Molecular Imaging of Endothelial Vascular Cell Adhesion Molecule-1 Expression and Inflammatory Cell Recruitment During Vasculogenesis and Ischemia-Mediated Arteriogenesis

Carolyn Z. Behm, MD; Beat A. Kaufmann, MD; Chad Carr, MD; Miles Lankford, BS; John M. Sanders, BS; C. Edward Rose, MD; Sanjiv Kaul, MD; Jonathan R. Lindner, MD

Background—Inflammatory responses contribute to vascular remodeling during tissue repair or ischemia. We hypothesized that inflammatory cell recruitment and endothelial cell activation during vasculogenesis and ischemia-mediated arteriogenesis could be temporally assessed by noninvasive molecular imaging.

Methods and Results—Contrast ultrasound perfusion imaging and molecular imaging with microbubbles targeted to activated neutrophils, α5-integrins, or vascular cell adhesion molecule (VCAM-1) were performed in murine models of vasculogenesis (subcutaneous matrigel) or hind-limb ischemia produced by arterial occlusion in wild-type or monocyte chemotactic protein-1–deficient mice. In subcutaneous matrigel plugs, perfusion advanced centripetally between days 3 and 10. On targeted imaging, signal enhancement from α5-integrins and VCAM-1 coincided with the earliest appearance of regional blood flow. Targeted imaging correlated temporally with histological evidence of channel formation by α5-integrin-positive monocytes, followed by the appearance of spindle-shaped cells lining the channels that expressed VCAM-1. In ischemic hind-limb tissue, skeletal muscle blood flow and arteriolar density increased progressively between days 2 and 21 after arterial ligation. Targeted imaging demonstrated early signal enhancement for neutrophils, monocyte α5-integrin, and VCAM-1 at day 2 when blood flow was very low (<20% control). The neutrophil signal declined precipitously between days 2 and 4, whereas VCAM-1 and monocyte signal persisted to day 7. In mice deficient for monocyte chemotactic protein-1, monocyte-targeted signal was severely reduced compared with wild-type mice (1.2 ± 0.6 versus 10.5 ± 8.8 video intensity units on day 4; *P* < 0.05), although flow responses were only mildly impaired.

Conclusions—Different components of the inflammatory response that participate in vascular development and remodeling can be assessed separately with targeted molecular imaging. (Circulation. 2008;117:2902-2911.)

Key Words: angiogenesis ■ echocardiography ■ imaging ■ inflammation

Inflammation contributes in many different ways to angiogenic processes during wound healing and tissue response to injury.1–8 Leukocytes serve as a source for proangiogenic peptides and proteases that participate in extracellular matrix remodeling.3,4 In certain circumstances, endothelial precursor cells may derive from monocyte lineage.5,6 In addition, proinflammatory endothelial cell adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) may act as a homing receptor for precursor cell populations and may favorably influence endothelial cell survival during vascular remodeling.7,8

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There also is strong evidence that recovery of blood flow in response to limb ischemia is critically dependent on the inflammatory response. Beneficial vascular remodeling in limb ischemia is enhanced by exogenously administered proinflammatory chemokines such as monocyte-chemotactic protein-1 (MCP-1),9 whereas remodeling is attenuated by monocyte depletion10 or by functional inhibition of endothelial cell adhesion molecules or MCP-1 signaling.11–13 Noninvasive imaging of key immune responses could potentially be used to study inflammation in vascular remodeling and how it can be modulated for therapeutic effect. Molecular imaging of inflammation with ultrasound has been achieved by surface modification of microbubble contrast agents.14–17 In this study, we hypothesized that targeted imaging of endothelial cell activation and inflammatory cell recruitment would herald vascular remodeling. This hypothesis was tested in murine models of de novo...
vessel formation (vasculogenesis) and ischemia-mediated arteriogenesis. Contrast agents were targeted to endothelial VCA-1, complement receptors expressed preferentially by activated neutrophils, or the α5-integrin component of the fibronectin receptor (α5β1) on monocytes. The last target was selected because of its role in both fibronectin-mediated attachment during vascular remodeling and its role for stimulating proangiogenic activity of monocytes.18

Methods

Study Design

The study was approved by the institutional Animal Research Committee. Growth factor–enriched matrigel was placed subcutaneously to study vasculogenesis in 18 wild-type C57Bl/6 male 8- to 10-week-old mice. Contrast-enhanced ultrasound (CEU) perfusion imaging of the matrigel plug and targeted imaging for α5-integrins, VCA-1, or neutrophil complement receptors were performed on day 3, 6, or 10 (n=6 for each time interval, 1 follow-up interval per animal). To study arteriogenesis, unilateral hind-limb ischemia was produced by interruption of iliac artery inflow. CEU perfusion imaging and targeted imaging of the proximal adductor muscles in the ischemic and control hind limbs were performed 2, 4, or 7 days after ligation (n=5 for each time interval). Late recovery of perfusion was assessed at day 21 in an additional 5 mice. Molecular imaging and perfusion imaging also were assessed in 5 MCP-1−/− mice (kindly provided by Dr Barrett Rollins, Dana-Farber Cancer Institute, Boston, Mass) 4 days after ligation. An additional 6 wild-type mice were used to validate the molecular imaging analysis method.

Matrigel Model

Matrigel (BD Biosciences, San Jose, Calif), composed of basement membrane extract from the Engelbreth-Holm-Swarm mouse sarcoma, was thawed at 4°C and enriched with fibroblast growth factor-2 (500 ng/mL; Sigma-Aldrich, St Louis, Mo) and heparin (64 U/mL). Enriched matrigel (1 mL) at 4°C was injected subcutaneously to form discrete ellipsoid plugs in the ventral abdominal wall. Imaging was performed in anesthetized mice at day 3, 6, or 10 (n=4 for each). In an additional 4 mice, the spatial extent of perfusion at either day 6 or 10 was characterized by the presence of intravascular fluorescent nanospheres. Approximately 1×1010 fluorescently labeled polystyrene spheres (Duke Scientific Corp, Fremont, Calif) with a mean diameter of 500 nm were injected intravenously. After 5 minutes, the plugs were excised, flash-frozen in liquid nitrogen, sectioned, and observed by microscopy (Axioskop2-FS, Carl Zeiss, Inc, Thornburg, NY) with fluorescent epi-illumination (530- to 560-nm excitation filter).

Hind-Limb Ischemia

Mice were anesthetized with an intraperitoneal injection (12.5 μL · g−1) of a solution containing ketamine hydrochloride (10 mg · mL−1), xylazine (1 mg · mL−1), and atropine (0.02 mg · mL−1). Body temperature was maintained at 37°C with a heating pad. A short segment of the distal common iliac artery encompassing the origin of the epigastric vessel was isolated and removed. The skin incision was closed, and animals recovered in a heated environment. Imaging studies were performed at day 2, 4, 7, or 21 after arterial ligation.

Microbubbles

For perfusion imaging, lipid-shelled decafluorobutane microbubbles were prepared by sonication of a gas-saturated aqueous suspension of 2 mg · mL−1 distearoylphosphatidylcholine and 1 mg · mL−1 polyoxethylene-40-stearate. For in vivo imaging of intravascular recruitment of activated neutrophils, microbubbles were targeted to complement receptors on activated leukocytes by addition of distearoylphosphatidylserine (0.3 mg · mL−1) to the aqueous suspension before sonication.16,17 Microbubbles targeted to α5-integrins or VCAM-1 were prepared from biotinylated microbubbles containing distearoylphosphatidylethanolamine-PEG(2000)biotin in the shell. Biotinylated rat monoclonal antibody against murine α5-integrin (SH10-27, BD Biosciences), VCAM-1 (MK2.7 purified from hybridoma), or isotype control antibody (R3-34, BD Biosciences) was conjugated to the surface of microbubbles as previously described.19 The mean diameters for targeted and control microbubbles were 2.7±0.3 and 2.9±0.4 μm, respectively.

Ultrasound

CEU imaging was performed with 2 different imaging modalities to optimize balance between sensitivity and resolution for the 2 target tissues. For matrigel imaging in which sensitivity for detecting very low levels of flow was paramount, power Doppler imaging (Sonos 5500, Philips Ultrasound, Bothell, Wash) was performed at 7.1 MHz with a mechanical index of 0.8, a pulse-repetition frequency of 2.4 KHz, and a medium packet size. This imaging modality did not provide sufficient spatial resolution to study regional hind-limb muscle imaging. Accordingly, in vivo limb imaging was performed with Contrast-Pulse Sequencing (Sequoia, Siemens Medical Systems, Mountain View, Calif), which balances microbubble sensitivity and spatial resolution. Imaging was performed at a centerline frequency of 7 MHz, a mechanical index of 1.6, and a dynamic range of 60 dB. The proximal hind-limb adductor muscles in the ischemic and contralateral control nonischemic limb were imaged in a transaxial plane midway between the inguinal fold and the knee.

Perfusion Imaging

Perfusion was assessed during continuous intravenous infusion of nontargeted lipid microbubbles at 1×1010 min−1 for matrigel and 2×1010 min−1 for hind-limb imaging (total volume ≤50 μL). Background images were acquired at 30 Hz. Intermittent imaging was performed by progressive prolongation of the pulsing interval from 0.2 to 12 seconds with an internal timer. Several frames were acquired at each pulsing interval. Averaged background frames were digitally subtracted from averaged contrast-enhanced frames at each pulsing interval. Pulsing interval versus video intensity (VI) data were fit to the following function: y=A+(1−e−b), where y is the VI at the pulsing interval t; A is the plateau VI, which is an index of microvascular blood volume; and β is the rate constant, which provides a measure of microvascular blood velocity.20 Microvascular blood flow was calculated by the product of A and β. For display purposes, parametric images of microvascular blood flow were created in which the value of A · β was displayed on a pixel-by-pixel basis. Because neovascularization of matrigel plugs progresses centripetally (from the outer surface toward the center),21 perfusion data were analyzed separately for the outer, middle, and central regions of the plug defined by a central and 2 surrounding concentric ellipsoid rings, all with equal radial width.

Targeted Imaging

Targeted imaging was performed as previously described after intravenous injection of control microbubbles or microbubbles targeted to VCAM-1, α5-integrin, or neutrophil complement receptors (5×1010 for matrigel and 1×1010 for hind-limb imaging).19 The order of injection was randomized. A single image reflecting only retained microbubbles was captured by creating the initial frame obtained 5 minutes after injection and subtracting averaged frames at a pulsing interval of 10 seconds obtained after destroying all microbubbles within the sector with several high–mechanical-index frames. Because the number of microbubbles retained is influenced by microbubble influx, the image representing retained microbubbles was normalized to microvascular blood flow. Data for matrigel were analyzed according to radial position as described above. Flow-normalized targeted imaging data were validated by comparison with the microbubble retention fraction derived from modeling time-intensity curves during continuous low–mechanical-index CEU from 6 additional animals undergoing iliac occlusion (see the online Data
Supplement). These studies demonstrated a good correlation between techniques in the ischemic hind-limb model.

**Histology**

Staining was performed on perfusion-fixed and paraffin-embedded sections. Hematoxylin and eosin staining was performed to evaluate inflammatory cell infiltration. For immunohistochemistry, an antigen retrieval protocol was performed, and primary antibody labeling was performed with sc1504 (VCAM-1), sc1506 (CD31), sc315 (flk-1), sc6593 (αv-integrin), and sc-6999 (CD14) (Santa Cruz Biotechnology, Santa Cruz, Calif). Neutrophils were labeled with 7/4 (ABD Serotec, Raleigh, NC). Biotinylated polyclonal secondary antibodies (Vector Laboratories, Burlingame, Calif) were used for VCAM-1 and CD31 detection, and staining was performed with a peroxidase kit (Vector Laboratories) and 3,3′-diaminobenzidine chromagen. For detection of the remaining antigens, fluorescent labeling was performed with secondary antibodies conjugated with Alexa Fluor 488 or 555 (Molecular Probes, Carlsbad, Calif). Capillary density and ratio to myocyte number in skeletal muscle were determined from CD31-stained tissue sectioned in the transaxial fiber plane. Analysis was blinded to animal identity.

**Statistical Analysis**

Unless otherwise specified, parametric data are expressed as mean±SD. Comparisons between targeted and control agent were performed with ANOVA. Follow-up comparisons were made for each animal group by use of a paired t test with Bonferroni adjustment for multiple comparisons for bubble type. A Wilcoxon signed-rank test was used for VCAM-1 in the hind limb because these data violated assumptions for the paired t test. Comparisons between MCP-1−/− and wild-type mice were made with an unequal t test; data for αv-integrin imaging were compared with an unequal variance version of the test. Blood flow data in each region of matrigel were assessed by an unpaired t test with Bonferroni adjustment for multiple comparisons for time interval. Tests for trends in hind-limb blood flow or microvascular flow components according to duration after arterial occlusion were made with Spearman’s rank correlation. Differences were considered significant at \( P<0.05 \) (2 sided).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Matrigel Neovascularization**

On histology, a dense inflammatory response was present at the interface between the matrigel surface and surrounding tissue 3 days after subcutaneous injection (Figure 1A). Linear penetrations of mononuclear cells were observed at the outer margin of the plug. These cellular penetrations were surrounded by focal clearing of matrigel material, consistent with early channel formation. At day 6, there was further penetration of the cellular projections toward the plug center (Figure 1B). The outer portions of the channels had enlarged and were partially lined with spindle-shaped cells, consistent with vascular channel maturation. At day 10, these channels were interconnected in a reticular network, and almost all were partially lined with spindle-shaped cells (Figure 1C). The centripetal growth of channels on histology correlated temporally with the inward advancement of the leading edge of perfusion on CEU (Figure 2). The peripheral or outer region of the matrigel was well perfused even at day 3. Flow to the middle region was initially low at day 3 but increased significantly by day 10. The late increase in perfusion in this segment was due primarily to an increase in microvascular blood volume (median A values of 18.7, 12.4, and 37.0 VI units [VL] on days 3, 6, and 10, respectively). Flow in this region was verified by the presence of intravenously injected nanospheres in this region on fluorescent microscopy at day 10. Flow to the central core region was minimal at all time intervals (Figure 2).

**Molecular Imaging of Matrigel Neovascularization**

According to CEU perfusion imaging data, the middle region of the matrigel provided an opportunity to temporally study de novo appearance of functional microvessels. In this region, there was robust molecular imaging signal from microbubbles targeted to αv-integrin on days 3 and 6 when flow was relatively low (Figure 3A). This signal subsequently diminished by day 10. Immunohistochemistry demonstrated αv-integrin staining predominantly on a subset of mononuclear cells infiltrating the matrigel material. Occasionally, spindle-shaped cells also stained positive for αv-integrin, although the intensity was weak and was localized primarily to the outer matrigel region. Signal enhancement for VCAM-1–targeted microbubbles in the middle region was initially low at day 3 but then increased on day 6 (Figure 3B). This signal originated primarily from the spindle-shaped cells that lined the vascular channels. Some of these cells also stained positive for flk-1 (vascular endothelial growth factor receptor-2) (Figure 3C), consistent with endothelial differentiation. Infiltrating monocytes positive for flk-1 were present but uncommon. Positive staining for CD14 was found not only on a portion of the monocytes in the matrigel but also on some spindle-shaped cells lining the vascular channels, suggesting a possible monocytic origin for some of these cells (Figure 3D). Signal enhancement from microbubbles selective for activated neutrophils was low (<3.0 VL) in the middle region at all time points. Likewise, polymononuclear cells that stained positive for anti-neutrophil monoclonal antibody 7/4 were sparse on histology. Signal for control nontargeted microbubbles was low (<2.0 U) at all time intervals, indicating that targeted microbubble signal was due to ligand-specific binding.

**Ischemic Hind-Limb Perfusion**

In mice undergoing arterial ligation, microvascular blood flow in the ischemic proximal hind-limb adductor muscle was reduced to 10% to 20% of that in the control nonischemic limb at 2 days and increased in a time-dependent fashion (Figure 4). The increase in ischemic limb perfusion was secondary to changes in both microvascular blood volume and blood velocity (Table). Immunohistochemistry for CD31 failed to detect any significant change in muscle capillary density over time (Figure 4), indicating that the increase in microvascular blood volume was due to restoration of precapillary hemodynamic conditions as described previously. However, enlargement of intramuscular arterioles and venules between days 2 and 7 was particularly prominent in transverse arterioles between major muscle fibers.

**Molecular Imaging of Hind-Limb Skeletal Muscle**

Molecular imaging with microbubbles targeted to activated neutrophils demonstrated selective signal enhancement in the
ischemic limb at day 2 that rapidly declined thereafter (Figure 5A). This signal correlated temporally with an abundance of polymorphonuclear leukocytes (positive staining with monoclonal antibody 7/4) in the perivascular and interfibrillar space at day 2 that was not present on subsequent days. Also present on day 2 were abundant \( \alpha_5 \)-integrin–positive mononuclear cells that continued to be detected on days 4 and 7 (Figure 5B). Likewise, targeted imaging signal for \( \alpha_5 \)-integrin in the ischemic limb was greatest at day 2 but remained significantly higher than signal from control microbubbles at days 4 and 7. Molecular imaging of VCAM-1 showed a similar pattern of robust signal enhancement in the ischemic limb at day 2 that persisted, albeit at a lower level, on subsequent days and correlated with endothelial expression of VCAM-1 in the intramuscular arterioles and venules on immunohistochemistry (Figure 5C).

Imaging was performed in MCP-1 \(^{-/-} \) mice 4 days after interruption of arterial inflow. This time interval was chosen on the basis of studies showing that genetic deletion of the MCP-1 receptor (CCR2) suppresses monocyte recruitment at day 4, before there is any detectable impairment in flow recovery.\(^{13} \) In the present study, there was a nonsignificant trend toward lower blood flow in the adductor muscles of the ischemic hind limb at day 4 in MCP-1 \(^{-/-} \) compared with wild-type mice (Figure 6). Signal enhancement from \( \alpha_5 \)-integrin–targeted microbubbles in the ischemic limb was severely attenuated in MCP-1 \(^{-/-} \) mice, whereas signal enhancement with neutrophil and VCAM-1–targeted microbubbles was only mildly reduced (Figure 6).

**Discussion**

In this study, we have demonstrated in models of vasculogenesis and ischemia-induced arteriogenesis that molecular
imaging can detect inflammatory processes that coincide with the appearance of functional microvessels, differentiate the cellular components of the inflammatory response to ischemia, and detect expression of specific adhesion molecules on monocytes and endothelial cells that participate in vascular development and/or remodeling. Data from MCP-1−/− mice also indicate that alterations of the inflammatory response that are associated with impaired vascular remodeling can be detected by molecular imaging.

Inflammation plays a complex and varied role in tissue response to peripheral arterial insufficiency. With severe acute ischemia-reperfusion injury, inflammation contributes to microvascular insufficiency and exacerbates tissue injury. On the other hand, the inflammatory response is a critical component for vascular remodeling that occurs in response to chronic limb ischemia and wound healing. Gene profiles of ischemic tissues have demonstrated upregulation of proinflammatory cytokines and their receptors within hours after interruption of arterial inflow. This response promotes neutrophil accumulation that peaks 24 to 48 hours after ischemic onset and resolves almost entirely within a few days. In the present study, this early neutrophil response was detected with a phosphatidylserine-containing contrast agent that exhibits complement-dependent attachment preferentially to activated neutrophils. Histology confirmed perivascular neutrophil infiltrations at the time of strong enhancement on targeted CEU. Although histology and targeted imaging correlated temporally, they do not necessarily provide the same information. Targeted microbubbles attach only to cells during active recruitment, which does not necessarily reflect total tissue accumulation at any given time. Targeted imaging in matrigel indicated that neutrophils probably play a minor role in de novo vasculogenesis in the adult animal. The neutrophilic response was prominent only at the interface between matrigel and surrounding connective tissue and was most consistent with a foreign body response. Otherwise, the neutrophil signal was weak at the leading edge of perfusion advancement.

Recruitment of monocytes in ischemic tissue has been shown to be a critical factor in vascular remodeling. In models of adult vasculogenesis such as matrigel models, monocytes participate in matrix remodeling and tube formation, provide a source for proangiogenic peptides, and can assume an endothelial phenotype when stimulated by growth factors. To detect monocytes with molecular imaging, we chose to target the α5-integrin subunit. Fibronectin engagement of α5β1 has been shown to induce angiogenic activity of monocytes through a CXR chemokine–dependent pathway. This same receptor-ligand pair also may synergistically enhance tyrosine kinase receptor signaling. By targeting the α5-integrin, we detected the presence of monocytes in matrigel, particularly at the leading edge of perfusion. These cells appeared to be responsible for channel formation. A subset of these cells also may have undergone transformation to an endothelial phenotype, suggested by the expression of CD14 and flk-1 in some of the monocytes and spindle-shaped cells lining the channels.

In animal models, monocyte recruitment in ischemic tissue peaks several days after interruption of limb blood flow. In mice, MCP-1 and its major receptor, CCR2, are upregulated within 24 hours of onset of limb ischemia. Manipulation...
The administration of MCP-1 and CCR2 has produced solid evidence of its functional role and the role of monocytes/macrophages in ischemia-mediated vascular remodeling. Administration of exogenous MCP-1 augments monocyte recruitment and accelerates arteriogenesis and flow recovery in rabbits undergoing femoral artery occlusion. Genetically modified mice deficient for either MCP-1 or CCR2 have an impaired monocyte response and less recovery of perfusion after interruption of arterial inflow. In this study, the molecular signal for monocyte α5-integrin at 4 days was markedly reduced in MCP-1−/− mice compared with wild-type mice. The relatively mild differences in blood flow in the 2 groups was likely due to the early time interval (4 days). Greater differences in flow would be expected at 7 to 21 days.

Interactions between VCAM-1 on the endothelium and integrins such as VLA-4 (α4β1) contribute to slow rolling and firm adhesion of monocytes and lymphocytes in the microcirculation. Targeted CEU in ischemic limbs detected VCAM-1 expression on the microvascular endothelium in a temporal pattern that paralleled monocyte α5-integrin signal. VCAM-1 expression by CEU preceded the period of maximal flow recovery. In the matrigel model of vasculogenesis, peak VCAM-1 signal on CEU again preceded the major change in blood flow but occurred later than the monocyte signal. In

Figure 3. Targeted CEU imaging data and examples of immunohistochemistry from middle matrigel region. Mean (±SEM) signal enhancement during molecular imaging for α5-integrin (A) and VCAM-1 (B) (bars) is displayed with perfusion data superimposed (triangles). For both, examples of targeted signal enhancement on CEU at day 6 are shown (color scale at bottom). Immunohistology with diaminobenzidine chromagen (brown) demonstrated α5-integrin present on infiltrating mononuclear cells. VCAM-1 signal originated primarily from the spindle-shaped cells lining the matrigel channels. Immunohistology for flk-1 (C; green) and CD14 (D; red) at day 6 demonstrated positive staining for spindle-shaped cells during channel formation and subsets of mononuclear cells. Scale bar=25 μm. *P<0.05 vs other time points for targeted imaging data.
this model, monocyte-mediated channel formation preceded the appearance of the spindle-shaped cells that stained positively for VCAM-1. Expression of VCAM-1 in this model may have reflected nonspecific endothelial activation in developing vessels, although there is evidence for a functional role of VCAM-1 for counteracting endothelial apoptosis or homing of VLA-4+ progenitor cells.7,8

The application of CEU for molecular imaging of inflammation during angiogenesis raises several important issues related to technique. Microbubbles are pure intravascular agents that, even in angiogenic microvessels, do not leave the vascular compartment.21 Hence, they are ideal for detecting the molecular signature only from endothelial cells or leukocytes rather than other nonvascular tissues that are accessible to diffusible contrast agents. On the other hand, microbubbles may not be suitable for other purposes for which targeting of nonvascular structures is required. It also is worth noting that our ability to perform multitarget imaging sessions was possible because of the rapid clearance of freely circulating microbubbles from the blood pool, which reduces study time for each agent, and the ability to destroy microbubbles, which allows subsequent injection of another targeted tracer.

The present study has several important limitations. The presence of a minimal amount of existing perfusion was necessary for microbubbles to reach their intended target. Hence, it may not be possible to detect the very earliest changes during vasculogenesis. The issue of how changes in perfusion can influence targeted tracer delivery has largely been neglected in angiogenesis imaging studies. We used a protocol that controlled for the amount of perfusion because the overall signal enhancement from targeted microbubbles reflects not only the retention fraction of agent but also the amount of tracer entering the tissue (determined by relative perfusion). For flow normalization, we used perfusion data from the total region of interest to avoid artificially high voxel values that would be generated in no-flow regions.

Validation of this method was hindered by a lack of adequate methods to quantify intraluminal expression of the target molecules. We demonstrated that flow-corrected molecular imaging data correlated well with microbubble retention fraction values that were calculated by modeling time-intensity curves during low-power CEU as previously described.32 Another limitation is that we were not able to correct for the regional or temporal differences in vascular

<table>
<thead>
<tr>
<th>Day 2 (n=5)</th>
<th>Day 4 (n=5)</th>
<th>Day 7 (n=5)</th>
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<td>Microvascular rate constant (β), s⁻¹</td>
<td>0.13±0.05</td>
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<tr>
<td>Microvascular volume (A value)</td>
<td>12±13</td>
<td>16±12</td>
<td>21±12</td>
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*P<0.05 for trend of increasing values over time for both parameters (Spearman’s ρ=0.46 and 0.55 for β and A value, respectively).
shear forces that can influence microbubble attachment. However, we are reassured by the lack of attachment of microbubbles bearing control nonsense ligands even at very low shear rates in this study and in previous studies. Finally, it should be noted that cellular identification was based on appearance and molecular expression profile. Controversy persists with regard to identifying the cellular origin and defining cells according to markers that change during differentiation and that may be common to monocytes, endothelial cells, pericytes, and circulating precursor cells.

Figure 5. Molecular imaging data for microbubbles targeted to activated neutrophils (A), α5-integrin (B), and VCAM-1 (C). For each molecular target, mean (±SEM) signal enhancement in the ischemic limb is shown for targeted and control microbubbles. Data are normalized to muscle blood flow. Corresponding examples of molecular imaging with targeted microbubbles at day 2 and immunohistochemistry for neutrophils (green), α5-integrin (red), and VCAM-1 (brown) at days 2 to 7 are shown to the right of each graph. *P<0.05 vs control microbubble signal.
Conclusions

We have demonstrated that different components of the inflammatory response that participate in vascular remodeling can be assessed separately with targeted molecular imaging. These studies represent an important step toward evaluating impaired vascular adaptive responses in disease states in which the cellular inflammatory response is altered and for evaluating proangiogenic strategies that rely in part on immune regulation.

Acknowledgments

We are grateful to Benjamin Kron for histological analysis and to Alexander Klibanov, PhD, for technical assistance. We also wish to thank Barrett J. Rollins, MD, PhD, for the opportunity to study MCP-1−/− mice.

Sources of Funding

This work was supported by grants R01-HL-074443, R01-HL-078610, and R01-DK-063508 to Dr Lindner from the National Institutes of Health, Bethesda, Md. Drs Behm and Carr were supported by fellowship grants from regional affiliates of the American Heart Association. Dr Kaufmann was supported by research grants from the Lichtenstein Foundation.

Disclosures

None.

References


Multiple protective mechanisms guard against severe ischemic injury caused by coronary and peripheral arterial disease. Structural remodeling of the microcirculation occurs when disease becomes so severe that autoregulation is exhausted and ischemia is produced by minimal exertion or at rest. Proliferation and enlargement of vessels at the arteriolar level lower degrees of ischemic burden. Understanding the key regulatory processes is critical for developing methods to modulate arteriogenesis, which is thought to be an important contributor to arteriogenesis and angiogenesis. The aim of this study was to determine whether cytokines can be best used as a therapeutic approach.

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CLINICAL PERSPECTIVE

Multiple protective mechanisms guard against severe ischemic injury caused by coronary and peripheral arterial disease. Structural remodeling of the microcirculation occurs when disease becomes so severe that autoregulation is exhausted and ischemia is produced by minimal exertion or at rest. Proliferation and enlargement of vessels at the arteriolar level lower total vascular resistance and promote flow distribution through microvascular collateral circuits. However, the ability to mount an arteriogenic response varies considerably between tissues, species, and even individual patients with similar degrees of ischemic burden. Understanding the key regulatory processes is critical for developing methods to modulate vascular remodeling as palliative therapy in patients with no other treatment options. Ischemia-mediated inflammation is thought to be an important contributor to arteriogenesis and angiogenesis. The aim of this study was to determine whether it is possible to use noninvasive ultrasound to evaluate key aspects of the inflammatory response involved in vascular remodeling. The results indicate that endothelial cell activation and monocyte recruitment, which herald vasculogenesis and arteriogenesis, can be quantified by molecular imaging. This approach shows great promise for studying the contributing role of different inflammatory cells in vascular remodeling and how manipulation of the proinflammatory cytokines can be best used as a therapeutic approach.
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Circulation. 2008;117:2902-2911; originally published online May 27, 2008;
doi: 10.1161/CIRCULATIONAHA.107.744037

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