Dynamics of Leukocyte-Platelet Adhesion in Whole Blood

By Henry M. Rinder, Jayne L. Bonan, Christine S. Rinder, Kenneth A. Ault, and Brian R. Smith

The dynamics of leukocyte-platelet adhesion and platelet-platelet interaction in whole blood are not well understood. Using different platelet agonists, we have studied the whole blood kinetics of these heterotypic and homotypic interactions, the relative abilities of different leukocyte subsets to participate in platelet adhesion, and the ligands responsible for adhesion. When platelet aggregation was inhibited by the Arg-Gly-Asp-Ser (RGDS) peptide, thrombin stimulation of whole blood resulted in platelet expression of granule membrane protein 140 (GMP-140) and, simultaneously, a marked increase in the percentage of monocytes and neutrophils (PMN) binding platelets, as well as an increase in the number of platelets per monocyte and PMN. Lymphocytes were unaffected. Monocytes bound more platelets and at an initially faster rate than PMN. This increase in monocyte and PMN adhesion to platelets was completely inhibited by the blocking monoclonal antibody (MoAb), G1, to GMP-140. When the combination of epinephrine and adenosine diphosphate (epi/ADP) was used as a less potent agonist in the presence of RGDS, GMP-140 expression per platelet was less, and while monocyte-platelet conjugates formed, PMN-platelet conjugates did not. With epi/ADP in the absence of RGDS, there was an immediate, marked decrease in the percentage of all leukocytes with bound platelets, simultaneous with an increase in the percentage of unbound platelet aggregates. As these platelet aggregates dissociated, the percentage of monocytes and PMN with adherent platelets increased, with monocytes again binding at a faster initial rate than PMN. This recovery of monocyte and PMN adhesion to platelets was also inhibited by the G1 MoAb. We conclude that: (1) monocytes and PMN bind activated platelets in whole blood through GMP-140; (2) monocytes have a competitive advantage over PMN in binding activated platelets, particularly when less potent platelet agonists are used; and (3) platelet aggregate formation initially competes unactivated platelets off leukocytes; subsequent aggregate dissociation allows the now activated platelets to adhere to monocytes and PMN through GMP-140. These studies further elucidate the dynamic interaction of blood cells and possible links between coagulative and inflammatory processes.

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MATERIALS AND METHODS

Antibodies. All monoclonal antibodies (MoAbs) were used as purified whole IgG, or IgM, except for the anti-GMP-140 MoAb designated “G1,” which was used both whole and as a Fab fragment. All experiments included irrelevant isotype-specific mouse MoAbs as negative controls. The MoAbs 1E3 and G1 (the latter kindly donated by Dr R. McEver, University of Oklahoma, Oklahoma City) are both specific for GMP-140/PADGEM, but only G1 inhibits adhesion of stimulated platelets to neutrophils and monocytes. SZ2 and P2 (AMAC, Inc, Westbrook, ME) recognize GPIb and GPIIb/IIIa, respectively. Anti-CD45 (HL6; Becton-Dickinson Immunocytometry Systems, San Jose, CA) recognizes a CD45 isof orm present on PMN, monocytes, and lymphocytes but neither erythroid cells nor platelets. The MoAb MMA (Leu-M1, Becton-Dickinson) recognizes CD15.

Thrombin stimulation of whole blood. Blood was drawn from normal human volunteers into a final concentration of sodium citrate, 0.38%. To prevent clotting through fibrinogen-GPIIb/IIIa binding, blood was then incubated with Arg-Gly-Asp-Ser (RGDS) peptide (Peninsula Laboratories, Inc, Belmont, CA) at a final concentration of 1 mg/mL. The sample was stimulated (time 0) with diluent or bovine thrombin (Sigma, St Louis, MO) at a final concentration of 0.1 U/mL at 37°C. The blood was kept at 37°C for the entire assay. Preliminary studies demonstrated no differences between samples that were agitated or stationary during the assay. For blocking studies with MoAbs, G1 Fab, 1E3, or isotype-matched control MoAb were preincubated with whole blood for 5 minutes before the addition of agonist. The concentrations of MoAbs used were found to be saturable at 1 μg/mL.

Epinephrine-adenosine diphosphate (epi/ADP) stimulation of whole blood. Blood was drawn into porcine heparin at a final concentration of 14 U/mL or citrate as described previously. In some experiments, RGDS was added at a final concentration of 1 mg/mL. One milliliter of blood was then incubated at 37°C with diluent or epinephrine HCl (Parke-Davis, Morris Plains, NJ) at a final concentration of 1 μmol/L for 10 minutes, followed by diluent or ADP (Sigma) at a final concentration of 5 μmol/L at 37°C.

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Addition of ADP was designated as time 0. Blood samples were kept at 37°C throughout the experiment. Studies performed at 22°C demonstrated similar results. MoAb blocking experiments were performed as above.

Sample preparation. At each sampling time point, 100 μL of blood was removed and incubated with paraformaldehyde (final concentration 1%) for 60 minutes, followed by addition of 1/8 vol/vol Tris-glycine solution (250 mmol/L Tris and 500 mmol/L glycine), as described previously. Preliminary experiments using isolated cell fractions demonstrated that paraformaldehyde fixation did not significantly change the percentages of leukocytes binding platelets. After 15 minutes, samples were washed three times in Tyrodes-HEPES buffer (HEPES 5 mmol/L, NaCl 140 mmol/L, KCl 2.7 mmol/L, dextrose 5.5 mmol/L, NaH₂PO₄ 0.42 mmol/L, and NaHCO₃ 12 mmol/L, pH 7.4). Samples were then resuspended in 200 μL of TH buffer. Each sample was divided in half, one part for fluorescent labeling of leukocyte-platelet conjugates and the other half for labeling of platelets for GMP-140 and measurement of platelet aggregates.

Fluorescence labeling. For determination of the percentage of platelets expressing GMP-140 and the percentage of platelet aggregates, 100 μL of sample was incubated with fluorescein isothiocyanate (FITC)-anti-GPIb (S2Z; AMAC) or FITC-anti-GPIIb/IIIa (P2; AMAC) and biotinylated anti-GMP-140 (1E3) MoAbs for 20 minutes, then washed and resuspended in 100 μL TH buffer. The sample was then incubated with saturating concentrations of phycoerythrin (PE)-avidin (Becton-Dickinson) for 20 minutes, washed, and resuspended in 300 μL TH buffer for fluorescence-activated cell sorter (FACS) analysis.

For leukocyte-platelet conjugate analysis, 100 μL of sample was incubated with saturating concentrations of FITC-conjugated anti-CD45 and biotinylated anti-GPIb or anti-GPIIb/IIIa MoAbs for 20 minutes, then washed and resuspended in 100 μL TH buffer. PE-avidin labeling and preparation for FACS analysis was performed as described previously.

Flow cytometry. Samples were analyzed on a FACScan flow cytometer (Becton-Dickinson) with data stored in list mode files. The determination of the percentage of platelets expressing GMP-140 and the percentage of platelet aggregates was performed as previously described.

The determination of leukocyte-platelet conjugates was performed as recently described. In brief, live gating on leukocyte-sized events was performed to exclude single platelets using a combination of forward and side scatter and positive anti-CD45 fluorescence. Leukocyte subsets, ie, monocytes, PMN, and lymphocytes, were distinguished from one another on the basis of these characteristics. An isotype-matched control antibody was used to set a threshold (95% of events below threshold) for positive platelet marker fluorescence. The percentage of platelet-marker–positive conjugates represents the percentage of leukocytes with at least one bound platelet. In addition, the mean platelet marker fluorescence per bound leukocyte was determined. This has been previously demonstrated to correspond in a semi-quantitative fashion to the number of platelets bound per leukocyte. Previous studies with fractionated cell populations have demonstrated that this fluorescence assay detects leukocytes with only a single platelet bound per leukocyte. Using either GPIb or GPIIb/IIIa as the platelet marker was found to yield equivalent results for both percentages of leukocyte-platelet binding and relative platelet fluorescence per bound leukocyte. Inert bead and image analysis studies have also established that measured leukocyte-platelet conjugates are not the result of simultaneous passage of unbound platelets and leukocytes through the detection chamber, confirming that this assay measures only platelets bound to leukocytes. An ACAS 570 microscope-based laser cytometer (Meridian Instruments, East Lansing, MI) was also used to analyze whole blood preparations and confirmed that dual fluorescent events represent leukocyte-platelet conjugates in stimulated and unstimulated samples.

All experiments were performed at least three times. Each of the figures presents data from a single representative experiment.

RESULTS

Studies of unstimulated whole blood. Heparinized and citrated samples showed identical results to one another and were analyzed together. In both heparinized and citrated whole blood transferred immediately to fixative after drawing, a significant portion of leukocytes at “baseline,” ie, before any manipulation, have at least one adherent platelet associated with them when assessed by the flow cytometric assay. This “baseline” percentage of leukocytes binding at least one platelet was averaged for 10 experiments, as shown in Table 1. Monocytes had the largest percentage of unactivated “baseline” binding, followed by PMN and lymphocytes. These numbers compare favorably with previously reported results of binding of unstimulated isolated cell fractions, except for monocytes, which showed a lesser bound percentage in whole blood compared with recombination of isolated cell fractions. These percentages were confirmed using dual immunofluorescence and direct visualization with the ACAS 570 laser cytometer. When unstimulated whole blood samples (in heparin or citrate) were incubated with 1 mg/mL of RGDS, the percentage of leukocytes with bound platelets was halved. Using platelet fluorescence as an estimate of the number of platelets bound per leukocyte, we found that the number of unactivated platelets per leukocyte at baseline in whole blood averaged one platelet per white blood cell, similar to previous studies with isolated cell fractions.

Thrombin stimulation of whole blood. Citrated whole blood was incubated with RGDS peptide at a final concentration of 1 μg/mL to prevent formation of platelet-platelet aggregates. After addition of diluent or thrombin (0.1 U/mL at time 0), samples were studied at various time points (Fig 1) while incubating at 37°C. Fig 1A demonstrates the percentage of monocytes, PMN, and lymphocytes binding platelets over time after addition of thrombin. Compared with the unactivated sample in Fig 1B (diluent alone added at time 0), thrombin stimulation caused a significant increase in the percentage of monocytes and PMN with adherent platelets but no change in lymphocyte-platelet adhesion. The initial slope of the curve over the

Table 1. Leukocyte-Platelet Adhesion in Whole Blood

<table>
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<tr>
<th>Anticoagulant</th>
<th>Baseline % of Leukocytes Binding Platelets*</th>
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<tr>
<td></td>
<td>baseline</td>
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<tr>
<td>Heparin or citrate</td>
<td>45.25 ± 3.20</td>
</tr>
<tr>
<td>Heparin + RGDS or citrate + RGDS</td>
<td>22.29 ± 5.23</td>
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*Mean ± standard deviation of the mean.
Whole blood, anticoagulated with citrate, was preincubated with RGDS peptide (1 mg/mL) and stimulated with thrombin 0.1 U/mL (A) or diluent (B) at time 0. The blood was kept at 37°C. Samples were obtained at the indicated time points and examined for the percentage of monocytes (□), neutrophils (+), and lymphocytes (△) with adherent platelets and for the percentage of unbound platelets expressing GMP-140 (★). The first 6 minutes for the percentage of leukocytes binding thrombin-activated platelets was 14.1%/min for monocytes, 5.1%/min for PMN, and 0.7%/min for lymphocytes, indicating that monocytes initially bound platelets at nearly three times the rate compared with PMN-platelet conjugate formation.

In addition to the percentages of monocytes and PMN with bound platelets after thrombin stimulation, Fig 1A also shows the simultaneous percentages of circulating (unbound) GMP-140-positive platelets. The percentage of free platelets expressing GMP-140 increased immediately after thrombin and corresponds with the initial increase in the percentage of monocytes and PMN with bound platelets. The percentage of free, activated platelets then decreased over the next 30 minutes while the percentage of monocytes and PMN with bound platelets continued to increase. The decrease in free activated platelets was not due to aggregate formation because RGDS completely inhibited platelet aggregation. In the unactivated sample (Fig 1B), a slight increase (7% to 18%) in the percentage of activated platelets late in the assay corresponded to a minimal rise in the percentage of monocytes binding platelets without any increase in the percentage of PMN or lymphocytes bound. Because CD15 has been postulated by one group of investigators to function as a receptor on monocytes and PMN for GMP-140 on activated platelets, we measured surface CD15 fluorescence on leukocyte subsets in this whole blood system. Thrombin stimulation had no effect on the surface density of leukocyte CD15, as measured by its mean fluorescence, shown in Table 2.

Figure 2 demonstrates an experiment similar to that of Fig 1 in which citrated whole blood with 1 mg/mL RGDS was preincubated with the Fab\(_\beta\) fragment of the anti–GMP-140 MoAb G1, at a concentration of 1 μg/mL. Thrombin stimulation of this sample showed no increase in the percentage of monocytes or PMN with adherent platelets.

### Table 2. Leukocyte CD15 Fluorescence After Agonist

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Monocytes</th>
<th>PMN</th>
<th>Lymph</th>
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<tr>
<td>Diluent</td>
<td>10.43 ± 0.16</td>
<td>373 ± 1.50</td>
<td>1.10 ± 0.12</td>
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<tr>
<td>Thrombin 0.1 U/mL</td>
<td>10.01 ± 0.20</td>
<td>380 ± 1.71</td>
<td>1.08 ± 0.10</td>
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</table>

*Ratio to control fluorescence ± standard error of the mean.
Thrombin stimulation of a control sample preincubated with the MoAb 1E3, which recognizes GMP-140 but has been shown not to block adhesion, resulted in increases in the percentage of monocytes and PMN with adherent platelets, similar to Fig 1A. In Fig 3, the mean platelet fluorescence per bound leukocyte was measured for the same experiment as Fig 1. This fluorescence corresponds in a relative fashion to the number of platelets bound to each leukocyte. Thrombin stimulation (Fig 3A) resulted in a gradual increase in the mean platelet fluorescence of monocytes and PMN with bound platelets; the final values for monocytes were more than twice as high as for PMN, indicating that more platelets bound per monocyte than per PMN. The platelet fluorescence per lymphocyte after thrombin and for all leukocytes in the control sample (diluent stimulation only, Fig 3B) did not change over time.

**Fig 3.** From the experiment detailed in Fig 1, the mean platelet fluorescence of each leukocyte-platelet conjugate was measured over time after thrombin (A) or diluent (B) stimulation. This fluorescence provides a semi-quantitative estimate of the number of platelets bound per leukocyte.

**Fig 4.** Whole blood anticoagulated with heparin was preincubated with RGDS (1 mg/mL). The sample was then incubated with epi 1 μmol/L for 10 minutes followed by ADP 5 μmol/L at time 0. (A) The percentage of leukocytes with bound platelets over the course of the assay. (B) The mean platelet fluorescence per bound leukocyte, an estimate of the number of platelets bound per leukocyte.

**epi/ADP stimulation of whole blood with RGDS peptide.** Formation of platelet aggregates was again inhibited by preincubating heparinized whole blood with RGDS peptide. The sample was incubated with epi for 10 minutes, followed by ADP stimulation at time 0 (Fig 4). Over the next 30 minutes, the percentage of monocytes with adherent platelets more than doubled (30% to 72%, Fig 4A), and the number of platelets bound per monocyte (as estimated by fluorescence in Fig 4B) also increased (200 to 850). However, unlike after thrombin, PMN-platelet conjugate formation did not increase either with respect to the percentage of cells binding platelets (21% to 24%, Fig 4A) or the number of platelets bound per cell (relative fluorescence 440 to 450, Fig 4B). Lymphocytes were again unaf-
fected. Although thrombin and epi/ADP resulted in similar percentages (72% v 69%) of free platelets expressing GMP-140 (Table 3), thrombin stimulation resulted in a fourfold higher surface expression of GMP-140 per platelet, as measured by fluorescence, when compared with the weaker agonist epi/ADP. Experiments performed using citrated whole blood yielded similar results.

**epi/ADP stimulation of whole blood without RGDS peptide.**

Figure 5 illustrates a representative study in which heparinized whole blood (without RGDS) at 37°C was incubated with 1 μmol/L epi followed by 5 μmol/L ADP at time 0 (Fig 5A) or no agonist (Fig 5B). RGDS peptide was not used to allow the formation of platelet-platelet aggregates. Because the combination of epi and ADP is a less potent platelet agonist than thrombin and there is little fibrin formation with epi/ADP, platelet-platelet interaction is reversible, and whole blood clotting does not occur despite the absence of RGDS. Figure 5A demonstrates the percentage of leukocytes with bound platelets over time after epi/ADP. The percentage of all leukocytes with bound platelets decreased markedly after ADP. The percentage of monocytes with adherent platelets subsequently increased over time, reaching a peak similar to that seen in Fig 4A. However, the percentage of PMN and lymphocytes with adherent platelets increased only slightly and did not fully recover to the preagonist level of binding. Figure 5B demonstrates that, in the absence of agonist, leukocyte-platelet binding changes minimally over the time of the assay, similar to Fig 1B. Experiments in which citrate was substituted for heparin as the anticoagulant and then stimulated with epi/ADP showed an identical pattern of leukocyte-platelet adhesion.

To further study this dynamic pattern of leukocyte-platelet adhesion after the addition of epi/ADP in a situation where platelet aggregate formation occurs, we simultaneously measured the percentages of: (1) free (not bound to leukocytes) GMP-140–positive platelets; (2) free platelet aggregates; and (3) leukocytes with bound platelets over time (Fig 6). In this experiment, 1 μmol/L epi was added to citrated whole blood 10 minutes before 5 μmol/L of ADP was added at time 0. Figure 6A demonstrates a slight decrease and a later increase in leukocyte-platelet binding after epi, and then a rapid decrease (like the experiment in Fig 5A) in the percentage of leukocytes with bound platelets after addition of ADP. The identical pattern of recovery of binding as observed in Fig 5 occurred in this representative experiment as well. Figure 6B demonstrates that immediately after ADP, the percentages of both free platelet aggregates and GMP-140–positive platelets increased dramatically. Furthermore, the peak percentages of platelet aggregates and GMP-140–positive platelets corresponded to the nadir of leukocyte-platelet binding. After this point, the percentage of free platelet aggregates and GMP-140–positive platelets then decreased as the simultaneous percentage of monocytes (and to a lesser extent PMN) with bound platelets increased.

In a separate experiment (Fig 7A), the inhibition of the recovery of leukocyte-platelet adhesion after epi/ADP was examined by preincubating whole blood (without RGDS) with the G1 MoAb to GMP-140 before stimulation with epi and ADP. In the presence of the G1-blocking MoAb, the agonist binding of leukocytes to platelets was unaffected. There was also no change in the immediate decrease in leukocyte-platelet adhesion after ADP. However, the recovery phase for leukocyte-platelet conjugates after the initial decrease in binding was nearly completely abolished. There was only a slight increase in the percentage of

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<th>Table 3. Expression of GMP-140 on Unbound Platelets</th>
<th>Platelet GMP-140 Expression</th>
<th>After Agonist</th>
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<tbody>
<tr>
<td>Agonist</td>
<td>Peak % of GMP-140 Positive Platelets</td>
<td>Mean GMP-140* Fluorescence</td>
</tr>
<tr>
<td>Epi/ADP</td>
<td>69</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>Thrombin</td>
<td>72</td>
<td>4.23 ± 0.42</td>
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*Ratio to control fluorescence ± standard error of the mean.
Fig 6. An experiment in which citrated whole blood in the absence of RGDS was stimulated with epi/ADP. Epinephrine was added at time \(-10\) minutes followed by ADP stimulation at time 0. (A) The percentage of leukocytes with bound platelets. (B) The simultaneous measurements of unbound GMP-140-positive platelets (B) and free platelet aggregates (C).

monocytes with adherent platelets, but this remained well below the preagonist levels. Platelet aggregate formation and dissociation was identical to that shown in Fig 6B, indicating that the G1 MoAb only blocked the GMP-140-dependent recovery of leukocyte-platelet adhesion and had no effect on platelet aggregates. In Fig 7B, the sample was preincubated with the nonblocking anti–GMP-140 MoAb 1E3. This demonstrated a pattern of binding in which there was no inhibition of the recovery of leukocyte-platelet adhesion over time, ie, identical to the experiment of Fig 6A.

DISCUSSION

Leukocyte-platelet adhesion is likely of physiologic importance not only for the targeting of both cell types to appropriate inflammatory and/or hemostatic sites but also for functional alterations in these cells. For example, metabolic cooperation between platelets and heterotypic blood cells has been postulated. Studies have demonstrated that platelets supply free cholesterol to monocytes for cholesteryl ester synthesis; such monocytes may then participate in foam cell generation seen in atherosclerotic lesions. Platelets have also been shown to provide prostaglandin endoperoxide H₂ to endothelial cells and lymphocytes for production of prostacyclin (PGI₂), which is a potent vasodilator and platelet inhibitor. Recent work has demonstrated that platelets synthesize leukotriene C₄ (LTC₄) from PMN-derived LTA₄; this cell-cell interaction for eicosanoid synthesis is aspirin-insensitive and independent of platelet activation. To further understand these interactions, model systems are necessary in which heterotypic and homotypic conjugate formation can occur together under circumstances similar to those found in vivo.
Isolated PMN and monocytes have been shown to bind activated and, to a lesser degree, unactivated platelet fractions. The kinetics of this adhesion and some responsible receptor-ligand pairs have been determined in vitro. However, the interactions between platelets and leukocytes have not previously been examined in whole blood. The current studies have determined the conditions under which heterotypic cell-platelet adhesion occurs in whole blood, the dynamic interaction between homotypic (platelet-platelet) and heterotypic (leukocyte-platelet) adhesion in whole blood, and the relative affinities of different leukocyte subsets for activated (GMP-140-positive) platelets.

Baseline studies demonstrated that unstimulated whole blood samples show both a minority of leukocytes with bound platelets and a low level of platelets bound per white blood cell. These results were similar to those observed when unstimulated isolated cell fractions were allowed to recombine in vitro; this included a similar percentage of both neutrophils and peripheral blood lymphocytes that bound resting platelets. However, monocytes in whole blood had a lower percentage of platelet binding (45%) compared with isolated cell fractions (87%) in which monocytes and platelets are recombined in the absence of red blood cells, plasma, and other leukocytes. In the whole blood assay, the presence and much higher concentration of other leukocytes that bind unactivated platelets, albeit less effectively than monocytes, would be expected to decrease monocyte-platelet adhesion when compared with the isolated fraction assay. It is also interesting to note that, in whole blood, RGDS peptide reduced the percentages of all leukocytes with adherent unactivated platelets by more than 50%, whereas in isolated cell fraction experiments, RGDS had no effect on unactivated platelet binding to leukocytes. One important difference between isolated cell fraction studies and these whole blood experiments is the lack of plasma fibrinogen in the former. Because RGDS inhibits fibrinogen binding to the GPIIb/IIIa integrin receptor, it is possible that fibrinogen-integrin interaction plays a role in unactivated platelet adhesion to leukocytes in whole blood. When isolated cell fractions are studied, only platelet and leukocyte-associated fibrinogen is available for such an interaction and thus the effect of added RGDS in isolated fractions is minimal. It is also possible that RGDS may lower the baseline percentage of leukocyte-platelet binding through inhibition of platelet-platelet interaction. If platelet-platelet conjugates are present on the leukocyte surface at baseline, their aggregate platelet marker fluorescence will determine the lower limits of detection of platelet binding, especially since there may be some fluorescent quenching by adjacent erythrocytes in this whole blood assay. If RGDS dissociates these platelet conjugates on the cell surface, then the aggregate fluorescence is decreased, lowering the measured percentage of leukocyte binding.

When platelets were stimulated with thrombin in whole blood under conditions that preclude formation of platelet aggregates (RGDS), our studies have shown that: (1) platelets rapidly express GMP-140; (2) monocytes rapidly bind platelets and continue to increase their cumulative platelet binding as the number of available circulating activated platelets decreases; (3) PMN bind platelets at about one third the rate of monocytes and in far fewer numbers of platelets per PMN; (4) lymphocyte-platelet adhesion is not changed by thrombin; and (5) all of the increased monocyte and PMN adhesion of platelets after thrombin is dependent on the epitope of platelet GMP-140, which is recognized by the G1 MoAb but not the epitope recognized by 1E3. These findings are consistent with data reported in isolated fraction experiments and present further evidence for a competitive advantage of monocytes over PMN in both the initial binding of GMP-140-positive platelets and the cumulative binding of platelets, ie, the eventual number of platelets bound per leukocyte. This advantage of monocytes over PMN is particularly striking in view of the log-higher concentration of PMN in whole blood. The advantage of monocytes over PMN is further supported by the increase in monocyte-platelet conjugate formation observed after epi/ADP with no increase for PMN-platelet adhesion. This competitive advantage of monocytes over PMN for platelet conjugate formation may be exaggerated when platelets are stimulated to express relatively less surface GMP-140 per platelet compared with the expression of this receptor on platelets after thrombin stimulation (Table 3).

The advantage of monocytes over PMN in binding activated platelets cannot be currently explained by data regarding the postulated receptor on PMN and monocytes for binding GMP-140. The identity of this receptor is controversial. Larsen et al have found evidence that CD15, a nonsialated penta-sugar not present on resting lymphocytes, is a leukocyte ligand for PADGEM/GMP-140. However, other investigators have not found conclusive evidence that CD15 is the GMP-140 receptor and have additionally shown that the leukocyte receptor for GMP-140 is likely a sialated glycoprotein, unlike CD15. Our studies suggest a competitive advantage of monocytes over PMN for adhesion to activated platelets, an intriguing finding in this regard because there is a 30-fold higher expression of CD15 per PMN compared with monocytes. Our data suggest either that CD15 is not the receptor for GMP-140-positive platelets or that qualitative differences between monocyte and PMN CD15 exist with respect to GMP-140 binding (a less likely alternative in view of the small size of the CD15 sugar).

To determine the competitive dynamics of platelet-platelet versus leukocyte-platelet adhesion in whole blood, epi/ADP in the absence of RGDS was used to cause reversible platelet aggregation. Our studies demonstrated that: (1) initial platelet activation resulted in preferential platelet-platelet adhesion with a simultaneous decrease in the percentage of all leukocytes with bound platelets; (2) as the platelet aggregates spontaneously dissociated, the activated platelets returned to heterotypic cell adhesion over time; (3) monocytes again had the advantage over PMN for this recovery of platelet adhesion; and (4) the G1 (blocking) MoAb, but not the 1E3 (nonblocking) MoAb, to GMP-140 prevented the recovery of leukocyte-platelet adhesion at a time when platelet aggregates were dissociating. These studies suggest that early after the addition of agonist, adherent platelets dissociate from monocytes, PMN, and lymphocytes to undergo homotypic binding (aggregation).
mediated by an RGDS binding site revealed by the agonist. As platelet aggregates dissociate, the GMP-140–positive platelets readhere to monocytes and to a lesser degree to PMN, but not to lymphocytes. This is consistent with studies demonstrating that resting lymphocytes do not bind GMP-140 or activated platelets. It is important to note that the setting in which the dynamics of leukocyte-platelet adhesion are determined in this study differs from in vivo conditions by several factors. Because these experiments were performed under relatively static conditions, the crucial effects of blood flow and shear rate are not taken into account when cell-cell interactions are measured. In addition, perturbations introduced by endothelial injury or inflammation are not accounted for in this study and may significantly affect the interactions between blood cells described. It will be instructive for future studies to determine similar leukocyte-platelet and homotypic platelet interactions under more physiologic conditions.

As noted earlier, metabolic cooperation has been shown to occur between platelets and heterotypic blood cells; presumably these interactions require some ability for these cells to adhere or at least remain in close proximity for a time. These whole blood studies have demonstrated that monocytes have a competitive advantage over PMN for binding to activated platelets and that platelet-platelet adhesion is initially favored over platelet-leukocyte adhesion after addition of a platelet agonist. Subsequent platelet aggregate dissociation allows GMP-140–expressing platelets to readhere to monocytes and, to a lesser extent, to PMN. The functional consequences of such adhesion are not yet known, but the dynamics of interaction between hemostatic and inflammatory cells provide a basis for studying relevant pathophysiologies.

REFERENCES

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