Collection and Sample Preparation}

Blood was collected by clean venipuncture with the use of siliconized Vacutainer tubes containing 1/10 vol of 200 µg/mL recombinant hirudin (CIBA-Geigy). Within 3 minutes of collection, 200 µL aliquots of blood were added to prewarmed siliconized cuvettes. Blood was incubated at 37°C for 5 minutes in the presence of vehicle or appropriately diluted antagonist(s) or blocking MAb(s). Afterward, collagen (1 µg/mL) or fMLP (10\textsuperscript{−6} mol/L) was added to induce platelet or leukocyte activation, respectively, and the samples were further incubated for 5 minutes, with stirring at 900 rpm. Thereafter, 5 µL blood was added to 45 µL HEPES-buffered saline containing appropriately diluted fluorescent MAbs. The samples were incubated at room temperature for 20 minutes and then diluted and mildly fixed with 0.5% (vol/vol) formaldehyde saline before measurement with use of a Coulter EPICS XL-MCL flow cytometer (Coulter Corp), as described previously.\textsuperscript{22}

\section*{Platelet-Poor Blood Preparation}

To confirm that the collagen preparation does not activate leukocytes, platelet-poor blood was prepared by using Percoll (Amersham Pharmacia Biotech) as described previously\textsuperscript{18} but omitting the step of red blood cell lysis. The lower layer (granulocytes and red blood cells) was resuspended with HEPES-buffered saline containing 1.25 mmol/L CaCl\textsubscript{2} and centrifuged at 800g for 10 minutes. The pellet was resuspended in the same buffer. Afterward, the platelet-poor blood was incubated with or without collagen as described above.

\section*{Flow Cytometric Analysis}

\subsection*{Platelet P-Selectin Expression}

The flow cytometric analysis of platelets in whole blood has been described previously.\textsuperscript{23} RPE-Cd62P fluorescence was monitored to obtain the percentage of P-selectin-positive platelets. The P-selectin–blocking MAb 9E1 did not interfere with platelet P-selectin measurements with the use of MAb AC1.2, as determined in separate experiments with collagen-stimulated platelets.

\subsection*{Leukocyte CD11b Expression}

The flow cytometric analysis of leukocyte CD11b expression in whole blood has been described previously.\textsuperscript{23} CD11b expression was determined as mean fluorescence intensity (MFI) in total leukocytes and leukocyte subpopulations and expressed in arbitrary units. However, the CD18-blocking MAb 6.5E interfered with CD11b measurements that made use of MAb BEAR 1.

\subsection*{Platelet-Leukocyte Aggregates}

PLA analysis has been described previously.\textsuperscript{22} The percentages of platelet-conjugated leukocytes in the total leukocyte population (PLA), lymphocytes (P-Lyms), monocytes (P-Mons), and polymorphonuclear cells (P-PMNs) were obtained.

\section*{Statistical Analysis}

Data are presented as mean±SEM. Individual measurements were compared with the Wilcoxon signed rank test (StatView 4.5, Abacus Concepts). A value of \(P<0.05\) was considered statistically significant.

\section*{Results}

\subsection*{Titration of Antagonists}

PAF (10\textsuperscript{−6} mol/L, \(n=4\)) increased P-selectin–positive platelets from 3.8±1.2% to 22.9±15.3%, and this effect was
completely blocked by SR27417 at $10^{-8}$ mol/L. PAF increased leukocyte CD11b MFI from 3.02 ± 0.51 to 5.44 ± 0.74, and this effect was abolished by $10^{-6}$ mol/L SR27417. Thus, $10^{-6}$ mol/L SR27417 was chosen to block the effects of PAF.

The TXA$_2$ analogue U-46619 ($10^{-6}$ mol/L, n=4) increased P-selectin–positive platelets from 1.8±0.5% to 95.5±0.7% and leukocyte CD11b MFI from 3.85±0.51 to 6.22±0.45. Both effects were abolished by the TXA$_2$ antagonist ICI 192.605 at $10^{-6}$ mol/L.

fMLP-Induced Leukocyte Activation
The leukocyte-specific agonist fMLP ($10^{-6}$ mol/L) increased leukocyte CD11b expression (MFI) from 2.88±0.38 to 6.89±0.51 ($P<0.05$, n=7). This effect was predominantly seen in polymorphonuclear leukocytes (PMNs; from 3.67±0.58 to 9.81±0.48, $P<0.05$), with only small increases among monocytes (from 2.43±0.38 to 2.78±0.20, $P<0.05$) and no effect in lymphocytes. Neither the PAF antagonist SR27417 ($10^{-6}$ mol/L) nor the 5-lipoxygenase inhibitor Zileuton ($10^{-6}$ mol/L) significantly influenced leukocyte CD11b expression in unstimulated samples (data not shown). The leukocyte responses to fMLP were partially inhibited by SR27417 ($P<0.05$) but not by Zileuton. Similar results were found in the presence of the GP Ib/IIIa antagonist SR121566 (data not shown).

fMLP-Induced Platelet Activation
Stimulation with fMLP ($10^{-6}$ mol/L) increased P-selectin–positive platelets from 2.5±0.1% to 5.1±0.6% ($P<0.05$). This leukocyte-dependent platelet activation was markedly inhibited by the PAF antagonist SR27417 ($10^{-6}$ mol/L) or the 5-lipoxygenase inhibitor Zileuton ($10^{-6}$ mol/L), which produced 87% and 94% inhibition, respectively ($P<0.05$). The combination of both inhibitors did not have additive effects (Figure 1A).

When the nonpeptide GP Ib/IIIa antagonist SR121566 ($10^{-6}$ mol/L) was used to minimize influences of platelet-platelet aggregation (n=7), fMLP failed to induce P-selectin expression in single platelets (Figure 1B).

To clarify whether the effect of Zileuton was related to 5-lipoxygenase inhibition, the FLAP inhibitor MK-886 ($10^{-5}$ mol/L) was also studied (n=6). As with Zileuton, MK-886 markedly inhibited fMLP-induced platelet P-selectin expression (86% inhibition). However, LTB$_4$, LTC$_4$, and LTD$_4$ ($10^{-7}$ mol/L) failed to increase platelet P-selectin expression in whole blood (n=5, data not shown).

fMLP-Induced PLA Formation
PLA formation was increased by fMLP ($10^{-6}$ mol/L) as a result of increases in P-PMNs (Figure 2). Neither the PAF antagonist SR27417 nor the 5-lipoxygenase inhibitor Zileuton influenced this response. Similar results were obtained in the presence of the GP Ib/IIIa antagonist SR121566 (data not shown).

Effects of O$_2^-$ in fMLP-Induced Leukocyte and Platelet Activation
To investigate the effects of O$_2^-$ in leukocyte-induced platelet activation, hirudinized blood was preincubated without or with 100 U/mL SOD, which did not influence leukocyte CD11b expression or PLA formation in either resting or fMLP-stimulated samples. SOD did not influence platelet P-selectin expression in resting samples (2.2±0.3% without
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Figure 3. Effect of collagen (1 μg/mL) on platelet P-selectin expression (A), leukocyte CD11b expression (B), and PLA formation (C) in the absence (open bars) or presence (stippled bars) of the GP IIb/IIIa inhibitor SR121566 (n = 7). Whole blood was preincubated without or with the TXA2 receptor antagonist ICI 192.605 (10^-6 mol/L) for 5 minutes, followed by further incubation and stirring for 5 minutes without or with collagen. *P < 0.05 compared with unstimulated samples; †P < 0.05 compared with corresponding collagen-stimulated samples.

and 2.3 ± 0.4% with SOD, P = 0.74; n = 7) but tended to inhibit fMLP-induced platelet P-selectin expression (3.1 ± 0.3% without and 2.6 ± 1.1% with SOD, P = 0.06).

Collagen-Induced Platelet P-Selectin Expression
Collagen markedly increased platelet P-selectin expression (Figure 3A, n = 7) in the absence (from 2.9 ± 0.2% to 24.4 ± 2.5%) and presence (from 1.6 ± 0.3% to 59.6 ± 8.3%) of the GP IIb/IIIa inhibitor SR121566. The enhancement by SR121566 is presumably due to blockage of platelet-platelet aggregation, which increases the numbers of single activated platelets. The effect of collagen was largely blocked by the TXA2 antagonist ICI 192.605, inasmuch as P-selectin positive platelets fell to 8.0 ± 1.5% in the absence and 2.9 ± 0.7% in the presence of the GP IIb/IIIa inhibitor.

Collagen-Induced Leukocyte CD11b Expression
The collagen preparation used did not increase CD11b expression of isolated PMNs in platelet-poor blood (2.58 ± 0.17 in the absence and 2.38 ± 0.20 in the presence of 1 μg/mL collagen, P = 0.11; n = 3). However, in stirred whole blood, collagen increased CD11b expression in total leukocytes (Figure 3B, n = 7), predominantly because of an increase among PMNs (from 4.01 ± 0.54 to 5.50 ± 0.60 in the absence and from 4.39 ± 0.67 to 5.58 ± 0.94 in the presence of the GP IIb/IIIa antagonist SR121566, respectively; P < 0.05 for both). The TXA2 antagonist ICI 192.605 reduced CD11b expression in unstimulated samples and attenuated the leukocyte response to collagen in the presence of the GP IIb/IIIa antagonist SR121566 (P < 0.05). However, in the absence of SR121566, ICI 192.605 reduced the basal CD11b expression but not the effects of collagen.

To investigate whether platelet-derived PAF mediates collagen-triggered leukocyte activation, blood samples were preincubated without or with the PAF antagonist SR27417 (10^-6 mol/L) and then further incubated in the absence or presence of 1 μg/mL collagen (n = 5). SR27417 did not influence platelet P-selectin expression or leukocyte CD11b expression in either resting or collagen-stimulated samples (data not shown).

Collagen-Induced PLA Formation
Collagen increased PLA formation markedly in the absence and in the presence of the GP IIb/IIIa antagonist SR121566 (Figure 3C). Leukocyte subpopulation analysis showed that P-PMMNs increased more markedly with GP IIb/IIIa blockade (from 14.5 ± 1.8% to 72.4 ± 5.2% without and from 22.2 ± 7.8% to 95.3 ± 1.2% with SR121566). The TXA2 antagonist ICI 192.605 partially inhibited collagen-induced PLA formation; this effect was seen in all leukocyte subpopulations (data not shown).

Influence of P-Selectin Blockade
To investigate the roles of direct cell-cell contact, whole blood was preincubated with blocking MAbs before fMLP or collagen stimulation. The nonspecific control antibody (MOPC21) did not influence any of the parameters studied (data not shown).

As expected, fMLP increased leukocyte CD11b expression, platelet P-selectin expression, and PLA formation without blocking reagents (Figure 4). The fMLP-induced increase of platelet P-selectin expression was not influenced by the anti-P-selectin MAb 9E1, although fMLP-induced PLA formation was markedly reduced.

Collagen markedly increased platelet P-selectin expression and PLA formation and also caused a mild increase in leukocyte CD11b expression (Figure 5). MAb 9E1 completely blocked collagen-induced PLA formation and attenuated collagen-induced leukocyte CD11b expression.

Effects of GP IIb/IIIa Blockade
The GP IIb/IIIa MAb RFGP56 and the nonpeptide GP IIb/IIIa inhibitor SR121566 did not significantly influence fMLP- or collagen-induced leukocyte CD11b expression. fMLP-induced P-selectin expression was inhibited by RFGP56 and SR121566 (Figure 4B), whereas fMLP-induced PLA formation was enhanced, or not altered (Figure 4C).

Both GP IIb/IIIa inhibitors enhanced collagen-induced platelet P-selectin expression (Figure 5A) and PLA formation (Figure 5C), presumably because of reduced platelet-platelet aggregate formation, leading to the retention of more activated platelets as single cells. Collagen-induced leukocyte CD11b expression was not significantly influenced by MAb RFGP56 but was inhibited by the nonpeptide GP IIb/IIIa inhibitor SR121566 (Figure 5B).
Ligand-Receptor Systems in fMLP- and Collagen-Induced PLA Formation

In the experiments shown in Figures 4 and 5, a blocking MAb cocktail containing 9E1, RFGP56, CD11b MAb44, and CD18 MAb 6.5E was also used to block the major ligand-receptor systems involved in PLA formation simultaneously. However, CD18 MAb 6.5E interfered with the flow cytometric measurements of CD11b, which limited the use of the cocktail to PLA analysis. The cocktail markedly reduced PLA formation in unstimulated but stirred samples (from 16.2 ± 1.5% to 3.8 ± 0.5%, \( P < 0.05 \)) and totally blocked fMLP-induced PLA formation (from 23.0 ± 1.8% to 4.3 ± 0.5%, \( P = 0.40 \) compared with unstimulated samples). MAb 9E1 alone caused partial inhibition (8.1 ± 1.8%, \( P < 0.05 \); Figure 4C). Similarly, the inhibitory cocktail totally blocked collagen-induced PLA formation. With collagen stimulation, PLA formation was equally blocked by 9E1 alone.

**Discussion**

The present study demonstrates platelet-leukocyte interactions under conditions that mimic the physiological state, ie, in stirred whole blood and with normal extracellular calcium levels. The results indicate that fMLP-activated leukocytes can induce platelet activation, ie, increased platelet P-selectin expression, and that this effect may be related to the generation of PAF, 5-lipoxygenase production, and superoxide anions. Furthermore, collagen-induced platelet activation can lead to leukocyte activation, seen as increased leukocyte CD11b expression; this response was not inhibited by blockade of TXA2 or PAF receptors. The present study also provides new evidence that P-selectin and GP IIb/IIIa are involved in cellular signaling during platelet-leukocyte cross talk.

Activated leukocytes may influence platelets via different mediators in different experimental settings.19,21,25,26 In the present investigation in stirred whole blood, fMLP-activated leukocytes increased platelet P-selectin expression, and this was inhibited by blockade of PAF receptors by SR27417 and \( \text{O}_2^- \) scavenging by SOD, suggesting the involvement of PAF and \( \text{O}_2^- \). Blockade of 5-lipoxygenase by either the 5-lipoxygenase inhibitor Zileuton or the FLAP inhibitor MK-886 inhibited fMLP-induced platelet activation. However, neither \( \text{LTB}_4 \), \( \text{LTC}_4 \), nor \( \text{LTD}_4 \) increased platelet P-selectin expression in whole blood. Thus, inhibition of leukocyte-platelet cross talk by 5-lipoxygenase blockade involves not only reduced leukothriene formation but also complex mechanisms. Several lines of evidence suggest that 5-lipoxygenase products may contribute to the cross talk. For instance, 5-hydroxyeicosatetraenoic acid and \( \text{LTB}_4 \) have been shown to enhance granulocyte PAF synthesis.27 Further investigation to clarify the mechanisms involved would be of considerable interest. Other leukocyte-derived mediators not investigated in the...
present study, such as neutrophil-released proteinases.\textsuperscript{19,26} may also be involved in the cross talk, albeit to a limited extent. Furthermore, PAF antagonism also inhibited fMLP-induced leukocyte CD11b expression, suggesting an autocrine effect of PAF.

Blockade of collagen-induced platelet P-selectin expression by the TXA\textsubscript{2} receptor antagonist ICI 192.605 confirms that collagen-induced platelet activation is dependent on TXA\textsubscript{2} synthesis. Our collagen preparation did not increase leukocyte CD11b expression in platelet-poor blood but did enhance leukocyte CD11b expression in whole blood. The TXA\textsubscript{2} receptor antagonist ICI 192.605 and the PAF antagonist SR27417 reduced leukocyte CD11b expression in unstimulated and collagen-stimulated samples. However, the leukocyte CD11b response to collagen was not reduced by ICI 192.605 or SR27417. This suggests that collagen induces leukocyte CD11b expression either via platelet-derived mediators other than TXA\textsubscript{2} or PAF (eg, platelet dense granule–released ADP\textsuperscript{20} and platelet-derived microparticles\textsuperscript{3}) or via cell-cell contact, as discussed below.

In the present study, collagen induced marked platelet activation and mild leukocyte activation, whereas fMLP induced marked leukocyte activation and mild platelet activation. Because collagen enhanced PLA formation more markedly than did fMLP, it seems as if PLA formation is more dependent on platelet activation than on leukocyte activation. This is quite reasonable, inasmuch as the major adhesion molecule involved in PLA formation, P-selectin, is expressed only on the surface of activated platelets, whereas its counterparts PSGL-1 and CD15 are constitutively expressed on leukocytes. Differential blockade of PLA formation with different stimuli and different blocking agents suggests that multiligand-receptor systems are involved in PLA formation under the present conditions. Our results are compatible with the idea that platelet activation–initiated PLA formation is entirely dependent on ligation via P-selectin, whereas leukocyte activation–initiated PLA formation involves ligation via P-selectin–PSGL-1/CD15 and GP Ib/IIa–fibrinogen–CD11b/CD18. In contrast, GP Ib/IIa blockade alone may enhance PLA formation, presumably because of the inhibition of platelet-platelet aggregation, which provides more activated platelets for heterotypic conjugation.

Enhancement or inhibition of PLA formation by blocking agents seemed to have only minor influences on fMLP-induced leukocyte CD11b expression, indicating that leukocyte-platelet cross talk is likely to depend on soluble mediators rather than direct cell-cell contact. Collagen-induced leukocyte CD11b expression was attenuated by blockade of PLA formation by the P-selectin–blocking MAb 9E1. Thus, direct cell-cell contact may contribute to platelet-induced leukocyte activation. However, this was apparently contradicted by the data obtained with the GP Ib/IIa inhibitor SR121566, which attenuated platelet-induced leukocyte CD11b expression despite enhanced collagen-induced PLA formation. Therefore, platelet-leukocyte cross talk seems to involve complex mechanisms, and further investigation is warranted to define the mechanism(s) that may mediate collagen-induced leukocyte CD11b expression.

Cellular signaling involves integrins as well as selectins.\textsuperscript{28,29} Blockade of selectins and integrins not only severs the ligations of cell-cell adhesion/conjugation but also affects intercellular signaling. The present findings reinforce previous evidence of selectin and integrin involvement in intercellular cross talk.\textsuperscript{13,14,30,31} We showed that GP Ib/IIa blockade inhibited fMLP-induced platelet P-selectin expression and that GP Ib/IIa blockade by SR121566 and P-selectin blockade by MAb 9E1 inhibited collagen-induced leukocyte CD11b expression. These findings indicate that GP Ib/IIa occupancy by antagonists may interfere with the inside-out signaling of platelets and that P-selectin blockade (if not simply by its inhibition of PLA formation) and GP Ib/IIa antagonists may inhibit intercellular signaling between platelets and leukocytes. Our data add new evidence for the involvement of the integrins and selectins in intracellular and intercellular signaling during cellular activation and interaction.\textsuperscript{28,29} Integrin- and selectin-linked cell signaling is complex and has not yet been well defined, but interest in this field is expanding. Perhaps it will be possible to identify integrin- or selectin-proximal signaling proteins as future drug targets.

It is well worth stressing that although platelet-leukocyte cross talk induces only mild platelet and leukocyte activation, the major physiological importance of this intercellular interaction may be the priming of platelets and leukocytes, leading to platelet and leukocyte hyperreactivity. Several antagonists also decreased platelet P-selectin expression, leukocyte CD11b expression, and PLA formation in unstimulated samples, suggesting that there is spontaneous platelet and leukocyte activation during incubation with stirring and that such spontaneous activation also involves multiple mediators.

In conclusion, the present study has provided strong support for the existence of platelet-leukocyte cross talk under physiological conditions in whole blood. The present study has demonstrated that several mediators are involved in the cross talk and that blockade of GP Ib/IIa or P-selectin may inhibit platelet-leukocyte cross talk.

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