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ORIGINAL CONTRIBUTION

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Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ mediate VSMC migration and are elevated during neointima formation in the rat aorta

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Abstract Neointima formation involves tissue expression of matrix proteins and growth factors. The role of $\alpha_v\beta_3$, but not $\alpha_v\beta_5$ integrin in vascular cells has been sufficiently investigated. The aim of the present study was to determine and compare the function of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in rat aortic (RASMC) and human coronary vascular smooth muscle cells (HCSMC) and to characterize their expression accompanying neointima formation *in vivo*. RASMC and HCSMC express $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin subunits. The $\alpha_v\beta_5$ integrin predominantly mediated adhesion of RASMCs to vitronectin and spreading on vitronectin via RGD-binding sequences. In contrast, the $\alpha_v\beta_3$ integrin did not contribute to the adhesion and spreading on fibronectin, vitronectin, gelatin or collagen I coated layers. PDGF-directed migration through gelatin coated membranes involved both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. Selective blocking antibodies for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ inhibited migration of RASMC and HCSMC by more than 60 % ($p < 0.01$). Integrin expression was studied in vivo in thoracic aorta of Sprague Dawley rats before and after balloon injury. In situ hybridization demonstrated low signals for α_v, β_3 and β_5 mRNA in uninjured aorta, which increased significantly at 14 days, localized predominantly in the neointima. Northern analysis of aorta after 14 days of injury also demonstrated an upregulation of α_v, β_3 and β_5 mRNA compared to uninjured aorta. Consistent with the increase in message levels, increased integrin protein expression was seen in the neointima after 7 and 14 days. This study provides evidence that $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are elevated during neointima formation in the rat and indicates a novel role for $\alpha_v\beta_5$ participating in mechanisms regulating smooth muscle cell migration.

Key words Integrins – neointima formation – migration – rat – vascular smooth muscle cells

Introduction

Vascular smooth muscle cell migration and production of extracellular matrix (ECM) are critical processes in neointima formation and atherosclerosis (34). Thus ECM is not only a physical component of the neointima, but dynamically regulates VSMC actions important to neointima development (2, 15, 27). Fibronectin, laminin, vitronectin and collagens are major constituents of the extracellular matrix. Fibronectin is one of the first matrix proteins laid down in response to vascular injury (11). Vitronectin has been recognized to accumulate in animal and human atherosclerotic plaques and stimulates migration of human VSMCs (3, 14). Recently, the extracellular matrix adhesion protein osteopontin was shown to be induced in the neointima and to regulate VSMC migration (28, 30). These results were relevant in vivo,

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since antibodies against osteopontin attenuated neointima formation (29).

A well-established integrin binding motif is the tripeptide arginine – glycine – aspartic acid (RGD) which is recognized by $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_{IIb}\beta_3$, and $\alpha_5\beta_1$ integrin receptors. This RGD motif is also present in the adhesion protein osteopontin and in extracellular matrix proteins like fibronectin and vitronectin. Both osteopontin and vitronectin bind to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ via their RGD motifs (28). Unlike the $\alpha_v\beta_5$ integrin, the $\alpha_v\beta_3$ integrin is also a ligand for other matrix or adhesion proteins such as fibronectin, fibrinogen, thrombin, van Willebrand factor (vWF), collagen, thrombospondin and others (12). $\alpha_v\beta_3$ is prominently expressed in endothelium and is also expressed in human atherosclerotic plaques (22). In addition, RGD peptides which compete for integrin binding have been shown to attenuate neointima and atherosclerotic development (7, 32, 33). Despite these observations demonstrating an important role for α_v and some of the β integrins in the response to vascular injury, and some recent investigations regarding the regulation of integrins by the influence of corticoids and growth factors (6, 21, 25), little is known about the regulation of integrin expression in VSMCs and their appearance during neointima formation. The purpose of the present investigation was to 1) assess and compare the roles of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in VSMC adhesion, spreading and migration and 2) investigate the expression of α_v, β_3 and β_5 integrins in injured rat arteries.

Materials and methods

Materials

Materials were obtained from the following suppliers: Dulbecco's modified Eagle's medium (DMEM), glutamine, antibiotics, Hepes, DMSO, PDGF-BB, fetal bovine serum (FBS), rat vitronectin, rat fibronectin, monoclonal antibody against smooth muscle α-actin from Sigma (St. Louis, MO), culture plastic ware from Becton Dickinson (Lincoln Park, NJ), the transwell chambers from Costar (Cambridge, MA), [γ^{32}P]ATP from ICN (Irvine, California). Sprague-Dawley rats were from Charles River (Wilmington, MA). Antibodies against human α_v (AB 1930), human β_3 (AB 1932), human $\alpha_v\beta_3$ (LM 609) and human β_5 (AB 1926) were purchased from Chemicon (Temecula, CA). Antibody against rat β_3 (clone F11) was from Pharmingen (San Diego, CA). Monoclonal antibodies against $\alpha_v\beta_5$ (P1F6) and α_v (VNR139) were from GIBCO BRL (Gaithersburg MD). Peptide hexamers GRGDSP and GRGESP were purchased from Bachem (Torrance, CA). The monoclonal osteopontin antibody MPOIIIB was from the University of Iowa, Iowa City, IA, under contract N01-HD-6-2915 from the NICHD.

Cell culture

Rat aortic smooth muscle cells (VSMCs) were prepared from thoracic aorta of 2–3 month old Sprague-Dawley rats using the explant technique (4, 17). Human coronary smooth muscle cells (HCSMCs) were purchased from Clonetics (San Diego, CA). Cells were cultured in DMEM containing 10 % FBS, 150 mmol/L Hepes, 100 µg/mL penicillin, 100 µg/mL streptomycin and 200 mmol/L glutamine. The purity and identity of the smooth muscle cell cultures were verified by using a monoclonal antibody against smooth muscle α-actin. For all experiments, early passaged (6 or less) rat VSMCs were grown to 60–70 % confluence.

Flow cytometry

Cells were incubated with primary antibody, washed, resuspended in the appropriate FITC-conjugated secondary antibody (Sigma) and analyzed for fluorescence on a FACSCAN flow cytometer (Becton Dickinson, San Jose, CA). The x and y axes represent log fluorescent intensity and cell number, respectively.

Migration

Chemotaxis experiments were performed as described previously (19). VSMC migration was examined in transwell cell culture chambers using a gelatin (conc. 0.1 %) coated polycarbonate membrane with 8 µm pores. The number of VSMCs per 320x high power field (HPF) that migrated after 4 h to the lower surface of the filters was determined microscopically. Four randomly chosen HPFs were counted per filter. Experiments were performed in duplicate or triplicate, and were repeated at least three times.

Adhesion

Adhesion assays were performed on adhesive substrates (rat fibronectin 50 µg/ml, rat vitronectin 10 µg/ml, gelatin 0.1 %, type I collagen 20 µg/ml) as described previously (25, 28).

Spreading

The rate of cell spreading was determined by number of attached and spread cells by measuring the number of cells that adhered to a specific substrate as described recently (25). Peptides or antibodies were given to cells after a minimal attachment time of 10 min. The number of spread cells was expressed as percent of total cells/high

power field. Extracellular matrix, antibody and peptide concentrations were identical to concentrations used in adhesion experiments.

Isolation and analysis of RNA

Total RNA was isolated from smooth muscle cells using the guanidinium-sodium acetate phenol-chloroform method (8). The cDNAs for rat α_v, rat β_3 and rat β_5 integrin were kindly provided by Dr. Gideon Rodan (Merck). The hybridization signals of the specific mRNAs of interest were normalized to those of CHOB, a constitutively expressed gene to correct for differences in loading or transfer (20). CHOB cDNA was originally isolated from Chinese hamster ovary cells that corresponds to an RNA ubiquitously expressed in mammalian tissues which does not exhibit regulation as a function of growth or development. Quantitation of Northern blots was performed by densitometric analysis using NIH Image 1.60 software for Macintosh personal computers.

Balloon injury

Male Sprague-Dawley rats weighing 280–300 g were used in this study. Animals received balloon injury to the aorta extending caudal to the renal artery and rostral to the left main carotid artery. Balloon-catheter injury was induced during ketamine (35 mg/kg IM) and xylazine anesthesia, as described previously (27). Two, fourteen and 35 days after balloon injury, animals (n = 6, at each time point) were sacrificed for histological examinations. RNA extractions of aortas were performed 14 days after injury (n = 6) in comparison to control animals (n = 6).

In situ hybridization

The plasmid with the cDNAs for rat α_v (0.95 kb fragment, position 1914–2879), rat β_3 (0.4 kB fragment, position 484–893) and rat β_5 integrins (0.7 kb fragment, position 1650–2289) were kindly provided by Dr. Gideon Rodan (Merck Laboratories). The cDNAs were linearized by digestion with restriction enzymes. Riboprobe was generated by transcription of linearized cDNA with T3- and T7-polymerase using digoxigenin-labeled UTP (Boehringer) as the substrate. In situ hybridization was performed on formalin-fixed, paraffin embedded 4 μm-thick sections of the aorta according to standard protocols, as described previously (18).

Immunohistochemistry

The labeled avidin-biotin method (LAB) was used for detection as described previously (1, 18). Anti-human α_v integrin rabbit antiserum, detecting the cytoplasmatic domain of the α_v integrin, anti-human β_5 integrin rabbit antiserum at a titer of 1:200 and an monoclonal antibody against α-smooth muscle actin at a titer 1:500 (Boehringer) were used in staining paraffin embedded aortic tissue. A monoclonal anti-rat β_3 integrin antibody (F11) at a titer of 1:100 was used in frozen sections from rat aorta. Nonimmune mouse and rabbit IgG was used as the control.

Statistics

Analysis of variance (ANOVA), and paired or unpaired *t* test were performed for statistical analysis, as appropriate. A P value less than 0.05 was considered to be statistically significant. Data were expressed as mean ± SEM, if not stated otherwise.

Results

α_v, β_3 and β_5 integrins are expressed in cultured VSMCs

VSMCs obtained from Sprague-Dawley rats consistently expressed $\alpha_v\beta_3$ and $\alpha_v\beta_5$ on the cell surface throughout passages 1 to 8 (data not shown). Flow cytometry also demonstrated expression of these integrins in human coronary smooth muscle cells. The mean channel fluorescences for HCSMCs were non-specific IgG 6.98, α_v integrin 134.51, β_3 integrin 112.50, β_5 integrin 76.8, which indicates the presence of these integrins on the surface of HCSMCs.

Adhesion studies

Rat VSMCs (30,000/well) adhered to vitronectin, fibronectin, collagen I and gelatin. Maximal adhesion was observed on plates coated with 10 μg/mL vitronectin, 20 μg/mL fibronectin, 20 μg/mL collagen I and 1 % gelatin (not shown). The effect of the β_3 integrin blocking antibody F11, the $\alpha_v\beta_5$ integrin blocking antibody P1F6, and peptide hexamers containing RGD or RGE on VSMC adhesion to these matrices was studied. Neither of these antibodies nor the RGE hexamers affected adhesion of VSMCs to fibronectin and gelatin (data not shown). A small, but significant inhibition was seen in the presence of RGD hexamers (100 μmol/L) in adhesion to fibronectin, and a decrease of 31 % of adhesion to gelatin (both $p < 0.05$, data not shown). In contrast, adhesion of VSMCs to vitronectin was markedly inhibited by P1F6 (50 μg/mL, $p < 0.01$) and by RGD hexamers (100 μmol/L,

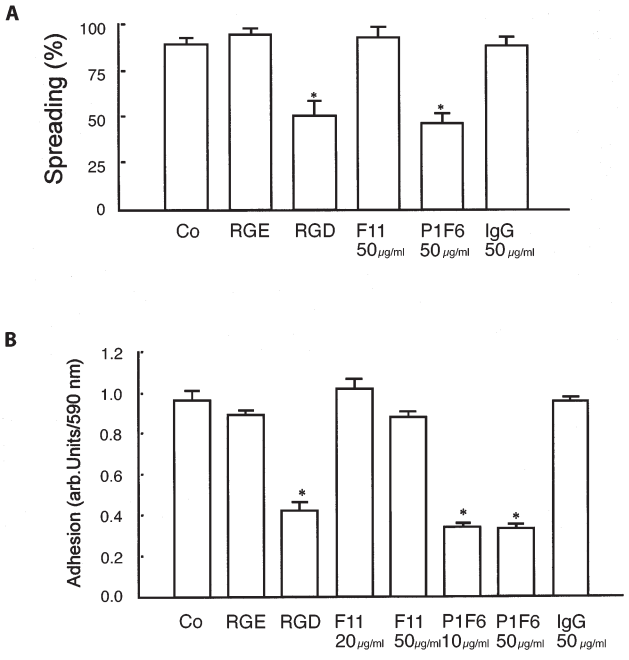

Fig. 1 **(A)** Cell spreading experiments were performed in the presence of specific blocking antibodies and peptides on vitronectin (10 µg/mL). Inhibition of VSMC spreading was determined in the presence of non-specific IgG (50 µg/mL), antibodies against β_3 (F11), $\alpha_v\beta_5$ (P1F6) and by RGD and RGE peptides. Experiments were performed in quadruplicate with cells from four different preparations. *$p < 0.05$ vs. IgG alone. **(B)** Adhesion of rat VSMCs to vitronectin (10 µg/mL). Inhibition of adhesion was tested in the presence of blocking antibodies against β_3 (F11), $\alpha_v\beta_5$ (P1F6) and by RGD and RGE peptide. *$p < 0.05$ vs. IgG alone, n = 8-10.

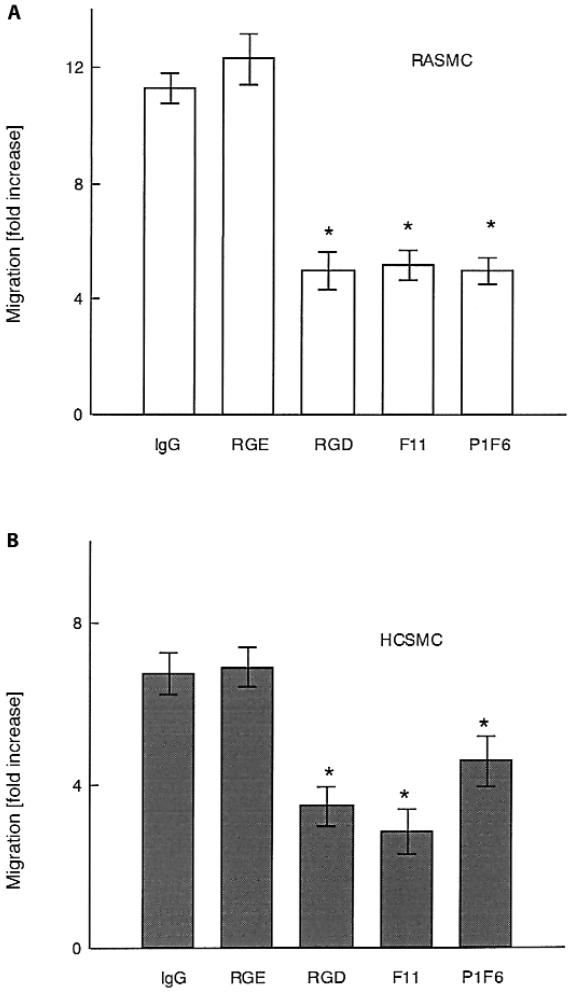

Fig. 2 **(A)** Migration of rat aortic VSMCs towards PDGF was inhibited by blocking antibodies against β_3 (F11, 50 µg/mL) and $\alpha_v\beta_5$ (P1F6, 20 µg/mL) and by RGD (100 µmol/L). PDGF-BB (10 ng/mL) induced a significant increase in migrated cells, which was not inhibited by IgG (50 µg/mL) or control peptide hexamer RGE (n = 6, mean ± SE, *$p < 0.01$ vs. PDGF + IgG). **(B)** Migration of human coronary VSMCs towards PDGF was inhibited by blocking antibodies against $\alpha_v\beta_3$ (LM 609, 20 µg/mL) and $\alpha_v\beta_5$ (P1F6, 20 µg/mL) and by RGD (100 µmol/L). PDGF-BB (10 ng/mL) induced a significant increase in migrated cells, which was not affected by IgG (50 µg/mL) or control peptide hexamer RGE (n = 5, *$p < 0.01$ vs. PDGF alone).

$p < 0.01$), whereas control RGE (GRGESP) did not affect VSMC adhesion (Fig. 1B). F11 (50 µg/mL) antibody, which inhibits β_3 integrin, did not affect VSMC adhesion to vitronectin (Fig. 1B). Neither RGD hexamers (GRGDSP) nor the tested antibodies inhibited adhesion to collagen I.

VSMC spreading studies

Spreading of rat VSMCs was determined on plates coated with 10 µg/mL vitronectin, 20 µg/mL fibronectin, 20 µg/mL collagen I and 1 % gelatin. Neither of the two blocking antibodies nor the RGD hexamers affected the spreading of VSMCs on fibronectin and collagen I (data not shown). The RGD hexamers inhibited cell spreading on gelatin by 60 % ($p < 0.01$ vs. RGE, not shown) and on vitronectin by 45 % ($p < 0.05$ vs. RGE, Fig. 1A). P1F6 (50 µg/mL) partially impaired the spreading on vitronectin by 50 % ($p < 0.01$, Fig. 1A), but did not affect spreading on the other matrices. The blocking antibody F11 did not affect spreading on the tested matrix proteins. Spreading of VSMCs on vitronectin was partially mediated by $\alpha_v\beta_5$ integrin presumably via its RGD binding sequences.

Migration mediated by $\alpha_v\beta_3$ and $\alpha_v\beta_5$

We performed migration experiments in a transwell chamber system using PDGF-BB (10 ng/mL) as the chemoattractant. VSMCs were pretreated for 30 min with a nonspecific IgG (50 µg/mL), F11 (50 µg/mL), P1F6 (25 µg/mL), RGD or RGE (both 100 µmol/L). Nonspecific IgG or RGE did not affect VSMC migration. In contrast the antibodies against $\alpha_v\beta_3$ and $\alpha_v\beta_5$, or the competing RGD peptide inhibited VSMC migration by more than 60 % (Fig. 2A). Parallel experiments were performed with human coronary smooth muscle cells (HCSMCs). An

antibody against human $\alpha_v\beta_3$ (LM 609, 40 μg/mL) and RGD inhibited PDGF-BB-induced migration by 50 % ($p < 0.01$). The antibody P1F6 (25 μg/mL) inhibited migration of HCSMCs by 35 % ($p < 0.05$) (Fig. 2B). These data indicate that migration of rat and human VSMC is mediated by both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins.

Expression of α_v, β_3 and β_5 integrin in normal and injured rat

Total mRNA was extracted from thoracic and abdominal aorta from sham-treated animals and animals 14 days after balloon injury. β_5 integrin mRNA was readily detectable in normal aorta and was increased in aortas after 14 days of injury (0.78 + 0.23 β_5/CHOB in normal aorta, 1.55 + 0.21 β_5/CHOB in injured aorta, $P < 0.05$, $n = 6$). α_v and β_3 integrin mRNAs were expressed at low levels in normal aorta (0.17 + 0.04 α_v/CHOB, 0.05 + 0.02 β_3/CHOB, $n = 6$ each); injury increased message levels for both of these integrins (0.24 + 0.02 α_v/CHOB, 0.13 + 0.03 β_3/CHOB, $n = 6$ each, $P < 0.05$).

In situ hybridization confirmed the increase in integrin message levels in injured aorta. α_v message was markedly detectable in the neointima and there were areas in the media and adventitia that expressed av mRNA in aortas 14 days post injury (Fig. 3A). There was substantially less digoxigenin labeling using the sense control for all three integrins (Fig. 3D-F). Similar results were seen with β_3 message levels (Fig. 3B). The β_5 message was also readily detectable in the neointima, but also appeared to be present throughout the media (Fig. 3C). In contrast, normal thoracic aorta sections showed no expression for α_v and β_3 mRNA, and very low levels of β_5 mRNA. In aortic sections obtained 2 d after injury, a modest increase in message levels was detected in the subendothelial and layer for all three integrins (not shown).

Immunohistochemistry revealed similar results as *in situ* hybridization. Normal aorta exhibits minimal immunoreactivity for β_5 integrin in the medial and the adventitial layer. The antibodies against α_v and β_3 did not reveal significant staining (not shown). No significant changes were observed in sections from aortas 2 d after injury. In rings 7 d post-injury, focal staining against α_v, β_3 and β_5 integrins was seen in the intimal lesion areas (not shown). After 14 d, staining with the α_v integrin antibody was prominent in the neointima, present in the media, and intense in the adventitia (Fig. 4B). The β_3 integrin antibody demonstrated staining in the neointima and media and relatively less in the adventitia (Fig. 4C). Staining with the antibody against β_5 integrin was intense in the neointima and adventitia and, similar to the distribution of α_v integrin, was also present in the media (Fig. 4D).

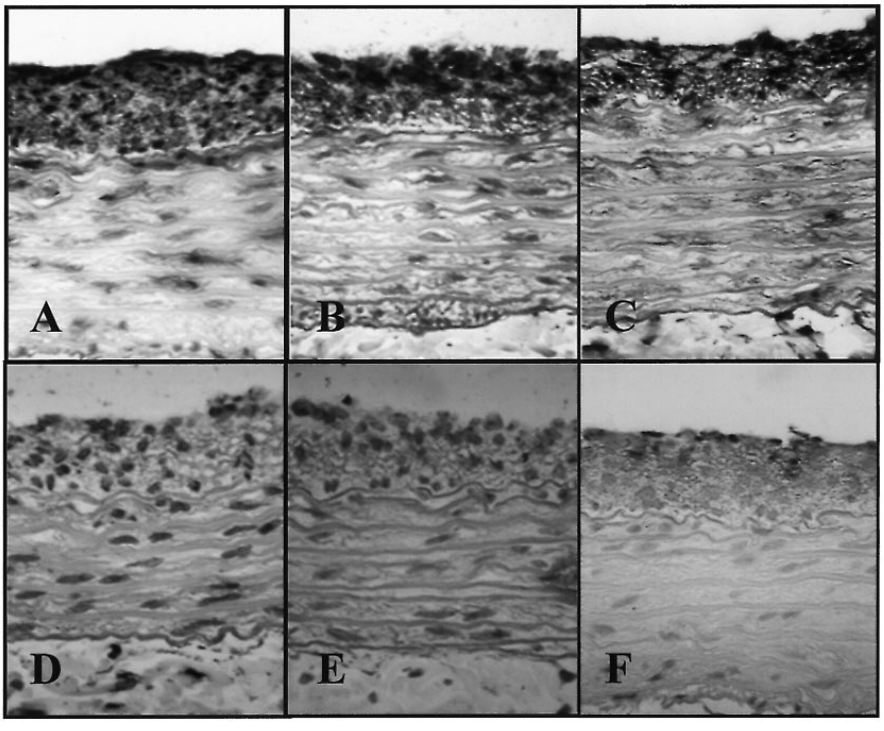

Fig. 3 **A** In situ hybridization of aorta 14 d after balloon injury detected by antisense riboprobes for β_3 integrin, **(B)** for α_v integrin, and **(C)** for β_5 integrin. **(D)** In situ hybridization of aorta 14 d after balloon injury with sense riboprobe for β_3 integrin, **(E)** sense riboprobe for α_v integrin, and **(F)** sense riboprobe for β_5 integrin (all magnification x200, counterstain hematoxylin).

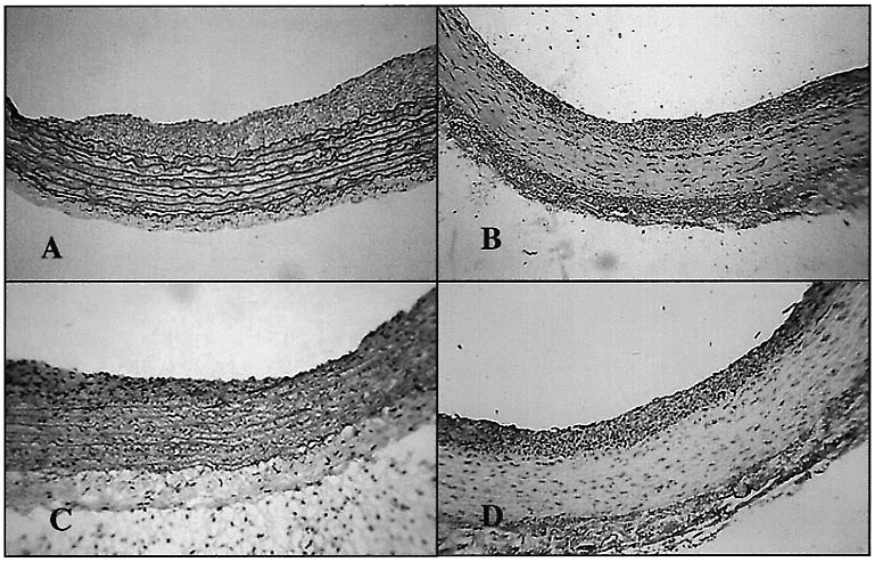

Fig. 4 Immunohistochemical analysis of frozen and paraffin sections of aorta 14 d after balloon injury stained with **(A)** non-specific IgG, **(B)** stained with antibody against α_v integrin, **(C)** frozen section of aorta 14 d after balloon injury stained with antibody against β_3 integrin (F11), **(D)** stained with antibody against β_5 integrin (Magnification 40x, counterstain hematoxylin).

Discussion

The identification of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in cultured human coronary artery cells and rat aortic smooth muscle cells is consistent with recent publications reporting the presence of both integrins in smooth muscle cells derived from rat, rabbit, porcine and human vasculature (12, 22, 24, 28, 30, 37). The present investigation demonstrates that 1) $\alpha_v\beta_5$ but not $\alpha_v\beta_3$ predominantly contributes to adhesion to and spreading on vitronectin in human and rat smooth muscle cells, 2) $\alpha_v\beta_5$ participates in PDGF-directed migration of human coronary and rat aortic VSMCs, and 3) all three integrin subunits are substantially upregulated in the rat neointima, induced by balloon denudation. These data suggest that $\alpha_v\beta_5$ as well as $\alpha_v\beta_3$ integrins play an important role in the vascular response to injury.

We found that mRNA levels of α_v, β_3 and β_5 integrins were detected in the subendothelial layer by medial cells at 2 d post-injury and were increased at 14 d with the highest expression seen in intimal cells. This time course of integrin expression in the neointima is consistent with the regulation of these genes by growth factors that promote neointima formation. It is likely that growth factors and cytokines as well as mechanical stress also contribute to enhanced integrin expression in vascular injury. A ligand for $\alpha_v\beta_3$ and $\alpha_v\beta_5$, osteopontin, is regulated by PDGF and other growth factors and is upregulated in neointimal lesions. Hoshiga and coworkers (22) described immunoreactivity for $\alpha_v\beta_3$ in neointima, some medial VSMCs and adventitia of human coronary artery specimens. Recently it has been demonstrated that vitaxin, a humanized monoclonal antibody against $\alpha_v\beta_3$, reduced neointimal hyperplasia and total vessel area after balloon injury in hypercholesterolemic rabbits (13). Also vitronectin protein was found to be markedly elevated in the intima of human atherosclerotic plaques. $\alpha_v\beta_5$ integrin was also shown to be expressed in the intima of these plaques in close association to vitronectin (14). These findings indicate that both integrins are involved in the pathophysiological mechanisms of vascular injury and atherosclerosis.

To determine the function of these integrins, we used blocking antibodies and RGD peptides to inhibit 1) adhesion to the matrix proteins collagen I, gelatin (denatured collagen I), fibronectin and vitronectin, 2) spreading of VSMCs on these matrices, and 3) PDGF directed migration. Adhesion of rat VSMCs to vitronectin was predominantly mediated by $\alpha_v\beta_5$, and not by $\alpha_v\beta_3$. Adhesion to fibronectin did not involve $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Linear RGD-peptides significantly reduced adhesion to vitronectin, but inhibited only slightly fibronectin binding. These data are in agreement with data in other cell types suggesting that cell adhesion to vitronectin is primarily $\alpha_v\beta_5$ dependent, while several integrins including $\alpha_v\beta_3$ and RGD-independent integrins mediate binding to fibronectin (23). We could not find a significant inhibition of adhesion to fibronectin by the blocking antibody F11 against β_3 integrins, although it has been shown that both of the ECM proteins fibronectin and vitronectin influence cell migration and spreading (15). Adhesion to collagen was not impaired in the presence of blocking antibodies, nor by RGD hexamers. This observation is partially in accordance with a previously published study (12), which demonstrated adhesion to collagen and fibronectin is predominantly mediated by β_1 integrins via non-RGD sequences, and may also account for the usage of linear RGD, which has been shown to have less affinity in integrin binding than cyclic RGD, thus serving

as a complete substitute for integrins.

Spreading experiments showed no effects of blocking $\alpha_v\beta_3$ function on VSMC spreading. There was a significant effect of P1F6 on cell spreading on vitronectin indicating that $\alpha_v\beta_5$ is also involved in cell spreading on this substrate. We could also show an RGD-dependent inhibition of VSMC adhesion and spreading on gelatin which was not mediated via $\alpha_v\beta_3$ or $\alpha_v\beta_5$. These data indicate that $\alpha_v\beta_5$ mediates adhesion to and spreading on vitronectin, whereas the integrin $\alpha_v\beta_3$ has no major effects on these VSMC functions on the tested matrices. However, it does not completely exclude the involvement of $\alpha_v\beta_3$ in cell spreading. PDGF-induced migration of rat and human VSMCs on a gelatin matrix was blocked by RGD peptide, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antibodies suggesting that both integrins are necessary for migration under the studied conditions. Since linear RGD peptides potently inhibited VSMC migration, we cannot exclude the involvement of other integrins, especially the RGD-dependent β_1 integrins $\alpha_v\beta_1$ and $\alpha_2\beta_1$. Skinner and coworkers have reported that $\alpha_2\beta_1$ is required for chemotaxis events in VSMCs, which is not expressed in medial smooth muscle cells (21, 35). Several reports have already demonstrated that $\alpha_v\beta_3$ regulates VSMC migration (2, 3, 30). The present finding that $\alpha_v\beta_5$ also is important in the regulation of VSMC migration is supported by a study in pancreatic tumor cells, which showed that motility of these cells was dependent on $\alpha_v\beta_5$ (26). These authors demonstrated that epidermal growth factor (EGF), which induced motility in these cells, did not alter expression of $\alpha_v\beta_5$, but enhanced common signaling pathways that regulate migration. Our observations are in accordance with a previously published report that found that non-directed migration of VSMCs was inhibited by an anti-β_3 antibody, and even more by incubation with a rabbit anti-human $\alpha_v\beta_{3/5}$ (anti-VNR) antibody in a scrape-wound injury in vitro assay (36). We have demonstrated that MAP kinase mediates both PDGF and A_{II} directed VSMC migration, since PD98059 is an inhibitor of this pathway, or antisense to MAP kinase substantially attenuated migration (19). Engagement of RGD-dependent integrins has been shown to result in-activation of focal adhesion kinase and MAP kinase (5, 9, 10, 31). Thus, the integrin effect on migration likely involves the MAP kinase pathway. The involvement of another intracellular pathway has been investigated by Bilato and Crow (2), who demonstrated that $\alpha_v\beta_3$ mediated activation leads to an increase of intracellular CAM kinase activity, which regulated PDGF-directed migration of VSMCs. The inhibition of $\alpha_v\beta_3$ and consecutively of the migration of VSMCs could be eliminated by elevation or restoration of intracellular CAM kinase activity in human and rat VSMCs (2).

Taken together with previous observations, the present investigation underscores the importance of integrin receptor-matrix interaction that appears increasingly important in the process of neointimal development, such as VSMC growth, adhesion and migration which follows vascular injury and repair.

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