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$\alpha_4\beta_1$ Integrin (VLA-4) Blockade Attenuates both Early and Late Leukocyte Recruitment and Neointimal Growth following Carotid Injury in Apolipoprotein E (–/–) Mice

Kurt G. Barringhaus[a] J. William Phillips[a] Jayant S. Thatte[b]
John M. Sanders[a] Ann C. Czarnik[a] Daniel K. Bennett[a] Klaus F. Ley[b,c]
Ian J. Sarembock[a,b]

[a]Department of Medicine, Cardiovascular Division, [b]Cardiovascular Research Center,
[c]Biomedical Engineering, University of Virginia Health System, Charlottesville, Va., USA

Key Words
Adhesion molecules · Arterial injury · Arteries · Atherosclerosis · Inflammation · Leukocytes · Restenosis

Abstract
Background: The $\alpha_4\beta_1$ integrin (VLA-4) supports rolling and firm adhesion of leukocytes to inflamed tissues via ligation of VCAM-1 or fibronectin expressed on the activated endothelium. We tested the hypothesis that VLA-4 mediates leukocyte recruitment and neointimal growth after arterial injury in the atherosclerosis-prone apolipoprotein E (ApoE)-deficient mouse. ***Methods:*** ApoE (–/–) mice fed a Western diet underwent air desiccation injury, and the expression patterns of VLA-4 and VCAM-1 were determined by immunohistochemistry (IHC). To determine the effect of targeted VLA-4 blockade on leukocyte recruitment and neointimal growth, ApoE (–/–) mice received an intraperitoneal injection of a VLA-4 neutralizing monoclonal antibody (PS/2) at the time of injury alone or over a prolonged administration course. Additional mice received an isotype control antibody. ***Results:*** IHC demonstrated a marked increase in VLA-4 expression 7 days following injury. Prolonged administration of PS/2 resulted in a 72% reduction ($p < 0.02$) in neointimal growth 28 days following injury. IHC revealed a marked 95% reduction in neutrophil recruitment at 7 days and a 48% reduction in macrophage recruitment 28 days following injury with prolonged PS/2 administration. ***Conclusions:*** Prolonged VLA-4 blockade reduces leukocyte recruitment and neointimal growth following air desiccation injury in ApoE (–/–) mice. These findings demonstrate an important role for VLA-4 in the response to arterial injury.

Introduction

Arterial injury initiates a cascade of cellular and molecular events designed to restore vascular integrity. However, amplification of this process may result in excessive neointimal growth and luminal narrowing. Monocytes and other leukocytes recruited to the arterial surface early after injury modulate this reparative response [1, 2]. Cellular adhesion molecules including P-selectin and

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Dr. Ian J. Sarembock
Cardiovascular Division, University of Virginia Health System
Box 800158
Charlottesville, VA 22908-0158 (USA)
Tel. +1 434 924 2420, Fax +1 434 982 0901, E-Mail ijs4s@virginia.edu

VCAM-1 and their respective ligands play a critical role in mediating leukocyte recruitment to atherosclerotic arteries by promoting leukocyte tethering, rolling and arrest on the endothelium [3, 4]. Arterial injury increases expression of these same inflammatory adhesion molecules concomitantly with enhanced leukocyte recruitment [5, 6]. We recently reported that injection of a P-selectin-blocking antibody markedly reduces macrophage entry and neointima formation following carotid wire arterial injury in the apolipoprotein E (ApoE) (–/–) mouse fed a Western diet [7].

The $\alpha_4\beta_1$ integrin (VLA-4) is expressed on monocytes, lymphocytes and at a low level on neutrophils, and supports both slow rolling and firm adhesion to the activated endothelium via ligation of VCAM-1 or fibronectin [8–10]. Huo et al. [11] demonstrated that blockade of VLA-4 with a neutralizing antibody reduced monocyte slow rolling and adhesion to the endothelium in the carotid artery of atherosclerosis-prone ApoE-deficient mice. In this study, we tested the hypothesis that cells expressing VLA-4 are recruited to the denuded arterial wall early after carotid arterial injury and that transient VLA-4 blockade would reduce neointimal growth following arterial injury. We utilized the ApoE-deficient mouse fed a Western diet, a widely accepted model of both accelerated atherosclerosis and arterial injury, to determine the expression patterns of VLA-4 and its ligand VCAM-1 early after carotid air desiccation injury. As VLA-4 gene deletion results in fetal death [12], we utilized a VLA-4 neutralizing antibody to determine whether or not targeted VLA-4 blockade would reduce leukocyte recruitment and neointimal growth after arterial injury.

Materials and Methods

Antibodies

The VLA-4 blocking monoclonal antibody (mAb) was purified from the supernatant of the clone PS/2 (IgG_{2b}), which was a generous gift from P.W. Kincade [13]. For the VLA-4 blockade experiments, the isotype control antibody was an IgG_{2b} that does not specifically bind murine antigens (LO-DNP-11; Serotec, Raleigh, N.C., USA). CD49d (VLA-4), CD106 (VCAM-1), CD3 and CD31 (PECAM) polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif., USA). Mac-2 antibody (M3/38) was purchased from Accurate Chemical and Scientific Corporation (Westerbury, N.Y., USA). The antineutrophil (7/4) antibody was purchased from Serotec. The α-actin antibody (1A4) was purchased from Dako Corporation (Carpinteria, Calif., USA). The FITC-conjugated anti-rat IgG_{2b} antibody (G15-337) was obtained from Pharmingen (San Diego, Calif., USA).

Arterial Injury

C57Bl6 ApoE (–/–) female mice (body weight 18–22 g) were purchased from Jackson Laboratories and used for all experiments. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and protocol approval was obtained from the Animal Research Committee of the University of Virginia Health System. Mice were anesthetized with ketamine (80 mg/kg) and xylazine (5 mg/kg). Air desiccation was performed as described by Simon et al. [14] with minor modification. Briefly, the right common and internal carotid arteries were dissected and hemostasis was maintained with microaneurysm clips. The right external carotid was ligated and a 30-gauge needle attached to PE-10 tubing was introduced through an arteriotomy. The cannula was attached to an angioplasty indeflator and the artery was distended at 2.5 atm for 30 s. The hub was then disconnected and a ventilation port was created with a 30-gauge needle in the proximal common carotid artery. Air desiccation was performed through the cannula at a continuous rate of 15 ml/min for 3 min. The cannula was then removed and the external carotid was ligated. The clips were removed and restoration of blood flow through the internal carotid artery was verified. Hemostasis was achieved prior to closure of the skin with surgical glue (Veterinary Products Laboratories, Phoenix, Ariz., USA).

Flow Cytometry

To determine the persistence of circulating antibody following intraperitoneal injection, peripheral blood from mice injected with 200 μg of PS/2 (n = 4) or isotype control (n = 4) was collected by left ventricular puncture into EDTA-containing microtainer tubes (Becton Dickinson, Franklin Lakes, N.J., USA) 4, 24 and 72 h following injection. Whole blood was centrifuged at 2,000 *g*, placed in lysis buffer for 10 min and again centrifuged at 2,000 *g*. Leukocytes were resuspended in PBS and incubated with an FITC-IgG_{2b} antibody for 10 min and then washed with PBS. Light scatter and fluorescence data were obtained on a Becton Dickinson FACSCalibur (Becton Dickinson) flow cytometer with a gain setting in the logarithmic mode, and data were analyzed with Cellquest software (Becton Dickinson).

Animal Protocol

Mice were commenced on a Western diet (21% fat, 0.15% cholesterol and 19.5% casein by weight; Harlan Teklad, Indianapolis, Ind., USA) 7 days prior to injury and maintained on this diet until the time of sacrifice. Mice were assigned to one of the following four treatment groups: (1) no injection (n = 8), (2) isotype control injection (200 μg i.p. on days 0, 3, 7 and 10; n = 7), (3) early PS/2 injection (200 μg i.p. on day 0 only; n = 8), and (4) prolonged PS/2 injection (200 μg i.p. on days 0, 3, 7 and 10; n = 8). Mice received the initial injection 1 h prior to arterial injury (day 0). The operator remained blinded to the treatment group. Carotid arteries were harvested 28 days after injury following anesthesia and then perfusion fixed at physiologic pressure with phosphate-buffered paraformaldehyde (4%, 0.1 *M*, pH 7.4) by left ventricular cannulation. Arteries were excised, dehydrated in ethanol and xylene, and embedded in paraffin. In addition, to determine the time course of VLA-4 and VCAM-1 expression, leukocyte recruitment, and the effect of VLA-4 blockade on leukocyte recruitment following arterial injury, carotids from mice receiving no injection (n = 5), isotype control injection (n = 5) and prolonged PS/2 injection (n = 5) were harvested on days 3 and 7.

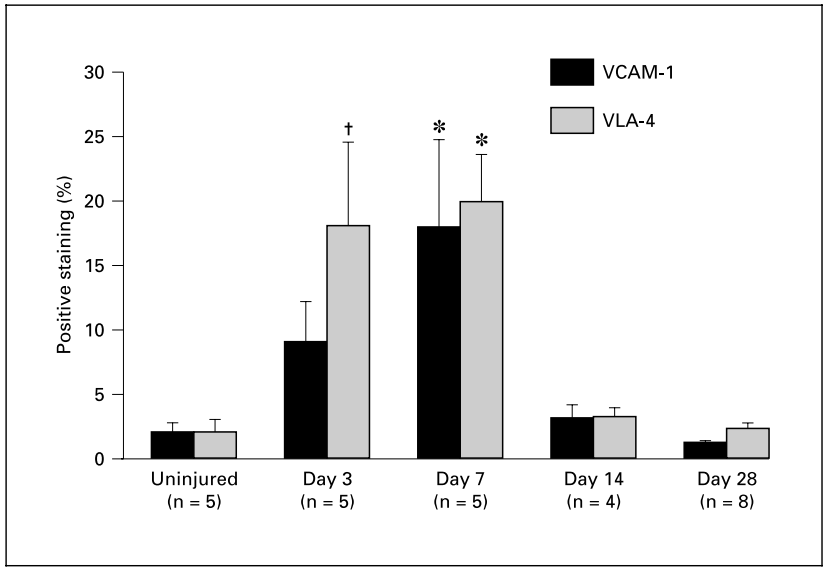

Fig. 1. Time course of expression of VLA-4 and VCAM-1 in the carotid artery following air desiccation injury as determined by IHC. Note that peak VLA-4 and VCAM-1 expression occurred 3–7 days following injury. * $p < 0.01$, † $p < 0.05$ compared with uninjured arteries.

Quantitative Histomorphometry and Immunohistochemistry

Following paraffin embedding, serial 5-μm-thick sections were obtained starting at the carotid bifurcation through 1,200 μm from the bifurcation. Six sequential sections at 150-μm intervals beginning 500 μm beyond the bifurcation were stained by the Movat technique [15]. Histomorphometry was performed as described previously to determine the external elastic lamina (EEL), internal elastic lamina (IEL), media and neointima areas of each vessel [16]. Immunohistochemistry (IHC) was performed utilizing the avidin-biotin technique (Vector Labs, Burlingame, Calif., USA) as previously described to characterize the expression patterns of VCAM-1 and VLA-4 after arterial injury in untreated mice. Neutrophils (7/4), T lymphocytes (CD3) and macrophages (Mac-2) were assessed by IHC in all treatment groups at 3, 7 and 28 days. IHC for α-actin (1A4) and PECAM (CD31) was performed at these time points as well. Semiquantitative analysis of the media and neointima was performed by a blinded investigator as described previously utilizing Image Pro Plus 3.0 (Media Cybernetics, Silver Springs, Md., USA). For each arterial section, the percentage area of positive staining was calculated based on the number of positively stained pixels in the vessel divided by the total number of pixels in the same area (mean ± SEM).

Complete Blood Counts and Lipid Levels

Peripheral blood was collected for automated testing performed by the University of Virginia Clinical Pathology Laboratory. Complete blood counts, automated differential leukocyte counts and lipid profiles were assessed at 28 days.

Statistical Analysis

Statistical analysis was performed using SPSS software (Chicago, Ill., USA). Histomorphometry data are reported as the number of carotid arteries in each group and the mean area ± SEM. Data were compared using one-way analysis of variance with Bonferroni corrections for multiple comparisons and Student's t test to evaluate two-tailed levels of significance.

Results

VLA-4 and VCAM-1 Are Increased Early after Carotid Air Desiccation Injury

To determine the expression patterns of VLA-4 and VCAM-1 after arterial injury, IHC was performed on sections from uninjured control arteries as well as injured arteries harvested 3, 7, 14 and 28 days after injury. Figure 1 illustrates an increase in VLA-4 and VCAM-1 expression in the vessel wall early after arterial injury, with peak expression occurring on day 7. Adjacent sections 7 days after injury revealed a predominance of neutrophils and α-actin-positive cells within the developing neointima (fig. 2). Few macrophages or T lymphocytes were seen at this time. Expression of both VLA-4 and VCAM-1 decreased by day 28, at which time a robust neointima was present consisting of α-actin-positive smooth muscle cells (SMCs) and macrophages. PECAM staining for endothelium, which was sparse at 3 days and incomplete 7 days after injury, was uniformly positive along the luminal surface at 28 days.

PS/2 Administration and Flow Cytometry

To determine the time course of PS/2 antibody binding to circulating leukocytes in vivo, ApoE (–/–) mice fed a Western diet were injected intraperitoneally with 200 μg of isotype control mAb or PS/2 mAb. Blood was obtained 4, 24 and 72 h following administration to determine the presence of bound antibody on peripheral leukocytes by FACS analysis utilizing an FITC-conjugated anti-rat sec-

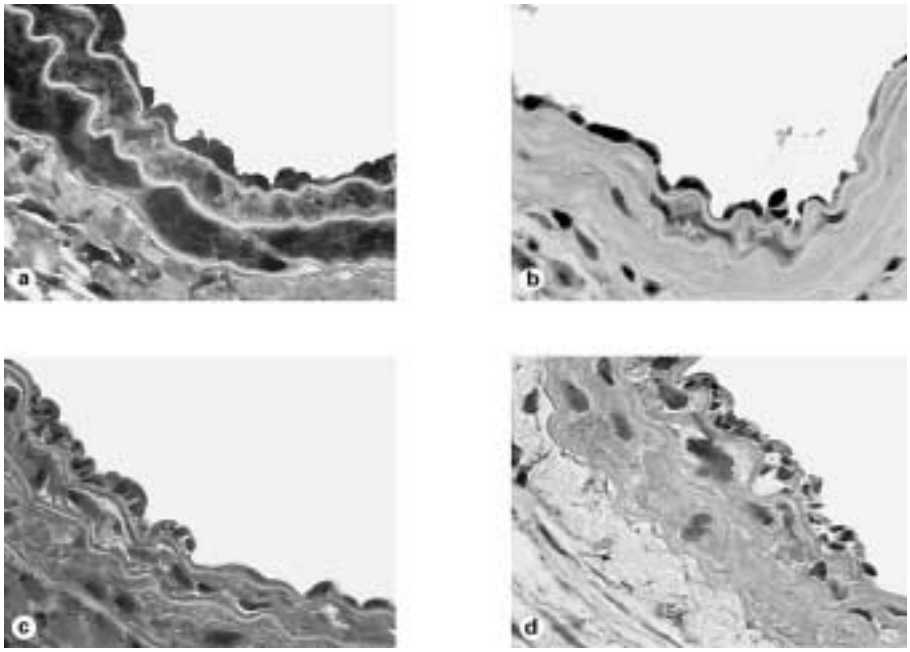

Fig. 2. IHC of serial sections 7 days following air desiccation injury of the right carotid artery. **a** VLA-4. **b** Neutrophil. **c** α-Actin. **d** Mac-2. Note the robust VLA-4 staining in both the media and developing neointima together with the presence of neutrophils and SMCs within the developing neointima. Note the paucity of macrophages in the vessel wall (**d**).

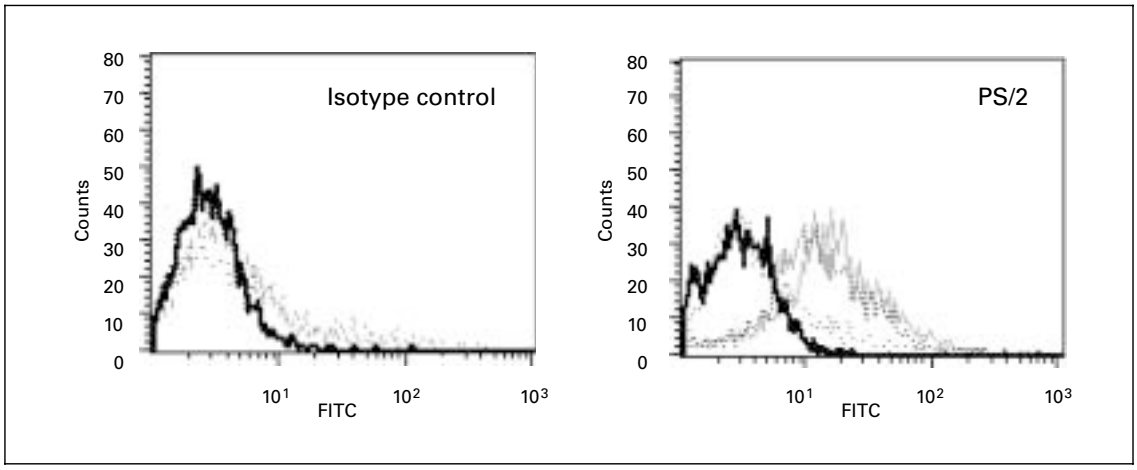

Fig. 3. Flow cytometry data demonstrating binding of PS/2 or isotype antibody to peripheral blood leukocytes following a 200-μg i.p. injection. Unstained leukocytes are represented by the solid black line. Experiments conducted 4 (solid gray), 24 (black dashed) and 72 h (gray dotted) following injection are also shown. Note that PS/2 binds abundantly 4 and 24 h following injection but with low frequency 72 h after injection. Mice injected with the isotype control antibody showed no specific binding at 4, 24 or 72 h.

ondary antibody. Figure 3 demonstrates an abundance of PS/2 binding to leukocytes 4 and 24 h following intraperitoneal injection. However, minimal PS/2 binding to leukocytes was seen 72 h following injection. Leukocytes from mice injected with isotype control antibody showed no specific staining at 4, 24 and 72 h.

VLA-4 Blockade Inhibits Neointimal Formation

ApoE (–/–) mice fed a Western diet for 1 week underwent carotid air desiccation injury 1 h after random assignment to no mAb injection, isotype control mAb injection, early (single-injection) PS/2 mAb administration or prolonged PS/2 mAb administration. Vessels were

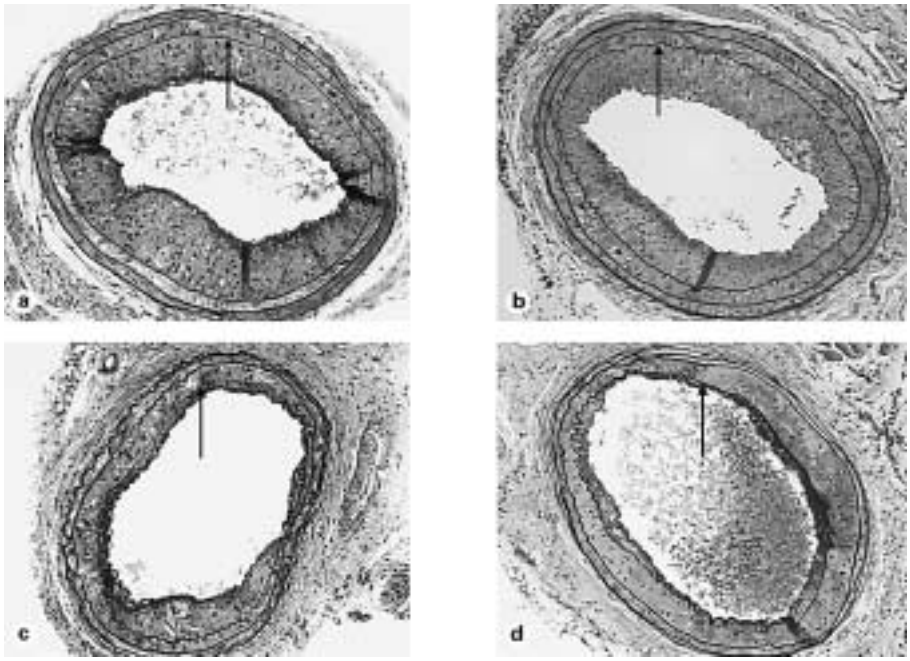

Fig. 4. Movat-stained sections of carotid arteries from ApoE (–/–) mice harvested 28 days following air desiccation injury. **a** No injection. **b** Isotype control. **c** Early PS/2. **d** Prolonged PS/2. Note the robust neointima formation in control mice (**a**, **b**). A significant 72% reduction in neointimal growth was seen with prolonged PS/2 administration. Arrows indicate the IEL.

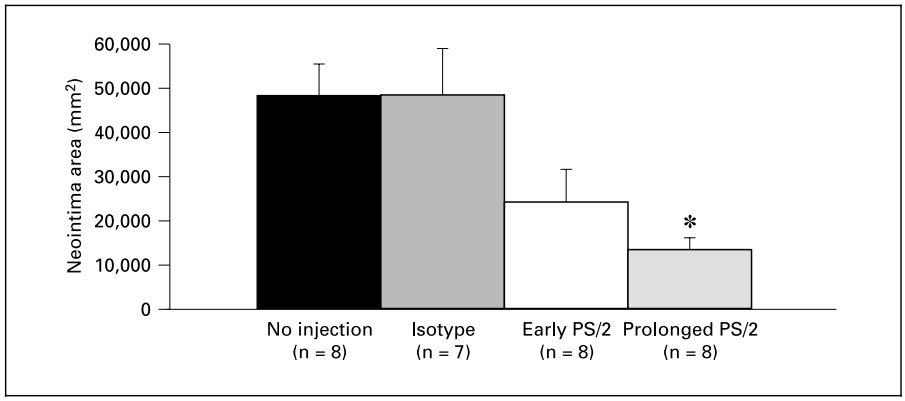

Fig. 5. Quantitative histomorphometry demonstrating a 72% reduction (* $p < 0.02$) in neointima area with prolonged PS/2 administration

Group	EEL, μm²	Media, μm²	Neointima, μm²
No injection	162,000 ± 11,000	47,000 ± 6,000	48,000 ± 7,300
Isotype control	157,000 ± 7,800	44,000 ± 4,200	48,000 ± 11,000
Early PS/2	127,000 ± 9,400	36,000 ± 4,500	24,000 ± 7,100
Prolonged PS/2	149,000 ± 9,900	50,000 ± 8,600	13,000 ± 3,200*

harvested 28 days following injury for assessment of intimal growth by quantitative histomorphometry. No difference was seen in neointima area between mice receiving no injection and mice receiving isotype control antibody (fig. 4, 5). In contrast, a dramatic reduction in neointima area was seen in mice receiving prolonged PS/2 mAb administration as compared with isotype control mice. Mice receiving a single injection of PS/2 mAb 1 h prior to injury demonstrated a modest reduction in neointimal growth that did not achieve statistical significance. EEL

a

Group	Neutrophil		Macrophage		T lymphocyte		α-actin	
	isotype control	PS/2	isotype control	PS/2	isotype control	PS/2	isotype control	PS/2
Day 3 (n = 5)	2.8 ± 2.4	0.01 ± 0.01	3.3 ± 0.5	2.5 ± 0.5	0.3 ± 0.06	0.1 ± 0.05	4.3 ± 1.0	7.4 ± 1.8
Day 7 (n = 5)	8.2 ± 2.8	0.4 ± 0.1*	6.6 ± 3.1	4.6 ± 1.4	0.2 ± 0.04	0.1 ± 0.03	7.8 ± 2.3	5.8 ± 2.0
Day 28 (n = 8)	0.2 ± 0.1	0.1 ± 0.1	12.9 ± 1.8	6.8 ± 2.1†	0.1 ± 0.04	0.1 ± 0.4	9.6 ± 2.3	8.8 ± 2.1

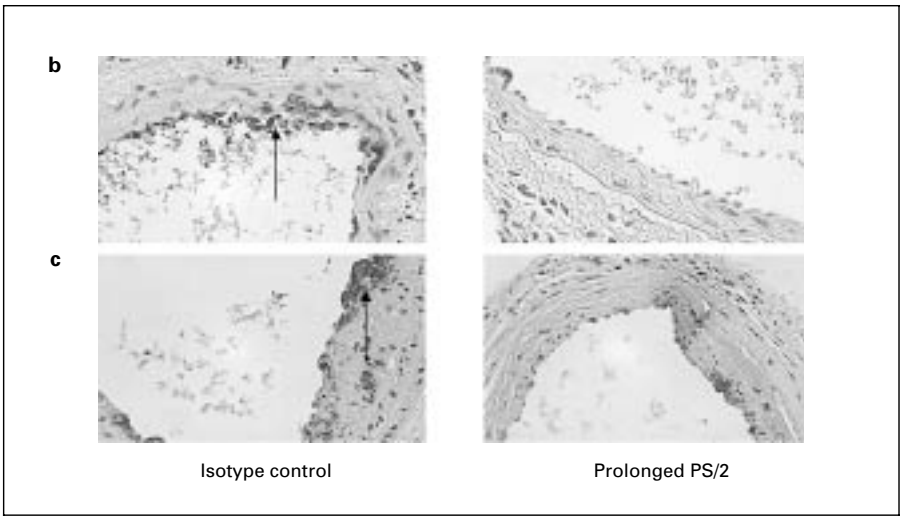

Fig. 6. a Semiquantitative IHC of injured vessels at 3, 7 and 28 days (percentage positive staining). * 95% reduction ($p < 0.02$). † 48% reduction ($p < 0.05$). **b**, **c** Representative sections demonstrating staining for neutrophils at 7 days (**b**) and macrophages at 28 days (**c**) after air desiccation injury in isotype control-treated and prolonged PS/2-treated mice. × 40. Note the significant reduction in early neutrophil recruitment (95% reduction, $p < 0.02$) and late macrophage recruitment (48% reduction, $p < 0.05$) in the PS/2-treated animals. Arrows demonstrate cells staining positive.

and media areas were similar among all treatment groups. No disruption of the IEL and EEL was noted in any of the sections analyzed, demonstrating similar degrees of arterial injury across treatment groups.

PS/2 Reduces Arterial Neutrophil and Macrophage Recruitment after Injury

To determine the effect of PS/2 injection on leukocyte recruitment and plaque composition, vessels were harvested 3, 7 and 28 days following injury from mice injected with either prolonged PS/2 antibody or isotype control antibody. IHC was performed on sections located 600 μm from the carotid bifurcation to assess the presence of neutrophils, macrophages, T lymphocytes and α-actin-positive SMCs. Figure 6a demonstrates that neutrophil recruitment to the neointima peaked at 7 days in the isotype control group and had declined by day 28. Neutrophil recruitment was minimal in PS/2-treated mice at all time points evaluated. At 7 days after injury, PS/2 mAb administration markedly reduced neutrophil recruitment as compared with isotype control antibody (fig. 6a, b). By 28 days after injury, few neutrophils were present in either the PS/2 mAb- or isotype control mAb-injected mice. Macrophage recruitment increased progressively to 28 days following injury in isotype control mice. PS/2 administration significantly reduced macrophage recruitment at 28 days as compared with isotype control mice (fig. 6a, c). Few T lymphocytes were noted at all time points studied in both the isotype control and PS/2 mice. No difference in α-actin staining was seen between the PS/2 and isotype control groups at any time point evaluated. PS/2 mAb administration had no effect on the cellular composition of the media at any time point studied (data not shown). IHC performed 28 days after injury demonstrated uniform PECAM staining and reendothelialization in all treatment groups (data not shown).

WBC Counts and Lipid Levels

Total leukocyte counts at sacrifice were similar between isotype control and PS/2-treated mice (3.3 ± 0.4 vs. 3.4 ± 0.4 × 10^6/ml, respectively). Similarly, no differences were seen in neutrophil [24 ± 3 vs. 22 ± 8%, p = not significant (NS)] and lymphocyte counts (76 ± 1 vs. 69 ± 4%, p = NS). Total cholesterol (34.4 ± 1.9 vs. 31.6

± 2.0 mmol/l, p = NS), LDL (30.3 ± 1.7 vs. 28.3 ± 1.4 mmol/l, p = NS), HDL (3.8 ± 0.12 vs. 3.1 ± 0.7 mmol/l, p = NS) and triglycerides (0.08 ± 0.01 vs. 0.02 ± 0.01 mmol/l, p = NS) were not different between isotype control and PS/2 mAb groups.

Discussion

The results from this study demonstrate an important role for VLA-4 in the response to arterial injury. We showed that cells expressing the $\alpha_4\beta_1$ integrin (VLA-4) are present in abundance in the developing neointima early after carotid artery air desiccation injury in the atherosclerosis-prone ApoE (–/–) mouse. IHC performed on serial sections revealed a predominance of neutrophils at peak VLA-4 expression. Furthermore, we demonstrated that prolonged blockade with a neutralizing antibody (PS/2) markedly reduces early neutrophil recruitment (95% reduction, $p < 0.02$), subsequent macrophage entry (48% reduction, $p < 0.05$), and neointimal growth 28 days after injury (72% reduction, $p < 0.02$).

These results challenge the findings and conclusions from a prior study by Labinaz et al. [17] evaluating the role of VLA-4 following arterial injury. In that study, a VLA-4 blocking antibody was administered once at the time of injury in a normolipemic porcine angioplasty model [17]. The authors concluded that VLA-4 blockade reduced late lumen loss but had no effect on neointimal growth. Several contrasting elements exist between these two studies. First, in the study of Labinaz et al. [17], normal Yorkshire swine were employed, which lack the atherogenic background of the ApoE (–/–) mouse. In our study, we investigated the role of VLA-4 in promoting neointimal growth following distension and denudation injury in the atherogenic environment created by ApoE gene deletion. Differences likely exist in the activation states of VLA-4 on circulating leukocytes of normal pigs and ApoE (–/–) mice, and the adhesive interaction with the VLA-4 counter-ligands therefore likely differ as well. We cannot exclude the possibility that ApoE deficiency itself amplifies the role of VLA-4, thereby resulting in a more substantial effect in this model. Second, the methods of injury in the two studies and the anticipated degree of neointimal hyperplasia as compared with vessel remodeling following injury differ dramatically. The results from Labinaz et al. [17] suggest a beneficial effect on vessel remodeling, but limited conclusions can be drawn regarding the effect of VLA-4 blockade on neointimal growth, the primary pathological result of arterial stent implantation. Additionally, Labinaz et al. [17] utilized an antibody directed against the human $\alpha_4\beta_1$ integrin and administered the antibody as a single injection at the time of injury alone. In the current study, we demonstrate that peak recruitment of VLA-4-expressing cells occurs 7 days after arterial injury in the mouse model. In agreement with Labinaz et al. [17], mice injected with the murine-specific VLA-4 blocking antibody at the time of injury alone demonstrated no statistically significant reduction in neointimal growth. However, prolonged VLA-4 blockade resulted in a marked reduction in neointimal area. Taken together, these findings suggest that prolonged VLA-4 blockade is required for optimal efficacy.

Leukocyte recruitment occurs early after arterial injury and is thought to play a prominent role in regulating neointimal hyperplasia. Prior studies have demonstrated the importance of VLA-4 and VCAM-1 in promoting leukocyte recruitment to the vessel wall across an intact endothelium. Kling et al. [18] demonstrated that administration of a VLA-4-blocking antibody inhibited monocyte and lymphocyte recruitment in electrically injured rabbit carotid arteries. This inhibition of leukocyte uptake into the arterial wall was associated with a trend towards decreased neutrophil recruitment 36 h after injury ($p = 0.08$). Oguchi et al. [19] demonstrated that administration of a VCAM-1-blocking antibody (1 μg/g i.v.) on alternate days reduced arterial macrophage accumulation and neointimal growth 21 days following the placement of a periadventitial cuff around the carotid in ApoE (–/–) mice. Neutrophil trafficking was not studied. The current study extends our understanding by demonstrating an important role for VLA-4 in mediating both neutrophil and macrophage recruitment after endothelial denuding injury.

Recently, Horvath et al. [20] demonstrated that balloon- and stent-injured arteries differ significantly in the inflammatory cell type recruited. Thus, blockade of both neutrophil and monocyte entry may have broad implications pertaining to arterial injury. The mouse carotid air desiccation model results in complete endothelial denudation together with distension injury of the media and initiates an early inflammatory response dominated by neutrophil infiltration with macrophage accumulation occurring later [14]. In the present study, prolonged VLA-4 blockade decreased both early neutrophil and late macrophage recruitment after carotid air desiccation injury. These findings are interesting and pathophysiologically relevant given the relatively high expression of $\alpha_4\beta_1$ on monocytes and lymphocytes as compared with quiescent neutrophils [8].

To our knowledge, this is the first report to demonstrate the importance of VLA-4 for neutrophil trafficking in vivo. Prior studies have established the importance of VLA-4 in mediating slow rolling and arrest of mononuclear cells [8, 9, 21]. More recently, in vitro data have demonstrated that VLA-4 can support neutrophil adhesion to VCAM-1 and thus may participate in mediating selectin-independent neutrophil arrest [22]. Clinical data also support a role for VLA-4 on neutrophils and monocytes. Ibbotson et al. [23] showed that the surface expression of VLA-4 on neutrophils is elevated in septic patients as compared with control patients. Further, neutrophils from septic patients as well as control neutrophils exposed to plasma from septic patients exhibit a markedly enhanced ability to roll and adhere to VCAM-1 under flow conditions [23]. Arefieva et al. [24] demonstrated that VLA-4 expression is increased on monocytes in patients who develop restenosis after coronary intervention; however, VLA-4 expression on neutrophils was not evaluated. Whether or not patients prone to developing restenosis exhibit enhanced VLA-4/VCAM-1-mediated neutrophil rolling and adhesion is yet to be determined.

In addition to its important cellular adhesion function, VLA-4 ligation plays an important role in signal transduction. Recent data suggest that VLA-4 engagement activates β_2 integrins on neutrophils and thereby promotes neutrophil adhesion to fibronectin [25]. VLA-4 ligation has also been shown to induce expression of reactive oxidant species by neutrophils [26] and TNF-α by monocytes [27]. Both mechanisms are thought to play important roles in the development of atherosclerosis and in the mechanical injury response. These molecular and cellular effects were not addressed in the current in vivo study. Additionally, VLA-4 is expressed in atherosclerotic lesions and plays a role in modulating the differentiation and contractile function of cultured SMCs [28, 29]. While we did see an increase in VLA-4 expression early after injury that colocalized with α-actin staining, we saw no difference in α-actin staining between isotype control-treated mice and mice receiving PS/2 at any time point. These findings do not exclude the possibility that $\alpha_4\beta_1$ expressed on SMCs plays an important role following arterial injury, but additional studies focusing on this specific question are required to clearly support this hypothesis.

We and others have demonstrated an important role for adhesion molecules and their ligands, including the selectins, selectin ligands and β_2 integrins, in mediating leukocyte arrest and neointimal growth following injury [14]. The $\alpha_4\beta_1$ integrin (VLA-4) is capable of supporting not only downstream leukocyte slow rolling and arrest but also early selectin-independent tethering. Our current study demonstrates that prolonged blockade of VLA-4 attenuates both early and late leukocyte recruitment and neointimal growth following arterial injury in the atherosclerosis-prone ApoE (–/–) mouse. These findings suggest an important role for VLA-4 in the response to vascular injury and further support the importance of this pathway for leukocyte recruitment to sites of vascular inflammation.

Acknowledgments

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