Molecular determinants of the prothrombogenic phenotype assumed by inflamed colonic venules

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Molecular determinants of the prothrombogenic phenotype assumed by inflamed colonic venules. Am J Physiol Gastrointest Liver Physiol 288: G920–G926, 2005. First published November 18, 2004; doi:10.1152/ajpgi.00371.2004.—Although platelets have been implicated in the pathogenesis of human inflammatory bowel diseases, little is known about the magnitude of platelet accumulation in the inflamed bowel, what regulates this process, and its relevance to the overall inflammatory response. In this study, intravital video microscopy was used to monitor the trafficking of platelets and leukocytes and vascular permeability in colonic venules during the development of colonic inflammation induced by 3% dextran sodium sulfate (DSS). Blocking antibodies directed against different adhesion molecules as well as P-selectin-deficient mice were used to define the adhesive determinants of DSS-induced platelet recruitment. DSS induced an accumulation of adherent platelets that was temporally correlated with the appearance of adherent leukocytes and with disease severity. Platelet adhesion and, to a lesser extent, leukocyte adhesion were attenuated by immunoblockade of P-selectin and its ligand P-selectin glycoprotein ligand-1 (PSGL-1), with contributions from both platelet- and endothelial cell-associated P-selectin. DSS induced a rapid and sustained increase in vascular permeability that was greatly attenuated in P-selectin-deficient mice. P-selectin bone marrow chimeras revealed that both endothelial cell- and platelet-associated P-selectin contribute to the P-selectin expression detected in the inflamed colonic microvasculature, with endothelial P-selectin making a larger contribution. Our findings indicate that colonic inflammation is associated with the induction of a prothrombogenic phenotype in the colonic microcirculation, with P-selectin and its ligand PSGL-1 playing a major role in the recruitment of platelets.

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There is increasing recognition that platelets are not only involved in hemostasis and thrombosis but can also modulate acute and chronic inflammatory responses. Indeed, there are several lines of evidence that support an intimate connection between the inflammatory and hemostatic systems in the chronically inflamed intestine. For example, patients with inflammatory bowel disease (IBD) exhibit thrombocytosis, and the elevated platelet count has been used as a marker of disease activity (5, 6). These patients also exhibit an increased risk of thromboembolism, and their platelets appear to circulate in an activated state and more readily form homotypic (platelet-platelet) and heterotypic (platelet-leukocyte) aggregates both in vitro and in vivo (5–7). The circulating activated platelets not only express more P-selectin on the cell surface but also shed this adhesion glycoprotein into blood plasma, which accounts for the much higher levels of circulating soluble P-selectin that are detected in IBD patients relative to healthy subjects (17, 34). In addition to the enhanced thrombogenic potential that exists in IBD, there is evidence that platelets represent an important potential source of inflammatory mediators that can modulate the activation state of inflammatory cells as well as vascular endothelial cells (24).

The microcirculation plays an important role in the recruitment of different circulating blood cell populations into inflamed tissue (22, 29). This role is achieved through phenotypic changes in vascular endothelial cells that lead to a more proadhesive surface, which allows for blood cell attachment and extravasation. Adhesion glycoproteins (e.g., P-selectin, ICAM-1) expressed on the surface of activated endothelial cells can mediate the recruitment of different blood cell populations, including leukocytes and platelets. This likely explains the growing body of evidence that the vascular endothelium in acutely or chronically inflamed tissue can assume both an inflammatory and prothrombogenic phenotype, leading to the recruitment of both leukocytes and platelets (4, 26, 32). The presence of capillary thrombi has been identified in biopsies from patients with IBD (11), suggesting that platelets are recruited during disease progression. Although clinical and experimental evidence indicates that there may be a pathogenic role for platelets in intestinal inflammation, relatively little is known about the nature, time course, and mechanisms underlying the accumulation of platelets in the inflamed intestine.

The overall objective of this study was to determine whether and how platelets accumulate in the colonic microvasculature during the development of inflammation in an animal model of experimental colitis. Platelet- and leukocyte-vessel wall interactions were monitored in murine colonic microvessels at different times following the induction of dextran sodium sulfate (DSS)-induced colitis. The kinetics of platelet and leukocyte adhesion in colonic venules was compared with the development of clinical signs of disease activity and colonic microvascular dysfunction (increased vascular permeability). The molecular determinants of the colitis-associated platelet and leukocyte adhesion were also evaluated. The study findings implicate P-selectin and its ligand P-selectin glycoprotein ligand-1 as major determinants of the platelet and leukocyte
adhesion observed in inflamed colonic venules and are consistent with clinical evidence showing a correlation between platelet activation and disease severity in intestinal inflammation.

MATERIALS AND METHODS

Mice. The animals used in the experiment were 6- to 8-wk-old male C57Bl/6J mice [wild-type (WT) control strain], CD45 congenic B6.SJL-PTPRCPEB/BOY mice (for bone marrow chimeras), and P-selectin knockout (P-sel−/−) mice (B6.129S7-SELPTMLBAY) developed on a C57Bl/6 background, obtained from Jackson Laboratory (Bar Harbor, ME). Animals were housed under specific pathogen-free conditions in standard cages and were fed standard laboratory chow and water ad libidum until the desired age. The experimental procedures were performed according to the criteria outlined in the National Institutes of Health (NIH) and were approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee.

Induction of colonic inflammation and assessment of inflammation. Colonic inflammation was induced by administration of 3% DSS (molecular mass, 40 kDa; ICN Biomedicals, Aurora, OH) in filter-protected (Millipore, Bedford, MA) drinking water for 6 days, although some mice were studied at days 2 and 4 on DSS. Control mice received the filtered water alone. Daily clinical assessment of DSS-treated animals included measurement of drinking volume and body weight, evaluation of stool consistency, and the presence of blood in the stools by a guiac paper test (Coloscreen; Helena Laboratories, Beaumont, TX) (12). A previously validated clinical disease activity index (28) ranging from 0 to 4 was calculated using the following parameters: stool consistency, presence or absence of fecal blood, and weight loss.

Platelet preparation and labeling. Platelets were derived from non-DSS-treated donors (either C57Bl/6/f or P-selectin-deficient mice) for administration to recipient DSS-treated mice. Donor mice were anesthetized using ketamine hydrochloride (150 mg/kg im) and xylazine (7.5 mg/kg im). As described previously (8), ~0.9 ml of blood were harvested via a catheter placed in the carotid artery. The blood was collected in polypropylene tubes containing 0.1 ml acid-citrate-dextrose buffer (Sigma-Aldrich, St. Louis, MO). Platelet-rich plasma was obtained by two sequential centrifugations (120 g for 8 min and 120 g for 3 min). The platelet-rich plasma was removed and centrifuged again at 550 g for 10 min, and the platelet pellet was resuspended in PBS, pH 7.4. Platelets were then incubated for 10 min at room temperature with the fluorochrome carboxyfluorescein diacetate (CFSE; 90 mM final concentration; Molecular Probes, Eugene, OR), which was protected from light until infused into a recipient animal. Leukocytes were labeled with rhodamine 6G (excitation: 525 nm, emission: 550 nm) required a Nikon filter block with an excitation filter (470–490 nm), a dichroic mirror (510 nm), and a barrier filter (520 nm). The microscopic images were received by a charge-coupled device video camera (XC-77; Hamamatsu Photonics) that was attached to an intensifier (C2400; Hamamatsu Photonics), projected onto a monitor (PVM-2030; Sony), and recorded on a videocassette recorder (BR-S601MU; JVC; Wayne, NJ) for off-line analysis. A video time-date generator (WJ810; Panasonic) displayed a stopwatch function onto the monitor. Vessel diameter was measured online using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX).

Single unbranched venules with diameters ranging between 25 and 50 μm and a length >150 μm were selected for this experiment. In each animal, three to six venules were examined randomly, and the results were calculated as the means of each parameter in all venules examined. CFSE-labeled platelets were infused into a recipient animal over 5 min and allowed to circulate for 5 min before recording. Adherent platelets were defined as the number of platelets remaining stationary for a period >2 s within the microcirculation. Other animals received rhodamine 6G over 5 min, followed by 5 min circulation time before observation of leukocytes. Adherent leukocytes were defined as the number remaining stationary for a period ≥30 s within the venules. Adherent cells were expressed as the number of cells per square millimeter of venular surface, calculated from diameter and length, assuming cylindrical vessel shape (25). The flux of rolling leukocytes was measured as the number of rolling leukocytes that passed a fixed vertical line in the vessel per minute.

Production of P-selectin chimeras. Bone marrow transfer (groups indicated by donor→recipient) was employed to create P-selectin chimeric mice wherein the genetic deficiency of P-selectin (P-sel−/−) is confined to either circulating blood cells (P-sel−/−→WT chimeras) or nonblood tissue (WT→P-sel−/−chimeras), with WT→WT marrow transfer used to produce control chimeras. Briefly, bone marrow cells were isolated from the femurs and tibias of donor (e.g., congenic WT mice expressing the CD45.1 isotype of CD45 or P-sel−/− mice that express CD45.2) and resuspended at 4 × 107 cells/ml in PBS. Recipient (P-sel−/− or CD45 congenic WT) mice were irradiated with two doses of 500–525 Rads, 3 h apart, after which 8 × 108 donor marrow cells in 200 μl PBS were injected into the femoral vein. The chimeras were kept in autoclaved cages, with 0.2% neomycin drinking water for 2 wk, after which normal drinking water was used. Flow cytometry was used to verify chimera reconstitution (usually requiring 6–8 wk) by staining for CD45.1 (CD45 congenic mice) and CD45.2 (expressed by C57Bl/6 mice or knockout mice on a C57Bl/6 background) expression on circulating leukocytes with an FITC-conjugated anti-CD45.1 antibody and a biotinylated anti-CD45.2 antibody with a streptavidin-PerCP secondary antibody (BD Pharmingen, San Diego, CA). This procedure normally yields >90% penetrance of the transferred marrow at 6 wk or longer after transplant.

Measurement of P-selectin expression in the colonic vasculature. The dual radiolabeled MAb technique was used to quantify P-selectin expression in the colonic vasculature, as previously described (14). The MAbs used for measurement of P-selectin expression were RB40.34, a binding rat immunoglobulin (IgG1) that is specific for mouse CD162P (P-selectin; BD Pharmingen), and P23, a nonbinding murine IgG1 directed against human P-selectin (Pharmacia-Upjohn, Kalamazoo, MI). The binding (RB40.34) and nonbinding (P23) MAbs were labeled with 125I and 14C (DuPont New England Nuclear Research Products, Boston, MA), respectively, using the iodogen method as described previously (14) and stored at 4°C. Mice were anesthetized as described above, and then the left jugular vein and carotid arteries were cannulated. A mixture (200-μl) of 125I-labeled RB40.34 MAb (10 μg) and 131I-labeled P23 MAb (1 μg) was administered through the jugular vein catheter. Five minutes after injection of the MAb mixture, a blood sample was obtained from the carotid artery. Immediately thereafter, the animal was rapidly exanguinated by jugular perfusion of bicarbonate-buffered saline, immediately followed by carotid perfusion with bicarbonate-buffered saline.
after severing the inferior vena cava at the thoracic level. The large bowel was harvested, weighed, and placed in a gamma-scintillation counter for radioactivity measurement.

The method for calculating P-selectin expression has been described previously (14). Briefly, activity of $^{125}$I and $^{131}$I (marking the binding MAb and the nonbinding MAb, respectively) in the tissue and in 50-μl samples of cell-free plasma was counted in a 14800 Wizard 3 counter (Wallac, Turku, Finland). The accumulated activity of each labeled MAb in the colon was expressed as the percentage of the injected activity per gram of tissue. P-selectin expression was then calculated by subtracting the accumulated activity per gram of tissue of the nonbinding MAb from the activity of the binding P-selectin MAb. This value, expressed as percentage of injected dose per gram of tissue, was converted to nanograms of MAb per gram of tissue by multiplying the above value by the total injected binding MAb.

**Vascular permeability measurements.** Albumin leakage, a quantitative index of endothelial barrier dysfunction, was measured in normal and inflamed colon. Albumin extravasation was quantified by fluorescence imaging of the leakage of FITC-labeled albumin from colonic venules (20–40 μm in diameter). After background fluorescence intensity was determined, the FITC-albumin was administered intravenously and allowed to circulate for 30 min. The fluorescence intensities (excitation wavelength, 420–490 nm; emission wavelength, 520 nm) within a specified segment of a colonic venule (Iv) and in a contiguous area of perivenular interstitium (Ii) were measured using a computer-assisted digital imaging processor (NIH Image 1.62 software). Venular albumin leakage was determined from the Ii-to-Iv ratio (after subtraction of background intensity), as previously described (20).

**Circulating platelet counts.** Blood samples were obtained from the tail in WT and P-sel$^{-/-}$ mice at 0, 2, 4, and 6 days of DSS treatment. Circulating platelet counts were measured using the Unopette system (Becton-Dickinson, Franklin Lakes, NJ).

**Experimental protocols.** Five series of experiments were performed employing the procedures outlined. One series was devoted to determining the time course and magnitude of platelet adhesion in colonic venules over 6 days of DSS exposure. In this series, the disease activity index, vascular albumin leakage, platelet adhesion, and leukocyte adhesion were measured in control mice and in mice receiving 3% DSS in drinking water for 2, 4, or 6 days. A second series of experiments addressed the adhesion molecules that mediate the platelet and leukocyte adhesion detected on inflamed and noninflamed colon. Albumin extravasation was quantified in colonic venules of noncolitic and colitic WT mice and in colitic P-selectin$^{-/-}$ mice.

**Statistics.** Standard statistical analyses, i.e., one-way ANOVA with Scheffé’s (post hoc) test, were applied to the data. All values are reported as means ± SE, (n = 5/group). Statistical significance was set at $P < 0.05$.

**RESULTS**

**Time course and magnitude of platelet adhesion.** Figure 1 summarizes the time course of changes in platelet (Fig. 1C) and leukocyte (Fig. 1D) adhesion in colonic venules during the development of DSS colitis and compares these responses to the corresponding changes in disease activity index (Fig. 1A). Although vascular permeability exhibited a large increase at day 2 that was sustained over the 6-day period, both platelet and leukocyte adhesion increased gradually over the 6 days on DSS. For platelet adhesion, the largest increment was noted between days 4 and 6 (Fig. 1C). The gradual rise in blood cell adhesion observed in colonic venules closely paralleled the progressive rise in disease activity index.

**Molecular determinants of platelet and leukocyte adhesion in inflamed colonic venules.** Figure 2 summarizes the responses of DSS-induced platelet and leukocyte adhesion (day 6) to blocking MAbs directed against P-selectin, PSGL-1, or GPIIb/IIIa under baseline conditions in noncolitic control mice, the number of platelets adhering in colonic venules was 25.5 ± 9.1 cells/mm$^2$ (Fig. 2A). However, it increased ~14-fold (361.7 ±
41.9 cells/mm²) on day 6 of DSS exposure. Treatment with a P-selectin blocking MAb profoundly reduced the DSS-induced platelet adhesion response (9.0 ± 4.8 cells/mm²). A significant, but less dramatic, reduction in platelet adhesion was noted after anti-PSGL-1 treatment (56.5 ± 10.4 cells/mm²). The GPIIb/IIIa MAb did not significantly affect DSS-induced platelet adhesion.

A similar pattern was noted for adherent leukocytes, although the attenuating actions of the P-selectin and PSGL-1 MAbs were less impressive (Fig. 2B). A minimal number of adherent leukocytes (1.8 ± 0.8 cells/mm²) was detected in venules of control (noncolitic) mice. On day 6 of DSS-induced inflammation, 440.2 ± 74.3 adherent leukocytes/mm² were detected. Immuneunneutralization of P-selectin resulted in a 44% (248.5 ± 17.3 cells/mm²) reduction, whereas PSGL-1 blockade reduced leukocyte adhesion by 42% (253.6 ± 21.4 cells/mm²). Blockade of GPIIb/IIa showed no effect on DSS-induced leukocyte adhesion (517.2 ± 41.2 cells/mm²).

Role of platelet vs. endothelial cell P-selectin in DSS-induced platelet adhesion. Figure 3 compares the platelet adhesion responses in WT and P-sel−/− mice with DSS-induced colonic inflammation that received fluorescent-labeled platelets harvested from either WT or P-sel−/− donors. Compared with control (noncolitic) WT mice receiving WT platelets, a large and significant increase (22-fold) in platelet adhesion was seen in DSS-treated WT mice receiving WT platelets. However, only a 2.5-fold increase in platelet adhesion (compared with control noncolitic mice) was observed when platelets from P-sel−/− mice were monitored in DSS-treated WT mice. An attenuated adhesion response (3.5-fold increase vs. controls) was also noted in DSS-treated P-sel−/− mice receiving WT platelets. No adherent platelets were detected in DSS-treated P-sel−/− mice receiving platelets from P-sel−/− donors.

Expression of P-selectin in the inflamed colonic vasculature. Figure 4 summarizes the values for P-selectin expression in different mice undergoing bone marrow transplantation 6–8 wk before exposure to 3% DSS for 6 days. In WT→WT chimeras, DSS treatment was associated with a significantly increased expression of P-selectin in the colonic vasculature compared with noncolitics (water-fed) controls. However, in WT→P-sel−/− chimeras (wherein the genetic deficiency of P-selectin was confined to the vascular wall), the colonic expression of P-selectin during DSS treatment was reduced to
control (noncolitic) levels. A significant attenuation of P-selectin expression was also noted in P-sel$^{-/-}$→WT chimeras (wherein the genetic deficiency of P-selectin was confined to circulating blood cells). However, the level of P-selectin expression remained significantly greater than the control noncolitic value.

**Role of P-selectin in DSS-induced endothelial barrier dysfunction.** Figure 5 compares the change in albumin leakage across colonic venules during DSS-induced inflammation (day 6) between WT and P-selectin-deficient mice. Although WT mice responded to DSS colitis with a large increase in venular permeability to albumin, the extravasation of albumin across colonic venules in P-sel$^{-/-}$ mice did not differ from that detected in WT noncolitic (control) mice.

Circulating platelet counts. Figure 6 demonstrates that blood platelet numbers did not change in either WT or P-sel-deficient mice for the first 4 days of DSS treatment. However, platelet counts began to fall in WT mice by day 6 of DSS treatment (not significant), which corresponded to peak platelet recruitment.

**DISCUSSION**

Although platelet activation has been widely implicated in the pathogenesis of chronic intestinal inflammation, only recently have efforts been made to dissect the molecular mechanisms underlying the initiation of platelet activation and to define the consequences of this response to microvascular function and systemic hemostasis. For example, platelets isolated from IBD patients activate neutrophils to enhance reactive oxygen metabolite production via a P-selectin-dependent mechanism (36) and activate intestinal microvascular endothelial cells to increase the expression of adhesion molecules (9). However, to date relatively little is known about the time course and magnitude of platelet accumulation within the microcirculation of the inflamed colon and the pathophysiological consequences of this platelet accumulation. Our study provides the first systematic analysis of the kinetics of platelet adhesion in inflamed colonic venules and its correlation to indexes of disease activity and endothelial dysfunction. In addition, the study addresses the role of some of the key adhesion glycoproteins in this response.

We found that platelet adhesion showed a gradual time-dependent increase during the development of colitis that closely paralleled the rise in leukocyte adhesion. It should be noted that the number of labeled platelets infused only represented ~10% of the total platelet population of the recipient mouse; therefore, the level of platelet adhesion measured is likely to be an underestimation and is comparable with other models of acute and chronic inflammation (4, 26, 27, 35, 37). Furthermore, the incremental changes (relative to noncolitic values) in platelet recruitment (30-fold at day 6) were much larger than those observed for leukocyte recruitment (10-fold at day 6). Circulating platelet numbers fell slightly in WT mice at day 6 of DSS, possibly due to the large microvascular recruitment of these cells. In addition, the increase in platelet adhesion measured during the course of DSS colitis was temporally correlated with a clinical index of disease severity, which is consistent with the clinical use of platelet activation and thrombocytosis as indexes of disease activity in human IBD (10).

On the basis of other studies of venular platelet adhesion (8, 19, 33), we sought to define the contribution of P-selectin and its ligand PSGL-1 and GPIIb/IIIa in the accumulation of platelets (and leukocytes) in the inflamed colon. GPIIb/IIIa did not contribute directly to either the platelet or leukocyte adhesion responses that were elicited by DSS colitis. However, because the blocking antibody was administered 30 min before observation, we cannot exclude the possibility that GPIIb/IIIa plays an earlier indirect role possibly by promoting the release of inflammatory/thrombogenic mediators such as CD40L. In contrast, the immunoneutralization studies strongly implicate P-selectin and PSGL-1 in mediating the DSS-induced platelet adhesion. These antibodies also attenuated the adhesion of leukocytes to inflamed colonic venules, in agreement with others (31, 40). P-selectin is expressed on both platelets and endothelial cells (1, 3), whereas PSGL-1 is densely expressed on unstimulated leukocytes and, to a smaller degree, on platelets (25- to 100-fold lower than on leukocytes) (15). Hence, several combinations of ligand-receptor interactions could explain the platelet-vessel wall interactions, i.e., platelet-associated PSGL-1 binding to endothelial cell-associated P-selectin, and/or platelet-associated P-selectin binding to PSGL-1 expressed on adherent leukocytes. Although there is limited in vivo support for the former ligand-receptor interactions (15), the latter scenario has been proposed in several models of inflammation (8).

To more clearly define the cell-specific contributions to the P-selectin-mediated platelet-vessel wall interactions observed...
in DSS colitis, we examined the ability of WT and P-selectin−/− platelets to adhere in colonic venules of WT and P-selectin−/− mice with DSS-induced colonic inflammation (Fig. 3). Adhesion of P-selectin-deficient platelets in DSS-treated P-selectin−/− recipients was negligible. This was not due to altered circulating platelet count in these knockout mice (Fig. 6). We found that both endothelial cell- and platelet-associated P-selectin are important in mediating these platelet adhesion responses, suggesting that both ligand-receptor interactions proposed above could be valid in our model. When taken together with our immunoneutralization studies, the major pathway appears to be platelet-associated P-selectin binding to PSGL-1 expressed on adherent leukocytes. We have recently reported that approximately one-half of the adherent leukocytes in colonic venules of mice with DSS colitis has platelets attached to their surface (39). Because immunoneutralization of P-selectin or PSGL-1 reduced leukocyte adhesion by 40–45% but almost completely attenuated platelet recruitment, it is plausible that all of the platelet-bearing leukocytes detach in the MAB-treated animals, leaving predominantly leukocytes that are recruited via a P-selectin/PSGL-1-independent pathway and that do not bear platelets.

It has previously been demonstrated (14, 30) that the expression of P-selectin as well as ICAM-1, VCAM-1, MAdCAM-1, and E-selectin is elevated in different models of murine colitis. To dissect the contributions of platelet- vs. endothelial cell-associated P-selectin to its expression within the inflamed colonic microvasculature, we created P-selectin chimeric mice wherein the genetic deficiency of P-selectin (P-sel−/−) was confined to either circulating blood cells (e.g., P-sel−/−/WT chimeras) or endothelial cells (WT→P-sel−/− chimeras). The observations that deficiency of P-selectin in endothelial cells alone completely abolished the DSS-induced “upregulation” of P-selectin detected by the dual radiolabeled MAb method, whereas P-selectin deficiency that was confined to platelets produced a substantial (but partial) attenuation of the expression response, are consistent with a model of P-selectin-dependent platelet recruitment that involves platelet P-selectin binding to PSGL-1 on adherent leukocytes, which, in turn, rely on endothelial cell P-selectin for their attachment. This influence of platelet accumulation on estimates of microvascular P-selectin expression is not confined to DSS colitis, because much of the increased expression of P-selectin detected in the postischemic intestine is also attributable to accumulated platelets (13).

An interesting finding in this study was the rapid onset of endothelial cell dysfunction that is induced by 3% DSS. The endothelial dysfunction, manifested as increased vascular permeability, increased about ninefold on day 2 and remained elevated at this level through day 6. Although this was temporally disassociated from disease severity and the recruitment of blood cells, it proved to be dependent on P-selectin expression, as indicated by the absence of DSS-induced albumin leakage on day 6 in P-selectin-deficient mice (Fig. 5). This suggests that the low-grade inflammation already present on day 2, as indicated by the small number of accumulated blood cells, was enough to initiate the elevated permeability that could be maintained by the increasing levels of adherent platelet and leukocytes. Because others have already shown that immunoneutralization of P-selectin significantly attenuates clinical and pathological scores in DSS-induced colonic inflammation (16), it is possible that the recruitment of adherent blood cells and the consequent microvascular dysfunction are critical early events in the pathogenesis of DSS-induced inflammation that ultimately lead to tissue injury and the clinical signs of colitis that are observed in this experimental model. However, it should be noted that the same group demonstrated that although P-selectin null mice exhibited a comparable histological protection and reduction of leukocyte recruitment, clinical scores were not reduced. Their findings that these mice demonstrated a significantly greater VCAM-1 expression and leukocyte infiltration of the lung suggest that compensatory mechanisms for the loss of P-selectin may have contributed to the observed responses.

The findings of this study may have some bearing on the mechanisms underlying the accelerated formation of platelet-leukocyte aggregates that has been reported for patients with intestinal inflammation, such as IBD (5, 18). Although such aggregates may form within the circulation due to the higher activation state of circulating platelets and leukocytes, it also appears likely that platelet-leukocyte aggregates are initially formed within inflamed colonic venules, where they are subsequently dislodged by shear forces generated from the movement of blood. Such a scenario has been described in postischemic mesenteric venules of hypercholesterolemic rats, where the formation of these platelet-leukocyte aggregates was shown to be P-selectin dependent (21). Although the platelet-leukocyte aggregate has been widely used as a surrogate marker of systemic inflammatory responses (23), the pathophysiological importance of this structure remains unclear. However, it appears likely that these aggregates may represent an important source of inflammatory mediators both in the systemic circulation and within distant regional vascular beds once the aggregate lodges in capillaries. These possibilities are consistent with evidence that patients with IBD are at increased risk of systemic thromboembolism that occurs in 5–10% of patients (2, 38).

In conclusion, this study demonstrates that the development of colonic inflammation is associated with the induction of a prothrombogenic phenotype in the colonic microcirculation. Our findings implicate P-selectin and its ligand PSGL-1 as major determinants of the platelet and leukocyte adhesion observed in inflamed colonic venules and are consistent with a mechanism whereby platelets accumulate on the venular wall by attaching to already adherent leukocytes. The study results are also consistent with clinical evidence showing a correlation between platelet activation and disease severity in intestinal inflammation. Further studies are necessary to determine the role of platelets in chronic models of intestinal inflammation.

**GRANTS**

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