Fibrinogen Mediates Leukocyte-Endothelium Bridging In Vivo at Low Shear Forces

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In addition to preserving hemostasis, fibrinogen assembly on leukocytes mediates inflammatory responses and may aberrantly contribute to vascular injury. In this study, we used real-time intravital video microscopy in exposed rabbit mesentery to investigate the potential role of fibrinogen on leukocyte adherence mechanisms, in vivo. At physiologic concentrations of 0.15 to 0.5 mg/mL, human fibrinogen dose-dependently enhanced by threefold to fivefold the adhesion of chemoattractant-stimulated mononuclear HL-60 cells to rabbit mesenteric endothelium, by acting as a bridging molecule between the two cell types. Fibrinogen-dependent intercellular bridging occurred in venules, but not in arterioles or capillaries (1), was optimal at reduced flow shear forces (range: 0.77 to 2.79 dyne/cm²) (2), and produced a firm attachment of mononuclear cells to endothelium, rather than transient rolling (3). Consistent with this model, rabbit fibrinogen failed to support human leukocyte adhesion, while human fibrinogen enhanced mononuclear cell attachment to rabbit endothelial cells in vitro, in a reaction indistinguishable from that observed with human endothelium. Antagonists of the recently described association of fibrinogen with intercellular adhesion molecule-1 (ICAM-1), including monoclonal antibodies (MoAbs) LB-2 or 2D5, or the fibrinogen γ2 peptide γAsn137,Ala138, blocked fibrinogen-dependent leukocyte-endothelium interaction in vitro or in vivo, respectively, while a control nonbinding antibody or the fibrinogen L10 peptide γLeu402-Val411 were ineffective. These data suggest that simultaneous assembly of fibrinogen on leukocytes and endothelial ICAM-1 provides a pathway of intercellular adhesion which may act in concert with β2 integrins to stabilize firm leukocyte attachment to endothelium, in vivo. Given the recognized role of fibrinogen as a major risk factor for atherosclerosis, this mechanism may directly contribute to thrombus formation and endothelial cell damage in vascular diseases. © 1996 by The American Society of Hematology.

It has been appreciated for more than 2 decades that blood clotting mechanisms contribute to inflammatory responses in vivo. Particularly, the use of systemically defibrinated animals has shown that fibrinogen plays a primary pathogenetic role in the formation of intraabdominal abscesses during purulent infections, in mediating severe intraglomerular tissue damage in acute nephrotoxic nephritis, and in promoting recruitment of inflammatory cells onto biomaterial implants. Furthermore, fibrinogen degradation products exhibited chemotactic and proinflammatory properties on polymorphonuclear leukocytes, and profoundly downmodulated lymphocyte effector functions.

Among the several membrane receptors that coordinate the assembly of fibrinogen on leukocytes, the inductive and high affinity recognition of fibrinogen by the myelo-monocytic integrin CD11b/CD18 (Mac-1, αMβ2) has been shown to play a primary role in a variety of vascular cell effector functions, including procoagulant activity, intracellular signaling with generation of second messengers, cytokine release, production of oxidative radicals, and intercellular adhesion. Specifically, fibrinogen has been shown to act as a bridging molecule to enhance leukocyte attachment to endothelial cells in vitro, through a simultaneous interaction with CD11b/CD18 on the leukocyte and intercellular adhesion molecule-1 (ICAM-1) on the endothelium. This novel recognition of fibrinogen by endothelial ICAM-1 has been independently substantiated in recent studies, with respect to cytoskeletal requirements for ligand binding, and its signaling role in vascular wall relaxation.

In view of the considerable interspecies homology and conservation of function between human and rabbit vascular cell adhesion molecules, it has recently become possible to examine the interaction between human leukocytes and rabbit endothelial cells in the mesenteric circulation at physiologic shear rates, in vivo. Using real-time intravital video microscopy in exposed rabbit mesentery, we have now re-investigated the potential contribution of fibrinogen to the multistep “adhesion cascade” of leukocyte-endothelium interaction, in vivo. We have found that fibrinogen mediates firm attachment of mononuclear cells to endothelium in a shear stress-dependent mechanism, and that this pathway of intercellular bridging was abrogated by monoclonal antibody (MoAb) or synthetic peptide antagonists of the ICAM-1–fibrinogen recognition.3-8

MATERIALS AND METHODS

Cells and cell culture. Promyelocytic HL-60 and monocytic THP-1 cell lines (American Type Culture Collection, Rockville, MD) were maintained in culture in complete RPMI 1640 medium (Bio-Whittaker, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Whittaker), 2 mMol/L L-glutamine, and 10 mMol/L HEPES. HL-60 cells were terminally differentiated to a mature monocytic phenotype by a 72-hour culture in the presence of 10 μMol/L 1,25-dihydroxy-vitamin D3, and 1.25 μMol/L indomethacin (Sigma Chemical Co, St Louis, MO), according to
published protocols. De novo induction of differentiation-specific monocytic markers, including CD11b/CD18, on vitamin D3-treated HL-60 cells was confirmed by flow cytometry, in agreement with previous observations. Published comparative studies showed that vitamin D3–differentiated HL-60 and monocytic THP-1 cells were indistinguishable for their ability to bind fibrinogen and adhere to human umbilical vein endothelial cells in a fibrinogen-dependent manner, thus providing reliable models for this pathway of intercellular bridging. Briefly, animals were anesthetized with a single intramedulary (i.m.) injection of ketamine (25 mg/kg) mixed with xylazine (Rompun, 5 mg/kg), and complete anesthesia was maintained during the entire procedure by intravenous administration of 0.5 to 1.0 mL of ketamine/xylazine mixture through an intravenous catheter placed in the marginal ear vein. After unilateral neck incision, the external jugular vein was exposed, followed by ligation of all visible branches. A proximal and a distal silk loops were placed and tied at the thoracic outlet and at the angle of the jaw, respectively. After venotomy with a n.11 blade, the vessel was flushed with M-199 medium containing 10 U/mL heparin prewarmed at 37°C, followed by excision in proximal to distal fashion, and immediately processed for endothelial cell culture. Cells were obtained under sterile conditions by collagenase treatment (0.2% Worthington type I collagenase in M-199 medium plus 1% FBS), washed, and plated in complete tissue culture medium containing M-199 plus 20% FBS, 100 μg/mL endothelial cell growth factor, 2 mM/L L-glutamine, 17.5 μU/mL heparin (Sigma; porcine intestinal mucosa) onto gelatin-coated 24-well tissue culture plates (Costar Corp, Cambridge, MA). Cells were maintained in culture for 4 to 6 passages, and functionally characterized as described previously.

Cell labeling and fibrinogen-dependent intercellular bridging. Vitamin D3–differentiated HL-60 cells (1 × 10^6/mL) in phosphate-buffered saline (PBS), pH 7.4, containing 0.01% glucose were fluorescently labeled with 20 μM/L carboxy fluorescein diacetate (CFDA; Molecular Probes, Eugene, OR) for 30 minutes at 22°C in the dark with intermittent agitation, as described previously. At the end of the incubation, the cells were washed, suspended in PBS, pH 7.4, containing 0.01% glucose at 1 × 10^7/mL, and kept in the dark at 22°C until use. To test the effect of fibrinogen on leukocyte-endothelium interaction in vivo, vitamin D3–treated, CFDA-labeled HL-60 cells at 0.2 to 1 × 10^7/mL were stimulated or treated with 10 μM/L of the chemotactic formyl-methionyl-leucyl phenylalanine (fMLP; Sigma) and incubated with increasing concentrations of plasma-purified human fibrinogen, for 20 minutes at 37°C before perfusion into the rabbit superior mesenteric artery (see below). fMLP-stimulation of HL-60 cells resulted in transient increased affinity of CD11b/CD18 for fibrinogen binding, in agreement with previous observations. In another series of experiments, aliquots (1 × 10^6/mL) of monocytic THP-1 or vitamin D3–differentiated HL-60 cells were labeled with 0.5 μCi/Cr (Amersham, Arlington Heights, IL) for 1.5 hours at 37°C, with a final incorporation of 3 to 7 cpm/cell. After washes in PBS, pH 7.4, cells were suspended in serum-free RPMI 1640, stimulated with 10 μM/L fMLP, and equilibrated with human or rabbit fibrinogen (300 μg/mL), or control protein transferrin (300 μg/mL) in the presence of 2.5 mM/L CaCl_2. After 20 minutes of incubation at 22°C, cells were separately added to confluent monolayers of human or rabbit endothelial cells for increasing time intervals between 10 and 90 minutes at 37°C. At the end of each incubation, wells were washed three times with serum-free RPMI 1640, and attached cells were solubilized in 20% sodium dodecyl sulfate (SDS) with determination of radioactivity in a scintillation β-counter. In inhibition experiments, monolayers of human or rabbit endothelial cells were incubated with 25 μg/mL aliquots of functionally blocking antibody–ICAM-1 MoAbs LB-2, control nonbinding MoAb 14E11 for 20 minutes at 37°C, before addition of ^51Cr-labeled THP-1 cells pre-equilibrated with human fibrinogen, and determination of leukocyte-endothelium interaction after a 30-minute interval at 37°C, as described above. The effect of fibrinogen peptide antagonists on monocytic cell adhesion to rabbit mesenteric endothelium in vivo was investigated as follows. Increasing concentrations (0 to 2.0 mg/mL) of fibrinogen γ-chain–derived synthetic peptides γ3 (Asn^17^-Ala^18), L10 (Leu^40^-Val^41), or a control peptide with the γ3 sequence synthesized in scrambled order were perfused in the rabbit mesentery circulation (see below) simultaneously with fMLP (10 μM/L)-stimulated, CFDA-labeled HL-60 cells, equilibrated with 300 μg/mL human fibrinogen before injection, as described above. Peptides were greater than 95% pure as judged by high-performance liquid chromatography purification. Endotoxin contamination in purified fibrinogen preparations or the fibrinogen-derived synthetic peptides used in this study was always less than 70 pg/mL, as measured by the Limulus Amebocyte assay, according to the manufacturer’s specifications (Associates of Cape Cod, Inc, Woods Hole, MA).

Adhesion assay. Ninety-six well microtiter plates (Costar) were coated with increasing concentrations of human or rabbit fibrinogen (0.4 to 50 μg/mL), or control protein transferrin, in PBS, pH 7.4, for 16 hours at 4°C. After washes in PBS, pH 7.4, wells were postcoated with 3% bovine serum albumin (BSA; Sigma) for 30 minutes at 37°C, rinsed, and incubated with 0.1 mL aliquots of fMLP-stimulated ^51Cr-labeled THP-1 cells (1 × 10^6/mL) for 45 minutes at 37°C. In some experiments, THP-1 cells were pre-equilibrated with anti-CD18 MoAb IB4 for 20 minutes at 22°C before addition to substrate-coated plates. At the end of the incubation, wells were washed three times with PBS, pH 7.4, and attached cells were solubilized in 20% SDS with determination of radioactivity in a scintillation β-counter.

Intravital microscopy studies. Real-time adhesion of vitamin D3–differentiated HL-60 cells to endothelium in vivo was assessed in the mesenteric circulation of New Zealand White rabbits, as described previously with the following modifications. Briefly, animals (1.2 to 1.5 kg body weight) were sedated with i.m. injection of 0.1 mL 0.1% droperidol and 0.002% fentanyl (Mallinckrodt Veterinary, Mundelein, IL) and orally administered with loperamide hydrochloride (2 mg/1.5 mL PBS) to reduce intestinal peristalsis. Surgical anesthesia was subsequently induced with 5 to 10 mL 20% urethane intravenously and animals were prepared for in vivo observation as described. The animals were placed on a heating pad (37°C) controlled by a rectal thermometer. A tracheal tube was placed to facilitate breathing. This was followed by insertion of catheters in the right carotid artery for continuous maintenance of blood pressure or to draw blood samples, and in the left jugular vein for continuous administration of saline containing anticoagulant PPACK at 5 μg/kg/min body weight. A mid-line abdominal incision was made and the terminal ileum was exteriorized and spread on a heated glass window (37°C) of the stage of a Leitz intravital microscope (Wetzlar, Germany). The exteriorized mesentery was superfused continuously with warm endotoxin-free isotonic saline solution, pH 7.4, and parts of the intestine which were not microscopically observed were covered with isotonic saline-soaked cotton pads. This was followed by cannulation of a side branch of the terminal superior mesenteric artery with a polyethylene catheter. CFDA-labeled HL-60 cells were injected through the cannulated artery as described. Venular blood flow was reduced by partial occlusion of the superior mesenteric artery using an inflatable balloon catheter, as described previously with slight modifications. After insertion of PE10 polyethylene catheter into the arterial side branch upstream from a vascular area chosen for microscopic observation, a balloon catheter was gently wrapped and tied around the main stem of the superior mesen-
tery artery (upstream of the cannulated side branch). The balloon catheter was attached to an air-filled syringe. Controlled inflation of the balloon resulted in regulating the blood flow through the mesentery artery. The collateral circulation through the ileum was also blocked using occluder clamps. Regulation of the blood flow in microvascular segment for short and intermittent duration of times had no effect on local hemodynamics and was reversible.25 Initial video recording were obtained to determine the baseline adhesion of CFDA-labeled HL-60 cells in the venules. Subsequently, the mesenteric blood flow was reduced by a regulated inflation of the balloon catheter followed by administration of injected HL-60 cells through the catheter downstream from the occlusion site. The balloon catheter was deflated between injections. CFDA-labeled HL-60 cells (0.2 to 0.5 × 107) injected under the various conditions tested, and passing through mesenteric vessels (35- to 60-μm diameter) in the downstream circulation, were made visible by stroboscopic epi-illumination using a video triggered Xenon lamp (Chadwick Helmuth, Mountain View, CA) and a Leitz Ploemopak epi-illuminator employing an 12 filter block. The images were recorded through a silicon-intensified tube camera (SIT768; Dage MTI, Michigan City, IN) using a ×10/0.13 water immersion objective (Nikon, Melville, NY) and a SVHS video recorder (JVC HC-6600; Japan). Video recordings were analyzed off-line by two individuals for assessment of HL-60 cell adhesion by counting the total number of adherent CFDA-labeled cells per venule. A cell was considered adherent to venular endothelium if it remained stationary for longer than 30 seconds. HL-60 cell adhesion before or after treatment with control, or fibrinogen γ-chain–derived synthetic peptides was separately determined by frame-by-frame analysis of video recorded images. The centerline blood flow velocity (Vc) in each venule was determined as 

\[ V_c = \frac{2 \times V_{max}}{2 + D_c/D_v}, \]

as previously described26 where \( V_{max} \) is the maximum velocity measured in each sample and \( D_c \) and \( D_v \) are the diameters of HL-60 cells and of the venules, respectively. The wall shear rate was calculated as previously described25 (\( V_c/1.6 \)) × (1/\( D_v \)) × 8 (s⁻¹), and shear stress acting on HL-60 cells was determined as: wall shear rate × 0.025 (dyne/cm²) where 0.025 is the approximate blood viscosity in Poise. For all experiments, data were expressed as number of adherent cells/venule. Statistical analysis of HL-60 cell adhesion to endothelium under the various conditions tested was performed by multiple comparisons of paired data by Student’s t-test using a statistical software package (Sigmastat, Jandel Scientific). \( P \) values of <.05 were considered statistically significant. All results are given as mean ± SD.

RESULTS

Fibrinogen-dependent leukocyte-endothelial cell interaction. Vitamin D₃–differentiated monocytic HL-60 cells18 did not constitutively adhere to rabbit endothelium, under physiologic and reduced conditions of blood flow (Fig 1). Stimulation of HL-60 cells with 10 μmol/L fMLP, which upregulates CD11b/CD18 ligand binding,7 or addition of 300 μg/mL of human fibrinogen, resulted, in detectable levels of monocytic cell adhesion to endothelium under reduced flow conditions (shear stress: 1.71 ± 0.47 dyne/cm²) (fMLP-stimulated: 1.55 ± 0.88 cells/venule; \( n = 7 \) venules in 4 rabbits) ie, \( n = 7/4; \) \( P = .002 \) unstimulated cells; fibrinogen-equilibrated cells: 1.14 ± 0.69/venule; \( n = 7/4; \) \( P = .011 \) untreated cells) (Fig 1). Under these conditions of shear stress (range: 0.77 to 2.79 dyne/cm²), binding of fibrinogen to fMLP-stimulated HL-60 cells resulted in a threefold to fivefold enhancement of adhesion of monocytic cells to venular endothelium (6.8 ± 3.03 cells/venule; \( n = 8/4; \) \( P = .004 \)), compared with control cells stimulated with fMLP alone (1.43 ± 0.97 cells/venule; \( n = 7/4 \)), or equilibrated with fibrinogen alone (Fig 1).

The enhancing effect of fibrinogen on monocytic cell-endothelium interaction in vivo was concentration-dependent (4.7 to 7.8 cells/venule, Fig 1) and regulated by flow shear forces. At physiological shear rates (11.67 ± 4.59 dyne/cm²; range 3.38 to 17.8 dyne/cm²), fibrinogen did not enhance monocytic cell adhesion to endothelium (0.57 ± 1.13 cells/venule), compared with control incubations stimulated with fMLP alone (fMLP-treated cells: 0.62 ± 1.4 cells/venule; fibrinogen-equilibrated cells: 0.51 ± 1.2 cells/venules; \( n = 5/3 \)) (Fig 1). On the other hand, HL-60 cells attached to venular endothelium in the presence of fibrinogen at reduced shear rates (68.2 ± 18.8 s⁻¹), did not disengage from the vessel wall upon increase in the venular shear rate (467 ± 183 s⁻¹) (data not shown). As morphologically characterized in Fig 2, fibrinogen mediated firm attachment of leukocytes to mesentery endothelial cells, and no leukocyte rolling was observed under these experimental conditions in the presence or in the absence of fibrinogen (Fig 2), and in agreement with previous observations27,28 (see Discussion). Finally, the enhancing effect of fibrinogen on leukocyte-endothelial cell interaction was exclusively observed in venules, but not in arterioles or capillaries (Fig 2).
The possibility that circulating rabbit fibrinogen may contribute to this pathway of intercellular adhesion was investigated. Monocytic THP-1 cells, which constitutively express the fibrinogen receptor CD11b/CD18 and bind fibrinogen in a reaction quantitatively indistinguishable from that of vitamin D₃--differentiated HL-60 cells, adhered to human fibrinogen-coated plates in a specific and concentration-dependent reaction (Fig 3), completely inhibited by anti-CD18 MoAb IB4 (not shown), in agreement with previous observations. In contrast, rabbit fibrinogen, or comparable concentrations of control protein transferrin, did not support human monocytic cell attachment, under the same experimental conditions (Fig 3).

ICAM-1-dependent leukocyte-endothelium bridging in vivo. Previous studies indicated that ICAM-1 acted as a novel endothelial cell fibrinogen receptor, mediating fibrinogen-dependent leukocyte adhesion and transendothelial cell migration. The potential role of ICAM-1 in fibrinogen-dependent leukocyte adhesion to rabbit endothelium in vitro and in vivo was investigated. First, and consistent with the in vivo data presented above, pre-equilibration with human fibrinogen enhanced by twofold to threefold and in a time-dependent manner the adhesion of monocytic THP-1 cells to monolayers of rabbit endothelial cells, compared with control incubation in the presence of transferrin (Fig 4A). This reaction was quantitatively indistinguishable from the enhancement of monocytic THP-1 cell adhesion to human endothelium mediated by fibrinogen (Fig 4A), in agreement with previous observations. Indistinguishable results were also obtained with vitamin D₃--differentiated HL-60 cells (not shown). Preincubation of human or rabbit endothelial cells with saturating concentrations of the functionally blocking anti-ICAM-1 MoAbs LB-2 or 2D5, completely inhibited the fibrinogen-dependent component of leukocyte adhesion to either endothelial cell type (Fig 4B). In contrast, comparable concentrations of control, nonbinding MoAb 14E11 were ineffective, under the same experimental conditions (Fig 4B). The potential role of ICAM-1 in fibrinogen-dependent intercellular bridging in vivo was also investigated. A synthetic peptide from the fibrinogen γ-chain sequence N₁₁₇QKIVNLKEKVAQLE₁₃₃ designated γ₃, bound to ICAM-1 transfectants and inhibited fibrinogen association with ICAM-1. Increasing concentrations of γ₃
cyto-endothelium interaction in vivo to background levels observed in the absence of fibrinogen (1.25 ± 0.847 cells/venule; n = 8/4; P < .001 v fibrinogen-containing samples) (Figs 5 and 6A). In contrast, comparable concentrations of a control scrambled peptide synthesized with the γ3 sequence in random order (5.1 ± 1.732 cells/venule; n = 11/4; P > .5 v control) (Figs 5 and 6B), or the γ-chain L10 peptide Leu402-Val411 (5.66 ± 1.03 cells/venule, n = 5/3; P > .5 v control), did not significantly diminish leukocyte-endothelium interaction, under the same experimental conditions (Figs 5 and 6).

**DISCUSSION**

In this study, we have shown that fibrinogen mediates attachment of monocytic cells to rabbit mesentery endothelium in vivo by acting as a bridging molecule between the two cells types. This pathway of intercellular adhesion was observed in venules, but not in arterioles or capillaries, was effective at reduced shear forces, and occurred through the recognition of the fibrinogen γ-chain sequence Asn117-Ala133 by ICAM-1 on the endothelium. Consistent with this model, rabbit fibrinogen did not mediate human leukocyte adhesion, whereas rabbit endothelial cells were indistinguishable from human endothelium for their ability to support fibrinogen-

![Image](https://via.placeholder.com/150)

**Fig 5.** Effect of fibrinogen γ-chain–derived peptides on HL-60 cell adhesion to endothelium in vivo. Increasing concentrations (0 to 2.0 mg/mL) of the synthetic peptides γ3 (Asn117-Ala133), control scrambled γ3, or L10 (Leu402-Val411), were injected into the superior mesenteric artery simultaneously with fMLP-stimulated, CFDA-labeled, HL-60 cells pre-equilibrated with 300 μg/mL of human fibrinogen. The effect of the various fibrinogen peptides on HL-60 cell adhesion to endothelium was determined under conditions of reduced venular flow achieved by partial occlusion of the superior mesenteric artery, and determined by frame-by-frame analysis of the recorded images. The results are expressed as percent inhibition of the number of HL-60 cells attached/venule as compared with untreated HL60 cells. Data represent the mean ± SD separately obtained from 11 venules in 4 rabbits.
dependent monocytic cell adhesion, in a reaction completely inhibited by anti–ICAM-1 blocking MoAbs LB-2 or 2D5.18

One of the most significant findings of our study was the ability of fibrinogen to mediate a firm type of attachment of monocytic cells to rabbit endothelium in vivo, as opposed to transient leukocyte rolling. Consistent with current models of “adhesion cascade” recapitulating leukocyte-endothelium interaction,6,17 this suggests that fibrinogen may cooperate with the recognition of β2 integrins in stabilizing secondary tight attachment of leukocytes to the vessel wall.16,17 As compared with other published in vivo studies, the relatively low level of monocytic cell adhesion observed here in the presence of fibrinogen may reflect the stringent experimental protocol selected for this study. Although vitamin D3–differentiated HL-60 cells possess a high-affinity fibrinogen receptor in the CD11b/CD18 integrin,7 these cells lack L-selectin which resulted in absence of leukocyte rolling under our experimental conditions, in agreement with previous observations.27,28 Consistent with this scheme, published studies showed that sialyl-Lewisx–dependent HL-60 cell rolling in comparable in vivo models was exclusively restricted to very early (<20 minutes) or very late (>2 hours) time intervals,27,28 in an experimental protocol entirely different from that used in the present study. Finally, no cytokine stimulation of rabbit endothelial cells was performed before to assess the effect of fibrinogen on leukocyte adhesion, thus resulting in a low level or absence of E-selectin expression in this model. Based on these considerations, it seems plausible to hypothesize that fibrinogen-dependent intercellular bridging may play an even more prominent role in localizing leukocytes to endothelium in vivo, when physiologic rolling mechanisms mediated by selectins/α4 integrin-sialyl-Lewisx interactions44,29,30 may contribute an initial transient leukocyte-endothelium tethering.

As anticipated previously, the ability of fibrinogen to mediate leukocyte-endothelium bridging depended on a novel recognition of fibrinogen by ICAM-1.8,18 In agreement with recent independent contributions,10,11 this was further substantiated here, where two different classes of antagonists of the ICAM-1–fibrinogen recognition, including anti–ICAM-1 MoAbs LB-2 or 2D5,18 or the synthetic peptide γ3 sequence Asn17-Ala17, blocked monocytic cell attachment to rabbit endothelial cells mediated by fibrinogen, in vitro or in vivo, respectively. Moreover, the inability of rabbit fibrinogen to support monocyte adherence further lends credibility to our model of intercellular bridging, in which human fibrinogen bound to the leukocyte surface was solely responsible for enhancing monocyte attachment to endothelium, in vitro or in vivo.

The structural topography of the γ3 sequence in fibrinogen may help explain the ability of this pathway to mediate intercellular adhesion under physiologic conditions, in vivo. Using epitope-mapped MoAbs, Ugarova et al12 showed that part of the γ3 sequence may not be accessible in native fibrinogen, while becoming exposed in the conformationally rearranged molecule, immobilized on plastic or bound to a cell-surface receptor. This opens the possibility that initial binding of fibrinogen to activated CD11b/CD18 on leukocytes may transduce conformational changes in the ligand thus exposing the γ3 sequence for a secondary interaction with ICAM-1 on the endothelium. Such sequential receptor cascade on vascular cells may explain the ability of fibrinogen to support leukocyte-endothelium bridging in a physiologic human plasma milieu, containing large concentrations of soluble, potentially competing human fibrinogen.8

The potential pathophysiologic relevance of these observations is highlighted by the prominent role of fibrinogen in vascular diseases,25 and its invariable participation in inflammatory responses, in vivo.2,4 The model of fibrinogen-dependent intercellular bridging in vitro,8,18 and in vivo (this study), may define a potential novel adhesive mechanism promoting leukocyte recruitment and accumulation at the vessel wall, as a prerequisite of inflammatory responses with...
vascular cell activation, leukocyte transendothelial migration, and cytokine release. On the other hand, this pathway may also aberrantly contribute to the onset and development of vascular injury and thrombus formation, invariably characterized by increased leukocyte attachment to endothelium, and complex hemodynamic alterations. Consistent with the mechanistic model of intercellular adhesion presented here, increased deposition of fibrinogen and increased expression of ICAM-1 have been shown on endothelial cells in all types of atherosclerotic lesions.

In summary, these findings demonstrate the existence of an alternative adhesive pathway of leukocyte-endothelium interaction in vivo, and propose a novel pathogenetic role of fibrinogen in the cellular contribution to vascular injury. Advanced antagonists of the fibrinogen-ICAM-1 recognition (ie, γ3 peptide, MoAb 2D5) may prove beneficial at reducing aberrant leukocyte accumulation at the vessel wall in vivo, without interfering with fibrinogen-directed primary and secondary hemostatic functions.

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Fibrinogen mediates leukocyte-endothelium bridging in vivo at low shear forces

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