Alterations of Arterial Physiology in Osteopontin-Null Mice

Daniel L. Myers, Kelley J. Harmon, Volkhard Lindner, Lucy Liaw

Objective—In this study, we characterized the effects of an osteopontin (OPN)-null mutation in normal arterial function and remodeling in a murine model.

Methods and Results—OPN-null mutant mice were compared with wild-type mice before and after carotid artery ligation. Before ligation, OPN-null mice had increased heart rate, lower blood pressure, and increased circulating lymphocytes compared with wild-type mice. OPN-null vessels also demonstrated greater compliance accompanied by a loosely organized collagen network. After carotid artery ligation, significant differences were also found in the remodeling response of OPN-null animals. At 4 days after ligation, leukocyte adhesion/invasion was diminished by 10-fold in OPN-null mice compared with wild-type mice. At 14 days after ligation, the ligated arteries of OPN-null mice had smaller neointimal lesions but greater constrictive remodeling compared with wild-type mice, resulting in similar lumen areas. Continued remodeling resulted in a similar morphological phenotype in both groups at 28 days.

Conclusions—These data show that endogenous OPN regulates normal vascular physiology and contributes to the vascular remodeling response by regulating vascular compliance and the inflammatory response. (Arterioscler Thromb Vasc Biol. 2003;23:1021-1028.)

Key Words: osteopontin ♦ cardiovasculature ♦ remodeling ♦ carotid artery ♦ flow cessation

Both genetic and physiological factors have been shown to affect vascular physiology and pathophysiology. Platelets, leukocytes, endothelial cells, and smooth muscle cells interact in a complex system regulated by plasma protein systems, cytokines, and physical forces such as pressure and shear stress. We previously found that inhibiting osteopontin (OPN) function after endothelial denudation led to a significant decrease in the extent of neointimal formation in rats. In this study, the arterial effects resulting from the removal of OPN (product of the spp1 gene) were examined.

OPN is a secreted integrin-binding protein that is present in small amounts in uninjured arteries but is abundantly expressed by smooth muscle cells, endothelial cells, and activated inflammatory cells in injured arteries. Abundant OPN expression has also been observed in human atherosclerotic lesions and thoracic aneurysms. The functional significance of this increased vascular expression was suggested by work demonstrating OPN activity as a chemotactic substrate for endothelial cells and vascular smooth muscle cells. Inhibitor of vascular smooth muscle calcification and a chemotactic/activator of inflammatory cells. In vivo studies confirmed these important functions of OPN during tissue remodeling and repair. Chiba et al. observed that hematopoietic cell overexpression of OPN resulted in increased atherosclerotic lesion formation. In OPN-null mice, recent data have indicated that the nonredundant functions of OPN in vivo involve widespread participation in tissue remodeling, inflammation, tumor progression, and angiogenesis. Defective tissue remodeling on an OPN-null background has been observed in tissues including the myocardium, bone, skin, kidney, lymph nodes, joints, and malignant tissues. Two phenotypes that characterize many of these studies are a change in the immune response and altered matrix remodeling. Therefore, the widespread participation of OPN in tissue remodeling is likely due to its unique functions in basic repair processes. In addition, the role of OPN as a physiological inhibitor of vascular calcification was demonstrated in studies showing that spontaneous vascular calcification in the matrix of Gla protein-null mice was significantly increased on an OPN-null background. Recently, constitutive overexpression of OPN in mice was observed to result in increased neointima formation after capping of the femoral artery. Interestingly, this overexpression of OPN in uninjured mice did not result in accumulation of leukocytes in the vessel wall, indicating that control of vascular inflammation is complex.

Our present study used a genetic OPN-null murine model to test the hypothesis that OPN regulates vascular remodeling in vivo. We had anticipated that smooth muscle cell contribution to neointimal lesion formation would be deficient on the OPN-null background. Our studies provide the first demonstration that endogenous OPN contributes to normal blood vessel structure and function and that OPN is indeed a
regulator of both constrictive remodeling and neointimal lesion formation. We provide evidence to support a model in which OPN might be mediating these effects through regulation of vascular compliance and/or the inflammatory response.

**Methods**

**Animals**

All animal protocols were approved by the Institutional Animal Care and Use Committee. The OPN-null mutant allele has been described and mice used in this study were 16- to 24-week-old males on a 129×Black Swiss hybrid background. Heterozygous breeding pairs were used to generate wild-type and OPN-null groups.

**Blood Count, Hemodynamics, and Vascular Compliance**

Blood (25 μL) was obtained by retro-orbital collection (wild type, n=10; OPN-null, n=10), diluted to 200 μL, and then analyzed by flow cytometry (Cell-Dyn 4000). Blood pressure measurements were made with a tail cuff (Visitech BP200; wild type, n=29; OPN-null, n=17). After a 1-week conditioning period, 30 measurements were made in a single session for 5 days. The initial 10 measurements were discarded, and the last 20 were averaged to give a single daily blood pressure measurement for each mouse. Blood flow was measured with a flow probe (Transonic) with a T160 computer interface (wild type, n=18; OPN-null, n=17). Animals were anesthetized (2 mL of a 2.5% solution of Avertin per 100 g IP), and the common carotid artery was exposed and placed within the flow probe (Transonic; 0.5 VB). Flow rate and heart rate were recorded simultaneously. Determination of small-vessel and murine carotid artery compliance have been previously described. Vascular compliance was measured with a standard micromanipulator and microinjector (Nashige, IM 300). The left carotid artery was isolated, suspended in lactated Ringer’s solution containing sodium nitroprusside (10 μmol/L), sutured closed at the distal end, and sealed proximally with a suture onto a microneedle. The carotid artery was then inflated to 50, 100, 150, 200, and 250 mm Hg. Vessel diameter was measured from digitized images at each pressure (wild type, n=4; OPN-null, n=4). Relative diameters were calculated as fold increases from the relaxed state. Extensibility was calculated as described by Li et al. For example, at 125 mm Hg; 

\[ \text{extensibility} = \frac{\text{diameter at 150 mm Hg} - \text{diameter at 100 mm Hg}}{\text{diameter at 100 mm Hg}} \times 100 \]

A similar experiment was performed on a segment of the descending aorta between the renal arteries and iliac bifurcation. The isolated segment was sutured closed at the proximal end and sealed distally with a suture onto a blunted 30-gauge needle. The aorta was then uniformly pressurized at a rate of 3 mL/min with a valve (Intraflow), and pressure was measured with a standard arterial-line pressure transducer. Vessel diameter was measured from digitized images at each pressure.

**Arterial Ligation Model**

Ligation of the left carotid arteries of mice (wild type, n=14 at 28 days, n=8 at 14 days, n=8 at 4 days, n=8 unmanipulated, and n=6 sham-manipulated; OPN-null, n=14 at 28 days, n=12 at 14 days, n=8 at 4 days, n=8 unmanipulated, and n=6 sham-manipulated) proximal to the internal/external carotid artery bifurcation was performed as previously described, with sham-operated mice used as controls. Bromodeoxyuridine (BrdU) injections (100 μL of 25 mg/mL BrdU solution) were given to each animal 15 hours and 1 hour before harvest. At the indicated times after ligation, tissues were perfusion-fixed with 4% paraformaldehyde in 0.1 mol/L sodium phosphate buffer, pH 7.3. The perfusate was allowed to drain from an incision in the left carotid artery just proximal to the ligation. Vessels were harvested and embedded in paraffin. At least 800 consecutive serial sections were cut from both arteries of each mouse (7 μm thick). For the right carotid artery, all analyzed sections were cut from the vessel distal to the brachiocephalic artery.

**Western Blot Analysis**

Single, ligated left carotid arteries were harvested and homogenized in lysis buffer (50 mmol/L Tris, pH 8.0; 0.1% sodium dodecyl sulfate; and 2.5 mmol/L NaCl). Total protein (100 μg/sample) was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5% gel) followed by transfer to a polyvinylidene difluoride membrane and immunoblotted with an anti-OPN antibody (2 k1, kindly provided by Dr T. Uede, Hokkaido University, Sapporo, Japan). Recombinant OPN was used as a positive control, and the relative amount of OPN per sample was quantified using IMAGEQUANT software and normalization to smooth muscle α-actin (clone 1A4, Sigma) levels. Triplicate analyses were performed.

**Morphometric Analysis**

Morphometric analysis was carried out on ligated left common carotid arteries from both wild-type and OPN-null mice, which were harvested at 14 and 28 days after ligation, as well as from unmanipulated and sham-manipulated controls. The contralateral right common carotid artery was analyzed from harvests 28 days after ligation, as well as unmanipulated and sham-manipulated arteries. Eight sections were collected at 280-μm intervals from the proximal 2.25 mm closest to the ligature and stained with orcein for analysis (NIH IMAGE software, version 1.60). The lumen surface, the perimeter of the neointima, and the perimeter of the tunica media were traced, yielding circumference and area of the lumen, internal elastic lamina (IEL), and external elastic lamina (EEL), respectively. Very small folds were not included in the IEL and EEL measurements. The medial area was calculated by subtracting the area defined by the IEL from the area defined by the EEL. Intimal area was calculated as the area between the lumen surface and IEL. Because the shape of the cross sections is often distorted because of sectioning and the neointima might be unevenly distributed, lumen area could not be measured directly. With the assumption that the EEL was circular, lumen area was calculated by subtracting medial area and intimal area from the total area calculated from the circumference of the EEL. No difference in vessel areas was observed between sham-manipulated and unmanipulated animals. Sections adjacent to the orcein-stained sections were stained with hematoxylin, and the numbers of nuclei in the media and intima for ligated, contralateral, and control arteries were counted by using software developed specifically for this project by Sentient Machines, which was validated to be consistent with manual counts. Lumen area of aortas of anesthetized, unmanipulated mice was also measured in situ by magnetic resonance angiography (Siemens Magnetom) with blood flow for contrast (wild type, n=2; OPN-null, n=2), and total artery size was quantified by direct measurement after surgical exposure of the carotid artery (wild type, n=2; OPN-null, n=2).

**Immunostaining**

A total of 8 sections, taken every 280 μm apart from each mouse harvested at 2 weeks after ligation, were analyzed for cell proliferation with an anti-BrdU antibody (ICN Biomedicals) at 1:2000 dilution, followed by a biotinylated secondary antibody at 1:500 dilution. Similarly, a total of 10 sections, taken every 280 μm apart from each mouse harvested at 4 days after ligation, were analyzed for leukocyte infiltration with a biotinylated anti-CD45 antibody (ICN Biomedicals) at 1:200 dilution. Sections were then treated with ABC reagent (Vector Labs), followed by incubation with 3,3′-diaminobenzidine (Sigma). Digitized images of CD45-stained vessels were analyzed (NIH IMAGE 1.60), and the staining was quantified by first thresholding all nonstained areas from the image and then measuring the remaining positively stained area. Sections directly adjacent to those analyzed for leukocyte infiltration were analyzed for apoptosis with a TUNEL assay kit (Clontech, Apo-alert DNA fragmentation assay). Collagen was visualized with Masson’s trichrome stain.
Vascular Characteristics of OPN-Null Mice

OPN-null mice are overtly similar to wild-type mice with respect to size, viability, and physical activity. Low levels of OPN expression in unmanipulated arteries have been previously described. Because the significance of this expression in unmanipulated arteries has been previously described,

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OPN-null carotid arteries demonstrate greater compliance than wild type at physiological pressures. Relative increases were calculated by normalization to relaxed size. B, Carotid arteries of OPN-null mice demonstrate greater extensibility than wild type at physiological pressures. Asterisks indicate differences between wild-type and OPN-null groups at P<0.05.

Figure 1. Loss of osteopontin (OPN) results in increased arterial wall compliance. Left carotid arteries were isolated from wild-type and OPN-null mice and then sutured such that intravascular pressure could be regulated. Diagneters were then measured from digitized images at indicated pressures. A, OPN-null carotid arteries demonstrate greater compliance than wild type at physiological pressures. Relative increases were calculated by normalization to relaxed size. B, Carotid arteries of OPN-null mice demonstrate greater extensibility than wild type at physiological pressures. Asterisks indicate differences between wild-type and OPN-null groups at P<0.05.

Results

Morphometric data were structured as a 2-factor (OPN status, harvest time) randomized design and then analyzed by Fisher’s least significant difference procedure. Two-factor ANOVA was performed on medial, intimal, lumen, and total area measurements. Each showed OPN to have a significant effect. The effect of OPN was then further clarified by Student’s t test. Remaining comparisons involving a single variable (OPN genotype) at a single time point were carried out with Student’s t test. For the blood flow studies, measurements were considered outliers when they were >3 SDs away from the mean and were excluded from analysis of blood flow rate. Most of the outliers were found to have heart rates <250 beats/min, which is consistent with the observations of Hart et al53 that heart rates below a certain point dramatically affect stroke volume. Thus, we considered flow measurements in mice with heart rates <250 beats/min to be invalid, because flow rate was correspondingly decreased. All data are reported as mean±SD.

Blood pressure and heart rate measurements were obtained by the tail-cuff method. Unexpectedly, we observed significantly lower blood pressures and increased heart rates in OPN-null mice. The systolic blood pressure in wild-type mice was 146±12.2 mm Hg compared with 130±9.8 mm Hg in the OPN-null mutants (P<0.001). Conversely, heart rate in the wild-type group was 57 beats/min compared with 490±57 beats/min in OPN-null mice (P<0.001). Blood pressure and heart rate measurements were obtained by the tail-cuff method. Unexpectedly, we observed significantly lower blood pressures and increased heart rates in OPN-null mice. The systolic blood pressure in wild-type mice was 146±12.2 mm Hg compared with 130±9.8 mm Hg in the OPN-null mutants (P<0.001). Conversely, heart rate in the wild-type group was 57 beats/min compared with 490±57 beats/min in OPN-null mice (P<0.001).

Conversely, heart rate in the wild-type group was 57 beats/min compared with 490±57 beats/min in the OPN-null group (P<0.001). Because increased heart rate often results from anemia, we suspected that characteristics of the arterial wall were related to the rate of blood flow and heart rate, and thus, we measured carotid artery wall compliance as a function of intravascular pressure. Left carotid arteries were isolated and then inflated to specific pressures. Vessels were digitally recorded at pressures from 50 mm Hg to 250 mm Hg. In OPN-null mice, we observed a very rapid increase in vessel wall diameter with increasing pressure, whereas carotid arteries from wild-type mice demonstrated similar increases in size only at significantly higher pressures (Figure 1). The percentage increases in vessel size at all pressures measured were greater in vessels from OPN-null mice compared with wild-type mice, and the responses of each carotid artery sample of a particular genotype were remarkably consistent. To exclude the possibility that increased compliance in OPN-null mice was unique to the carotid artery, a similar experiment was...
performed on segments of abdominal aorta. The aorta demonstrated a similar increase in compliance in OPN-null animals compared with the wild type. Aortic compliance data are available as an online supplement (please see http://atvb.ahajournals.org). Interestingly, the compliance data predict OPN-null arteries to be larger than those of the wild type at physiological pressures, which was not observed in situ. We interpret this apparent inconsistency as supportive evidence of increased vascular tone in OPN-null mice as a compensatory mechanism for increased compliance.

We examined vessels histologically in an effort to determine the origin of the difference in compliance. Arterial cross sections were stained with Masson’s trichrome and orcein to visualize collagen and elastin, respectively (Figure 2). No difference in the elastic fibers was noted between groups (data not shown), but the collagenous matrix was organized much more loosely in the OPN-null mice, particularly in the adventitial region.

Expression of OPN After Blood Flow Cessation in Carotid Arteries

To quantify the temporal induction of OPN protein after carotid artery ligation, vessels from OPN-null or wild-type mice were collected at 4 and 14 days after ligation. Sham-manipulated vessels were collected as control samples. Single, ligated left carotid arteries from each group were homogenized and then analyzed by Western blotting for the presence of OPN. OPN protein in each vessel was quantified by densitometric comparison and normalized to smooth muscle α-actin levels. In vessels from wild-type mice, low levels of OPN were detected in unmanipulated carotid arteries (relative amount, 1.0±0.44). OPN protein was increased at 4 days after ligation (relative amount, 1.8±0.68) and was abundant at 2 weeks after ligation (relative amount, 8.5±3.5). No OPN was detected in any arterial samples from OPN-null mice (data not shown).

Vascular Remodeling Response Is Regulated by OPN

We used the carotid artery ligation model to test the hypothesis that OPN regulates vascular remodeling responses. Arteries in groups of wild-type and OPN-null mice were ligated, with sham manipulation and unmanipulated mice as controls. Carotid arteries were then harvested at 14 and 28 days after ligation, as well as from unmanipulated mice. Eight sections from the proximal 2.25 mm closest to the ligature were analyzed from each mouse. No difference in vessel areas was found between unmanipulated and sham-manipulated arteries (data not shown). Consistent with their increased compliance on isolation from the body, the unmanipulated carotid arteries in OPN-null mice were significantly smaller than those in wild-type counterparts (Table 1). After ligation, the ligated left carotid artery in all mice showed negative remodeling (decrease in total area) while the contralateral right artery underwent positive remodeling (increase in total area) after left carotid artery ligation.

The ligated left carotid artery of OPN-null mice showed significantly less neointima formation and greater contractive remodeling (as evidenced by decreased total area) at 14

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**TABLE 1. Morphometric Analysis of the Ligated Left Carotid Artery**

|               | Control 14 Days | Control 28 Days | Wild-type | 0.016±0.002 | 0.023±0.005 | 0.027±0.006 | NA | 0.02±0.024 | 0.017±0.033 |
|---------------|-----------------|-----------------|-----------|-------------|-------------|-------------|    |            |             |
| Medial Area   |                 |                 | OPN null  | 0.016±0.002 | 0.021±0.007 | 0.023±0.005 | NA | 0.005±0.003 | 0.015±0.022 |
|               |                 |                 | *P* (WT/null) | 0.375 | **0.040** | <0.001 | 0.003 | 0.659 |
| Lumen Area    |                 |                 | Wild-type | 0.064±0.008 | 0.022±0.018 | 0.019±0.001 | 0.081±0.009 | 0.065±0.032 | 0.061±0.025 |
|               |                 |                 | OPN null  | 0.057±0.008 | 0.019±0.013 | 0.024±0.019 | 0.074±0.009 | 0.05±0.025 | 0.061±0.032 |
|               |                 |                 | *P* (WT/null) | **0.002** | 0.420 | 0.087 | 0.005 | 0.024 | 0.785 |

Values presented are average areas (mm²)±SD. NA indicates values too small to be precisely measured.

Medial, intimal, lumen, and total areas from left carotid arteries harvested 14 and 28 days after left carotid artery ligation and from left carotid arteries harvested from unmanipulated mice are presented (control). Data from sham-manipulated mice did not significantly differ from unmanipulated at any time point. Two-way ANOVA (OPN/harvest time) indicated OPN to have a significant effect that was then clarified with the presented Student *t* tests. Bold values indicate statistical significance. At 14 days after ligation, OPN null mice demonstrated greater contractive remodeling (as indicated by a decrease in total area) and less neointima formation than wild-type mice. At 28 days after ligation, with the exception of medial area, the remodeled arteries of OPN null mice did not differ from wild-type.
days after ligation compared with that of wild-type mice (Table 1). Interestingly, the negative remodeling occurred such that the lumen area was no longer different between groups. At 28 days after ligation, additional remodeling resulted in similar intimal, lumen, and total areas between wild-type and OPN-null mice. At this time, only medial area demonstrated a significant difference, and it was smaller in the OPN-null than in wild-type vessels. Thus, although the carotid arteries of OPN-null mice displayed different remodeling responses, 28-day remodeled arteries were morphologically similar in both groups.

The right common carotid artery of all mice showed compensatory increases in lumen area (P<0.001) and total area (P<0.001), with no significant changes in medial or intimal areas compared with unmanipulated vessels (Table 2). Consistent with having increased compliance, the actual change in lumen area was greater in OPN-null mice.

**Rich Inflammatory Infiltrate After Carotid Artery Ligation**

Because OPN has been shown to control the influx of inflammatory cells in vivo, we examined whether early inflammation was quantitatively different between OPN-null and wild-type animals. Using an antibody against the panleukocytic marker CD45, we performed immunostaining on sections of carotid arteries from both groups collected 4 days after ligation. In sections from wild-type animals, CD45-positive leukocytes were abundant in the region with the largest neointima but rare in the remainder of the vessel (Figure 3). Analysis of OPN-null animals revealed few leukocytes in any region along the entire length of the carotid artery. Quantification of the area of positive immunostaining of wild-type mice showed a 10-fold increase over that of OPN-null mice. Additionally, we sought to determine whether the observed difference in neointima formation at 14 days between wild-type and OPN-null mice could be explained by altered cell proliferation or apoptosis. At 14 days after ligation, no significant difference in cell number (hematoxylin staining), cell proliferation (BrdU incorporation), or apoptosis (TUNEL assay) was observed (data not shown).

**Discussion**

Both gene products and physical forces have been shown to affect vascular physiology and pathophysiology. In this study, the loss of OPN was shown to alter hemodynamics and arterial mechanics. Because normal, wild-type mice express very little OPN in blood vessels, our finding of significant changes in normal blood vessel physiology on the OPN-null background was unanticipated. Compared with wild type, we observed unmanipulated OPN-null mice to have more circulating lymphocytes, lower systolic blood pressure, increased heart rate, greater arterial compliance, and reduced blood flow at similar heart rates. These observations are consistent with previous work that has observed increased systolic pressure associated with stiffened vessels. Although increased compliance can increase the lumen area of resistance vessels, thus reducing peripheral resistance/blood pres-

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**Table 2. Morphometric Analysis of the Contralateral Right Carotid Artery in Response to Ligation of the Left Carotid Artery**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>28 Days</th>
<th>P (Control/28 Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medial Area</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.018±0.004</td>
<td>0.019–0.005</td>
<td>0.931</td>
</tr>
<tr>
<td>OPN null</td>
<td>0.017–0.004</td>
<td>0.017–0.004</td>
<td>0.673</td>
</tr>
<tr>
<td>P (WT/null)</td>
<td>0.271</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td><strong>Intimal Area</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPN null</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (WT/null)</td>
<td>0.001</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td><strong>Lumen Area</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.074±0.019</td>
<td>0.106±0.018</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OPN null</td>
<td>0.051±0.012</td>
<td>0.098±0.018</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P (WT/null)</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td><strong>Total Area</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>OPN null</td>
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<tr>
<td>P (WT/null)</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

Values presented are average areas (mm²)±SD. NA indicates values that were too small to be precisely measured.

**Figure 3.** Leukocyte infiltration is deficient on an osteopontin (OPN)-null background. Representative sections of wild-type and OPN-null vessels harvested 4 days after ligation in regions close to the ligation are shown. Sections were stained with anti-CD45 panleukocyte antibody. Note absence of leukocytes in the OPN-null section. Arrows indicate regions of leukocyte influx. Bar=500 µm.
Figure 4. Model of altered hemodynamics in OPN-null mice. Structural changes noted on the OPN-null background include disorganized collagen and increased vessel wall compliance. OPN-null mice compensate for increased vascular compliance with increased vascular tone and reduced stroke volume with increased heart rate.

Our data support the following model of OPN activity in the normal vessel wall (Figure 4). Because OPN-null mice are similar to the wild type in size, physical activity, and oxygen-carrying capacity, blood flow is similar in both groups. Because of the lack of OPN, vessel wall compliance is greater, resulting in decreased systolic pressure. Although the OPN-null mice attempt to compensate for increased compliance with increased smooth muscle cell tone, this compensation is incomplete, as evidenced by lower blood pressure (arterial pressure = \[\text{mean arterial pressure} \times \text{[compliance/time constant]}\]), we observed increased compliance in the presence of decreased cardiac output, suggesting that the lowered systolic pressure observed in the OPN-null mice of this study might reflect lowered mean arterial pressure. Interestingly, the differences in arterial diameters predicted by our compliance data were not observed in an in situ comparison of OPN-null mice with wild-type mice. One possible interpretation of this inconsistency is supportive evidence of a compensatory increase in vascular tone in OPN-null mice.

As we had hypothesized, we found that the remodeling response of OPN-null vessels to cessation of blood flow was distinct from that in wild-type vessels. The response to carotid artery ligation has been shown to be mediated by factors affecting inflammation, vascular tone, plasma proteins, cytoskeletal components, and hormones/growth factors. After ligation, OPN-null mice demonstrated greater initial constrictive remodeling and less neointima formation compared with wild type. The observation of decreased neointima formation in the response of OPN-null mice to carotid artery ligation is consistent with our previous observation of decreased neointima formation after endothelial denudation in rats treated with anti-OPN antibody.

Because it was possible that the increased constrictive remodeling reflected an endothelium-dependent response, we reexamined our earlier data of anti-OPN antibody infusion after balloon catheter denudation. Similarly, we found significantly increased constrictive remodeling in the anti-OPN-treated group compared with wild type (authors' unpublished data). This suggests that blocking OPN function might be a means to increase vascular compliance. Another consistent finding between the endothelial denudation model and the carotid artery ligation model was that we could not detect changes in cell proliferation, apoptosis, or cell density.

OPN-null mice, despite having more circulating lymphocytes, did not demonstrate the early leukocyte infiltration that characterized the response of wild-type mice. OPN has been verified as an important mediator of immune cell migration and activity, and leukocyte invasion has been observed to be an important determinant of neointima formation. For example, decreased leukocyte infiltration was associated with reduced neointimal size in P-selectin–null mice and endothelial nitric oxide synthase–transgenic mice. Conversely, endothelial nitric oxide synthase–deficient mice demonstrated greater neointima formation in the presence of increased leukocyte infiltration, and fas ligand–deficient mice showed increased neointima formation preceded by increased leukocyte infiltration. Although our study supports a positive association between leukocyte infiltration and neointimal lesion size, we also observed decreased neointimal lesion size to be associated with increased constrictive remodeling in OPN-null mice. Interestingly, this altered remodeling occurred in such a way to maintain the remodeled lumen size. Although we hypothesized that loss of an immune cell modulator and chemottrant would directly alter the vascular injury response of OPN-null mice, the altered response might be secondary to the unexpected difference in hemodynamics/vessel mechanics. This alternative explanation is supported by several studies that have observed an association of inflammation with increased blood pressure.
In summary, the regulation of vascular function is multifactorial. This study demonstrated OPN to have a role in determining the hemodynamics/vessel mechanics of normal arteries as well as remodeled vascular phenotypes. Our data, combined with that of others, suggest alteration of collagen structure might be the mechanism by which compliance is changed in OPN-null mice. We are currently seeking to distinguish which of the observed effects are primary and secondary due to loss of OPN.

Acknowledgments
This work was supported by a grant from the American Heart Association to L.L. (0250150N). The authors wish to thank Dr Bill Cotter (Sentient Machines, Wells, Me) for development of software for data analysis; Jane Mitchell, MT (ASCP), (Maine Medical Center Research Institute) for her help with blood cell analysis; Sheila Smith, RT (Maine Medical Center, Portland, Me) for her help with magnetic resonance imaging; and Elizabeth Hoering for her help in data collection. We also thank Dr W. Robert Taylor and Dr Abdolreza Agaiehtra (Emory University, Atlanta, Ga) for their assistance in the interpretation of the arterial compliance data.

References


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Arterioscler Thromb Vasc Biol. 2003;23:1021-1028; originally published online April 24, 2003; doi: 10.1161/01.ATV.0000073312.34450.16
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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Table I. The loss of osteopontin results in altered circulating blood cells.

Comparison of complete blood count and differential cell counts of unmanipulated animals shows OPN null mice to have significantly more lymphocytes than wild type mice. No significant difference in other values was noted. Values presented are average ± S.D. The p value listed represents a comparison in each population between the wild type and OPN null values. RBC: red blood cells, HGB: hemoglobin, HCT: hematocrit, PLT: platelets, WBC: white blood cells, NEU: neutrophils, LYM: lymphocytes, MONO: monocytes, EOS: eosinophils, BASO: basophils, ND: not defined.