Molecular Identification of the Cross-reacting Epitope on $\alpha_M\beta_2$ Integrin I Domain Recognized by Anti-$\alpha_{IIb}\beta_3$ Monoclonal Antibody 7E3 and Its Involvement in Leukocyte Adherence*

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The monoclonal antibody (mAb) 7E3 directed to the platelet integrin $\alpha_{IIb}\beta_3$ was tested for its cross-reactivity with the homologous leukocyte integrin $\alpha_M\beta_2$. Nested recombinant fragments of $\alpha_M$ I domain were expressed as glutathione S-transferase fusion proteins and analyzed for antibody recognition. In enzyme-linked immunosorbent assay, mAb 7E3 bound $\alpha_M$ I domain fragments containing the amino-terminal sequence Cys$^{128}$-Ser$^{172}$, whereas the carboxyl-terminal region Leu$^{73}$-Pro$^{391}$ was ineffective. A synthetic peptide designated R1.1 and duplicating the $\alpha_M$ sequence G$^{127}$CPQEDSLAFLIDG-SGSIIPHFDF$^{150}$ bound mAb 7E3. In contrast, the adjacent $\alpha_M$ region F$^{150}$RRMKEFVSMQELKKSKTLFS$^{172}$ or a control peptide with a scrambled R1.1 sequence was not recognized by mAb 7E3. Binding of mAb 7E3 to $\alpha_M$ I domain blocked monocyte and neutrophil adhesion to immobilized fibrinogen and fibrinogen-dependent leukocyte-endothelium bridging, indistinguishably from bona fide anti-$\beta_2$ mAb IB4. In contrast, leukocyte binding to stable transfecants expressing intercellular adhesion molecule-1 was not affected by mAb 7E3. Balloon-mediated injury of iliofemoral arteries in rabbits resulted in prominent deposition of fibrinogen and increased monocyte adhesion to the injured vessel, in a reaction inhibited by mAb 7E3, but unaffected by control mAb 14E11. Through its cross-reactivity between $\alpha_{IIb}\beta_3$ and $\alpha_M\beta_2$, mAb 7E3 may initiate a new class of integrin antagonists, capable of simultaneously targeting platelet and leukocyte adhesion mechanisms in vascular injury.

Hemostasis and immune-inflammatory responses (1) are maintained by the adhesive interactions mediated by integrins $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) on platelets (2) and $\alpha_M\beta_2$ (Mac-1) on leukocytes (3). Despite their critical role in vascular cell homeostasis and signaling (4), platelet and leukocyte adhesion mechanisms participate in the pathogenesis of vascular injury. This is emphasized by the role of $\alpha_M\beta_2$ in platelet aggregation and thrombus formation (5, 6) and of $\alpha_M\beta_2$ in leukocyte recruitment (3), procoagulant activity (7), and reperfusion injury. Considerable effort has been devoted to the identification of molecular antagonists of $\alpha_{IIb}\beta_3$ (9) and $\alpha_M\beta_2$ (10), capable of disrupting aberrant platelet and monocyte adherence mechanisms. In this context, administration of anti-$\alpha_{IIb}\beta_3$ mAb 7E3 (11) reduced the incidence of mortality, myocardial infarction, and other emergency procedures in patients at risk of cardiovascular ischemic disease (12, 13).

In previous studies, it was also reported that mAb 7E3 unexpectedly cross-reacted with the active conformation of $\alpha_M\beta_2$, induced on monocytes by inflammatory stimuli (14) or Mn$^{2+}$ ions (15). These observations were recently independently confirmed with direct binding studies of mAb 7E3 to $\alpha_M\beta_2$ transfecants (16), whereas a ~200-aminio acid-inserted "I" domain in $\alpha_M$ (17) was provisionally implicated in this cross-reactivity (18).

In this study, we sought to re-investigate the molecular basis of mAb 7E3 cross-reactivity with $\alpha_M\beta_2$ and its potential relevance to leukocyte adhesion, in vivo. We found that mAb 7E3 recognizes a discrete region in $\alpha_M$ I domain (17), which is critically involved in monocyte adherence to fibrinogen in vitro and in balloon-injured arteries, ex vivo.

MATERIALS AND METHODS

Cell Culture and mAbs—Polymorphonuclear leukocytes (PMN) were isolated from acid citrate dextrose-anticoagulated blood drawn from normal informed volunteers by Ficoll-Hypaque (Amersham Pharmacia Biotech) centrifugation and dextran sedimentation, as described (19). PMN were suspended in serum-free RPMI 1640 medium (BioWhittaker) containing 10% heat-inactivated fetal bovine serum (Gemini Bioproducts, Calabasas, CA), 2 mM L-glutamine (Gemini), and 10 $^{-5}$ 2-mercaptoethanol (Eastman Kodak Co.). Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase treatment, maintained in medium 199 (BioWhittaker) containing 10% fetal bovine serum and endothelial cell growth factor, and plated onto gelatinized tissue culture plates (Costar Corp., Cambridge, MA). Cells were used between passages 2 and 4. Chinese hamster ovary cells stably transfected with the cDNA of intercellular adhesion molecule-1 (ICAM-1) were established in recombinant human interferon-γ (Mab Thera, Charseville, MD) at a concentration of 1 x 10$^5$/ml. The monocytic cell line THP-1 (American Type Culture Collection, Manassas, VA) was maintained in complete RPMI 1640 medium (BioWhittaker) containing 10% heat-inactivated fetal bovine serum (Gemini Bioproducts, Calabasas, CA), 2 mM L-glutamine (Gemini), and 10 $^{-5}$ 2-mercaptoethanol (Eastman Kodak Co.). Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase treatment, maintained in medium 199 (BioWhittaker) plus 20% fetal bovine serum and endothelial cell growth factor, and plated onto gelatinized tissue culture plates (Costar Corp., Cambridge, MA). Cells were used between passages 2 and 4. Chinese hamster ovary cells stably transfected with the cDNA of intercellular adhesion molecule-1 (ICAM-1) were established and characterized previously (20). Anti-$\alpha_{IIb}\beta_3$ mAb 7E3 was generously provided by Dr. Barry Coller (Mount Sinai Medical Center, New York) and characterized previously for its cross-reactivity with $\alpha_M\beta_2$ (14, 16). Anti-$\beta_2$ mAb IB4 was from ATCC. Nonbinding mAb 14E11 was used as a control.

Recombinant $\alpha_M$ I Domain Fragments—The map of the various $\alpha_M$ I domain fragments used in these studies is shown in Fig. 1. For these experiments, the full-length $\alpha_M$ cDNA was amplified by PCR in the

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presence of a forward oligonucleotide 5’-TGCTTCAAGGAGATGTYC-GAC-3’ and four distinct reverse oligonucleotides 5’-AGAAGACAAGGTTTGGAC-3’ (R1), 5’-CTGGCCCGTTGTTGTC-3’ (R2), 5’-CTCATATCCAAAGGATGUCG-3’ (R3), and 5’-CCGGTTCGATGGATG-3’ (R4). The fragment R1 (Fig. 1), lacking the amino-terminal R1 sequence

Fig. 1. Map of recombinant α1I domain fragments. The indicated I domain fragments were generated by PCR using full-length α1I cDNA, gel-purified, and directionally cloned in the prokaryotic expression vector pGEX-2T with transformation in BL-21 E. coli strain.

300 μCi of 51CrNaO4 (NEN Life Science Products) for 1 h at 37 °C, washed, and suspended in serum-free RPMI 1640 at a concentration of 2 x 10^6/ml. Two-hundred μl aliquots of cell suspension were stimulated with 1 μM fMLP (Sigma), treated with the various mAbs at 20 μg/ml for 15 min at 22 °C, and further incubated with or without fibrinogen (200 μg/ml in PBS) at 22 °C. After 10–25 min at 37 °C, 51Cr-labeled mAbs were added to PBMC or HUVEC or ICAM-1 transfectants for 45 min at 22 °C, and after washes attached cells were solubilized in 20% SDS with determination of radioactivity in a scintillation β-counter. The number of attached cells was calculated by dividing the counts/min observed by the counts/min/cell (21). Alternately, HUVEC monolayers were preincubated with mAbs IB4, 7E3, or control mAb 14E11 for 30 min at 22 °C, washed, and mixed with 51Cr-labeled fMLP-stimulated THP-1 cells before determination of fibrinogen-dependent intercellular bridging, as described above. In other experiments, microtiter wells were coated with human or rabbit (Sigma) fibrinogen at 10 μg/ml for 1 h at 4 °C and blocked with 3% gelatin for 30 min at 37 °C. Wells were incubated with fMLP-stimulated 51Cr-labeled THP-1 cells (1 x 10^7/ml) preincubated with 20 μg/ml mAbs 7E3, IB4, or control mAb 14E11 for 10 min at 22 °C with determination of cell adhesion after a 45-min incubation at 22 °C.

Animal Procedures—Adult New Zealand White rabbits (3–5 kg weight) were given free access to water and rabbit chow and were housed in a facility with alternating light and dark cycles. Animal care complied with the “Principles of Laboratory Animal Care” (National Academy of Sciences, Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 80–23, revised 1985). The Harvard Medical Area Standing Committee on Animals approved the experimental protocol. For surgical procedures, animals were anesthetized with ketamine (25 mg/kg, Ketalar™, Parke-Davis) and xylazine (5 mg/kg, Rompun™, Mobay, Shawnee, KS) administered by intramuscular injection, supplemented with intravenous doses of the mixture as needed. The iliofemoral arterial segment was exposed bilaterally via extended groin incisions, and all side branches proximal to the femoral bifurcation were ligated to create an isolated segment (22). The superficial femoral artery was then cannulated, and a 2-French Fogarty balloon embolectomy catheter (American Edwards, Anasco, Puerto Rico) was inserted. The catheter tip was passed into the terminal aorta and the balloon inflated and withdrawn three times to denude the vessel. After removal of the catheter, the superior femoral artery was ligated and antegrade flow re-established via the deep femoral branch. Wounds were closed surgically and the animals allowed to recover. At sacrifice (5–6 days after balloon injury) animals were re-anesthetized as described and systemically heparinized with a 1000-unit intravenous bolus. The groin wounds were re-opened and the femoral artery was re-exposed. The animal was then euthanized with an intravenous overdose of sodium pentobarbital. The abdominal aorta and inferior vena cava were cannulated and the distal arterial tree perfused at 80–120 mm Hg with 500 ml of heparinized (10 units/ml) lactated Ringer’s solution (Baxter Healthcare Corp., Deerfield, IL) with continuous venous drainage. The femoral arteries were re-cannulated with 20-gauge stainless steel catheters and the entire adventitia harvested and placed in PBS.

Ex Vivo Monocyte Adhesion—The freshly excised arterial segments were flushed gently with PBS prewarmed to 37 °C, and a microvascular clamp was placed at the origin of each external iliac artery. 51Cr-Labeled THP-1 cells (1 x 10^7/ml) were stimulated with 1 μM fMLP in the presence of 2.5 μM CaCl2, equilibrated with control mAb 14E11 or mAb 7E3 (10–25 μg/ml) for 30 min at 22 °C, and infused to fill each vessel segment. Paired arteries in each case were treated with mAb 7E3 versus control mAb 14E11. The external surface of the filled arteries was rinsed copiously with PBS and the vessels placed in PBS at 37 °C for 1 h. At the end of the incubation, a 5-mm segment of proximal aorta (not exposed to THP-1 cells intraluminally) was excised for measurement of background counts. The clamps were then removed, and each vessel was flushed gently with 3 ml of PBS at 1 ml/min by timed hand injection. The external surface was again rinsed and 5-mm segments cut (two from each iliofemoral segment) for scintillation counting. Segments of the remaining tissue were embedded in optimal cutting temperature compound (Bayer, West Haven, CT) and snap-frozen in 2-methylbutane cooled in liquid nitrogen for immunohistochemical analysis. Tissue was prepared for scintillation counting by fully clearing each specimen and embedding each specimen in 1% Tissue-Tek X-100, 10% SDS overnight at 22 °C. The following day an equal volume of scintillant was added, and activity determination was carried out in a scintillation counter.

Immunohistochemistry—Snap-frozen tissue was cut into 6-μm-thick cross-sections and adhered to glass slides coated with 0.25% gelatin and 0.025% chromium potassium sulfate (Cr3O5SO6, Sigma). Tissue was fixed in acetone at −20 °C for 10 min, quenched in 3% H2O2, and
I domain fragments containing the linked immunosorbent assay, mAb 7E3 bound all recombinant 2036 factor X peptide KDGLGEYG (indicated boxyl terminus with these findings, mAb 7E3 failed to react with three car

Laboratories Inc. Burlingame, CA) for 2 h at 2 °C. Negative controls characterized in previous studies (19) was applied for 1 h at 2 °C, 7.4, plus 1% fetal bovine serum. A mouse anti-fibrinogen antibody isotype control (IgG1). Sections were incubated with an avidin-biotin complex (ABC kit, Vector) followed by 3-amino-9-carbazole. Slides were counterstained with Mayer's hematoxylin (Sigma) followed by streptavidin-alkaline phosphatase plus p-nitrophenyl phosphate. After washes, absorbance was quantitated at 405.

FIG. 2. Epitope mapping of mAb 7E3. A, I domain fragments. The indicated αM I domain fragments were immobilized onto 96-well microtiter plates for 18 h at 4 °C, blocked with 3% gelatin, and incubated with 20 μg/ml control mAb 14E11, anti-β2 mAb IB4, or mAb 7E3 followed by biotin-conjugated rabbit anti-mouse IgG and streptavidin-alkaline phosphatase plus p-nitrophenyl phosphate. After washes, absorbance was quantitated at 405.

FIG. 3. Effect of mAb 7E3 on β2 integrin-ICAM-1 recognition. 51Cr-Labeled PMN or THP-1 cells (1 × 10^6/ml) were stimulated with 1 μM fMLP and incubated with 20 μg/ml of the indicated mAbs for 15 min at 22 °C. Cells were added to monolayers of resting HUVEC or stable ICAM-1 transfectants in the presence of 2.5 mM CaCl_2 for 45 min at 22 °C. After washes, attached cells were solubilized in 20% SDS and counted in a scintillation counter. Data are the mean ± S.D. of at least two independent experiments.

RESULTS

Epitope Mapping of mAb 7E3 on αM I Domain—In enzyme-linked immunosorbent assay, mAb 7E3 bound all recombinant I domain fragments containing the αM sequence Cys128–Pro291 (Fig. 2A). The minimal αM fragment reacting with mAb 7E3 contained the R1 sequence Cys128–Ser172 (Fig. 2A). Consistent with these findings, mAb 7E3 failed to react with three carboxyl terminus αM I domain fragments lacking the R1 region and designated R1(–) (Leu173–Pro291), B5 (Leu173–Asn232), and B4 (Ala233–Pro291), or with control, non-IGPTG-induced, bacterial lysate (Fig. 2A). In peptide mapping experiments, mAb 7E3 bound the R1.1 sequence G127CPQEDSDIAFLIDGSGSIIPHD150 (R1.1), R1.1-homologous sequence V107EDYPVDIYYLMDLSYSMKDDL128 in β3 integrin (Beta3), or an unrelated factor X sequence KDGLGEGY (control) did not associate with mAb 7E3 (Fig. 2B). Similarly, synthetic peptides duplicating the adjacent I domain region F170RMKEFVST-VMEQLKKSHTPS172 (R1.2), the R1.1-homologous sequence V107EDYPVDIYYLMDLSYSMKDDL128 in β3 integrin (Beta3), or an unrelated factor X sequence KDGLGEGY (control) did not associate with mAb 7E3 (Fig. 2B). In control experiments, mAb 14E11 or anti-β2 (CD18) mAb IB4 did not recognize any αM I domain fragments and did not associate with β2*, β3, or factor-X-derived peptides in enzyme-linked immunosorbent assay (Fig. 2, A and B).

Differential Regulation of Leukocyte Adhesion by mAb 7E3—The effect of mAb 7E3 on αMβ2 recognition of ICAM-1 (1) was first investigated. Preincubation of fMLP-stimulated PMN or monocyte THP-1 with mAb 7E3 or control mAb 14E11 did not reduce the attachment of these cells to monolayers of resting HUVEC or ICAM-1 transfectants (Fig. 3). In contrast, anti-β2 mAb IB4 nearly completely abrogated PMN adhesion to HUVEC or ICAM-1 transfectants, and significantly inhibited THP-1 cell attachment to either cell type, under the same experimental conditions (Fig. 3).

A potential effect of mAb 7E3 on αMβ2 recognition of fibrinogen (7) was next investigated. In adhesion assays, mAb 7E3 completely inhibited the attachment of fMLP-stimulated THP-1 cells to immobilized human or rabbit fibrinogen (Fig. 4A). Similar results were obtained with anti-β2 mAb IB4, whereas control mAb 14E11 was ineffective (Fig. 4A). The effect of mAb 7E3 on fibrinogen-dependent leukocyte-endothelium bridging (21) was also investigated. Consistent with previous observations (21), fibrinogen enhanced the adhesion of fMLP-stimulated PMN or THP-1 cells to HUVEC or ICAM-1 transfectants by 2–4.4-fold (Fig. 4B, legend), as compared with control incubation reactions in the absence of fibrinogen (Fig. 3). Under these experimental conditions, mAb 7E3 inhibited the fibrinogen-dependent enhancement of PMN or THP-1 cell adhesion to HUVEC or ICAM-1 transfectants (Fig. 4B). In contrast, fibrinogen-independent attachment of PMN or THP-1 cells to either cell type was unaffected by mAb 7E3 (Fig. 4B,
**FIG. 4.** Effect of mAb 7E3 on α5β2 recognition of fibrinogen. A, adherence to fibrinogen. Plastic microtiter plates were coated with 10 μg/ml human or rabbit fibrinogen for 18 h at 4 °C, washed, and blocked with 3% gelatin. 51Cr-labeled THP-1 cells (1 × 10⁶/ml) were stimulated with 1 μM fMLP, incubated with the indicated mAbs at 20 μg/ml for 10 min at 22 °C, and added to fibrinogen-coated plates for an additional 45-min incubation at 22 °C before determination of cell adhesion. B, fibrinogen-dependent leukocyte-endothelium bridging. The experimental procedures are essentially the same as described in the legend to Fig. 3, except that fMLP-stimulated PMN or THP-1 cells were incubated with the various mAbs and mixed with 200 μg/ml fibrinogen, and 2.5 mM CaCl₂ before addition to monolayers of resting HUVEC or ICAM-1 transfectants. Adherent THP-1 cells to HUVEC or ICAM-1 transfectants in the absence of fibrinogen were 18,997 ± 2,453 and 20,115 ± 6,900, respectively. Adherent PMN to HUVEC or ICAM-1 transfectants in the absence of fibrinogen were 14,183 ± 1531 and 24,233 ± 2201, respectively. For both panels, data are the mean ± S.D. of two independent experiments in duplicate.

In control experiments, anti-β₂ mAb IB4 inhibited fibrinogen-dependent and –independent intercellular adhesion, whereas control mAb 14E11 was ineffective (Fig. 4B). Finally, preincubation of HUVEC monolayers with mAb 7E3 followed by washes and addition of 51Cr-labeled THP-1 cells failed to reduce fibrinogen-dependent intercellular bridging, thus ruling out a potential role of HUVEC α5β2 recognition of fibrinogen in this interaction, and in agreement with previous observations (21).

**FIG. 5.** Effect of mAb 7E3 on fibrinogen-dependent monocyte adhesion to balloon-injured vessels. The right and left iliofemoral arteries of rabbits were injured with a balloon catheter with isolation of the arterial circulation 6 days after injury. fMLP (1 μM)-stimulated 51Cr-labeled THP-1 cells were equilibrated with 25 μg/ml control mAb 14E11 or mAb 7E3 for 30 min at 22 °C in the presence of 2.5 mM CaCl₂ before injection in the isolated injured vessels. After a 45-min incubation at 22 °C, vessels were flushed, minced and radioactivity associated under the various experimental conditions was determined in a scintillation counter. The degree of THP-1 cell adhesion to noninjured aorta in the absence of mAb is indicated (Background). Data are the mean ± S.E. of three independent experiments.

In this study, we have shown that anti-α₅β₃ mAb 7E3, an integrin antagonist currently used in clinical practice (11), binds a discrete region of α₅I domain and inhibits fibrinogen-mediated leukocyte adhesion in vitro, and in balloon-injured arteries of rabbits, ex vivo.

Although of paramount importance for normal hemostasis (22), platelet aggregation mechanisms maintained by α₅β₃ may precipitate thrombus formation and acute ischemic cardiovascular emergencies (5, 6). Among integrin antagonists capable of targeting platelet adherence mechanisms, anti-α₅β₃ mAb 7E3 significantly reduced mortality and emergency procedures in patients undergoing acute coronary intervention (12, 13). At the molecular level, it was also shown that mAb 7E3 possessed an unusual pattern of antigen recognition, which included, in addition to α₅I, the related integrin α₅β₃ (9), and the active form of the leukocyte integrin α₅β₂ (14, 16). This was potentially relevant to the beneficial effect of mAb 7E3 in vivo, because inhibition of α₅β₃ reduced neointimal hyperplasia in models of vascular injury (24), and α₅β₂-dependent leukocyte adherence contributed to monocyte recruitment and reperfusion injury (8).

Here, mAb 7E3 bound a cross-reacting epitope in the aminoterminal R1 region Cys₁₂⁸-Ser₁⁷² of α₅I-domain, which was further narrowed to the R1.1 peptide sequence G¹²⁹C-PQEDSDIAFLIDGSGSIIPHDF¹⁵⁰. The most salient feature of this motif is the presence of the amino acids Asp¹⁴⁰-Ser¹⁴²-Ser¹⁴⁴, which comprise the first group of oxygenated residues of the metal ion-dependent adhesion site (MIDAS) on α₅I domain (17). Experimental evidence obtained with homology models, mutagenesis, and divalent ion binding studies suggests
that a MIDAS-like motif containing the DXXSXS motif, in which X is a nonconserved amino acid, is structurally and functionally present in all integrin β subunits (25–28). Intriguingly, mAbs raised against this region in β3-inhibited fibrinogen binding to the receptor (29), and a synthetic peptide duplicating the MIDAS-like motif bind ligand and divalent ions (30). Under our experimental conditions, a β3-derived peptide containing the MIDAS homology motif failed to associate with mAb 7E3. This is consistent with the inability of mAb 7E3 to recognize the isolated β3 subunit (31) and suggests that conformational and/or divalent ion-dependent changes in αmβ2 are required to form a high affinity antibody binding interface (17). Whether or not mAb 7E3 recognizes MIDAS-like structures in other integrin β subunits is currently not known. However the data presented here suggest that the antigenic accessibility of this shared motif may be modulated by receptor-specific conformational changes and/or the requirement of additional contact site(s) in the α/β heterodimer. This complex pattern of multiple integrin recognition may not be unique of mAb 7E3, because other function blocking anti-αmβ2 mAbs, i.e. 25E11, have been shown to cross-react with αmβ2 (32).

The next question addressed by this study was the potential physiologic relevance of mAb 7E3 cross-reactivity with αmβ2. Consistent with the critical role of αm I domain in ligand binding (17, 18, 33, 34), engagement of the mAb 7E3 cross-reacting epitope suppressed the receptor recognition of fibrinogen, in agreement with recent observations (16). This resulted in inhibition of leukocyte adherence to immobilized fibrinogen and of fibrinogen-dependent leukocyte-endothelium bridging (21), indistinguishably from bona fide anti-β2 integrin mAb IB4. In contrast, at variance with a recent study (16), mAb 7E3 failed to reduce the αmβ2 recognition of ICAM-1 (1, 3). Although differences in protocol may account for this discrepancy, experimental evidence with epitope-mapped mAbs (33), and peptide inhibition studies (35), suggests that the ICAM-1- and fibrinogen-binding sites on αm I domain are physically distinct and nonoverlapping.

An in vivo model of vascular damage further underscored the relevance of mAb 7E3 targeting of monocyte-fibrinogen interaction. In these studies, balloon-mediated injury of the iliofemoral arteries in rabbits resulted in prominent deposition of fibrinogen as detected by immunohistochemistry and in agreement with previous studies (36). In addition to promoting increased procoagulant activity (37), this translated in our study in prominent monocyte attachment to the injured vessel, in a reaction specifically inhibited by mAb 7E3. Consistent with the importance of leukocyte adherence in vascular disease (38), this suggests that fibrinogen deposited on atherosclerotic lesions (39), and balloon-injured arteries (36), may provide an ideal substrate for leukocyte recruitment. In turn, this may further exacerbate vascular damage by promoting increased IL-1 (40) and tissue factor (41) gene expression, chemotaxis (42), and release of oxidative radicals (43). Whether or not the inhibition of monocyte adhesion by mAb 7E3 contributes to its protective effect in ischemic disease (12, 13) is currently not known. However, the data presented here are consistent with a model in which mAb 7E3 blockade of β3 integrins on platelets and endothelium, and αmβ2 on leukocytes may simultaneously inhibit multiple cell adherence pathways at the interface between thrombosis and inflammation (44).

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