Resistance artery remodeling in deoxycorticosterone acetate-salt hypertension is dependent on vascular inflammation: evidence from m-CSF-deficient mice

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The role of inflammation through recruitment, activation, survival, and proliferation of mononuclear phagocytes in the vascular wall has been increasingly recognized in atherosclerosis and hypertension (18). This proinflammatory state is mediated through increased expression of several mediators, including leukocyte adhesion molecules, chemokines, and specific growth factors, and may be triggered by vasoactive agents such as endothelin-1 (ET-1) and angiotensin II (ANG II) (4, 22).

Macrophage colony-stimulating factor (m-CSF) regulates the differentiation, proliferation, and survival of macrophages (20). Osteopetrotic mice are homozygous for a naturally occurring recessive frame shift mutation (Op) in the m-CSF gene leading to absence of m-CSF expression (30, 31). As a consequence, these mice have a defect in osteoclast development, and deficiency in monocyte and macrophage number (21). m-CSF deficiency plays a protective role on the vasculature of atherosclerotic and hypertensive animals (7, 24), through effects on inflammatory pathways [nuclear factor-κB (NF-κB) and adhesion molecules such as vascular cell adhesion molecule (VCAM)-1] or via reduced generation of reactive oxygen species (ROS). However, whether inflammation plays an important role and m-CSF deficiency confers vascular protective effects to a form of hypertension with an important ET-1-dependent component is unknown. We used the deoxycorticosterone acetate (DOCA)-salt hypertensive model, which has an ET-1-dependent component (14, 16), to test the hypothesis that m-CSF deficiency could exert vasculoprotective effects in this hypertensive model.

METHODS

Animals. The present study was performed under 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 op/CSF-11), purchased from Jackson Labs (Bar Harbor, ME), were cross-bred to obtain mice homozygous for the CSF-1 Op allele (Op/Op, B6C3Fe-a/a-CSF-1/CSF-11). Op−/− and Op+/− littermates had no distinguishing phenotypic features, whereas Op/Op mice were identified by the absence of tooth eruption, because of which they had to be fed a soft nutritionally balanced Transgenic Dough diet (BioServ, Frenchtown, NJ). Mice were unilaterally nephrectomized (UniNx). In 3- to 4-mo-old mice, silicone rubber impregnated with DOCA (200 mg/mouse) was implanted subcutaneously and mice were offered 1% saline to drink. Control mice received a silicone rubber implant without DOCA and were offered tap water. To dissect the role of blood pressure (BP) elevation, we infused a pressor dose of norepinephrine (3.8 μg·kg−1·min−1) with Alzet osmostic minipumps (Durect, Cupertino, CA) in a parallel set of animals of all genotypes, as described elsewhere (10). After surgery systolic BP (SBP) was measured weekly by the tail-cuff method, and after 2 wk of treatment mice were killed by decapitation.

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Genotyping. All animals were genotyped at 6 wk of age by tail biopsy and PCR (1, 7) to distinguish Op/Op, Op/+ , and +/+ mice.

Preparation and study of arterial function. Second-order branches of mesenteric artery (~2 mm in length with internal diameter ~150–250 μm) were dissected and mounted on a pressurized myograph (7). Briefly, vessels were equilibrated (60 min, 45 mmHg of intraluminal pressure) in warmed, oxygenated (95% air-5% CO2) physiological salt solution (PSS, pH 7.4) containing (mmol/l) 120 NaCl, 25 NaHCO3, 4.7 KCl, 1.18 KH2PO4, 1.18 MgSO4, 2.5 CaCl2, 0.026 EDTA, and 5.5 glucose. Endothelin-dependent relaxations were assessed by measuring dilatory responses in norepinephrine-precontracted vessels (5 × 10−5 mol/l) to cumulative increasing concentrations of acetylcholine (10−9–10−4 mol/l). To evaluate NO bioavailability, acetylcholine concentration-response curves were repeated after 20-min incubation with the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 10−4 mol/l). Concentration-response curves to norepinephrine (10−7–10−4 mol/l) and ET-1 (10−10–10−7 mol/l) were performed to evaluate vascular contractility.
and calculated as a percentage of basal values. Thereafter, vessels were perfused with Ca²⁺-free PSS containing 10 mmol/l EGTA for 30 min to eliminate any tone, and lumen and media dimensions were measured. Concentrations causing half-maximal response (EC₅₀ values) were expressed as geometric means with 95% confidence intervals (CI).

Measurement of vascular reduced nicotinamide adenine dinucleotide phosphate oxidase and basal superoxide activity. Vascular superoxide (O₂⁻) and reduced nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase activity were measured by chemiluminescence with lucigenin and NAD(P)H on mesenteric artery segments as previously described (2).

Laser confocal microscopy. Laser confocal microscopy (Zeiss LSM 510 system, Carl Zeiss MicroImaging, Thornwood, NY) was performed on mesenteric resistance arteries as previously described (23). Briefly, fixed pressurized vessels were incubated with macrophage-specific antigen MOMA-2 antibody (Serotec, Raleigh, NC) for 16 h at 4°C. After being washed, vessels were incubated with 200 μg/ml Alexa Fluor 647 anti-rat IgG (Molecular Probes, Eugene, OR) for 30 min at 37°C. α-Actin staining was achieved by incubation with 10 μmol/l phalloidin (Molecular Probes) for 30 min at room temperature. MOMA-2 staining was quantified with Northern Eclipse and expressed as a percentage of MOMA-2/total surface area.

NF-κB activation assay. Mesenteric artery proteins were extracted from frozen tissue as described above and in Ref. 2. Thereafter, NF-κB activation was quantified by using a TransAM kit to measure activated p50 subunit of NF-κB as previously described (7) according to the manufacturer’s protocol (Active Motif, Carlsbad, CA).

Western blot analysis. Mesenteric artery proteins were extracted from frozen tissue as previously described (23). Thereafter, samples were separated by SDS-PAGE, transferred to nitrocellulose membranes, and incubated with specific antibody to VCAM-1 (Santa Cruz Biotechnology, Santa Cruz, CA). Bands were visualized by chemiluminescence (Pierce Biotechnology, Rockford, IL) and were quantified by densitometry (Northern Eclipse 5.0, EMPIX Imaging, Mississauga, ON, Canada).

Data analysis. Results are presented as means ± SE, except for EC₅₀ values, which are presented as means with 95% CI. Comparisons between groups were made by two-way ANOVA or repeated-measures one-way ANOVA followed by a Student-Newman-Keuls test, as appropriate. P < 0.05 was considered statistically significant.

RESULTS

Physiological parameters. Body weight was significantly lower in Op/Op mice than in +/+ and Op/+ mice and was unaffected by DOCA-salt treatment. DOCA-salt treatment significantly increased SBP only in +/+ and Op/+ mice (Table 1).

Vascular function of mesenteric resistance arteries. Vasodilation to acetylcholine was significantly impaired in DOCA-salt +/+ and Op/+ mice compared with UniNx mice. No impairment of endothelium-dependent vasorelaxation was observed in DOCA-salt Op/Op mice (Fig. 1, A–D) and in noradrenaline-infused mice of all genotypes (Fig. 1E). Relaxation to acetylcholine was significantly impaired by the nitric oxide synthase inhibitor L-NAME in DOCA-salt and UniNx +/+ and Op/+ mice but to a lesser extent in DOCA-salt and UniNx Op/Op mice (Fig. 2). Endothelium-independent relaxation to sodium nitroprusside was similar in all groups (Supplemental Fig. 1; the online version of this article contains these supplemental data). Norepinephrine contractile responses were significantly enhanced in DOCA-salt +/+ and Op/+ mice, whereas no effect was observed in DOCA-salt Op/Op mice compared with UniNx mice (Fig. 3). ET-1 contractile responses were similar in all groups (Fig. 4).

Mesenteric resistance artery morphology. DOCA-salt treatment resulted in increased mesenteric resistance artery media-to-lumen ratio (M/L) and media cross-sectional area (MCSA), indicating hypertrophic remodeling of these vessels, only in +/+ and Op/+ mice compared with untreated UniNx +/+ and Op/+ mice (Table 1). Media thickness, M/L, and MCSA were similar in DOCA-salt treated and UniNx Op/Op mice and in all genotypes of norepinephrine-infused mice (Table 1).

Vascular superoxide and NAD(P)H oxidase activity. DOCA-salt treatment increased both O₂⁻ generation and NAD(P)H oxidase activity in mesenteric arteries in +/+ mice, whereas it only increased NAD(P)H oxidase activity in Op/+ mice (Fig. 5). Neither O₂⁻ generation nor NAD(P)H oxidase...
activity of mesenteric arteries was affected by DOCA-salt treatment in Op/Op mice (Fig. 5).

Vascular inflammatory markers. Macrophage infiltration in mesenteric resistance arteries was significantly increased only in DOCA-salt-treated /H11001/H11001 and Op/H11001/H11001 mice compared with UniNx littermates (Fig. 6A), whereas in DOCA-salt Op/Op mice there was no significant change. On the other hand, DOCA-salt treatment activated NF-/H9260 in mesenteric arteries only in /H11001/H11001 mice (Fig. 6B). Probably resulting from NF-/H9260 activation, VCAM-1 expression was increased only by DOCA-salt in mesenteric arteries in /H11001/H11001 mice (Fig. 6C).

DISCUSSION

m-CSF deficiency in the present study was associated with attenuated BP rise and vascular remodeling, endothelial dysfunction, NAD(P)H oxidase activation, and vascular inflammation in mesenteric resistance arteries from DOCA-salt hypertensive mice, a model of hypertension that has a significant ET-1-dependent component (13, 16). These results, taken together with our previously published findings on the role of inflammation in ANG II-mediated effects (7), suggest that inflammation may be an important mediator of vascular remodeling effects of potent vasoactive agents such as ET-1 and ANG II.

In hypertension, the vascular changes that result in decreased lumen size and increased M/L may be structural, functional, or mechanical and may be caused by different local agents or by hormonal factors (12). In DOCA-salt +/- and Op/+ mice, remodeled resistance vessels had increased MCSA, indicating the presence of hypertrophic remodeling, which has been considered a hallmark of ET-1-dependent hypertension (6, 8, 9).

There is abundant evidence that increased ROS production by NAD(P)H oxidase in hypertension may contribute to vascular injury by promoting vascular smooth muscle cell growth, extracellular matrix deposition, activation of matrix metalloproteinases, inflammation, endothelial dysfunction, and increased vascular tone (5, 29). We (2, 28) and others (3) have shown that NAD(P)H oxidase activity, one of the main sources of O2•− in the blood vessel wall, is enhanced in ET-1-dependent hypertension. However, our results indicate that BP elevation may not depend on NAD(P)H oxidase activity and
increased ROS generation in the model examined in this study, since we found increased SBP in DOCA-salt Op/+ mice in the absence of increased NAD(P)H oxidase activity. This agrees with previous findings from our laboratory in some other models of hypertension (2, 7).

In addition to altered vascular structure, DOCA-salt-treated Op/+ and Op/Op mice exhibited impaired endothelial function compared with all UniNx mice and DOCA-salt treated Op/Op mice. Endothelial dysfunction could be a consequence of decreased NO bioavailability due to increased ROS production. To assess this, mesenteric resistance vessels from DOCA-salt +/+ and Op/+ mice were incubated with L-NAME and were found to have significant further impairment of acetylcholine-induced relaxation, which was abrogated by L-NAME. This suggests that reduced NO production or bioavailability, which may participate in DOCA-salt-induced endothelial dysfunction (17, 19, 27), was not prominent in the present experiment paradigm in +/+ mice and were found to have significant further impairment of acetylcholine-induced relaxation, which was abrogated by L-NAME. This suggests that reduced NO production or bioavailability, which may participate in DOCA-salt-induced endothelial dysfunction (17, 19, 27), was not prominent in the present experiment paradigm in +/+ and Op/+ mice, since the significant L-NAME-induced effect found on acetylcholine-induced relaxation demonstrates the presence of abundant NO production by nitric oxide synthase in resistance vessels from these mice. However, impaired endothelial function was not observed in DOCA-salt-treated Op/Op mice. Furthermore, L-NAME did not abrogate acetylcholine-induced relaxation, but reduced it only partially. Thus L-NAME was less effective on Op/Op mice, whether UniNx- or DOCA-salt treated, than on UniNx or DOCA-salt +/+ and Op/+ mice. This suggests that in Op/Op mice there may be a switch from NO-mediated acetylcholine-stimulated endothelium-dependent relaxation to endothelium-derived hyperpolarizing factor-mediated relaxation. This result is paradoxical, since it would be expected that m-CSF-dependent cells (macrophages) would contribute to macrophage-derived NAD(P)H oxidase and ROS formation in the vascular wall (26) and to decreased bioavailability of NO leading to endothelial dysfunction, which should be less evident in Op/Op mice, which is not the case.

Oxidative stress has been shown to be linked to a proinflammatory state that includes upregulation of adhesion molecules (VCAM-1 and ICAM-1) and chemotactic molecules such as monocyte chemoattractant peptide-1 (11). Indeed, DOCA-salt treatment induced a proinflammatory state as demonstrated by increased macrophage infiltration and increased activity of transcription factor NF-κB, and its downstream target VCAM-1 in +/+ mice and to a lesser extent in Op/+ mice. The absence of DOCA-salt-induced activation of NAD(P)H oxidase and vascular NF-κB and VCAM-1 expression in Op/Op mice supports the important role of inflammation in this model of hypertension, probably a consequence in part of the action of ET-1, which we previously showed (15, 25) contributes to remodeling of resistance vessels in this hypertensive model.

Mesenteric resistance arteries from DOCA-salt-treated +/+ and Op/+ mice showed enhanced contractility to norepinephrine that was not observed in DOCA-salt Op/Op mice. Increased responsiveness to norepinephrine and impaired endothelium-dependent relaxation contribute to increased periph-

Fig. 4. Mesenteric resistance artery concentration-response curves to endothelin-1 in +/+ mice (A), in Op/+ mice (B), in Op/Op mice (C), and in all mice (D). Open and closed symbols represent UniNx and DOCA-salt mice, respectively. Results for dose-response curves are means ± SE, and EC50 values are presented as geometric means (95% CI). Results were analyzed by 1-way repeated-measures ANOVA followed by Newman-Keuls test; n = 6 or 7 animals/group.
eral resistance and, together with the structural and functional vascular changes, can ultimately affect BP, all of which were significantly increased only in DOCA-salt Op/Op mice and not in DOCA-salt Op/Op mice. This may explain in part the differences in BP elevation observed and the blunting of BP elevation in Op/Op mice. Additionally, data obtained from the norepinephrine-infused mice suggest that the observed vascular changes of mesenteric resistance arteries were merely due to SBP elevation since norepinephrine-induced hypertensive mice did not exhibit altered vascular morphology and function in any genotype.

In conclusion, this is the first report that m-CSF deficiency resulting in reduced vascular inflammation plays an important protective effect in the deleterious vascular effects of DOCA-salt and potentially of ET-1, which has been shown to be a major mediator in this model, on remodeling, ROS generation, and inflammation in mesenteric resistance arteries. These findings support the hypothesis that vascular inflammation may be one of the factors that participate in mechanisms leading to remodeling of resistance vessels in experimental hypertension.

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Fig. 5. Vascular O$_2^•$ (A) and reduced nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase (B) activity in mesenteric arteries. Open and filled bars represent UniNx and DOCA-salt mice, respectively. Results are means ± SE and were analyzed by 2-way ANOVA followed by Newman-Keuls test; n = 5–7 animals/group. *P < 0.05 vs. UniNx littermates.

Fig. 6. Vascular inflammation in mesenteric arteries of DOCA-salt and UniNx mice. A: quantification of macrophage infiltration into the media of mesenteric arteries of all groups. Data are expressed as % of MOMA-2/total surface area. B: nuclear factor-κB (NF-κB) p50 subunit activation in mesenteric resistance arteries as determined by ELISA. C: vascular expression of vascular cell adhesion molecule (VCAM)-1 (top) and β-actin (bottom) in mesenteric arteries of DOCA-salt and UniNx mice. Results are presented as means ± SE and were analyzed by 2-way ANOVA followed by Newman-Keuls test; n = 4 or 5 animals/group. *P < 0.01 vs. UniNx littermates; †P < 0.05 vs. UniNx littermates.
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