Endothelial cell P-selectin mediates a proinflammatory and prothrombogenic phenotype in cerebral venules of sickle cell transgenic mice

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Wood, Katherine C., Robert P. Hebbel, and D. Neil Granger. Endothelial cell P-selectin mediates a proinflammatory and prothrombogenic phenotype in cerebral venules of sickle cell transgenic mice. Am J Physiol Heart Circ Physiol 286: H1608–H1614, 2004. First published January 15, 2004; 10.1152/ajpheart.01056.2003.—Whereas the adhesion of leukocytes and erythrocytes to vascular endothelium has been implicated in the vasocclusive events associated with sickle cell disease, the role of platelet-vessel wall interactions in this process remains undefined. The objectives of this study were to: 1) determine whether the adhesion of platelets and leukocytes in cerebral venules differs between sickle cell transgenic (S/S) mice and their wild-type (WT) counterparts (C57Bl/6) under both resting and posthypoxic conditions, and 2) define the contributions of P-selectin to these adhesion processes. Animals were anesthetized, and platelet and leukocyte interactions with endothelial cells of cerebral postcapillary venules were monitored and quantified using intravital fluorescence microscopy in WT, βS, and chimeric mice produced by transplanting bone marrow from WT or βS mice into WT or P-selectin-deficient (P-sel−/−) mice. Platelet and leukocyte adhesion to endothelial cells in both unstimulated and posthypoxic βS mice were significantly elevated over WT levels. Chimeric mice involving bone marrow transfer from βS mice to P-sel−/− mice exhibited a profound attenuation of both platelet and leukocyte adhesion compared with βS bone marrow transfer to WT mice. These findings indicate that βS mice assume both an inflammatory and prothrombogenic phenotype, with endothelial cell P-selectin playing a major role in mediating these microvascular responses.

platelet; leukocyte; brain; bone marrow transplant

SICKLE CELL DISEASE (SCD) is a painful and life-threatening condition that involves multiple organ pathology, including acute chest syndrome and stroke. Studies on the pathogenesis of SCD have revealed that the vasculature is a principal target of the disease (3, 14) and that the affected tissues assume an inflammatory phenotype (7, 12, 21). Enhanced cytokine production (8, 23), oxidative stress (18, 26, 28), altered endothelium-dependent vasorelaxation (19), and increased endothelial cell adhesion molecule expression (9, 32, 33) are manifestations of the inflammatory state that occurs during SCD. Recent studies using the sickle transgenic mouse model have revealed increased adhesive interactions between circulating erythrocytes and leukocytes with the walls of postcapillary venules, which may impede microvascular perfusion and promote vasocclusive crises. These adhesion responses appear to involve both direct adhesive interactions of the circulating blood cells with venular endothelium and indirect interactions that involve the binding of erythrocytes to leukocytes already adherent to venular endothelium (40). A role for P-selectin in mediating leukocyte and erythrocyte interactions in mice with SCD has been demonstrated using both blocking monoclonal antibodies (18) and P-selectin knockout mice (40).

There is a growing body of evidence that implicates platelets in the vasculopathy of both small and large blood vessels observed in SCD. Platelets from SCD patients exhibit an abnormal phenotype marked by surface-mobilized, activation-dependent antigens and microparticle morphology (37, 46). Increased circulating levels of platelet products (thrombospondin, IL-1) have been noted in SCD patients and have been implicated in the abnormal adhesion of SCD erythrocytes (2, 4, 34). It has been demonstrated that antibodies directed against the thrombospondin receptor (reticulocyte CD36) dramatically inhibit platelet supernatant-mediated adhesion of SCD erythrocytes to monolayers of cultured endothelial cells (4, 34). Furthermore, studies of large vessel cerebral arterial disease in SCD stroke patients have revealed hyperplastic arterial vessels with overlying thrombi (10). Despite evidence that SCD patients are more prone to thrombus formation (16, 25, 43, 45) and its consequences (e.g., stroke), there has been no reported effort to determine whether circulating SCD platelets, like erythrocytes and leukocytes, exhibit enhanced adhesive interactions within the microvasculature. This possibility appears tenable inasmuch as P-selectin, which mediates the binding of erythrocytes to leukocytes already adherent to venular endothelium (40). A role for P-selectin in mediating leukocyte and erythrocyte interactions in mice with SCD has been demonstrated using both blocking monoclonal antibodies (18) and P-selectin knockout mice (40).

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I) determine whether SCD is associated with platelet and/or leukocyte adhesion in cerebral postcapillary venules under both unstimulated conditions and following hypoxia-reoxygenation (H/R), 2) define the contribution of P-selectin to the adhesion of platelets and leukocytes in the cerebral microcirculation of βS mice, and 3) determine the relative contributions of endothelial cells versus platelets in mediating the prothrombogenic phenotype that develops in cerebral venules during SCD.

MATERIALS AND METHODS

**Animals.** WT C57BL/6 mice, CD45 congenic B6.SJL-PTPRCPEP/BOY mice (which express CD45.1), and B6.129S7-sELPTMLBAY (P-selectin−/−) mice on a C57BL/6 background were obtained from Jackson Laboratories (Bar Harbor, ME). βS mice and their negative controls (C57BL/6) were provided by Dr. Robert P. Hebbel, Dept. of Medicine, University of Minnesota. The βS mice, developed on a mixed genetic background (FVB/N, 129, DBA/2, C57BL/6, and Black Swiss) (29), are homozygous for knockout of murine α-globin and heterozygous for knockout of murine β-globin and have one copy of the linked transgenes for human α- and human β-globins. The αS mice were 6–8 mo old and housed under specific pathogen-free conditions. The mice were maintained on standard laboratory chow and fed ad libitum until immediately before the experiments.

**Chimeras.** Four combinations of bone marrow chimeras were produced. WT/WT chimeras were WT animals (CD45.2-positive leukocytes) that received bone marrow cells from WT C57 congenic mice (CD45.1-positive leukocytes). This resulted in a significant increase of leukocytes expressing CD45.1 (of donor origin) from <5% in WT to >90% in the WT/WT chimeras, allowing verification of proper chimaera reconstitution, as previously described (38). βS/WT chimeras were produced by transplanting bone marrow from βS mice (CD45.1-positive leukocytes) into WT mice (CD45.2-positive leukocytes). WT/βS chimeras were produced by transplanting bone marrow from WT mice (CD45.2-positive leukocytes) into βS mice (CD45.1-positive leukocytes). Previous studies have shown that βS/WT chimeras assume the sickle phenotype a few weeks after transplantation of the βS marrow (40). Likewise, WT/βS chimeras assumed a normal (WT) phenotype a few weeks after transplantation of WT marrow into βS mice. In some experiments, βS bone marrow (CD45.1-positive leukocytes) was transferred into P-selectin−/− mice (CD45.2-positive leukocytes) yielding βS/P-selectin−/− chimeras with intact P-selectin on βS blood cells and a P-selectin deficient vessel wall.

Bone marrow cells were isolated from the femurs and tibias of donor mice and resuspended at 4 × 10^7 cells/ml phosphate-buffered saline (PBS). Recipient mice were irradiated with two doses of 500–525 rad, 3 h apart, after which 8 × 10^7 donor bone marrow cells in 200 μl of PBS were administered via the femoral vein. The chimeras were kept in autoclaved cages, with 0.2% neomycin sulfate (Sigma) in the drinking water for 2 wk, after which normal drinking water was used. Seven weeks after reconstitution, only chimeric mice demonstrating 90% or better conversion of leukocyte antigen to donor phenotype were subjected to 2 h hypoxia, followed by 4 h reoxygenation, and prepared for intravital microscopy using the closed cranial window preparation. Flow cytometry was used to verify chimaera reconstitution in all animals by staining for CD45.1 expression on window preparation. Flow cytometry was used to verify chimera reconstitution, and prepared for intravital microscopy using the closed cranial window. The mice were maintained on standard laboratory chow and fed ad libitum until immediately before the experiments.

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**Surgical protocol.** All animals were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and pentobarbitol (50 mg/kg). The left femoral artery was cannulated for monitoring mean arterial blood pressure (BP-1, World Precision Instruments) and sampling arterial blood for blood gas analysis (Omni-Modular System), whereas the femoral vein was cannulated for intravenous administration of carboxyfluorescein succinimidyl ester (CFSE, Molecular Probes; Eugene, OR)-labeled platelets and/or acridine orange (Sigma, St. Louis, MO) for in vivo labeling of leukocytes. Core body temperature was maintained at 35 ± 0.5°C. The experimental procedures described above were reviewed and approved by the Louisiana State University Health Sciences Center-Shreveport Institutional Animal Care and Use Committee and performed according to the criteria outlined in the National Institutes of Health guidelines.

**Experimental preparation.** Both male and female mice were studied. Mice were maintained on normal mouse chow until 6 h before the experiment. Mice subjected to hypoxia-reoxygenation were placed individually in a hypobaric chamber (10% O2, 0.05% CO2, balance nitrogen) for 2 h and then returned to normoxic conditions for 1.5 h before the induction of anesthesia. Mice used for baseline (unstimulated) experiments were handled identically but the hypoxic insult was omitted. Lidocaine (1%) was used for local anesthesia. All mice were tracheostomized and mechanically ventilated (Harvard Rodent Ventilator model 683; South Natick, MA) with room air. The head of each mouse was fixed in a stereotaxic frame, and the left parietal bone was exposed by a longitudinal midline skin incision. Two polyethylene-50 tubes (Clay Adams; Parsippany, NJ) were fixed on the skull with ethyl cyanoacrylate (Elmer’s Products; Columbus, OH), and a closed cranial window was constructed. A craniectomy was made by using a high-speed microdrill (Fine Science Tools; Foster City, CA), the dura was carefully reflected, and a coverslip (glass, affixed with a quick-setting self-curing acrylic resin (Biobond, Japan), was used to render the window air-tight. Artificial cerebrospinal fluid (Na+ 147.8 meq/l, K+ 3.0 meq/l, Mg2+ 2.3 meq/l, Ca2+ 2.3 meq/l, Cl− 135.2 meq/l, HCO3− 19.61 meq/l, lactate 1.67 meq/l, phosphate 1.11 mmol/l, and glucose 3.9 mmol/l) filled the space beneath the cranial window. The animals were allowed to stabilize for 30 min before observation of postcapillary venules under an upright Nikon Xaprophot microscope (HLX64610). Venular branches of the anterior, superior, and middle cerebral arteries were scanned, and images of 1-min duration were videotaped using a video cassette recorder (BR-S601MU, JVC; Japan) for each of 5 nonoverlapping 300-μm venular segments (20–80 μm diameter). A time-date recorder (WJ810, Panasonic) displayed a stopwatch function onto the video screen.

**Circulating cell-endothelial cell interactions.** Monitoring the activity of circulating cells with each other and the endothelium in the cerebral microcirculation required fluorescent labeling of cells: leukocytes were labeled in vivo with acridine orange (0.05% in 100 μl), and platelets were labeled ex vivo with CFSE (90 μM). The following manipulations, which have been shown to have no significant effect on the activity or viability of isolated platelets as assessed by flow cytometry (36), were used to isolate and label donor platelets from WT and βS mice. Platelets were isolated from whole blood obtained via the carotid artery of donor mice. The blood was collected in a polypropylene tube containing 0.1 ml acid citrate dextrose (Sigma) buffer and then centrifuged twice at 120 g for 8 and 3 min. Between centrifugations, platelet-rich plasma (PRP) was removed by pipette and placed in a clean polypropylene tube. The PRP was centrifuged at 550 g for 10 min to pellet the platelets. The platelet pellet was gently resuspended in 1,500 μl PBS (pH 7.4) and incubated with the fluorescent dye CFSE for 10 min at room temperature. The platelets were then centrifuged for 10 min at 550 g, resuspended in 500 μl PBS, and protected from light until use. Contamination of the platelet suspension by leukocytes was evaluated from a 25-μl sample and did not exceed 0.01%. Leukocytes were stained by the addition of 465 μg 3% citric acid and 10 μl 1% crystal violet (Sigma) and quantified with the aid of a hemocytometer and light microscope. Platelets (100 × 10^3) were infused over 5 min, yielding ~5% of the total platelet count. Infused platelets were allowed to circulate for a period of 5 min before being videotaped. 

Estimation of pseudoshear rate in venules were obtained using measurements of venular diameter (Dv) and the maximal velocity of flowing platelets (Vp) according to the formulation: pseudoshear rate = (Vp/1.6)/Dv × 8 (39).
**Intravital fluorescence microscopy.** Platelets were visualized, as previously described (6), with a Nikon Xanaphot upright microscope assisted by a silicon intensified target camera (C-2400, Hamamatsu Photonics). Cerebral venules were epi-illuminated, observed through a 20× long distance objective lens (Leitz Weitzler), and recorded on videotape using a videocassette recorder (BRS601MU, JVC). A video time/date generator projected the time, date, and stopwatch function on the monitor (diagonal 50.6 cm, PVM-2030, Sony Trinitron). CFSE (excitation: 490 nm, emission: 518 nm) and acridine orange (excitation: 500 nm, emission: 526 nm) visualization required a filter block with an excitation filter (B-2A, Nikon) for 450–490 nm, a dichroic mirror for 510 nm, and a barrier filter of 520 nm. The cerebral surface was scanned for five nonoverlapping venular segments per animal, and each was recorded for 1 min. Platelet and leukocyte adhesion were quantified separately within cerebral venules.

**Video analysis.** Leukocyte and platelet interactions with the endothelium were determined by offline analysis of videotaped images as previously described (5). Nonoverlapping venular segments of 300 μm length with diameters ranging between 20 and 80 μm (51.1 mean diameter) were chosen for quantification of adherent cells. Platelets and leukocytes were considered adherent if they arrested on the vascular endothelium ≥2 s. Platelet adherence was expressed as the number of cells per square millimeter of venular surface, calculated from diameter and length, assuming cylindrical vessel shape (24).

**Statistical analysis.** All values are reported as means ± SE. ANOVA with the Fisher’s post hoc test was used to compare groups, with statistical significance set at *P* < 0.05.

**RESULTS**

Comparisons of arterial blood pressure (79.8 ± 3.1 vs. 76.9 ± 3.1 mmHg), venular diameter (52.2 ± 2.7 vs. 53.1 ± 2.6 μm), and venular pseudoshear rate (400.4 ± 40.5 vs. 305.4 ± 29.7 s⁻¹) between unstimulated WT and βS mice revealed no statistically significant differences. Figure 1 illustrates that the number of adherent leukocytes in the cerebral venules of unstimulated βS mice (60 ± 11 cells/mm²) was approximately twice the adhesion detected in their unstimulated WT counterparts (34 ± 5 cells/mm², *P* = 0.047). This finding is consistent with previous reports of a twofold increase in leukocyte adhesion in cremasteric postcapillary venules of βS mice.

Major objectives of this study were to determine whether larger numbers of adherent platelets are observed in cerebral venules of βS mice (compared with WT) and to define the concentration of platelets versus endothelial cells to any platelet-endothelial vessel wall interactions that are elicited in venules of βS mice. When platelets isolated from βS donors were monitored in venules of βS recipients (βS → βS), significant platelet adhesion (654 ± 240 cells/mm²) was noted, and the intensity of adhesion was significantly higher than that observed when WT platelets were monitored in WT recipients (WT → WT; 147 ± 35 cells/mm², *P* = 0.03) (Fig. 2). When platelets isolated from WT donors were infused into βS recipients (WT → βS), platelet adhesion (454 ± 141 cells/mm²) was similar to that noted in βS controls (βS → βS). However, when platelets isolated from βS donors were infused into WT recipients (βS → WT), platelet adhesion (101 ± 50 cells/mm²) was similar to that noted in WT controls (WT → WT; 147 ± 35 cells/mm²), i.e., minimal platelet adhesion was observed. These experiments revealed that platelet adhesion in unstimulated βS mice is significantly increased compared with WT mice, and this thrombogenic response is endothelial cell dependent.

Another objective of the study was to determine whether 2 h of whole body hypoxia (10% O₂-0.05% CO₂, balance nitrogen) followed by 4 h reoxygenation (room air) alters the adhesion of leukocytes and platelets in the cerebral venules of βS and WT mice (Figs. 3 and 4). WT mice challenged with H/R exhibited leukocyte (27 ± 6 vs. 34 ± 5 cells/mm²) and platelet (124 ±
44 vs. 147 ± 35 cells/mm²) adhesion responses that are similar to those seen in normoxic WT mice. The response of leukocyte adhesion to H/R in controls is consistent with published reports that address H/R-induced responses in cremasteric venules of SCD mice. When βS mice were exposed to H/R, platelet adhesion was unaffected compared with their normoxic, unstimulated counterparts (571 ± 171 vs. 654 ± 240 cells/mm²). Although leukocyte adhesion was enhanced by H/R in βS mice, this response was not statistically significant (86 ± 18 vs. 59 ± 10 cells/mm²).

The role of endothelial cell P-selectin in mediating the leukocyte and platelet adhesion responses to H/R in βS mice was addressed in the bone marrow of chimeric mice (Figs. 5 and 6). Transfer of marrow from βS mice into WT mice (βS/WT) yielded significantly higher H/R-induced leukocyte

Fig. 3. Hypoxia-reoxygenation (H/R)-induced leukocyte adhesion in βS mice. Effects of H/R on leukocyte adhesion in cerebral postcapillary venules of WT and βS mice are shown. Mean responses of leukocytes adherent for ≥2 s. 15 to 16 animals were studied in each group. *Significant differences, P < 0.05 (one-way ANOVA and Fisher’s test).

Fig. 4. H/R-stimulated platelet adhesion is similar to baseline adhesion in βS mice. The effects of H/R on platelet adhesion in cerebral postcapillary venules of WT and βS mice are shown. Mean responses of platelets adherent for ≥2 s. Six to 8 animals were studied in each group. *Significant differences, P < 0.05 (one-way ANOVA and Fisher’s test).

Fig. 5. Endothelial cell-associated P-selectin mediates H/R-stimulated leukocyte adhesion in βS chimeras. Effects of H/R (2 h hypoxia, 4 h reoxygenation) on leukocyte-endothelial (L/E) adhesion in cerebral postcapillary venules of WT control chimeras (WT/WT), βS chimeras (βS/WT), and βS/P-selectin−/− chimeras (βS/P-selectin−/−) are shown. Mean responses of leukocytes adherent for ≥2 s. Five to 7 animals were studied in each group. *Significant difference versus WT/WT; **significant difference versus βS/WT, P < 0.05 (one-way ANOVA and Fisher’s test).

Fig. 6. Endothelial cell-associated P-selectin mediates H/R-stimulated platelet adhesion in βS chimeras. Effects of H/R (2 h hypoxia, 4 h reoxygenation) on platelet-endothelial cell (P/E) adhesion in postcapillary cerebral venules of WT control chimeras (WT/WT), βS chimeras (βS/WT), and βS/P-selectin−/− chimeras (βS/P-selectin−/−) are shown. Mean responses of WT platelets adherent for ≥2 s. Five animals were studied in each group. *Significant difference versus WT/WT; **significant difference versus βS/WT, P < 0.05 (one-way ANOVA and Fisher’s test).
(67 ± 12 vs. 24 ± 10 cells/mm², *P* = 0.01) and platelet (914 ± 198 vs. 175 ± 79 cells/mm², *P* = 0.002) adhesion responses compared with WT mice receiving bone marrow from WT mice (WT/WT). However, the transfer of bone marrow from β⁵ mouse into P-selectin⁻/⁻ mice (β⁵/P-sel⁻/⁻) yielded leukocyte (30 ± 7 vs. 24 ± 10 cells/mm²) and platelet (284 ± 53 vs. 175 ± 79 cells/mm²) adhesion responses to H/R that were no different from those noted in WT/WT chimeras and were significantly reduced compared with leukocyte (67 ± 12 cells/mm², *P* = 0.03) and platelet (914 ± 198 cells/mm², *P* = 0.004) adhesion responses observed in β⁵/WT chimeras. These observations are consistent with the transfer of both the inflammatory and prothrombogenic phenotypes into WT mice following transplantation of bone marrow derived from β⁵ mice.

In addition, the blunted adhesion responses of platelets and leukocytes in β⁵/P-selectin⁻/⁻ chimeric mice strongly implicates endothelial cell-associated P-selectin in these adhesion responses.

**DISCUSSION**

There is a growing body of evidence indicating that acute and chronic inflammatory conditions are associated with microvascular dysfunction, which is characterized by endothelial cells that assume both an inflammatory and prothrombogenic phenotype. For example, the recruitment of rolling and adherent leukocytes in postcapillary venules exposed to ischemia and reperfusion is accompanied by an intense accumulation of rolling and firmly adherent platelets (24). Colocalization of adherent platelets and leukocytes has been observed in response to transient retinal ischemia (17, 27, 39), as well as in venules of experimental animals with chronic arterial hypertension (1, 13, 22, 35). The results of the present study indicate that sickle cell disease is another pathological condition that produces both an inflammatory and prothrombogenic state in the microcirculation.

The results of our study demonstrate that SCD affects the cerebral microcirculation by 1) promoting the adhesion of platelets and leukocytes to venular endothelium, 2) inducing platelet and leukocyte adhesion responses that are P-selectin dependent, and 3) eliciting platelet and leukocyte responses that result primarily from changes in the function/adhesivity of endothelial cells, rather than circulating blood cells. The endothelial P-selectin-dependent inflammatory and prothrombogenic phenotype that we have observed in the cerebral microcirculation of β⁵ mice may explain, at least in part, the greater vulnerability of the brain to ischemic strokes in patients with SCD. This appears tenable in view of several published reports that implicate the recruitment of platelets and leukocytes in the brain injury and tissue dysfunction that is associated with strokes (11, 41, 42).

Our observation that leukocytes adhere in the cerebral microcirculation of β⁵ mice is consistent with published reports from other laboratories describing enhanced baseline and H/R- or tumor necrosis factor-α-stimulated leukocyte adhesion in cremasteric venules of SCD mice (18, 40). Our data show an approximate twofold increase in baseline leukocyte adhesion in cerebral venules of β⁵ mice (Fig. 1) that is not significantly enhanced by 2 h hypoxia followed by 4 h reoxygenation (Fig. 3). Kaul and Hebbel (18) reported a doubling of leukocyte adhesion in cremasteric venules of β⁵ mice. Similarly, Turhan and associates (40) noted that baseline and tumor necrosis factor-α-stimulated leukocyte rolling and adhesion in postcapillary and collecting venules of the cremaster muscle of sickle cell transgenic mice are nearly twice those observed in WT controls. The quantitatively similar leukocyte adhesion responses of the brain and cremaster circulations to experimental SCD raises the possibility that this inflammatory phenotype is exhibited in a variety of different vascular beds.

Previous intravital microscopic studies of leukocyte adhesion in cremasteric venules of mice with experimental SCD have demonstrated significant attenuation of adhesion following administration of a P-selectin blocking monoclonal antibody (18). These observations, coupled to reports of elevated expression of P-selectin on circulating endothelial cells isolated from patients with SCD (33) and recent data that describe an increased expression of P-selectin in different regional vascular beds of β⁵ mice (44), implicate endothelial cell P-selectin as a key mediator of the leukocyte recruitment associated with experimental SCD. The results of the present study support and significantly extend these observations. We observed that bone marrow transfer from β⁵ mice into P-selectin⁻/⁻ mice yields a leukocyte adhesion response that is comparable to that observed in WT mice receiving bone marrow from other WT mice but is significantly lower than the adhesion response seen when bone marrow is transferred from β⁵ mice into WT mice. Our findings indicate that P-selectin expressed by cerebral venular endothelium is critical for the recruitment of adherent leukocytes in β⁵ chimeric mice. An important and novel observation in the present study is that endothelial cell-associated P-selectin also mediates platelet recruitment observed in cerebral venules of β⁵ mice. Therefore, our observations suggest that therapeutic application of P-selectin-directed interventions may be useful in blunting both the inflammatory and prothrombogenic responses that are elicited in the cerebral circulation by SCD.

In addition to demonstrating that SCD cerebral venules exhibit enhanced platelet interactions with the vascular wall, our studies also provide novel insight into the relative contributions of endothelial cell versus platelet activation in SCD prothrombogenic responses. Our data show an approximate fourfold increase in baseline platelet adhesion in cerebral venules of β⁵ mice (Fig. 2) that is not significantly enhanced by 2 h hypoxia followed by 4 h reoxygenation (Fig. 4). Moreover, we demonstrate that β⁵ platelets adhere comparably to WT platelets in WT mice, whereas the adhesion of WT platelets is comparable to β⁵ platelets in β⁵ mice (Fig. 2). These observations indicate that β⁵ mice exhibit a prothrombogenic phenotype that largely reflects changes in the adhesivity of the venular wall rather than circulating platelets. This possibility is supported by our findings in the β⁵ chimeric mice that were created to determine whether the elevated platelet adhesion noted in the cerebral microvasculature of β⁵ mice, similar to leukocyte adhesion, is mediated by vascular P-selectin. Complete inhibition of platelet adhesion was noted in chimeric mice wherein the vessel wall (recipient mice) was deficient in P-selectin while circulating platelets (from β⁵ marrow donor) retained the capacity to express P-selectin (Fig. 6). Whereas endothelial cells clearly play a major role in promoting the adhesion of platelets as well as leukocytes in β⁵ chimeric mice,
the exact nature of this endothelial cell-dependent response remains unclear. One possible explanation relates to reports describing an oxidative stress in the vasculature of SCD mice (3, 26). This oxidative stress may contribute to a P-selectin involved, proteolytic environment on the endothelial cell surface that facilitates the capture of both platelets and leukocytes, as previously demonstrated in other experimental models (6, 15).

GRANTS

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