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The Role of Adhesion Molecules in Human Leukocyte Attachment to Porcine Vascular Endothelium: Implications for Xenotransplantation

Lisa A. Robinson,*† Li Li Tu,* Douglas A. Steeber,* Ori Preis,* Jeffrey L. Platt,*‡‡ and Thomas F. Tedder**

Many obstacles still prevent successful xenotransplantation of porcine donor organs. When hyperacute rejection is averted, transplanted pig organs are subject to acute vascular and cellular rejection. In autologous systems, leukocyte recruitment into inflamed tissues involves selectins, integrins, and Ig family members. To determine whether these mechanisms allow human leukocytes to effectively enter porcine grafts, the pathways by which human leukocytes adhere to TNF-α-stimulated porcine aortic endothelium were examined under static and physiologic flow conditions. L-selectin and E-selectin had overlapping functions in neutrophil capture and rolling, whereas Ab blockade of E-selectin and the β₂ integrins inhibited firm arrest of rolling neutrophils. Combined blockade of selectins and β₂ integrins resulted in negligible human neutrophil attachment to pig endothelium. Lymphocyte attachment to porcine endothelium was primarily L-selectin mediated, whereas β₁ integrin and VCAM-1/very late Ag-4 (VLA-4) interactions promoted static adhesion. Concurrent β₂ integrin, VLA-4, VCAM-1, and L-selectin blockade completely inhibited lymphocyte attachment. Thus, interactions between leukocyte-endothelial cell adhesion receptor pairs remained remarkably intact across the human-porcine species barrier. Moreover, disrupting the adhesion cascade may impair the ability of human leukocytes to infiltrate a transplanted porcine organ during rejection.


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Abbreviations used in this paper: PSGL-1, P-selectin glycoprotein ligand-1; VLA-4, very late Ag-4; PAEC, porcine aortic endothelial cells; L-IgM, L-selectin/murine IgM fusion protein.
porcine E-selectin in monkey COS cells promotes human neutrophil binding (31). Immobilization of a recombinant soluble form of porcine VCAM-1 on plastic also supports attachment of a human lymphoid cell line (32). Despite these studies, however, the extent to which adhesion molecules support human leukocyte interactions with porcine vascular endothelium remains generally uncharacterized.

To assess how leukocyte and vascular adhesion molecules might contribute to leukocyte entry into tissues following transplantation, the quantitative ability of human leukocytes to bind to porcine vascular endothelium was examined. In addition, the degree to which the selectins, integrins, and their ligands contribute to human leukocyte adhesion to pig endothelium was assessed using specific function-blocking mAbs. Remarkably, interactions between human adhesion molecules and their corresponding porcine counterreceptors remained strikingly intact across the human-porcine species barrier.

Materials and Methods

Tissue culture
Porcine aortic endothelial cells (PAEC) were harvested and characterized as previously described (33). PAEC were passaged serially and maintained in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM L-glutamine, and 25 mM HEPES (all from Life Technologies). In all experiments, PAEC were used between passages 4 and 9. Cells to be used in adhesion assays were plated on glass microscope slides (Gold Seal, Portsmouth, NH) within a 22-mm² area demarcated with a hydrophobic slide marker and grown to confluence. Confluent PAEC monolayers were stimulated for 6 h in culture medium containing 200 U/ml recombinant human TNF-α (Genzyme, Cambridge, MA).

Leukocyte isolation
Neutrophils were isolated by density gradient centrifugation of heparinized human blood from normal volunteers using Mono-Poly Resolving Medium (ICN Biomedicals, Costa Mesa, CA). Human lymphocytes were isolated by density gradient centrifugation using Lymphoprep (Nycomed Pharma, Oslo, Norway). All cells were kept on ice until utilized for adhesion assays. All protocols were approved by the Human Use Committee of Duke University.

Reagents and Abs
Abs used in these studies included: purified leukocyte adhesion molecule (LAM)–1 mAb (IgG1) directed against the lectin domain of human L-selectin (34); purified LAM–1 mAb (IgG1) directed against the consensus repeat domain of human L-selectin (35); the 4B4 anti-human CD29 mAb (VLA β-chain, IgG1) (36); the H52 anti-human CD18 mAb (LFA-1 β-chain, IgG1, Developmental Studies Hybridoma Bank, Iowa City, IA) (37); purified PL-1 anti-human PSGL-1 mAb (IgG1) (16, 18); the HAE2d anti-human and porcine VCAM-1 mAb (38); and purified MAB2124 anti-human and porcine E-selectin mAb (Chemicon International, Temecula, CA) (39). Purified mAbs were used at a final concentration of 10 μg/ml whereas other mAbs were used as ascites fluid diluted to final concentrations found to be at least fivefold in excess of saturation (usually 1/100 dilutions). To identify the presence of a ligand for L-selectin, a human L-selectin/murine IgM fusion protein (L’IgM), consisting of the extracellular domains of human L-selectin fused with the CH2, CH3, and CH4 domains of murine IgM heavy chain, was employed (L.T., M. Delahunty, and T.F.T., unpublished data). Supernatant fluid from L’IgM-cDNA-transfected COS cells was concentrated 50-fold and used at 50 μl to 10 μl PAEC for staining. Secondary Abs used in these studies included goat anti-mouse IgM Ab (Southern Biotechnology Associates, Birmingham, AL), goat anti-mouse IgG Ab (Caltag, South San Francisco, CA), and horseradish peroxidase-conjugated rabbit anti-mouse Ig Ab (Dako, Carpinteria, CA), used at final concentrations 40 μg/ml, 14 μg/ml, and 1/100 dilutions, respectively.

Flow cytometry
PAEC were stimulated for 6 h with TNF-α, then lifted from the culture flask using 1.5 mM EDTA in Dulbecco’s PBS (Life Technologies). Cells were washed in PBS containing 0.9 mM CaCl2, 0.5 mM MgCl2, and 2% bovine calf serum (PBS-FCS). Diluted HAE2d and MAB2124 mAbs and L’IgM were added to the cells before they were incubated at 4°C for 20 min. Cells were washed, then incubated with goat anti-mouse IgG or IgM Abs for 20 min at 4°C. Cells were washed, resuspended in 2% formaldehyde in PBS-FCS, and analyzed by flow cytometry using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). At least 10,000 cells were analyzed for each sample, with fluorescence intensity shown on a three-decade log scale.

PAEC whole cell ELISA
Cell surface expression of E-selectin and VCAM-1 by activated PAEC was assessed by growing PAEC to confluence in gelatin-coated 96-well plates (Costar, Cambridge, MA). PAEC were activated with 200 U/ml TNF-α for 0.5, 2, 4, 6, 14, or 24 h before the medium was decanted and the plates were gently washed with cold DMEM containing 10% FCS. The cells were incubated for 45 min at room temperature with HAE2d or MAB2124 mAb in DMEM containing 10% rabbit serum (Sigma) and 5% FCS. The plates were then washed three times with cold DMEM containing 10% FCS, and incubated for 30 min at room temperature with a secondary horseradish peroxidase-conjugated rabbit anti-mouse IgG Ab. Plates were washed five times with PBS containing 1% BSA and once with PBS. The developing solution, containing hydrogen peroxide and o-phenylenediamine in citrate buffer, was added and the optical density of the reaction product was quantitated at 492 nm light.

In vitro adhesion assays
Leukocyte adhesion assays were performed as previously described (38). Briefly, PAEC were grown to confluence on glass slides, stimulated with TNF-α, washed, and incubated for 20 min at 37°C with medium or HAE2d or MAB2124 mAbs. Neutrophils or lymphocytes (106) were resuspended in 200 μl of culture medium either alone or containing mAb at the appropriate dilutions. For assays done at 37°C, neutrophils and lymphocytes were maintained at room temperature until use, whereas for experiments done at 4°C, leukocytes were kept on ice after isolation. The leukocyte suspension was added to the endothelial monolayer under either static (gentle rocking at 10 cycles/min) or horizontal rotating (64 rpm) conditions. After a 30-min incubation period, the medium was tipped off, and the slides were fixed vertically in 2.4% glutaraldehyde (Polysciences, Warrington, PA) in PBS, pH 7.4. The number of leukocytes adherent to the endothelial monolayer was determined by counting between 4 and 12 × 400 microscopic fields that were located at half-radius distances from the center of the monolayers. Results were expressed as mean values ± SEM. Endothelial cell monolayers were confluent both at the beginning and at the end of the experiments.

Hydrodynamic flow chamber experiments
Adhesion of human neutrophils to TNF-α-stimulated PAEC under physiologic flow conditions was assessed as described (40). Briefly, PAEC were grown to confluence on 25-mm round glass coverslips (Fisher Scientific, Pittsburgh, PA), activated with TNF-α for 6 h, placed in a parallel plate laminar flow chamber, and mounted on the stage of an inverted phase-contrast microscope (Olympus, Lake Success, NY). Neutrophils were stored at 4°C until just before use, then resuspended in PBS containing 0.75 mM CaCl2, 0.75 mM MgCl2, and 0.5% BSA at a cell density of 106 cells/ml. In experiments involving blocking mAbs, either neutrophils or endothelium were preincubated for 5-20 min with various combinations of mAbs. The neutrophil suspensions were drawn through the chamber for a 10-min period at a wall shear stress of 1.85 dynes/cm² via a syringe pump (Harvard Apparatus, South Natick, MA). Neutrophil-endothelial interactions were recorded using a charged-coupled device (CCD) videocamera (Hitachi Denshi, Tokyo, Japan) and a Sony SuperVHS videorecorder. At the end of the 10-min neutrophil perfusion period, multiple ×100 fields were recorded for at least 30 s. The total number of adherent neutrophils (rolling + firmly adherent) was determined by analyzing the videotapes. Stable adhesion was defined as firm attachment for a minimum of 30 s. The rolling fraction was calculated by dividing the number of rolling neutrophils in any given field by the total number of adherent neutrophils. A minimum of ten 0.16-mm² fields were analyzed for each experiment. The data from multiple experiments were pooled.

Statistical analysis
Results are expressed as mean values ± SEM unless indicated otherwise. Student’s t test was used to determine the level of significance of differences in mean values between treatment groups.
**Results**

*Activation-induced expression of vascular adhesion molecules by PAEC*

The ability of TNF-α-activated PAEC to express E-selectin, VCAM-1, and vascular ligand(s) for L-selectin was assessed. L-selectin ligand expression by PAEC was assessed using a fusion protein, consisting of the extracellular domains of human L-selectin and the constant region of mouse IgM, termed L’IgM. Quiescent PAEC bound L’IgM at low levels as determined by flow cytometry analysis relative to staining with an unreactive mouse IgM control mAb (Fig. 1A). However, TNF-α-activated PAEC bound L’IgM at significantly higher levels. L’IgM binding was reduced to background levels by treating quiescent or activated PAEC with EDTA to block L-selectin binding (data not shown). Abs to E-selectin and VCAM-1 failed to label quiescent PAEC, but TNF-α activation up-regulated surface expression of these receptors (Fig. 1, B–D). Maximal E-selectin expression occurred at 4 h of activation and decreased thereafter (Fig. 1D). VCAM-1 expression was maximal at 6 h and remained at that level for 24 h (Fig. 1D). Therefore, adhesion molecule expression by TNF-α-activated PAEC mimics what has been shown for human vascular endothelium (38, 41, 42).

**Human neutrophil adhesion to PAEC**

The binding of human neutrophils to PAEC monolayers was assessed under both static and rotating conditions. Leukocyte attachment in static assays mimics the firm adhesive interactions that occur subsequent to initial leukocyte capture and rolling. In contrast, the shear force induced by 64 rpm rotation optimizes the assay to visualize selectin-medicated leukocyte attachment to vascular endothelium which may mimic the in vivo conditions whereby circulating leukocytes are initially captured by the endothelium (38). Quiescent PAEC supported minimal human neutrophil adhesion at 37°C under both static and rotating conditions (Fig. 2 and Fig. 3, A and B). However, PAEC activation with TNF-α enhanced neutrophil binding by greater than 10-fold (Figs. 2 and 3).

Blockade of neutrophil L-selectin function under static conditions caused a mere 14% decrease in neutrophil binding (Fig. 3A). Blocking endothelial E-selectin or neutrophil PSGL-1 functions under static conditions reduced neutrophil binding by 53% and 41%, respectively (Figs. 2 and 3A). It is likely that P-selectin plays a role in neutrophil adhesion in these assays since the PL-1 (anti-PSGL-1) mAb used in these experiments predominantly inhibits P-selectin and L-selectin binding to neutrophils rather than E-selectin function under these conditions. Blocking neutrophil CD18 function resulted in a 44% reduction in adhesion, whereas blockade of all selectin-ligand interactions resulted in a 67% decrease in binding of neutrophils. Inhibiting selectin and CD18 function reduced neutrophil binding to the level seen with quiescent endothelium (91% inhibition; Fig. 3A, p < 0.01).

When adhesion assays were performed under rotating conditions at 37°C, L-selectin-mediated neutrophil binding to PAEC was revealed, as LAM1–3 mAb inhibited neutrophil binding by 71% (Fig. 3B). E-selectin mAb blockade inhibited neutrophil binding to 23% of control levels (Fig. 3B). Under rotating conditions, PSGL-1 was less important for neutrophil binding to PAEC, as mAb against this structure inhibited binding by only 21% (Fig. 3B). Although blocking CD18 function under both rotating and static conditions resulted in a 41% reduction in adhesion (Fig. 3, A and B), the contribution of this molecule relative to that of other individual adhesion receptors was greater in static assays. Inhibition of the selectins and their ligands resulted in nearly complete inhibition of neutrophil binding, with modest additional benefit conferred by CD18 blockade (Fig. 3B, p < 0.05).

To further dissect the function of the three selectins during different phases of leukocyte recruitment, additional assays were performed at 4°C. At this temperature, L-selectin endoproteolytic release from the cell surface is minimal and β2 integrins are nonfunctional (38). When the assays were performed with rotation at 4°C, blocking L-selectin function inhibited neutrophil attachment by 95% (Fig. 3D), compared with only 53% inhibition under static conditions (Fig. 3C). mAb reactive with E-selectin did not reduce neutrophil binding under static conditions at 4°C (Fig. 3C), but diminished binding by 31% during rotation (Fig. 3D). mAb reactive with PSGL-1 blocked neutrophil binding under both static and rotating conditions at 4°C (46% and 56%; Fig. 3, C and D).
Not surprisingly, anti-CD18 mAb did not inhibit human neutrophil binding to porcine endothelium under either static or rotating conditions at 4°C (Fig. 3, C and D).

**Human lymphocyte adhesion to activated PAEC**

Lymphocyte binding to PAEC, like neutrophil binding, was dramatically enhanced after PAEC activation with TNF-α (Figs. 4 and 5). When the binding assays were performed at 37°C under static conditions, blocking L-selectin function inhibited lymphocyte binding by 32% (Fig. 5A). Blocking CD29, VCAM-1, and CD18 function reduced lymphocyte binding by 61%, 43%, and 39%, respectively (Fig. 5A). Simultaneous blockade of CD18, VLA-4, and VCAM-1 reduced lymphocyte binding to baseline levels, with L-selectin blockade conferring some additional benefit (Fig. 5A; \( p < 0.01 \)). Blocking L-selectin function inhibited lymphocyte binding by 63% under rotating conditions (Fig. 5B). Under rotating conditions, blocking CD29, VCAM-1, and CD18 function inhibited adhesion by 60%, 50%, and 43%, respectively (Fig. 5B). Blockade of CD29, VCAM-1, and CD18 diminished lymphocyte binding by 89%, whereas additional L-selectin blockade further reduced binding to 5% of control levels under rotating conditions (Fig. 5B, \( p < 0.05 \)). At 4°C, blocking L-selectin function inhibited human lymphocyte adhesion to activated PAEC by 50% and 85% under static and rotating conditions, respectively (Fig. 5, C and D). Blocking CD18 and CD29 function did not affect binding under static or rotating conditions.
The fact that blocking VCAM-1 function had no effect on adhesion at 4°C under rotating conditions (Fig. 5D), despite the functional ability of VCAM-1 to bind to VLA-4 at 4°C, suggests that L-selectin is the primary mediator of initial lymphocyte capture in these assays. The ability of anti-L-selectin mAb to also diminish binding by 50% under static conditions (Fig. 5C) suggests that initial L-selectin-mediated capture is prerequisite to establishment of subsequent integrin-mediated stable adhesion in this assay system.

**Human neutrophil adhesion to PAEC monolayers under physiologic flow**

The ability of human neutrophils to bind PAEC under physiologic flow conditions was assessed by passing human neutrophil suspensions through a parallel-plate flow chamber over adherent PAEC monolayers at 37°C. A physiologic wall shear stress of 1.85 dynes/cm² was used for all experiments. In these assays, the total number of adherent neutrophils (rolling + firmly adherent cells) was determined after a 10-min period of chamber perfusion. The rolling fraction was determined by dividing the number of rolling neutrophils by the total number of firmly adherent neutrophils. In these assays, TNF-α activation of PAEC increased neutrophil adhesion by 200-fold: 208 ± 14 neutrophils/0.16 mm² vs 1.1 ± 0.2 neutrophils/0.16 mm² for unstimulated PAEC monolayers (p < 0.01, Fig. 6). Rolling neutrophils accounted for 14% of the attached cells. Although incubating neutrophils with a control non-function blocking L-selectin mAb did not significantly affect the total number of adherent cells, the rolling fraction fell. However,

![FIGURE 4](image_url)

**FIGURE 4.** Human lymphocyte adhesion to cytokine-activated PAEC. Confluent PAEC monolayers were cultured on slides with either medium alone (quiescent) or TNF-α for 6 h. PAEC were incubated with an anti-VCAM-1 mAb (V) whereas human lymphocytes were incubated with a control mAb (LAM1–14) or function blocking mAbs reactive with L-selectin (L), CD18, CD29, or combinations of these mAbs. Subsequently, the human lymphocytes were incubated with the PAEC monolayers under static conditions at 37°C in the presence of the indicated mAbs. Nonadherent lymphocytes were removed by gentle washing. Photographs are representative of the results shown in Fig. 5A (×400).

![FIGURE 5](image_url)

**FIGURE 5.** Inhibition of human lymphocyte adhesion to PAEC. Human lymphocytes and PAEC were treated as described in Fig. 4 except the adhesion assays were conducted at 37°C under static conditions (A), at 37°C under nonstatic conditions (B), at 4°C under static conditions (C), and at 4°C under nonstatic conditions (D). The number of lymphocytes adherent to endothelial monolayers was determined by counting between 4 and 12 × 400 microscopic fields for each slide. Values represent the mean ± SEM of results from at least three experiments under each set of conditions. * Differences between activated PAEC that were untreated (no mAb) and all other groups were significant, p < 0.01.
the absolute number of rolling cells was small, and the control mAb may have exerted steric effects, spatially interfering with selectin-binding sites involved in rolling.

Pretreatment of neutrophils with anti-L-selectin mAb resulted in 56% fewer attached neutrophils (Fig. 6) and reduced the rolling fraction to 2.1% (p < 0.01). Blocking E-selectin function diminished neutrophil attachment by 53% (Fig. 6) and reduced the rolling fraction to 2.4% (p < 0.01). Blocking PSGL-1 function reduced attachment by 42% (Fig. 6) and reduced the rolling fraction to 7.9% (p < 0.01). Combined blockade of L-selectin, E-selectin, and PSGL-1 reduced binding to 23% of control levels (Fig. 6). Blocking neutrophil CD18 function in these assays had a less pronounced effect on neutrophil adhesion (25% inhibition; Fig. 6), but blocking anti-L-selectin mAbs, and assembled onto a parallel plate flow chamber. Endothelial monolayers were perfused for 10 min at a wall shear stress of 1.85 dynes/cm² with neutrophil suspensions (10⁶ cells/ml) in the presence of the indicated mAbs. At the end of the 10-min period, the total number of adherent neutrophils, comprised of the sum of rolling (open bars) and stably adherent (shaded bars) neutrophils, per 0.16-mm² microscopic field was determined by counting a minimum of 10 fields of view. Results are expressed as the mean ± SEM for three independent experiments for each mAb treatment, except for experiments using the PSGL-1 (PL-1) mAb alone, where n = 2. * Differences between activated PAEC that were untreated (no mAb) and all other groups were significant, p < 0.01.

Discussion

Our results indicate that 1) TNF-α activation of porcine vascular endothelium enhances surface expression of E-selectin, VCAM-1, and L-selectin ligand(s) and 2) human neutrophils and lymphocytes bind extensively to cytokine-activated PAEC in an adhesion receptor-dependent manner. In fact, the pattern and kinetics of E-selectin, VCAM-1, and L-selectin ligand expression by PAEC (Fig. 1) were similar to those described previously for cultured human umbilical vein endothelial cells (38, 39, 43). Under static and flow conditions, binding of both human neutrophils and lymphocytes to porcine endothelial monolayers was inhibited by mAbs directed against pig or human adhesion molecules (Figs. 2–6), demonstrating that interactions between specific human adhesion receptors and their porcine counterreceptors are well conserved. Although not directly compared in these studies, human neutrophil binding to activated porcine vascular endothelium under physiologic flow conditions (Fig. 6) and in static and nonstatic adhesion assays (Fig. 3) was of roughly the same order of magnitude as previously described in studies of human neutrophil attraction to TNF-α-activated human vascular endothelium under corresponding conditions (38, 44). The same appears to be true for the binding of human lymphocytes to both activated porcine and human vascular endothelium (38). That the selectins and integrins may mediate human leukocyte adhesion to PAEC on a scale of the same order of magnitude as that observed for human leukocytes interacting with human endothelial cells (38) suggests that these interactions may be well preserved in vivo following xenotransplantation.

The current studies significantly extend previous studies examining the molecular events that occur during human leukocyte interactions with porcine endothelial cells (30–32, 45, 46). As shown in the current studies, interactions between human or porcine L-, E-, and P-selectins and their ligands are consistent with the highly conserved nature of selectin binding across species (35, 45, 47, 48). Previous studies by two groups have shown that porcine E-selectin is 71–75% homologous with human E-selectin and that when overexpressed in COS cells it would mediate some level of human neutrophil adhesion (31, 45). In addition, a chimeric molecule consisting of the lectin domain of porcine E-selectin and the epidermal growth factor domain of human E-selectin fused to the human IgG constant region would bind porcine granulocytes and a subpopulation of porcine lymphocytes (45). The ability of human leukocyte integrins to bind porcine Ig superfamiliy molecules has also been described (30, 32, 46). Although mouse LFA-1 does not bind to human ICAM-1 (49), human LFA-1 interacts efficiently with porcine ICAMs (Figs. 3, 5, and 6). Others have shown VLA-4-dependent binding of a human lymphoid cell line to both recombinant porcine VCAM-1 bound to plastic as well as VCAM-1 expressed by cytokine-stimulated PAEC (32, 46). In those studies, an anti-VLA-4 mAb blocked adhesion of human T cells to stimulated PAEC by 60–65% in static assays. Therefore, the current studies provide qualitative evidence for the compatibility of human and porcine adhesion receptors and are the first to quantitate the sequential contributions of individual adhesion molecules in a physiologic flow setting.

The selectins act in concert to mediate the initial capture of free-flowing leukocytes from the circulation under shear conditions (12). Under nonstatic or hydrodynamic flow conditions, preincubation of human neutrophils with anti-L-selectin mAb reduced by 56–71% the total number of neutrophils adherent to porcine endothelium (Figs. 3 and 6). In a similar fashion, exposure of the endothelium to an anti-E-selectin mAb reduced neutrophil binding by 53–77% (Figs. 3 and 6), and reduced rolling by 83%. Although P-selectin function was not examined directly because function-blocking anti-porcine P-selectin mAbs were not identified, P-selectin function was assessed by treating human neutrophils with a mAb that blocks the P-selectin binding site of PSGL-1. This anti-PSGL-1 mAb reduced neutrophil binding less dramatically, a 21–42% decrease (Figs. 3 and 6), although P-selectin function is likely to be more critical at early time points following PAEC activation.

**FIGURE 6.** Adhesion of human neutrophils to activated porcine endothelium under physiologic conditions of flow. Confluent monolayers of PAEC grown on coverslips were activated with TNF-α for 6 h, cultured with medium or anti-E-selectin mAbs, and assembled onto a parallel plate flow chamber. Endothelial monolayers were perfused for 10 min at a wall shear stress of 1.85 dynes/cm² with neutophil suspensions (10⁶ cells/ml) in the presence of the indicated mAbs. At the end of the 10-min period, the total number of adherent neutrophils, comprised of the sum of rolling (open bars) and stably adherent (shaded bars) neutrophils, per 0.16-mm² microscopic field was determined by counting a minimum of 10 fields of view. Results are expressed as the mean ± SEM for three independent experiments for each mAb treatment, except for experiments using the PSGL-1 (PL-1) mAb alone, where n = 2. * Differences between activated PAEC that were untreated (no mAb) and all other groups were significant, p < 0.01.
as occurs with human endothelium (50). Nonetheless, the combined blockade of L-selectin, E-selectin, and PSGL-1 function produced the greatest effect, decreasing total neutrophil adhesion by 77–96% (Figs. 3 and 6). By contrast, lymphocyte attachment under shear conditions was predominantly L-selectin-mediated (Fig. 5). These results suggest that the three selectins act in concert to promote the initial capture of circulating leukocytes and their subsequent rolling on activated porcine endothelium as occurs with human endothelium at sites of inflammation.

The combined blockade of L-selectin, E-selectin, PSGL-1, and CD18 function drastically decreased human neutrophil adhesion to activated PAEC by >90% (Figs. 3 and 6). Lymphocyte static adhesion under shear conditions was predominantly promoted by VCAM-1/VLA-4 and β2 integrin interactions (Fig. 5). Combined mAb blockade of CD18, VLA-4, VCAM-1, and L-selectin essentially attenuated all (85–95%) lymphocyte attachment to PAEC (Fig. 5). Surprisingly, CD18 blockade alone only partially inhibited neutrophil attachment in parallel plate flow chamber experiments (Fig. 6). However, E-selectin may function synergistically with the β2 integrins to stabilize human neutrophil attachment to porcine endothelium under these conditions, since E-selectin blockade significantly impaired neutrophil attachment to PAEC in static binding assays (Fig. 3). By contrast, E-selectin blockade did not significantly inhibit neutrophil attachment at 4°C, a temperature at which CD18 is nonfunctional. This finding suggests that a component of E-selectin-mediated attachment is dependent on CD18 function. Therefore, it is likely that E-selectin and CD18 ligands cooperate to mediate firm leukocyte adhesion. This would explain why the simultaneous blockade of both E-selectin and CD18 significantly impaired the ability of rolling neutrophils to stably arrest in hydrodynamic flow assays (Fig. 6). This result is also in agreement with the recent finding that a specific E-selectin mAb can block neutrophil recruitment into an inflamed peritoneum without affecting neutrophil capture or rolling in vitro or in vivo (51). In addition, mice lacking E-selectin show normal rolling but reduced arrest of leukocytes on cytokine-activated microvascular endothelium (52). Thus, E-selectin may participate in steps of the adhesion cascade that extend well beyond initial rolling.

Many questions regarding the role of adhesion molecules in allograft rejection remain unanswered, and still less is known about their involvement in xenograft rejection. The current studies lay at which CD18 is nonfunctional. This finding suggests that a component of E-selectin-mediated attachment is dependent on CD18 function. Therefore, it is likely that E-selectin and CD18 ligands cooperate to mediate firm leukocyte adhesion. This would explain why the simultaneous blockade of both E-selectin and CD18 significantly impaired the ability of rolling neutrophils to stably arrest in hydrodynamic flow assays (Fig. 6). This result is also in agreement with the recent finding that a specific E-selectin mAb can block neutrophil recruitment into an inflamed peritoneum without affecting neutrophil capture or rolling in vitro or in vivo (51). In addition, mice lacking E-selectin show normal rolling but reduced arrest of leukocytes on cytokine-activated microvascular endothelium (52). Thus, E-selectin may participate in steps of the adhesion cascade that extend well beyond initial rolling.

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