Neutrophil-dependent Acute Lung Injury

Requirement for P-selectin (GMP-140)

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Abstract

Rapid translocation of P-selectin (GMP-140) from cytoplasmic granules to the cell membrane of endothelial cells promotes adhesive interactions with neutrophils which, when activated, damage the endothelium. The role of P-selectin in lung vascular endothelial injury in rats after systemic activation of complement by intravenous infusion of cobra venom factor has been assessed. Within 5–10 min after cobra venom factor infusion, the pulmonary vasculature demonstrated immunohistochemical expression of an epitope that reacts with anti-human P-selectin. Monoclonal antibody to human P-selectin blocked in vitro adherence of rat or human platelets (activated with thrombin) to neutrophils and was demonstrated to react with thrombin-activated rat platelets. The antibody did not react with rat neutrophils. In vivo, the antibody had strongly protective effects against cobra venom factor–induced pulmonary vascular injury as determined by permeability changes and hemorrhage. In parallel, lung myeloperoxidase content was greatly reduced and, by transmission electron microscopy, there was markedly diminished adherence of neutrophils to the pulmonary vascular endothelium and much diminished injury of endothelial cells, as defined by hemorrhage. These data indicate that anti-human P-selectin reacts with a pulmonary vascular antigen in rats and that this antigen is essential for the full expression of lung injury. (*J. Clin. Invest.* 1992. 90:1600–1607.) Key words: endothelial cells • P-selectin • lung • neutrophils • complement

Introduction

Neutrophils are key participants in the development of tissue injury in a variety of acute inflammatory diseases. It has recently been proposed that neutrophil recruitment to sites of inflammation occurs in several discrete steps involving: (*a*) rolling of the cells on the activated endothelium of the blood vessel wall; (*b*) activation of neutrophils; (*c*) firm adhesion of neutrophils to the endothelium; and (*d*) migration of neutrophils into the surrounding tissue (1–6). This process involves multiple cell adhesion molecules expressed on the surfaces of neutrophils and endothelial cells. Rolling of leukocytes is believed to be mediated by members of the selectin family, L-selectin (LECAM-1, LAM-1, Mel-14) expressed on the neutrophil, and E-selectin (ELAM-1) and P-selectin (GMP-140, PADGEM) expressed on the endothelial cell (5–9). Direct experimental evidence has been obtained for the roles of L-selectin (10–14) and E-selectin (15) in development of the inflammatory response in vivo. Firm adhesion and extravascular migration of neutrophils appears to require engagement of the neutrophil $\beta 2$ integrins, LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), which bind to their counter ligand, intercellular adhesion model (ICAM-1,2) on endothelial cells (16–22). There is direct evidence for the role of $\beta 2$ integrins and ICAM-1,2 in the in vivo development of the inflammatory response (16–20, 23). Indeed, the key role of $\beta 2$ integrins has been shown in patients with a defect in the common subunit of the $\beta 2$ integrins (CD18), in which case there is a profound inability to recruit neutrophils to inflammatory sites, the outcome of which is development of uncontained bacterial infections (23).

Although in vitro studies have implicated P-selectin in mediating the rolling of leukocytes on activated endothelium (1–9, 24), its role in the inflammatory response in vivo has not yet been demonstrated. P-selectin is stored in intracellular granules in endothelial cells (and platelets) and can be rapidly mobilized to the surface within minutes of activation of endothelial cells by thrombin, histamine, and oxygen radicals or peroxides (25–27). The current studies were designed to investigate the possible role of P-selectin in acute lung injury due to systemic complement activation produced by vascular infusion into rats of the cobra venom factor (CVF).[1] Injury in this model develops rapidly and is known to be dependent on toxic oxygen products generated from neutrophils (28). The following studies provide the first direct in vivo evidence that a rat epitope reactive with anti-human P-selectin is required for the full development of tissue injury during the acute inflammatory response in lung.

Methods

Antibodies to P-selectin. RBF/DnJ mice were immunized once with freshly isolated thrombin-activated human platelets and twice with "outdated platelets" (> 8 d old) which were further purified by differ-

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1. *Abbreviations used in this paper:* CVF, cobra venom factor; MPO, myeloperoxidase.

ential centrifugation. The mice were then twice immunized with partially purified human P-selectin. A hybridoma clone producing an IgG_1 antibody that blocked adhesion of thrombin-activated human platelets to human neutrophils was the source of a monoclonal antibody termed PB1.3. Another antibody was termed PNB1.6. This antibody was obtained from a mouse immunized three times with thrombin-activated platelets. For analysis in immunoblotting, intact platelets were boiled in nonreducing SDS-PAGE sample buffer and subjected to electrophoresis in an 8–16% gel gradient, and then transferred to nitrocellulose. The blot was treated with 1% BSA in PBS and then incubated with appropriate antibody. The blot was washed, incubated with goat anti-mouse Ig conjugated with alkaline phosphatase, and then visualized with bromochloroindolyl phosphate/nitroblue tetrazolium. For the assay for P-selectin–mediated adhesion of thrombin-activated platelets to human neutrophils, the fluid phase assay was used as recently described (29), with the exception that, before additional of isolated human neutrophils, the thrombin-activated platelets were incubated at room temperature for 20 min with various concentrations of the PB1.3 and PNB1.6 antibodies.

Flow cytometric analysis of binding of anti-P-selectin to rat platelets. Rat platelets were isolated by the method previously described for the isolation of human platelets (30, 31). For activation with thrombin, the isolated platelets at a concentration of 5×10^8/ml were incubated for 20 min at room temperature without stirring with human thrombin (Sigma Chemical Co., St. Louis, MO) at 0.25 U/ml in the presence of 4 mM Ca^{++}. At the end of the incubation period, EDTA at a final concentration of 10 mM was added and the cell sample was centrifuged. The activated rat platelets were washed once in Tyrode-Hepes buffer, pH 7.2, containing 10 mM EDTA, and then resuspended to a concentration of 2×10^8/ml. A second aliquot of the same rat platelets was not activated with thrombin and the platelets were suspended in the same buffer at the same concentration. Platelets (0.15 ml), either thrombin activated or nonactivated, were incubated for 15 min at room temperature with buffer, anti-human P-selectin (PB1.3), or with an isotype matched murine monoclonal antibody, antipenicillin, which was obtained from Calbiochem Corp. (La Jolla, CA). All antibody concentrations were at 33 μg/ml. All incubations were also done in the presence of 10% normal rat serum. The platelets were washed once and then incubated for 10 min with R-phycoerythrin-conjugated goat anti-mouse IgG_1 (Southern Biotechnology Associates, Inc., Birmingham, AL) at a concentration of 1 μg/ml in the same buffer. Finally, the platelets were pelleted and then resuspended in 1 ml and subjected to flow cytometry on a FACScan® (Becton Dickinson & Co., Mountain View, CA).

CVF model of lung injury. Specific pathogen-free, adult (250–350 g), male Long-Evans rats were used. 20 U of CVF/kg body wt together with an aliquot of ^{125}I-BSA (0.5 μCi) were injected intravenously as a bolus infusion (28). Animals were anesthetized with ketamine hydrochloride (100 mg/kg) (Parke Davis and Co., Morris Plains, NJ) and exsanguinated via the posterior vena cava 30 min after infusion of CVF. Negative control animals (receiving PBS, pH 7.4) were similarly treated but CVF was omitted in the intravenously infused PBS. The lung vasculature was then perfused through the right cardiac ventricle with 10 ml of PBS. The lungs were removed and the amount of radioactivity remaining within the tissue was assessed with a gamma scintillation counter. Lung damage was defined by the increased lung vascular permeability (as determined by the ratio of ^{125}I-BSA radioactivity present within lung tissue to the amount of radioactivity present in 1.0 ml of venous blood obtained at the time of death) and by hemorrhage (^{51}Cr-red blood cell radioactivity, as described below). Protection from lung injury was calculated using the following equation:

$$\text{protection (\%)} = 100 \times 1 - \frac{\text{test value} - \text{PBS value}}{\text{positive value} - \text{PBS value}}.$$

Tissue myeloperoxidase activity. To assess the use of tissue myeloperoxidase (MPO) activity as a measure of neutrophil influx, known numbers of glycogen-elicited rat peritoneal neutrophils were added to normal rat lungs, the tissue homogenized and extracted, and standard curves produced (15). Lung samples were homogenized with a homogenizer (Polytron; Tekmar Co., Cincinnati, OH) (4×10 s at a setting of 4) using 6 ml of homogenization buffer (50 mM phosphate, pH 6.0) and subjected to centrifugation (3,000 *g*, 30 min) at 4°C. MPO activity in supernatants was assayed by measuring the change in absorbance (460 nm) resulting from decomposition of H_2O_2 in the presence of *o*-dianisidine (15).

Morphological evaluation of lungs. Light microscopy and transmission electron microscopy were carried out on lungs of animals 20 min after intravenous infusion of CVF, using plastic-embedded tissues (15).

Immunoperoxidase analysis of lung. At the times indicated after infusion of CVF and killing of rats, lungs were inflated with warm OCT and snap-frozen in liquid nitrogen. Frozen sections of lung were obtained and mounted on poly-L-lysine–coated glass slides. The slides were then incubated with PB1.3 antibody (1.0 ng/ml) for 45 min at 25°C, washed with PBS, and reacted with the biotin/avidin-peroxidase system as recently described (15). Tissue sections were then counterstained with hematoxylin and permanently mounted with coverslips.

Results

In vitro characterization of anti-P-selectin. For the in vivo studies, the two murine monoclonal antibodies (PB1.3 and PNB1.6) to human P-selectin were used. Both antibodies were of the IgG_1 isotype and each reacted in the ELISA assay with human recombinant P-selectin and purified P-selectin, as well as with P-selectin on thrombin-activated platelets. In contrast, neither antibody reacted with recombinant E- or L-selectin (data not shown). As shown in Fig. 1 (*left panel, B* and *C*), immunoblotting revealed that both monoclonal antibodies recognized a single protein from human platelets with a molecular mass of 140 kD, as previously described for P-selectin (25–27). One of the antibodies, PB1.3 (anti-P-selectin/blocking IgG_1, clone 352, lane *C*) blocked the P-selectin–mediated adhesion of human platelets (activated by thrombin) to human neutrophils, while the second antibody, PNB1.6 (anti-P-selectin/nonblocking/IgG_1, clone P6H6), did not (Fig. 1, *right panel*). Of particular importance for this study, PB1.3 also inhibited adhesion to human neutrophils of thrombin-activated rat platelets (Fig. 1, *right panel*) and rabbit platelets (not shown). These data suggested that PB1.3 recognizes a conserved P-selectin epitope and that the antibody might be useful in the analysis of P-selectin function in animal models of inflammatory injury.

PB1.3 was demonstrated to react with thrombin-activated but not with unstimulated rat platelets, as shown in Fig. 2. In both *A* (nonactivated platelets) and *B* (thrombin-activated platelets), the platelets were treated in the absence of antibody (none) or in the presence of an unrelated mouse IgG_1 isotope-matched control antibody, or with PB1.3 anti-P-selectin. The horizontal axis is a logarithmic scale of fluorescence (two divisions being equal to one log unit) while the vertical axis represents linear cell count. Nonactivated platelets showed little if any fluorescence (Fig. 2 *A*) while thrombin-activated platelets showed greatly increased fluorescence after incubation with PB1.3, whereas activated platelets treated with the isotype-matched control antibody or with buffer showed much less fluorescence intensity. In data not shown, neutrophils isolated from fresh rat blood failed to react with PB1.3, as assessed in flow cytometry.

In vivo protective effects of anti-P-human-selectin in CVF-induced lung injury. For the in vivo experiments 20 U CVF/kg

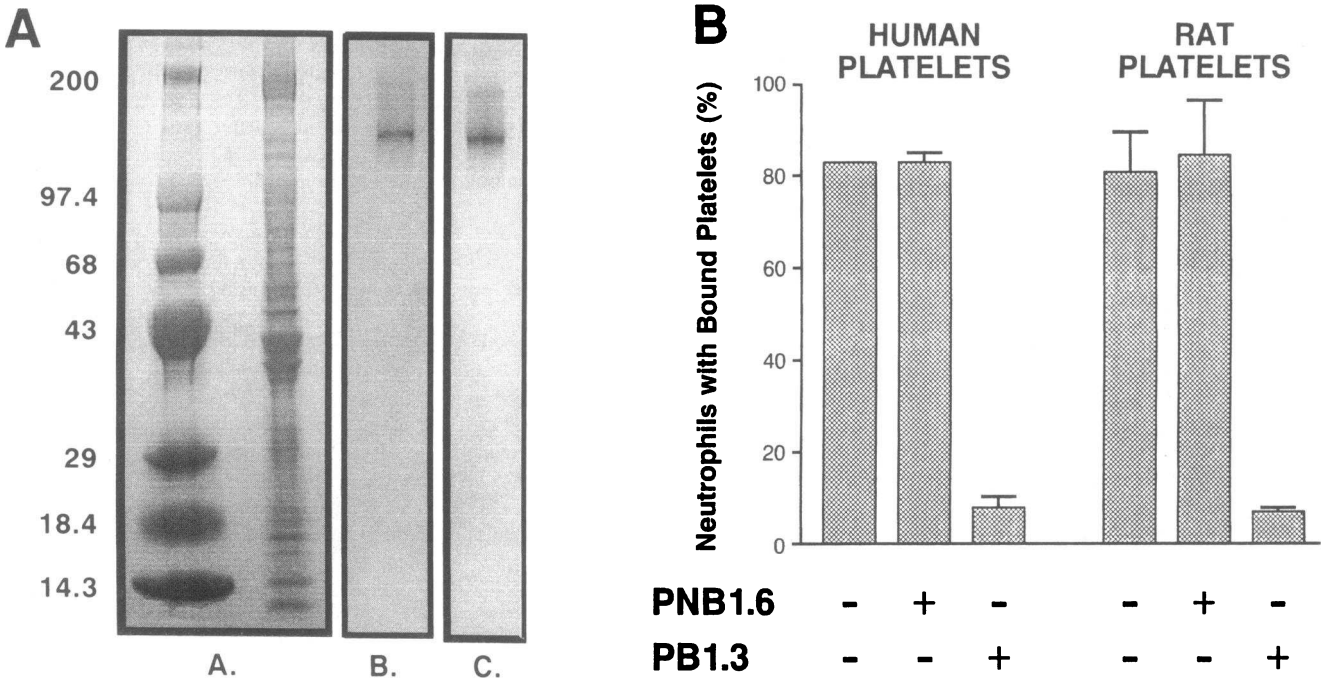


Figure 1. Characterization of monoclonal antibodies (PB1.3 and PNB1.6) to P-selectin. Left panel (*A*). *A*, Coomassie blue-stained molecular weight standards (*left*) and total extract of human platelet proteins (*right*). *B*, Immunoblotting of platelet proteins with monoclonal antibody, PNB1.6 (nonblocking). *C*, Immunoblotting of platelet proteins with monoclonal antibody, PB1.3 (blocking). Both monoclonal antibodies recognized a protein of 140 kD in the solubilized platelet preparation. Right panel (*B*). Fluid phase assay for P-selectin mediated adhesion of thrombin-activated platelets to human neutrophils. As indicated, PNB1.6 failed to inhibit binding of either human or rat activated platelets to human neutrophils, while PB1.3 inhibited adhesion of both human and rat platelets to neutrophils, with maximal inhibition by PB1.3 at 0.1 μg/ml and 10 μg/ml, respectively (data not shown).

body wt were infused intravenously into 300-g adult male Long-Evans rats. This resulted in rapid intrapulmonary, intravascular sequestration of blood neutrophils, with resulting damage of interstitial capillary endothelial cells at points of physical contact of endothelial cells and neutrophils (28). When used, clones PB1.3 and PNB1.6 murine monoclonal antibodies to human P-selectin were infused intravenously in varying amounts (as indicated) together with CVF, in total vol of 0.5 ml. Negative control animals received 0.5 ml intravenous infusion of PBS. In all cases the infusion material also contained trace amounts of ^{125}I-bovine serum albumin and homologous

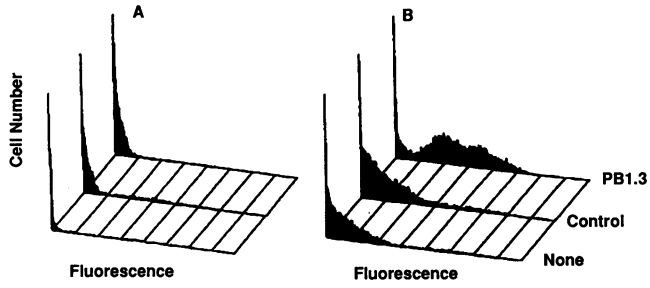


Figure 2. Flow cytometric analysis of nonstimulated (*A*) and thrombin-stimulated (*B*) rat platelets. The horizontal axis refers to intensity (on a log scale) of fluorescence while the vertical axis is linear cell number. Platelets were incubated with buffer (none), irrelevant isotype-matched murine monoclonal antibody (control), or with PB1.3 anti-human P-selectin. The indicator was a phycoerythrin-conjugated anti-murine IgG_1.

^{51}Cr-red blood cells. The parameters of lung injury (leakage of albumin and extravasation of red blood cells) were determined at 30 min according to established techniques (15, 28, 30). Injury was calculated by subtracting the negative control values (in animals injected with PBS) from each of the positive control values in the untreated (but CVF-injected) animals and also from the positive control values in the treated (and CVF-injected) animals. The results of various interventions on parameters of acute lung injury after infusion of CVF are shown in Fig. 3, *A* and *B*. The data points, which are indicated by the absence (10 μg injected) of infused clone PB1.3 in animals receiving CVF in PBS, served as the reference positive control values. When 100 μg PB1.3 antibody was infused with the CVF and compared to animals receiving CVF with PBS, there were reductions in permeability and hemorrhage of 19.2% (P = .002) (*A*) and 37.5% (P = .001) (*B*), respectively; with 200 μg PB1.3, the parameters of injury fell by 50.8% ($P < .001$) and 70.0% ($P < .001$), respectively; with 400 μg PB1.3 the parameters showed no further reduction, 47.5% ($P < .001$) and 70.1% ($P < .001$), respectively. Coinfusion of 200 μg PNB1.6) (the nonblocking anti-P-selectin) together with CVF failed to cause any reduction in the parameters of lung injury when compared to the values in untreated CVF positive controls (Fig. 3, *A* and *B*).

Effects of anti-human P-selectin antibodies on lung MPO content and morphological changes. Lungs from companion sets of animals were homogenized, sonicated, and the MPO measured to obtain an estimate of the neutrophil content in lung. As shown in Fig. 4, treatment with 100, 200, or 400 μg PB1.3 reduced MPO content below the values in the reference

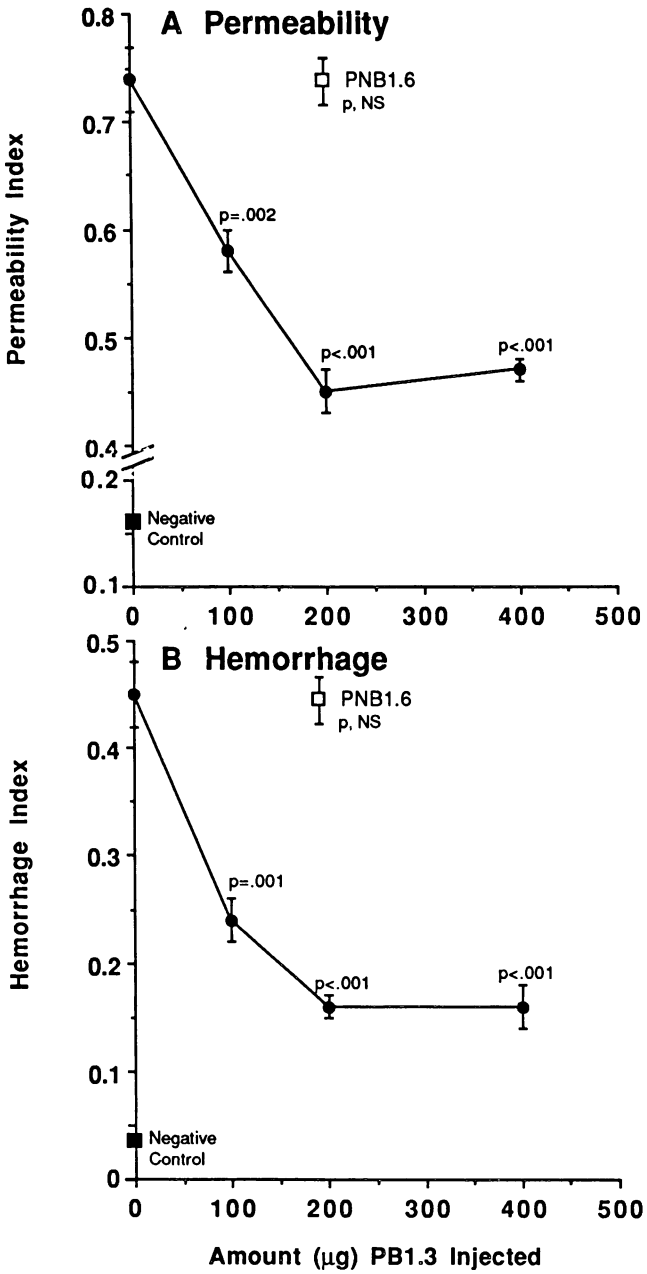


Figure 3. Anti-human P-selectin-related protection against acute lung injury in rats after systemic activation of complement by intravenous infusion of CVF, 20 U/kg body wt. Negative controls (not shown) received PBS alone while positive controls received CVF in PBS, or CVF with 200 μg PNB1.6 nonblocking anti-P-selectin or 100–400 μg PB1.3 blocking anti-P-selectin. Both anti-P-selectin antibodies were of the IgG_1 subclass. At time 0 animals were also injected intravenously with ^{125}I-BSA and ^{51}Cr-red blood cells, each in amounts containing 800,000 cpm. Lung injury was quantitated by vascular permeability (*A*) and by hemorrhage (*B*). At 30 min after infusion of CVF, animals were killed by exsanguination, the lung vasculature flushed with 10 ml PBS, and radioactivity in the lungs compared (as a ratio) to radioactivity in 1.0 ml blood obtained from the inferior vena cava at the time of death. One-way analysis of variance for permeability and hemorrhage measurements revealed $P < .001$. Negative and positive control values for permeability and hemorrhage were 0.16±0.01 and 0.74±0.03, and 0.04±0.003 and 0.45±0.03, respectively. *P* values were derived by comparing each group receiving PB1.3 or PNB1.6 to the positive control group receiving CVF in PBS. (For each data point, $n = 4–8$.)

(untreated) positive control group (infused with CVF and PBS) by 26% ($P = .024$), 41% ($P < .01$), and 50% ($P < .001$), respectively. In animals injected with 200 μg of the nonblocking anti-P-selectin (PNB1.6), there was no reduction in MPO content (Fig. 4), consistent with the inability of this antibody to protect against CVF-induced lung injury (Fig. 3). The protective effects of the blocking anti-P-selectin, PB1.3, correlated with its ability to interfere with neutrophil accumulation in lung tissue. Light and transmission electron microscopic examination of lung sections confirmed that treatment with PB1.3 resulted in reduced accumulation of neutrophils within the pulmonary interstitial capillaries, diminished adherence of neutrophils to endothelial cells, and greatly reduced hemorrhage (Fig. 5). In none of the transmission electron micrographs were platelets observed within the neutrophil aggregates.

Lung vascular expression after infusion of CVF of an epitope reactive with anti-human P-selectin after infusion of CVF. To investigate lung expression of an epitope reactive with anti-human P-selectin after intravenous injection of CVF, an additional group of rats was infused with CVF and animals killed at times 0, 5, 10, 15, 20, and 60 min later. Lungs were snap frozen, and sections obtained and examined for presence of reactivity by immunohistochemical techniques, using PB1.3 antibody. The results are shown in Fig. 6. Little detectable reactivity in the pulmonary vasculature was found at time 0 (*a*), whereas staining was clearly evident as early as 5 min (*b*) and

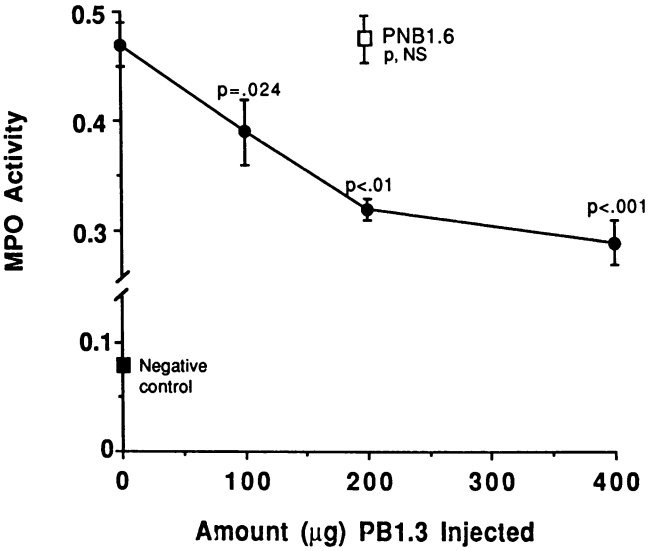
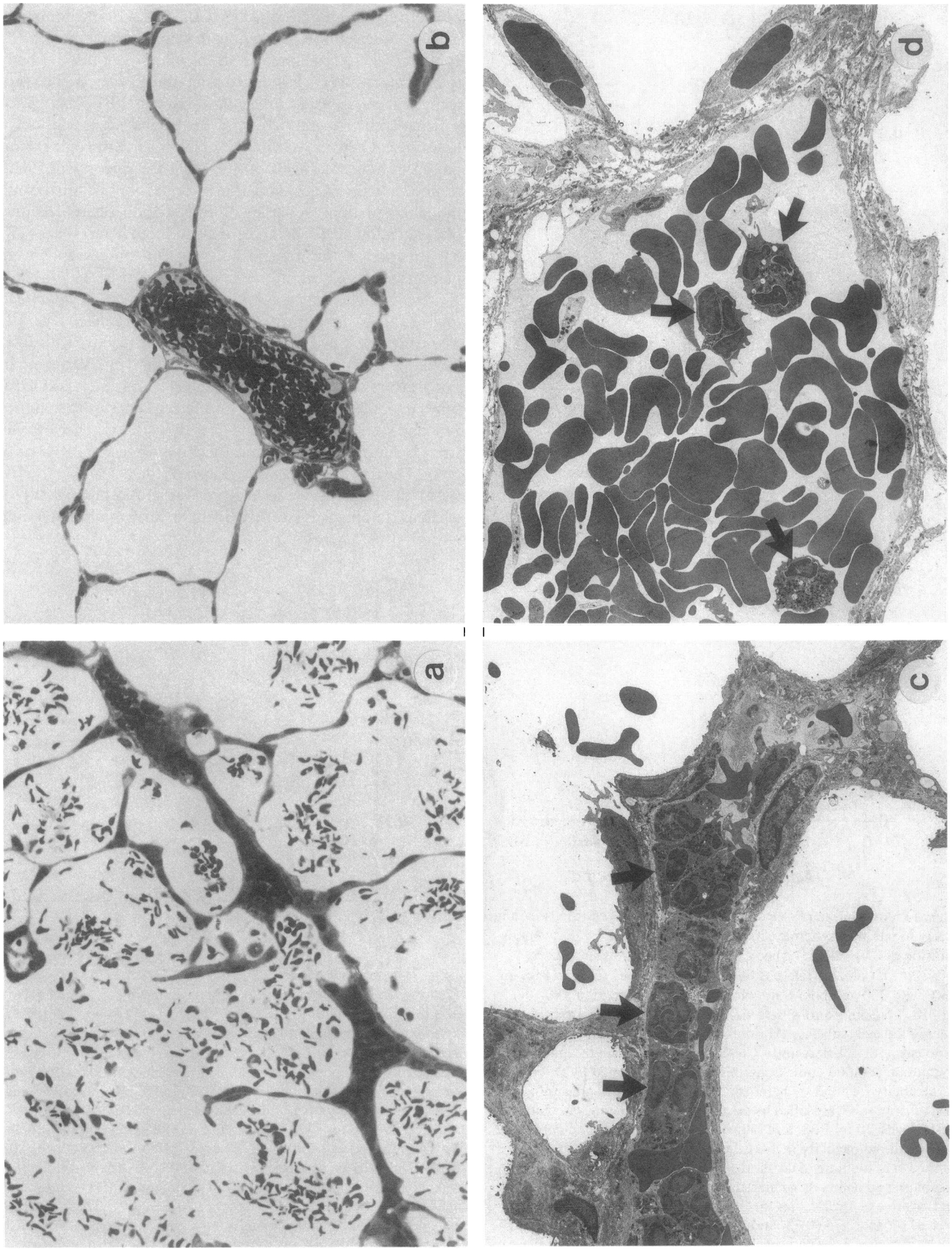


Figure 4. Lung MPO content in animals receiving an intravenous injection of CVF with PBS, 200 μg PNB1.6, or 100–400 μg PB1.3, followed by killing at 30 min. Lungs were homogenized and sonicated as described elsewhere (15). MPO activity in supernatant fluids was measured by the change (per minute) in optical density (at 460 nm) resulting from the decomposition of H_2O_2 in the presence of *o*-dianisidine. The mean negative control MPO value of 0.08±0.01 in PBS-infused rats rose to 0.47±0.02 in the CVF-PBS infused rats. In animals also treated with 200 μg PNB1.6, the MPO value was 0.49±0.03. In CVF-injected animals also treated with PB1.3, there was a dose-dependent reduction (26–50%) in lung content of MPO. One-way analysis of variance of these data revealed $P < .001$. These *P* values were determined by comparisons of each group to the positive control group (CVF in PBS). Percent reduction was calculated after first subtracting the background negative control value from all values of CVF-injected animals. (For each group, $n = 5$).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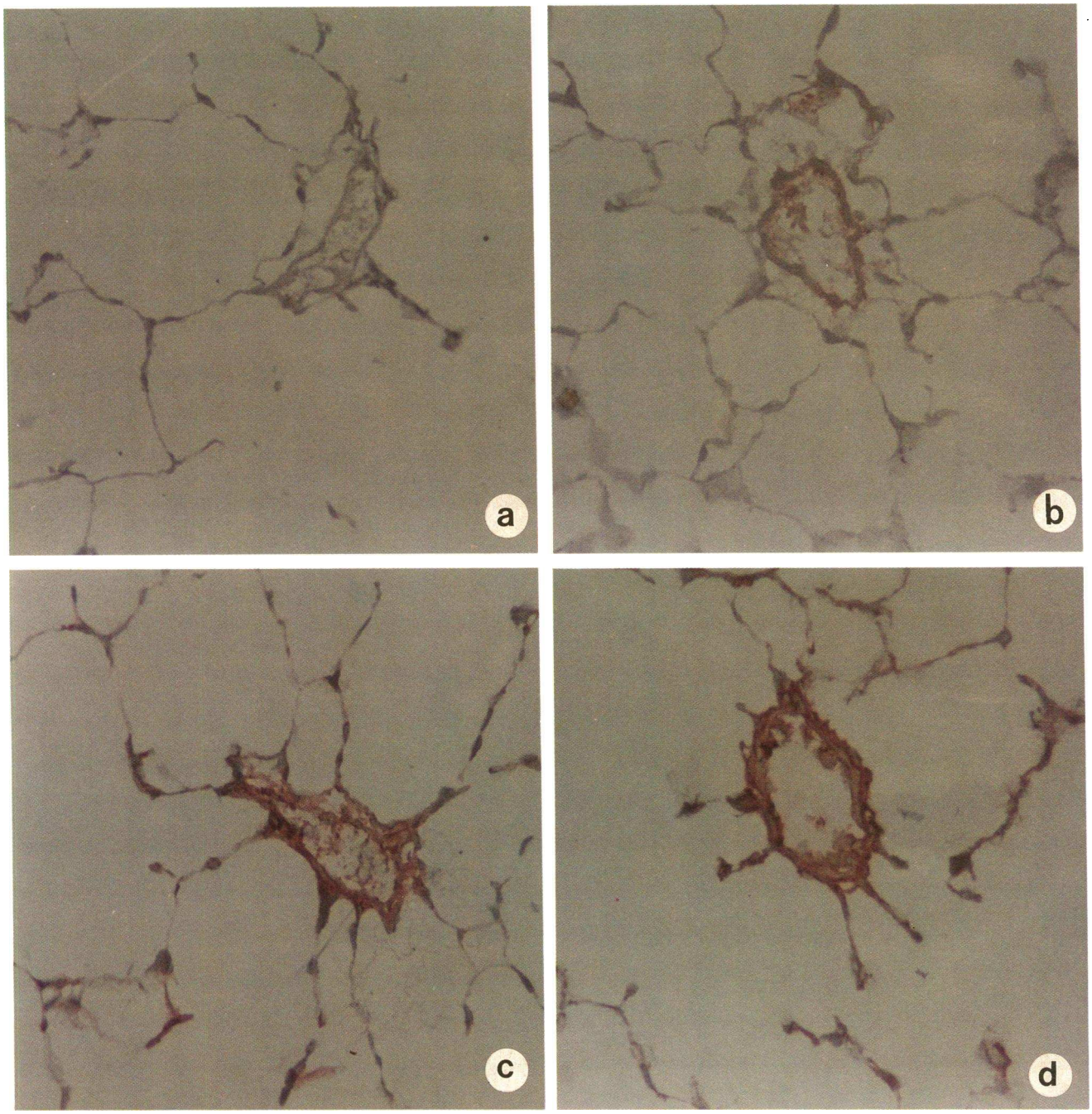

Figure 6. Immunohistochemical analysis of pulmonary vasculature for reactivity with anti-human P-selectin. Rats were infused with 20 U CVF/kg body wt and killed 0, 5, 15, and 20 min later. Lungs were harvested, inflated with OCT, snap frozen, and tissue sections stained for reactivity with anti-human P-selectin PB1.3. At time zero (*a*) little if any reactivity was evident. However, at 5 min (*b*) endothelial staining in postcapillary venules was evident. By 15 and 20 min (*c* and *d*), the venular staining was more intense and the septal capillaries were also reactive. (All magnifications, 150.)

Figure 5. Light and transmission electron micrographs of CVF-induced acute lung injury at 20 min in rats treated with 200 μg nonblocking antibody (PNB1.6) (*a* and *c*) or with blocking antibody (PB1.3) to human P-selectin (*b* and *d*). In animals treated with PNB1.6, vascular injury was indicated by extensive intraalveolar hemorrhage (*a*), associated with intravascular aggregates of neutrophils (*c, arrows*) in close contrast with endothelial cells. In contrast, in animals treated with PB1.6, intraalveolar hemorrhage was absent (*b*) and intravascular neutrophils showed little contact with the endothelium (*d, arrows*). (*a* and *b*, plastic-embedded sections stained with toluidine blue, X160; *c* and *d*, uranyl acetate, lead citrate stained, X2; 250.)

was increased at 15 and 20 min (*c* and *d*, respectively) after infusion of CVF. The pattern of staining involved pulmonary venules (*b–d*) and septal areas (*c* and *d*), the latter being in a pattern consistent with staining of interstitial capillaries. At 60 min staining had largely disappeared (data not shown). Although a P-selectin is presumably present in intracellular storage granules of endothelial cells before CVF infusion, it is apparent that mobilization of an epitope reactive with anti-human P-selectin to the surface of the endothelium dramatically enhanced its reactivity with PB1.3 antibody in tissue sections. Alternatively, it is also possible that a P-selectin-related material released by platelets had become absorbed to the endothelial surfaces, accounting for the immunohistochemical results.

Discussion

These data provide strong evidence for an in vivo biological role in the acute inflammatory response of a rat epitope reactive with anti-human P-selectin. P-selectin (GMP-140) is stored in alpha granules of platelets and in Weibel-Palade granules of endothelial cells, permitting its rapid translocation (within 5–10 min) to the surface of the cell after stimulation. Based on these facts, it could be predicted that a role for P-selectin would be expected in the early phases of an acute inflammatory response. In the CVF model of acute lung injury, because of the rapidity of the intrapulmonary vascular damage due to oxidants generated by complement-activated neutrophils, it was reasoned that expression of a P-selectin-like material might be key in this model of lung injury. Vascular injury in this model is associated with the intimate physical contact of neutrophils with pulmonary vascular endothelial cells; little if any transmigration of neutrophils occurs beyond the confines of the endothelium (28). The immunohistochemical analyses on sections of lung tissue confirmed rapid vascular expression of material reactive with PB1.3. It is possible that the reactivity visualized in Fig. 6 is due to translocation of a P-selectin from Weibel-Palade granules to the surfaces of endothelial cells; alternatively, there remains the possibility that platelets could have released P-selectin which then became physically associated with the surface of endothelial cells. A definitive resolution of this question is not currently possible. The early appearance of endothelial reactivity with PB1.3 after infusion of CVF is in striking contrast to the immune complex–induced intrapulmonary expression of E-selectin, requiring several hours. In the IgG immune complex model, the tissue accumulation of neutrophils is a rather slowly developing process, with neutrophil influx accelerating at 2.5 and 3 h, a time at which there is also rapidly developing expression of E-selectin in the pulmonary microvasculature (15, 32). In studies to date, the application of PB1.3 anti–P-selectin has failed to block the influx of neutrophils and attenuate injury in this E-selectin-dependent model of lung injury (Mulligan, M. S., and P. Ward, unpublished observations). It is not surprising that, in the CVF-induced model of lung injury, in which the events are compressed into a much shorter time frame (30 min), a role for a P-selectin has been identified. This very short time frame suggests that participation of E-selectin would not be likely.

The ability of PB1.3 anti-human P-selectin to reduce the sequestration of neutrophils and the attendant injury suggests that a rat P-selectin-like material may play a key role in this type of inflammatory injury. The inability of PNB1.6 to block injury is concordant with its inability to block P-selectin–mediated adhesion of activated platelets to neutrophils (as described above). In the CVF model of lung injury the role of platelets in intravascular aggregation of neutrophils is unclear. The transmission electron micrographs do not provide evidence for the physical presence of platelets which might act as "bridging" for neutrophil adhesiveness via P-selectin. Whether platelets are involved in the intravascular aggregation of neutrophils is not known. This raises the possibility that, via P-selectin, platelets activated by complement-generated anaphylatoxins may participate in the formation of neutrophil aggregates in vivo. Again, the role of platelets in the pathogenesis of CVF-induced lung injury cannot be determined at present.

Since anti-human P-selectin did not prevent the full extent of pulmonary vascular injury after infusion of CVF, it is possible that other constitutively expressed adhesion-promoting molecules could also be functioning to promote adhesion between endothelial cells and neutrophils. These could include endothelial ICAM-1 and the neutrophil $\beta 2$ integrins. The mediator(s) responsible for the upregulation of P-selectin on pulmonary vascular endothelial cells in the lung CVF model of lung injury has (have) not been identified, although it is known in this model that the levels of plasma histamine rise > 20-fold within 5 min after infusion of CVF (33). Histamine is a well-documented inducer of P-selectin expression on the surfaces of endothelial cells (24), and the time course of P-selectin expression (Fig. 5) would be consistent with the time course for the rise in plasma histamine after the infusion of CVF (33). The data in this study provide evidence for the role of a rat P-selectin-like material in the acute inflammatory response. P-selectin has been shown to recognize a carbohydrate ligand, sialylated Lewis^x, found on neutrophil glycoproteins and glycolipids (5, 6, 34). Thus, it is likely that P-selectin interaction with glycoprotein (or glycolipid) ligands plays an important role in initiation of the acute inflammatory response. Studies are in progress to define further the nature of the rat epitope that is reactive with anti-human P-selectin.

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