Reduced Vascular Remodeling, Endothelial Dysfunction, and Oxidative Stress in Resistance Arteries of Angiotensin II–Infused Macrophage Colony-Stimulating Factor–Deficient Mice

Evidence for a Role in Inflammation in Angiotensin-Induced Vascular Injury

Carolina De Ciuceis, Farhad Amiri, Pascal Brassard, Dierk H. Endemann, Rhian M. Touyz, Ernesto L. Schiffrin

Objective—Angiotensin (Ang) II-induced vascular damage may be partially mediated by reactive oxygen species generation and inflammation. Homozygous osteopetrotic mice (Op/Op), deficient in macrophage colony-stimulating factor (m-CSF), exhibit reduced inflammation. We therefore investigated Ang II effects on vascular structure, function, and oxidant stress generation in this model.

Methods and Results—Adult Op/Op, heterozygous (Op/+), and wild type (+/+ ) mice underwent 14-day Ang II (1000 ng/kg per minute) or saline infusion. Blood pressure (BP) was assessed by radiotelemetry, mesenteric resistance artery vascular reactivity was studied on a pressurized myograph, and vascular superoxide and NAD(P)H oxidase activity by lucigenin chemiluminescence. Ang II increased BP in Op/+ and +/+ mice but not in Op/Op. Ang II-treated Op/+ and +/+ mice showed reduced acetylcholine-mediated relaxation (maximal relaxation, respectively, 64% and 67% versus 84% and 93% in respective controls; \( P<0.05 \)), which was unaffected by L-NAME. Ang II-infused Op/Op mice arteries showed significantly less endothelial dysfunction than vehicle-infused counterparts (maximal relaxation 87% versus 96% in shams). Resistance arteries from Ang II-infused +/+ and Op/+ mice had significantly increased media-to-lumen ratio and media thickness, neither of which was altered in Op/Op mice compared with untreated littermates. Vascular media cross-sectional area, NAD(P)H oxidase activity and expression, and vascular cell adhesion molecule (VCAM)-1 expression were significantly increased by Ang II only in +/+ mice (\( P<0.05 \)).

Conclusions—m-CSF–deficient mice (Op/Op) developed less endothelial dysfunction, vascular remodeling, and oxidative stress induced by Ang II than +/+ littermates, suggesting a critical role of m-CSF and proinflammatory mediators in Ang II-induced vascular injury. (Arterioscler Thromb Vasc Biol. 2005;25:2106-2113.)

Key Words: hypertension ■ macrophages ■ reactive oxygen species

Inflammation plays an important pathophysiological role in the development and progression of atherosclerosis, hypertension, and other conditions associated with vascular damage.1 Macrophage colony-stimulating factor (m-CSF) functions as a chemotactic factor for monocytes, regulates effector functions of mature monocytes and macrophages, and modulates inflammatory responses by stimulating the production of other cytokines, adhesion molecules, and growth factors.2,3 Macrophages have the ability to secrete various cytokines, including tumor necrosis factor (TNF)-\( \alpha \) that can ultimately influence vascular inflammation.4 Mice deficient in m-CSF, the result of a spontaneously occurring osteopetrotic mutation within the m-CSF gene, possess macrophage deficiency, monocytopenia, and defective bone formation.5–7 Recently, it was demonstrated that m-CSF–deficient mice fed an atherogenic diet or crossed into a hypercholesterolemic apolipoprotein E-null background have significant reduction in atherosclerotic lesions.8,9 However, whether reduced macrophage number as a result of the osteopetrotic mutation confers microvascular protection in hypertension remains to be determined. These mice may represent a good model to better understand mechanisms leading to vascular injury mediated by oxidative stress and inflammation in hypertension associated with the activation of the renin-angiotensin system.
Indeed, angiotensin (Ang II) exerts pleiotropic actions contributing to vascular damage\textsuperscript{10} by modulating cytokine release\textsuperscript{11} and proinflammatory transcription factors such as NFκB,\textsuperscript{12} which in turn regulate adhesion molecule expression (vascular cell adhesion molecule [VCAM]-1 and intercellular adhesion molecule-1 [ICAM]-1).\textsuperscript{13} All these can induce inflammation within the vascular wall, deposition of extracellular matrix (ECM), and hypertrophy and/or hyperplasia of vascular smooth muscle cells (VSMCs).\textsuperscript{10} In addition, reactive oxygen species (ROS) act as signaling molecules modulating vascular tone and structural changes to the microcirculation,\textsuperscript{14} as well as the development and progression of atherosclerosis.\textsuperscript{15} NAD(P)H oxidase is the major source of vascular ROS and is expressed in endothelial cells, VSMCs, fibroblasts, and monocyte/macrophages.\textsuperscript{16,17} Humoral agents, such as Ang II or endothelin-1, and inflammatory mediators can modulate basal superoxide generation (O$_2^-$), as well as NAD(P)H oxidase activity and expression of NAD(P)H oxidase subunits.\textsuperscript{18}

We hypothesized that m-CSF deficiency and, consequently, reduced vascular macrophage recruitment, would be associated with reduced endothelial dysfunction, oxidative stress, and vascular inflammation leading to blunted remodeling of mesenteric resistance arteries in Ang II-induced hypertension, underlining the pivotal role of macrophage-mediated inflammation in vascular disease. We therefore assessed effects of Ang II on the development of hypertension, vascular structure and function, NAD(P)H oxidase activity, and inflammation of mesenteric resistance arteries from osteopetrotic mice.

**Methods**

**Animals**

Experiments were conducted using protocols approved by the Animal Care Committee of the Clinical Research Institute of Montreal and performed according to recommendations of the Canadian Council of Animal Care. Heterozygous m-CSF mice (Op/+), B6C3Fe-a/a–Csf-1<sup>19</sup>/Csf-1<sup>1</sup>) purchased from Jackson Labs (Bar Harbor, ME), were cross-bred to obtain mice homozygous for the Csf-1<sup>19</sup> op allele (Op/Op, B6C3Fe-a/a–CSF-1<sup>19</sup>/CSF-1<sup>1</sup>), purchased from Jackson Labs, and wild-type littermates (+/+, B6C3Fe-a/a–Csf-1<sup>19</sup>/Csf-1<sup>1</sup> +/–). Op/+ and wild-type littermates (+/+ or +/–) littermates had no distinguishing phenotypic features, whereas Op/Op mice were identified by the absence of osteopetrotic features, whereas Op/+ mice were identified by the presence of tooth eruption and were fed soft nutritionally balanced Transgenic Dough diet (BioServ), whereas +/+ and Op/+ were fed regular rodent diet (Charles River Laboratories) (Table I, available online at http://atsb.ahajournals.org/).

Under anesthesia with 5% isoflurane, radiotelemetry transmitters (TA11PA-C20; Data Sciences International) were surgically implanted in animals, and systolic blood pressure (BP), diastolic BP, and mean BP measured, as described previously.\textsuperscript{19} One week thereafter, Alzet osmotic mini-pumps (Durect Corp) containing vehicle or le–Ang II (1000 ng/kg per minute, 14 days subcutaneous, Calbiochem) were implanted in 12- to 16-week-old mice. The dose was chosen after infusing +/+ mice with different doses of Ang II, and determining which was the lowest dose that consistently elevated systolic BP over 2 weeks to 50 mm Hg above basal level. At the end of the study, mice were killed humanely.

**Genotyping**

All animals were genotyped at 15 days of age by tail biopsy and polymerase chain reaction (PCR) as described elsewhere,\textsuperscript{20} to distinguish Op/Op, Op/+ and +/+ mice.

**Preparation and Study of Mesenteric Arteries**

Second-order branches of mesenteric artery (~2 mm in length with internal diameter ~150 to 250 μm) were dissected and mounted on a pressurized myograph as previously described.\textsuperscript{21} Briefly, vessels were equilibrated (60 minutes, 45 mm Hg of intraluminal pressure) in warmed oxygenated (95% air–5% CO$_2$) physiological salt solution (PSS) (pH 7.4) containing (mmol/L): NaCl, 120; NaHCO$_3$, 25; KCl, 4.7; K$_2$HPO$_4$, 1.18; MgSO$_4$, 1.18; CaCl$_2$, 2.5; EDTA, 0.026; and glucose, 5.5. Vessels were considered viable when they constricted ≥60% of their resting lumen diameter in response to extraluminal application 125 mmol/L KCl plus 10$^-3$ mol/L norepinephrine (NE) (Sigma Chemicals). Endothelium-dependent relaxation was assessed by measuring the dilatatory response to acetylcholine (Ach) (10$^-10$ to 10$^-4$ mol/L) in NE preconstricted vessels (5 × 10$^-3$ mol/L). NO availability was evaluated by Ach concentration-response curve repeated after 20-minute incubation with NO synthase inhibitor N$^+$-nitro-l-arginine methyl ester (l-NAME, 10$^-4$ mol/L). Endothelium-independent relaxation was assessed with sodium nitroprusside (SNP) (10$^-7$ to 10$^-5$ mol/L) in NE preconstricted vessels. Concentration-response curves to NE (10$^-7$ to 10$^-1$ mol/L) and Ang II (10$^-6$ to 10$^-4$ mol/L) were performed to evaluate vascular contractility.

**Vascular O$_2^-$ and NAD(P)H Oxidase Activity**

Vascular O$_2^-$ and NAD(P)H oxidase activity of aorta and mesenteric arteries was assessed using lucigenin chemiluminescence assay as previously described.\textsuperscript{22}

**Laser Confocal Microscopy**

Laser confocal microscopy was performed on mesenteric resistance arteries as previously described.\textsuperscript{23} Briefly, fixed pressurized vessels were incubated with MOMA-2 antibody (Serotec) for 16 hours at 4°C. After washing, vessels were incubated with 200 µg/mL Alexa Fluor 647 anti-rat IgG (Molecular Probes Inc) for 1 hour at room temperature. For the final 30 minutes of incubation, 10 µmol/L phalloidin (Molecular Probes) was added to stain α-actin. Vessels were studied by laser confocal immunofluorescence microscopy with a Zeiss LSM 510 system. MOMA-2 antibody present in vessels was quantified by imaging (Northern Eclipse program, EMPIX Imaging Inc) by an investigator unaware of groups, and expressed as percentage of Moma-2 total surface area.

**Western Blotting**

Proteins were extracted from frozen tissue as previously described.\textsuperscript{10} Thereafter, samples were separated by SDS-PAGE, transferred to nitrocellulose membranes, and incubated with specific antibody to VCAM-1 (Santa Cruz Biotechnology), p47phox, or gp91phox (gifts from Dr Mark T. Quinn, Montana State University, Bozeman). Signal was revealed with chemiluminescence, visualized autoradiographically and subsequently membranes were stripped (Pierce Biotechnology) and reprobed with β-actin (Sigma Chemicals) to verify equal loading. Optical density of bands was quantified by AlphaEase (Alpha Innotech Corporation) and expressed as arbitrary units.

**NFκB Activation Assay**

Proteins were extracted from frozen tissue as described.\textsuperscript{18} Thereafter, NFκB activation was quantified by using TransAM kit to measure activated p50 subunit of NFκB as previously described\textsuperscript{24} and manufacturer’s protocol (Active Motif).

**Data Analysis**

Results are means±SEM, with n indicating number of animals. Two-way repeated measures ANOVAs were used to evaluate differences in radiotelemetry results and concentration-response curves among groups. Two-way ANOVA was performed to evaluate all other parameters, followed by Bonferroni post-hoc analysis. Area under the curve was calculated for dose-response curves with SigmaPlot (SPSS Inc). P<0.05 was considered statistically significant.
Results

Physiological Parameters
The osteopetrotic phenotype included stunted growth, bone sclerosis, domed skull, and absence of tooth eruption. Body weight was significantly lower in Op/Op mice compared with +/+ and Op/+ counterparts (Table 1). Because of bone sclerosis, relative tissue weights were significantly increased in Op/Op animals compared with +/+ and Op/+ mice. Both +/+ and Op/+ mice exhibited significantly enhanced relative heart weight and left ventricular weight after Ang II infusion, whereas no differences were observed between Ang II and untreated Op/Op mice. BP of all groups was similar at baseline and increased significantly after 14-day Ang II infusion only in +/+ and Op/+ mice, but was unaffected in Op/Op mice (Figure 1).

Vascular Structure
Controls from different genotypes showed similar structural parameters for both aorta and mesenteric resistance arteries (Table 2). In Op/+ mice, Ang II infusion resulted in significantly smaller internal diameter of resistance arteries when compared with vehicle-infused counterparts (P<0.001). Both mesenteric resistance arteries and aorta from Ang II-infused +/+ and Op/+ mice exhibited increased media thickness and media-to-lumen ratio (M/L), whereas media cross-sectional area (MCSA) was increased by Ang II only in +/+ but not Op/+ mice, suggesting hypertrophic remodeling in +/+ and eutrophic remodeling in Op/+ mice. MCSA was significantly smaller in Ang II-infused Op/+ and Op/Op than Ang II-infused +/+ mice. Lumen diameter, media thickness, M/L, and MCSA were similar in Ang II-infused Op/Op mice relative to vehicle-infused Op/Op counterparts.

Function of Mesenteric Resistance Arteries
Vasodilatation to Ach was significantly impaired in all mice receiving Ang II infusion compared with vehicle-infused mice (Figure 2A and 2B). However, Ach-induced relaxation in Ang II-infused Op/Op mice was less attenuated compared with vehicle-infused Op/Op counterparts. Indeed, untreated +/+ and Op/+ mice, and Ang II-infused Op/Op mice had similar relaxation responses to Ach. Relaxation to Ach was significantly impaired after NO synthase inhibition with L-NAME in vehicle-infused mice of all genotypes, and in Op/Op mice receiving Ang II (Figure 3A and 3B). Response to Ach was unaltered by L-NAME in Ang II-infused +/+ and Op/+ mice (Figure 3B). Endothelium independent relaxation responses to SNP were similar in all groups (Figure 2C and 2D).

Significantly increased maximum response to NE was observed in Ang II-infused compared with vehicle-infused +/+ mice, whereas Ang II did not alter NE-induced constriction in Op/+ or Op/Op mice as demonstrated both by dose-response curves and area under the curve (Figure 2E and 2F). Vascular reactivity to exogenous Ang II was not significantly affected by Ang II infusion in any genotype (data not shown). Op/Op mice exhibited a trend to enhanced maximum response to Ang II compared with the other genotypes, but this did not achieve statistical significance.
Vascular \( \cdot O_2^- \) and NAD(P)H Oxidase Activity and Subunit Expression

\( \cdot O_2^- \) levels were unaltered in the untreated mice of all groups, whereas Ang II significantly increased vascular \( \cdot O_2^- \) levels in both aorta and mesenteric arteries of \( +/+ \) and Op/+ mice (Figure 4A and 4B). Activity of NAD(P)H oxidase in aorta was significantly increased in Ang II-infused \( +/+ \) and Op/+ compared with untreated littermates (Figure 4C), whereas in mesenteric arteries Ang II only significantly increased NAD(P)H oxidase activity in \( +/+ \) mice (Figure 4D). Additionally, mesenteric artery NAD(P)H oxidase was unchanged by Ang II infusion in Op/Op, with an intermediate result in Op/+ compared with untreated littermates.

Expression of gp91 phox and p47 phox was significantly increased in aorta and mesenteric arteries only in Ang II-infused Op/+ mice compared with vehicle-treated Op/+ mice, whereas no differences were observed among the other genotypes between Ang II- and vehicle-infused mice.

### Table 2. Morphological Characteristics of Aorta and Mesenteric Resistance Arteries

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(+/+)</th>
<th>(+/+) Ang II</th>
<th>Op/+</th>
<th>Op/+ Ang II</th>
<th>Op/Op</th>
<th>Op/Op Ang II</th>
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</thead>
<tbody>
<tr>
<td>Aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen, ( \mu)m</td>
<td>552±37</td>
<td>530±5</td>
<td>578±33</td>
<td>547±21</td>
<td>478±16</td>
<td>507±36</td>
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<tr>
<td>Media, ( \mu)m</td>
<td>54.2±3.7</td>
<td>71.3±6.3</td>
<td>50.6±1.5</td>
<td>63.9±3.1</td>
<td>47.6±2.7</td>
<td>54.4±4.2</td>
</tr>
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<td>M/L, %</td>
<td>9.8±0.3</td>
<td>13.4±1.1</td>
<td>8.9±0.7</td>
<td>11.8±0.9</td>
<td>10.0±0.5</td>
<td>10.8±1.1</td>
</tr>
<tr>
<td>MCSA, ( \mu)m² (×1000)</td>
<td>98.2±5.9</td>
<td>139.6±23.6</td>
<td>98.5±4.3</td>
<td>121.9±7.2</td>
<td>92.1±5.3</td>
<td>107.7±9.4</td>
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<tr>
<td>Mesenteric Vessels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen, ( \mu)m</td>
<td>221±7</td>
<td>209±6</td>
<td>231±9</td>
<td>191±5†</td>
<td>204±11</td>
<td>204±9</td>
</tr>
<tr>
<td>Media, ( \mu)m</td>
<td>12.7±0.3</td>
<td>16.0±0.5†</td>
<td>12.7±0.3</td>
<td>14.3±0.5*</td>
<td>11.3±0.3</td>
<td>12.4±0.5</td>
</tr>
<tr>
<td>M/L, %</td>
<td>5.8±0.1</td>
<td>7.7±0.1†</td>
<td>5.5±0.2</td>
<td>7.5±0.2†</td>
<td>5.6±0.3</td>
<td>6.1±0.3</td>
</tr>
<tr>
<td>MCSA, ( \mu)m² (×1000)</td>
<td>9.4±0.5</td>
<td>11.3±6.3‡</td>
<td>9.7±4.8</td>
<td>9.2±5.0</td>
<td>7.6±5.1</td>
<td>8.4±5.4</td>
</tr>
</tbody>
</table>

Values are means±SEM; \( n=6–8 \) mice per group.

*\( P<0.05 \) vs nontreated littermates; †\( P<0.001 \) vs nontreated littermates; ‡\( P<0.01 \) vs nontreated littermates.

**Figure 2.** Mesenteric resistance artery endothelial function and vascular reactivity to norepinephrine (NE). Concentration-response curves to (A) acetylcholine (Ach) and (C) sodium nitroprusside (SNP) in all groups. Relaxation responses are % increase in lumen after NE precontraction. E, Concentration-response curves to NE, in which constriction responses are percentage of maximal contraction to KCl+NE. Area under curve concentration-response curves to Ach (B), SNP (D), and NE (F). Values are means±SEM. \( n=6 \) to 8 animals per group. Squares represent \( +/+ \) mice, circles represent Op/+ mice, triangles represent Op/Op mice, whereas open and closed symbols represent vehicle- and Ang II-infused mice, respectively. *\( P<0.05 \) between vehicle and Ang II-infused mice; †\( P<0.01 \) between vehicle and Ang II–infused mice; ‡\( P<0.001 \) between vehicle and Ang II–infused mice.
Vascular Macrophage Infiltration and Proinflammatory Mediators

In mesenteric resistance arteries, Ang II induced significant macrophage infiltration only in \(^{+/+}\) mice when compared with vehicle-treated mice (Figure 5A). Mesenteric artery macrophage infiltration was unchanged by Ang II infusion in \(\text{Op/Op}\), with an intermediate result in \(\text{Op/+}\) compared with untreated littermates. Similarly to mesenteric arteries, Ang II-induced macrophage infiltration in aorta of \(+/+\) mice (data not shown).

Ang II-induced activation of NF-\(\kappa\)B was found in aorta and mesenteric resistance arteries only in \(+/+\) mice (Figure 5B and 5C). As a possible consequence of NF-\(\kappa\)B activation, VCAM-1 expression in aorta and mesenteric resistance arteries was significantly increased only in Ang II-treated \(+/+\) mice compared with vehicle-treated littermates (Figure 5D and 5E).

Discussion

We demonstrate here for the first time to our knowledge that a reduction in macrophage number caused by m-CSF-deficiency is associated with attenuated Ang II-induced BP increase and mesenteric resistance artery remodeling, endothelial dysfunction, \(\cdot\text{O}_2^−\) generation, NAD(P)H oxidase activation, and vascular inflammation.

Osteopetrotic mice are characterized by generalized deficiency of various macrophage subpopulations. They have reduced m-CSF–dependent vascular macrophage numbers, particularly in the subendothelial and myocardial interstitium.\(^{26}\) Our findings extend previous data indicating that inflammation and macrophage-generated oxidative stress are involved in vascular remodeling induced by Ang II.\(^{27,28}\) Vascular changes in hypertension are associated with humoral and mechanical factors that modulate signaling events, resulting in abnormal function, media growth, ECM deposition, and inflammation.\(^{29,30}\) Indeed, \(+/+\) and \(\text{Op/+}\) mice exhibited greater M/L and media thickness of both mesenteric resistance arteries and aorta after a 14-day Ang II infusion compared with vehicle-treated littermates. Whereas Ang II-infused \(+/+\) mice underwent hypertrophic remodeling,\(^{29,30}\) as suggested by an increased MCSA, inward eutrophic remodeling\(^{29,30}\) was observed in Ang II-treated Op/+ mice, as demonstrated by the presence of higher M/L and narrower external and internal diameters, without significant increase...
in MCSA. These findings suggest that inflammation is an important regulator of Ang II-mediated vascular effects and agree with previous data demonstrating that Ang II induces hypertrophic remodeling. They also suggest that in face of slightly blunted inflammatory responses in Op/Op mice, remodeling cannot achieve hypertrophic characteristics, and is eutrophic. Eutrophic remodeling has been suggested to result from persistent vasoconstriction and low-grade ECM deposition in which the vasoconstricted state of the small artery becomes embedded, in the absence of a growth response. Previous data reported that Op/Op mice, which are phenotypically normal, had fewer monocytes/macrophages and develop less extensive atherosclerosis compared with /+ littermates. Vascular macrophage infiltration was significantly reduced in Op/Op mice when compared with /+ mice after Ang II infusion. Ang II-dependent NAD(P)H oxidase activity was absent in Op/Op mice, increased in /+, with an intermediate result in Op/+, whereas gp91phox and p47phox expression were significantly increased only in Ang II-infused /+ mice. These data suggest that a reduced inflammatory and oxidative stress response to Ang II may be involved in the differences between the 3 genotypes.

A major reduction in the presence of macrophages in the vascular wall, as indicated by MOMA-2 staining, could result in attenuated activation of intracellular pathways and inflammatory stimuli, involving cytokines and growth factors that contribute to vascular remodeling. The structure of mesenteric arteries, \( \cdot O_2^- \) generation, NAD(P)H oxidase activity and inflammation were unaltered in Op/Op. However, only vascular structure was affected by 14-day Ang II infusion in Op/Op when compared with /+ where vascular structure, \( \cdot O_2^- \) generation, NAD(P)H oxidase, and inflammation were all affected. Additionally, in Op/Op mice both aorta and mesenteric resistance arteries exhibited a similar trend to increase \( \cdot O_2^- \) generation in response to Ang II infusion, albeit not significantly, thus suggesting that the basal machinery of \( \cdot O_2^- \) generation is intact and responsive to Ang II in Op/Op mice but to a lesser degree possibly because of reduced response to vascular inflammatory mediators.

It is widely accepted that hypertension is associated with arterial structural alterations. Nevertheless, beneficial vascular effects beyond BP lowering have been noted with inhibition of the renin-angiotensin system. Pressure-independent mechanisms are able to induce vascular remodeling after long-term infusion of nonpressor doses of Ang II. Hypertension can result in vascular damage in part through increased ROS production that promotes VSMC proliferation.
and hypertrophy, and collagen deposition leading to arterial remodeling. We and others have found that major reduction in inflammatory responses, as indicated by reduced NFκB activation, VCAM-1 expression, and oxidative stress generation in Op/Op and Op/+ mice resulting from m-CSF deficiency may exert a vascular protective effect, even if significant BP rise occurs in Op/+ mice.

Increased ROS production by Ang II is involved in the mechanisms leading to vascular remodeling. Increased ROS in hypertension impairs endothelium-dependent vascular relaxation by reducing NO bioavailability and increasing the vascular contractile response. Ang II-induced endothelial dysfunction was less marked in Op/Op mice than in vessels from Ang II-treated +/+ and Op/+ animals in which endothelial function was clearly impaired. Furthermore, impaired Ach-induced relaxation of resistance arteries from both Ang II-infused +/+ and Op/+ mice was unaltered by incubation with 1-NAM, indicating reduced availability of NO. 1-NAM, however, significantly reduced Ach-induced relaxation of osteopetrotic mice. Thus, Op/Op mice had persistent vascular NO bioavailability associated with absence of activation of NAD(P)H oxidase and increased -O2· generation even when exposed to a dose of Ang II that induced endothelial dysfunction in +/+ and Op/+ mice.

Liu et al recently demonstrated in vivo a role of Ang II-stimulated NAD(P)H oxidase in ICAM-1 expression, macrophage infiltration, and vascular hypertrophy independently of BP elevation. However, in the present study Ang II failed to activate NAD(P)H oxidase in Op/+ mice even though BP was elevated, suggesting that this Ang II-induced activation is pressure-independent. This conclusion is supported by the absence of NADPH oxidase activation that is found with similar elevation of systolic BP with NE infusion. Macrophages infiltrating the adventitia or the media of blood vessels may mediate oxidative stress generated by NAD(P)H oxidase also in response to elevated BP, which may explain blunting of activation of NAD(P)H oxidase in Ang II-infused Op/Op mice. However, pathways linking Ang II to signaling molecules modulating vascular NAD(P)H oxidase remains to be completely clarified.

Hypertrophied mesenteric resistance vessels from Ang II-treated +/+ mice showed enhanced contractility in response to NE, which was not observed in Op/+ and Op/Op mice. The fact that BP increased (only slightly albeit not significantly relative to basal, but also not significantly differently from the other genotypes), that mild endothelial dysfunction did develop (minor compared with that in the other genotypes), and that vasoconstrictor responses of ex vivo small arteries to Ang II tended to be greater in Ang II-infused Op/Op mice suggest that changes in Ang II receptors are not downregulated and do not play a role in the blunted effects found in osteopetrotic mice in response to Ang II infusion, but that other factors may be involved. These could include deficiency in response of inflammatory mediators. Ang II stimulates vasoactive agents such as endothelin-1 and aldosterone, which could have an effect on BP and vasoconstrictor responses in m-CSF–deficient mice, and elevated BP could contribute to the mild endothelial dysfunction found in these mice.

The role played by the macrophage NAD(P)H oxidase versus vascular NAD(P)H oxidase in Ang II–induced hypertension and vascular remodeling remains unclear. Although it has been demonstrated that the effects of Ang II on vascular remodeling are mediated through generation of ROS, the exact source of free radicals, in a majority originating from vascular (endothelial, adventitial and VSMC) and macrophage NAD(P)H oxidase, has not yet been defined. Knockout of the various NAD(P)H subunits have not been tissue- or cell-specific. It is thus been impossible to determine the relative contributions of the various tissue NAD(P)H oxidases in the generation of O2·−. However, our results suggest that macrophage NAD(P)H oxidase is an important source of ROS stimulated by Ang II.

In summary, the present study provides the first in vivo evidence of the critical role played by m-CSF– and macrophage-dependent inflammatory processes in the development of vascular injury induced by a hypertensive dose of Ang II. Our findings suggest that mice deficient in monocyte/macrophage cell lineage are protected from Ang II-induced vascular injury, which is evidence in favor of a role of proinflammatory and prooxidant properties of Ang II in the pathogenesis of vascular disease.

Acknowledgments

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References


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Table I. Comparison of ingredients found in normal and transgenic dough diet given to wild type (+/+) and heterozygous (Op/+), and osteopetrotic (Op/Op) mice, respectively.

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<th>Ingredient</th>
<th>Normal Diet (+/+ and Op+)</th>
<th>Transgenic Dough Op/Op</th>
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<tr>
<td>Vitamin D (IU/kg)</td>
<td>2200</td>
<td>1500</td>
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<tr>
<td>Vitamin E (IU/kg)</td>
<td>90</td>
<td>112.5</td>
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<td>Vitamin A (IU/kg)</td>
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<td>6000</td>
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<td>Physiological Fuel Value (kcal/g)</td>
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<td>Fat (%)</td>
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