Mechanisms of platelet and leukocyte recruitment in experimental colitis


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Both leukocytes and platelets accumulate in the colonic microvasculature during experimental colitis, leading to microvascular dysfunction and tissue injury. The objective of this study was to determine whether the recruitment of leukocytes and platelets in inflamed colonic venules are codependent processes. The rolling and adherence of leukocytes and platelets in colonic venules of mice with dextran sodium sulfate (DSS)-induced colitis were monitored by intravitral videomicroscopy. DSS elicited an increased recruitment of both rolling and adherent leukocytes and platelets. DSS-colitic mice rendered thrombocytopenic with anti-platelet serum exhibited profound reductions in leukocyte adhesion. Neutropenia, induced with anti-neutrophil serum, significantly reduced the adhesion of leukocytes and the accumulation of platelet-leukocyte aggregates while greatly enhancing the number of platelets that roll and adhere directly to venular endothelial cells. The enhanced platelet adhesion associated with neutropenia was mediated by platelet P-selectin interactions with endothelial cell P-selectin glycoprotein ligand (PSGL-1). DSS colitis was also associated with an increased expression of PSGL-1 in the colonic vasculature. These findings indicate that the recruitment of leukocytes and platelets in inflamed colonic venules are codependent processes.

Platelets; leukocytes; intravitral microscopy; P-selectin; P-selectin glycoprotein ligand-1

It is now well recognized that the number and function of circulating platelets are altered in patients with inflammatory bowel disease (IBD) (9). The thrombocytosis associated with IBD is accompanied by an increased expression of activation markers, such as P-selectin and CD40 ligand, on platelets and an increased appearance of platelet-leukocyte aggregates in venous blood draining the inflamed bowel (16, 27). Studies in animal models of IBD have revealed that platelets accumulate within venules of the inflamed colon (22), with platelets binding directly to venular endothelium and to leukocytes that are already adherent to the vessel wall (29). Although the pathophysiological significance of platelet accumulation in the inflamed bowel remains poorly understood, there is evidence suggesting that platelets may contribute to the thrombosis that has been described in the bowel wall during human IBD and that platelets may amplify the inflammatory response (3, 7, 11, 36). A role for platelets in the amplification of gut inflammation is supported by reports describing an enhanced production of superoxide by isolated neutrophils when incubated with platelets isolated from patients with ulcerative colitis, an effect that is blocked by a P-selectin MAb (28). Further support is provided by the observation that coculture of the P-selectin-positive platelets from IBD patients with monolayers of human intestinal microvascular endothelial cells (HIMEC) results in increased IL-8 production and expression of VCAM-1 and ICAM-1 on HIMEC (8). These studies demonstrate the ability of platelets derived from IBD patients to activate both neutrophils and endothelial cells, thereby amplifying the inflammatory response.

Relatively little is known about the factors that regulate platelet recruitment into the inflamed bowel. In murine colitis induced by dextran sodium sulfate (DSS), the accumulation of adherent platelets in venules is temporally correlated with the appearance of adherent leukocytes and with disease severity (22). With the use of blocking antibodies and adhesion molecule-deficient mice, it was also revealed that the recruitment of both platelets and leukocytes in venules of DSS-colitic mice are largely mediated by P-selectin:P-selectin glycoprotein ligand-1 (PSGL-1) interactions. The parallel reductions in platelet and leukocyte accumulation noted in inflamed colonic venules following adhesion-molecule blockade and with other interventions suggest that the recruitment of platelets and leukocytes may be interdependent processes (22, 29, 31). Such interdependence of blood-cell recruitment has been previously demonstrated in intestinal venules exposed to ischemia-reperfusion, wherein thrombocytopenia attenuates the recruitment of adherent leukocytes and neutrophilia reduces platelet adhesion (5, 26). Consequently, a major objective of the present study was to determine whether the accumulation of platelets in colonic venules during experimental IBD is dependent on neutrophils and whether neutrophil recruitment is dependent on platelets.

Previous studies of platelet adhesion in inflamed colonic venules implicate P-selectin:PSGL-1 interactions in this recruitment process (22). Although an increased expression of P-selectin in the colonic vasculature has been demonstrated in colitic mice (31), it remains unclear whether the increased P-selectin expression on platelets previously described in human IBD is recapitulated in experimental colitis. Although it has been proposed that P-selectin-dependent platelet accumulation in colitic venules reflects the binding of platelet P-selectin to PSGL-1 that is constitutively expressed on adherent leukocytes (18), recent studies indicate that activated endothelial cells also have the capacity to express PSGL-1 (6, 25). This raises the possibility that PSGL-1 may be upregulated on the surface of colonic venules during experimental IBD and that this expression may mediate platelet recruitment via a leukocyte-indepen-
dent mechanism. Hence, another objective of this study was to quantify the expression of PSGL-1 in the colon during DSS colitis and to determine whether this adhesion molecule can sustain platelet adhesion in the absence of neutrophils.

**MATERIALS AND METHODS**

**Mice.** The animals used in the experiments were 6- to 8-wk-old male C57BL/6J mice [wild-type (WT) control strain], P-selectin knockout (P-selectin−/−), and PSGL-1−/− mice (all developed on a C57BL/6J background), obtained from Jackson Laboratory (Bar Harbor, ME). All experimental procedures were performed according to the criteria outlined by the National Institutes of Health. The experiments were approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee.

**Induction of colitis.** Mice were fed 3% DSS (molecular weight 40 kDa; ICN Biomedicals, Aurora, OH) dissolved in filter-purified (Millipore, Bedford, MA) drinking water ad libitum for 7 days (days 0–6) (23). Control mice received filtered water only. In a previous study, we (30) have demonstrated that in DSS-induced colitis, the severity of inflammation is DSS load dependent and that a critical DSS load ≥30 mg DSS/g body wt is required to reliably induce colitis in the C57BL/6J strain. Therefore, mice that did not meet the DSS load criteria were excluded from the study. All experiments were performed on day 6 of DSS administration.

**Platelet preparation.** Platelets were isolated from untreated donor mouse (WT, P-selectin−/−, or PSGL-1−/− mice) by using a series of centrifugation steps as described previously (4, 31). Finally, platelets were labeled with the fluorochrome carboxyfluorescein diacetate succinimidyl ester (CFSE, 90 mM final concentration; Molecular Probes, Eugene, OR) and were protected from light. Leukocytes accounted for <0.01% of the cells in the platelet suspension. The number of labeled platelets obtained from one donor mouse was sufficient for infusion into two recipients.

**Surgical preparation.** Animals were anesthetized with ketamine hydrochloride (150 mg/kg im) and xylazine (7.5 mg/kg im). The right carotid artery was cannulated for blood-pressure monitoring by using a disposable pressure transducer (Cobe Laboratories, Lakewood, CO) attached to a pressure monitor (BP-1; World Precision Instruments, Sarasota, FL). The right jugular vein was cannulated for infusion of rhodamine-6G (Sigma-Aldrich) for leukocyte labeling and for subsequent infusion of CFSE-labeled platelets. A laparotomy was performed, and the proximal large bowel (initial 2–3 cm adjacent to the cecum) was exteriorized and continuously superfused at 37°C with bicarbonate-buffered saline solution (pH 7.4).

**Intravital fluorescence microscopy.** Platelets and leukocytes in the colonic microcirculation were visualized with an inverted Nikon microscope (Nikon, Tokyo, Japan) equipped with a 75-W XBO xenon lamp, as described previously (29). The mice received rhodamine-6G over 5 min, which was allowed to circulate for 5 min. Subsequently, 100 × 10⁶ CFSE-labeled platelets were infused over 5 min and were allowed to circulate for 5 min before intravital observation. After the circulation time, the interactions of both leukocytes (excitation, 525 nm; emission, 555 nm) and platelets (excitation, 490 nm; emission, 518 nm) in colonic venules were recorded simultaneously, which required a Nikon filter block with an excitation filter (470–490 nm), a dichroic mirror (510 nm), and a barrier filter (520 nm). Five randomly selected postcapillary venules (20- to 40-μm diameter) in each colon preparation were recorded for 1 min each.

**Offline video analysis.** Platelets and leukocytes were classified according to their interaction with the venular wall as free-flowing, rolling (when cells were slower than centreline blood flow), or adherent (when cells remain stationary for ≥30 s). We determined whether a platelet or leukocyte was adherent directly to the endothelium or attached to the vessel wall by binding to each other. Platelet and leukocyte rolling were expressed as number of rolling cells per second per millimeter of vessel diameter, and their adherence was expressed as the number of cells per square millimeter of venular surface, calculated from diameter and length, assuming cylindrical vessel shape (20). Platelet interactions were termed as “platelet-endothelial” or “platelet-leukocyte” on the basis of whether the labeled platelets were interacting directly with vascular endothelium or with rolling or adherent leukocytes, respectively. Leukocyte interactions were termed “platelet-free” or “platelet-bearing” on the basis of whether the leukocytes were not associated with labeled platelets or were interacting with labeled platelets, respectively.

**In vivo measurement of PSGL-1 expression in the colonic vasculature.** The expression of PSGL-1 on vascular endothelial cells was measured in WT mice on day 6 of H₂O or DSS treatment with the use of the dual-radiolabeled MAb technique as described elsewhere in detail (12, 33). The MAb used for in vivo assessment of PSGL-1 expression were 2PH1, a purified binding ovalbumin-conjugated peptide covering amino acids 42–60 of mouse PSGL-1 [20 μg anti-PSGL-1 (CD162) per mouse; BD Pharmingen, San Diego, CA] and P-23, a nonspecific, nonbinding murine IgG1 directed against human P-selectin (provided by Dr Donald C. Anderson, Pharmacia-Upjohn, Kalamazoo, MI). The binding (2PH1) and nonbinding (P-23) MABs were labeled with 125I and 131I, respectively (DuPont-NEN, Boston, MA) with the iodogen method as described previously (13, 15). Receptor levels in the various vascular beds were expressed as nanograms of MAb per gram of tissue (12, 33).

**Analysis of platelet surface P-selectin and PSGL-1 by fluorescence-activated cell sorter.** To confirm the absence or presence of platelet-associated surface P-selectin and PSGL-1, flow cytometry was performed on whole blood diluted 1:10 with PBS. Samples were incubated with fluorescein isothiocyanate (FITC)-labeled anti-mouse P-selectin (rat anti-mouse CD62P, clone RB4.34), or a combination of phycoerythrin-labeled anti-mouse PSGL-1 (purified rat anti-mouse CD162, clone 2PH1) and FITC-labeled rat anti-mouse GPIbIIa (the latter was used to positively identify the platelet population) (BD Pharmingen). Cells were incubated with the antibodies, washed, and resuspended for analysis by using a FACSCalibur flow cytometer with CellQuest software (Becton Dickinson, Mountain View, CA). The platelet population was gated according to size and expression of GPIbIIa.

**Experimental protocols.** In the first series of experiments, we determined the contribution of platelets to the rolling and adhesion of leukocytes in DSS-treated mice rendered thrombocytopenic with an intraperitoneal injection of anti-platelet serum (APS; 125 μg/kg body wt) 24 h before intravital microscopy (n = 6). Treatment with APS reduced the number of circulating platelets by >92% without significantly reducing the number of circulating leukocytes, as confirmed by manual measurements of cells with a hemocytometer. In addition, we assessed the effects of neutrophil depletion on leukocyte and platelet adhesion in DSS colitis. Antineutrophil serum (ANS) (RB68C5, kindly provided by Stephen B. Pruett, Louisiana State University Health Care System, Shreveport, LA) was administered intraperitoneally 24 h before intravital microscopy (n = 6). Treatment with ANS reduced the number of circulating neutrophils by >95% while reducing the number of circulating platelets by 15%. Additional groups of neutropenic DSS-colitic mice were also treated intravenously with blocking MABs (BD Pharmingen) directed against either P-selectin (RB40.34, 2 mg/kg, n = 6) or PSGL-1 (2PH1; 2 mg/kg, n = 6) 30 min before intravital recording. In two additional groups, colitic neutropenic mice received CFSE-labeled platelets from P-selectin−/− (n = 6) or PSGL-1−/− (n = 5) mice instead of the WT platelets. The results were compared with data from 10 untreated DSS mice and 5 control mice receiving water.

In a second series of experiments, PSGL-1 expression in the colonic vasculature was quantified in control (n = 7) and DSS-colitic (n = 7) mice by using the dual-radiolabeled MAB method. A third series of experiments was performed to quantify the expression of P-selectin and PSGL-1 on platelets isolated from untreated and DSS-treated mice at day 6 by using flow cytometry.
**RESULTS**

Leukocyte recruitment in experimental colitis is platelet dependent. Figure 1 summarizes the changes in rolling (Fig. 1A) and adherent (Fig. 1B) leukocytes that occur in response to DSS treatment and illustrates how thrombocytopenia induced by APS alters the leukocyte responses. DSS elicited significant increases in the number of rolling and adherent leukocytes. APS treatment significantly reduced the DSS colitis-associated recruitment of both rolling and adherent leukocytes, suggesting that platelets contribute to the leukocyte response.

Neutropenia reduces platelet-leukocyte interactions while enhancing platelet-endothelial cell interactions. Figure 2 summarizes the changes in the rolling (Fig. 2A) as well as the adherence of platelets (Fig. 2B) in colonic venules during DSS colitis in the presence and absence of neutropenia. Most of the platelets that rolled (Fig. 2A) and firmly adhered (Fig. 2B) in colonic venules during DSS colitis were attached to leukocytes, with few changes in the number of platelets rolling or being firmly adherent directly on the endothelium. Neutropenia resulted in significant increases in the rolling and adherence of platelets directly on venular endothelium while reducing the interactions between platelets and leukocytes.

DSS-treated mice showed a significantly enhanced recruitment of rolling (Fig. 3A) and firmly adherent (Fig. 3B) leukocytes, with ~30% of rolling leukocytes and slightly more than half of the adherent leukocytes bearing platelets, and the remainder were free of attached CFSE-labeled platelets. Neutropenia attenuated the DSS-induced recruitment of both rolling and adherent leukocytes, with reductions noted for both platelet-free and platelet-bearing leukocytes.

Platelet-endothelial rolling and adhesion is dependent on P-selectin and PSGL-1. To define the molecular determinants of the enhanced platelet-endothelial rolling and adherence noted in DSS-colitic mice that were rendered neutropenic with ANS, the adhesion responses were studied following treatment with P-selectin and PSGL-1 MAbs. Figure 4 demonstrates that the ANS-enhanced platelet-endothelial cell interactions were largely abolished following administration of either the P-selectin or PSGL-1 MAb. Similar reductions in platelet-endothelial rolling (Fig. 4A) and adherence (Fig. 4B) were noted in ANS-treated mice receiving fluorescently labeled platelets from P-selectin−/− mice, rather than WT platelets. However, no significant attenuation was observed in mice receiving platelets from PSGL-1−/− mice. Administration of either the P-selectin or PSGL-1 MAb abolished the low residual leukocyte rolling and adherence that occurred in ANS-treated colitic mice (data not shown). No such reductions were noted in ANS-treated mice receiving fluorescently labeled platelets from either P-selectin−/− or PSGL-1−/− mice (data not shown).

Expression of PSGL-1 in the colonic vasculature during DSS-induced inflammation. The expression of PSGL-1 on colonic endothelial cells was measured in the proximal and distal colon of control (H2O) and 3% DSS-treated WT mice. PSGL-1 expression was significantly increased (compared with H2O controls) in both vascular beds in response to DSS treatment (Fig. 5).

P-selectin, but not PSGL-1, exhibits an increased expression on platelets from DSS colitic mice. Flow-cytometric measurements of P-selectin and PSGL-1 expression on platelets isolated from mice with DSS colitis revealed a highly significant 50-fold increase in P-selectin expression compared with their water-treated counterparts (Fig. 6). The expression of PSGL-1, on the other hand, was not detected on platelets from mice treated with either water or DSS (Fig. 6).

**DISCUSSION**

There is emerging evidence that supports an intimate connection between inflammatory and hemostatic mechanisms in different inflammatory diseases, including IBD (10). Indeed, the evidence supporting a link between inflammatory and thrombotic processes suggests that thrombosis is involved either in the initiation and/or progression of IBD (17, 34). Studies of human IBD and animal models of colitis have revealed an association between the recruitment of platelets and leukocytes in the inflamed bowel that is manifested by the
appearance of platelet-leukocyte aggregates in mesenteric and systemic venous blood, as well as by the binding of platelets to adherent leukocytes in colonic venules (3, 16, 29). Although these cell-cell interactions have been described in several reports, the codependency of platelet and leukocyte recruitment in microvessels of the inflamed colon has not been previously addressed. The present study reveals that platelets exert a profound influence on the recruitment of leukocytes in DSS-induced colitis and that the number of circulating neutrophils also influences the nature and magnitude of platelet adhesion with both endothelial cells and leukocytes. Our findings provide novel insights into how adhesion processes related to inflammation and coagulation combine to amplify the inflammatory phenotype that is assumed by the colonic vasculature during experimental colitis.

Using intravital microscopy to simultaneously monitor the trafficking of fluorescently labeled platelets and leukocytes in DSS colitis, we noted that the overwhelming majority of platelets accumulate on the venular wall by attaching to rolling or firmly adherent leukocytes, with a smaller percentage of platelets binding directly to venular endothelium. The platelet-leukocyte complexes that we visualized in colonic microvessels likely reflect a precursor of the free-flowing platelet-leukocyte aggregates that have been detected at increased levels in the blood of patients with IBD and other inflammatory and thrombotic states, including acute coronary syndromes, stroke, and type 1 diabetes mellitus (35). The pathophysiological significance of platelet-leukocyte aggregates remains poorly understood; however, it has been suggested that aggregates may represent an important source of inflammatory mediators that can sustain or amplify inflammatory responses (7).

The presence of rolling and firmly adherent platelet-leukocyte aggregates in colonic venules during DSS colitis was profoundly reduced in mice rendered neutropenic, suggesting that neutrophils are the dominant leukocyte population binding to platelets in this model. Monocytes and, to a lesser extent, T-lymphocytes are also known to form aggregates with platelets (19, 21), which may account for the residual platelet-leukocyte interactions observed in neutropenic colitic mice. Another interesting and potentially important consequence of

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**Fig. 2.** Rolling (A) and firmly adherent (B) platelets in postcapillary venules of WT mice after 7 days on H2O (n = 5), WT mice on DSS (n = 10), and WT mice on DSS + antineutrophil serum (ANS) 24 h before intravital microscopy (n = 6). Platelet interactions are defined as platelet-leukocyte (platelets bound to leukocytes) and platelet-endothelial (platelets bound directly to endothelium) interactions. *P < 0.05 relative to H2O; #P < 0.005 relative to WT mice treated with DSS.

**Fig. 3.** Rolling (A) and firmly adherent (B) leukocytes in postcapillary venules of WT mice after 7 days on H2O (n = 5), WT mice on DSS (n = 10), and WT mice on DSS + ANS 24 h before intravital microscopy (n = 6). Types of leukocyte interactions are separated into number of platelet-bearing vs. platelet-free rolling and adherent leukocytes. *P < 0.05 relative to H2O; #P < 0.005 relative to WT mice treated with DSS.
neutropenia in the DSS model is the remarkable increase in the number of platelets that directly bind to venular endothelial cells. One possible explanation is that activated neutrophils, via adhesion-dependent or -independent mechanisms, tend to promote an anti-thrombogenic phenotype in endothelial cells. Therefore, in the absence of circulating neutrophils, platelet adherence is increased. This appears unlikely because activated neutrophils liberate a variety of substances (e.g., superoxide, proteases) that are more likely to enhance platelet-endothelial cell adhesion (1). Another possibility is that the neutrophils that adhere to endothelial cells occupy or cover receptors that would normally sustain the adhesion of platelets. Support for this possibility comes from our effort to evaluate the contribution of P-selectin:PSGL-1 interactions to the platelet and leukocyte adhesion that occurs in our model. The neutropenia-induced enhancement of platelet-endothelial cell adhesion was completely prevented by treatment with blocking antibodies directed against P-selectin and its receptor, PSGL-1. Furthermore, although platelets from WT and PSGL-1−/− mice exhibited comparably high levels of platelet-endothelial cell adhesion in venules of neutropenic mice, P-selectin-deficient platelets exhibited minimal adhesion to endothelial cells. These findings, coupled to our flow-cytometric data demonstrating increased P-selectin, but not PSGL-1, expression on circulating platelets in DSS-treated mice, suggest that the platelet-endothelial cell adhesion that is revealed in neutropenic mice with DSS colitis is mediated by platelet P-selectin binding to PSGL-1 on endothelial cells.

PSGL-1 has received much attention as a leukocyte receptor for platelet P-selectin. Until recently, the expression of PSGL-1 on endothelial cells has been considered to be minimal and unimportant. However, a recent report (25) described PSGL-1

Fig. 4. Rolling (A) and adhering (B) platelets in post-capillary venules of WT mice after 7 days on H2O (n = 5), WT mice on DSS (n = 10), WT mice on DSS + ANS (n = 6), DSS in WT + ANS + blocking monoclonal antibodies for either P-selectin (n = 6) or P-selectin glycoprotein ligand-1 (PSGL-1; n = 6), and DSS in WT + ANS + infusion of platelets from either P-selectin−/− (n = 6) or PSGL-1−/− (n = 5) mice. Shown are number of platelets bound to leukocytes (platelet-leukocyte interactions) and those bound directly to endothelium (platelet-endothelial interactions). *P < 0.05 relative to H2O; #P < 0.05 relative to WT mice treated with DSS; and &P < 0.05 relative to WT-DSS mice treated with ANS 24 h before intravital microscopy.
expression in venules of mesenteric lymph nodes and small intestine in mice and demonstrated a major role for endothelial PSGL-1 in the development of chronic murine ileitis. A subsequent study demonstrated that PSGL-1 is constitutively expressed on monolayers of human umbilical vein endothelial cells (HUVEC), allowing HUVEC to sustain the tethering and firm adhesion of platelets as well as monocytes (6). The present study provides the first quantitative evidence of PSGL-1 expression in the intact colonic vasculature and demonstrates that DSS colitis is associated with a highly significant increase in PSGL-1 expression in the colonic vasculature. The PSGL-1 upregulation noted in our DSS model contrasts the results from HUVEC monolayers that indicate significant constitutive PSGL-1 expression, with no change in expression following cytokine challenge (6). We cannot exclude the possibility that the elevated vascular PSGL-1 expression is due to adherent leukocyte remnants that express PSGL-1, rather than an upregulation of endothelial PSGL-1. Although leukocyte fragments are not detectable with our current methodologies, we have previously used bone-marrow chimeras to obtain preliminary evidence in an LPS model that supports the possibility that endothelial PSGL-1 levels can be upregulated (32). Additional work is needed to define the factors that regulate the expression of endothelial PSGL-1 during gut inflammation.

Another novel finding of the present study is the dramatic inhibitory effect of thrombocytopenia (induced by APS treatment) on the rolling and firm adherence of leukocytes in inflamed colonic venules. This observation suggests that platelets play a major role in inducing the inflammatory phenotype that is assumed by leukocytes and/or venular endothelial cells during DSS colitis. There are several possible explanations for this dependency of leukocyte adhesion on platelets. One possibility that was offered to explain the dependency of ischemia-reperfusion-induced leukocyte adhesion on platelets in rat mesenteric vessels was the binding of leukocytes (using PSGL-1) to a P-selectin platform created by platelets that adhere to venular endothelium (26). This explanation seems less likely in the DSS model because so few platelets were seen to adhere directly to venular endothelium. Another possibility is that the binding of platelets to leukocytes in colonic venules results in an enhanced leukocyte-activation state that increases the avidity and/or expression of adhesion molecules on the leukocytes. This possibility is consistent with a report describing enhanced superoxide production by isolated neutrophils that are incubated with platelets isolated from patients with ulcerative colitis (28). Interestingly, a recent study highlighted the role of platelet-neutrophil interactions in promoting neutrophil extracellular trap (NET) formation (2). These NETs are a potential source of proteases that could contribute to the vascular injury during colitis and may help explain how blocking P-selectin abrogates the DSS-induced increase in vascular permeability (22). However, it remains unclear whether NETs are generated in the inflamed colonic microvasculature and whether P-selectin:PSGL-1 interactions support their formation.

Finally, mediators (e.g., cytokines, chemokines) released by activated platelets may enhance the activation state of venular endothelial cells, leading to a further increase in endothelial cell adhesion molecule expression and more leukocyte adhesion. Support for this possibility is provided by the observation that coculture of the P-selectin-positive platelets from IBD patients with monolayers of HIMEC results in increased IL-8 production and expression of VCAM-1 and ICAM-1 on HIMEC (8). It remains to be determined whether any or all of these mechanisms underlie the dependency of leukocyte adhesion on platelets in inflamed colonic venules.

In conclusion, the results of this study indicate that the recruitment of leukocytes and platelets in inflamed colonic venules are interdependent processes. The accumulation of both blood cell populations in colonic venules involves the
interaction between P-selectin, expressed on endothelial cells and platelets, with PSGL-1, expressed on the surface of leukocytes and endothelial cells. Studies on the efficacy of P-selectin blockade in reducing both the inflammatory response and clinical manifestation of colitis have yielded contrasting results, with immunoblockade producing a better outcome than P-selectin-deficient mice, perhaps due to genetic compensations in the mutant mice (14). Although clinical indices of disease severity have not been evaluated in PSGL-1-deficient mice, PSGL-1 antibody treatment significantly attenuates disease activity as well as inflammation in DSS colitis (22, 24). Our findings, coupled to these published observations, indicate that platelets and P-selectin:PSGL-1 adhesive interactions may represent a novel and effective therapeutic target for modulation of the inflammatory cell infiltration associated with IBD.

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REFERENCES