Adventitia-derived nitric oxide in rat aortas exposed to endotoxin: cell origin and functional consequences

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Kleschyov, Andrei L., Bernard Muller, Thérèse Keravis, Marie-Elisabeth Stoeckel, and Jean-Claude Stoclet. Adventitia-derived nitric oxide in rat aortas exposed to endotoxin: cell origin and functional consequences. Am J Physiol Heart Circ Physiol 279: H2743–H2751, 2000.—The role of adventitial cells in bacterial lipopolysaccharide (LPS)-induced vascular nitric oxide (NO) overproduction has been largely ignored. In rat aortas exposed to LPS in vitro or in vivo, it was found that adventitia contained the major part of NO synthase (NOS)-2 protein (Western blot and immunohistochemistry) and generated the largest amount of NO (electron paramagnetic resonance spin trapping). NOS-2 immunoreactive cells were mainly resident macrophages at an early stage (5 h, in vitro or in vivo) and fibroblasts at a later stage (20 h, in vitro). Adventitial NOS-2 activity largely accounted for 1) the relaxing effect of L-arginine in rings exposed to LPS in vivo, 2) generation of an “NO store” revealed by N-acetylcysteine-induced relaxation, and 3) formation of protein-bound dinitrosyl iron complexes in the medial layer of aortic rings exposed to LPS in vitro. In conclusion, the adventitia is a powerful source of NO triggered by LPS in the rat aorta. This novel source of NO has an important impact on smooth muscle function and might be implicated in various inflammatory diseases.

adventitial fibroblasts; adventitial macrophages; dinitrosyl iron complexes; electron paramagnetic resonance spin trapping

BLOOD VESSELS EXPOSED TO bacterial lipopolysaccharide (LPS) express inducible nitric oxide (NO) synthase (NOS)-2 and produce large amounts of NO, leading to a dramatic decrease of vascular contractility. In this situation, it is generally assumed that NO is produced by NOS-2 expressed in the endothelium and especially in the vascular smooth muscle cells (VSMC) themselves (14, 23, 25). This assumption is mainly based on the idea that VSMC are the major cell type of the vascular wall and that, like endothelial cells (12), they express NOS-2 in cell culture after proinflammatory stimuli (4, 7). The outer blood vessel layer (tunica adventitia) contains various cell types, including fibroblasts and resident macrophages (20), which are known to express NOS-2 in response to proinflammatory cytokines and/or LPS (16, 28). Recent studies performed in our and other laboratories suggest that the adventitia is an important source of NO in LPS-exposed blood vessels (13, 30). However, direct evidence of potential NOS-2 protein expression and NO production in the adventitia is still lacking. The cell type(s) that expresses NOS-2 as well as the potential functional role of adventitial NO are also illusive.

LPS-induced downregulation of vascular contractility mainly depends on ongoing synthesis of NO via the l-arginine/NOS-2-dependent pathway (8, 10, 14, 23). The high level of NOS-2 activity can lead to the formation of an NO store that, under the influence of thiols, releases NO and contributes to a further decrease of contractility (18). One candidate for such an NO store are protein-bound dinitrosyl iron complexes (DNIC) (18, 19), the formation of which is associated with overproduction of NO (11). The localizations of both vasoactive NO stores and DNIC, as well as the potential sources of NO for their generation in blood vessels, are not fully elucidated.

The purpose of the present study was to examine the degree of expression and the potential functional consequences of adventitial NOS-2 activity. In the rat aorta exposed to LPS, either in vitro or in vivo, we demonstrate that the main part of NOS-2 expression and activity can be attributed to the resident macrophages and fibroblasts within the adventitia. Furthermore, we provide evidence that adventitial NOS-2 activity largely accounts for the LPS-induced downregulatory effect on vascular contraction and potential generation of DNIC in the medial layer.

METHODS

Materials. Reagents were from Sigma (Saint Quentin-Falavier, France) unless otherwise indicated.
Aortic preparations and induction of NOS-2. Animal studies were conducted in accordance with the guidelines of the Declaration of Helsinki (authorization no. 01918 given by the French Ministry of Agriculture). Male Wistar rats (11–13 wk) bred in our institute from genitors provided by Iffa Credo (Abresles, France) were killed by cervical dislocation, and thoracic aortas were removed and cleaned of adherent tissues under sterile conditions. Freshly isolated aortas were used as the control. For the in vivo model of NOS-2 induction, rats were treated with LPS (Escherichia coli 055:B5; Difco, Detroit, MI; 40 mg/kg ip) 5 h before they were killed. For the in vitro model, intact aortas or medial layers were incubated for 5 or 20 h in the presence of LPS (10 μg/ml) at 37°C in modified Eagle’s medium (MEM; GIBCO) without addition of serum in an incubator gassed with 95% air-5% CO₂. Some aortas were incubated with LPS in the presence of 10 μM dexamethasone. Before Western blot analysis, NO spin trapping, and contractile studies, the endothelium was removed by gently rubbing the aortic intimal surface.

Removal of adventitia. In some aortas, adventitial layers were separated from medial layers according to a previously described technique (3), either before or after exposure to LPS. The thoracic aorta was cleaned of adherent adipose tissue and collateral vessels. After careful cleaning, the adventitial and medial layers could be distinguished at both ends of the aorta under magnification. The dissection was started from the aortic arch-end by splitting of the aortic wall with the use of two microsurgery forceps and gentle peeling of the adventitia toward the distal end. When removal of adventitia is performed by a skilled person, the medial layer rings display reproducible contractile properties.

Western blotting. Samples of adventitia and media stored at −80°C were powdered in liquid nitrogen. Tissue powder was homogenized at 4°C in 250 mmol/l NaCl, 25 mmol/l Tris-HCl (pH 7.5), 5 mmol/l EDTA (pH 8.0), 1 mmol/l Pefabloc, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 2 μg/ml pepstatin (Boehringer) and then sonicated and centrifuged (2 × 10 min at 12,000 g at 4°C). Soluble proteins (200 μg/lane) were heated for 5 min at 95°C in Laemmli buffer and electrophoresed along with controls for NOS-2 (1 μg protein of mouse macrophage lystate), NOS-3 (2 μg protein of human endothelial lystate), and NOS-1 (2 μg protein of rat pituitary lystate) on SDS-8% polyacrylamide gels; transferred to nitrocellulose membranes; and processed for immunoblotting with a polyclonal rabbit antibody against NOS-2 (1:5,000 dilution from Transduction Laboratories, Lexington, KY). A monoclonal mouse antibody against NOS-1 (1:250 dilution from Transduction Laboratories) was used for analysis of NOS-1 expression in tissue powder homogenized in the above-mentioned buffer supplemented with 1% SDS. Anti-rabbit or anti-mouse IgG horseradish peroxidase conjugates were used as secondary antibodies (1:5,000 dilution; Promega). The immobilized antigens were detected with the use of an enhanced chemiluminescence assay kit (Amersham). Densitometric analysis was performed using Starwise software (Imstar, Paris, France). The signal intensity data are expressed in arbitrary units as means ± SE of three experiments.

Immunohistochemistry. Aortas were fixed in freshly prepared 4% paraformaldehyde in 0.1 M PBS, pH 7.4, for 10–15 h. Morphological characterization of NOS-2-expressing cells was performed on immunoperoxidase-labeled semithin sections. Double labeling with NOS-2 and resident macrophage-specific antibodies was performed by immunofluorescence on frozen sections. For semithin sections, 5-mm segments from three control aortas and three aortas incubated for 20 h with LPS were dehydrated in graded alcohols and conventionally embedded in an araldite-epon mixture. Sections, 1–2 μm thick, were prepared on a cryostat microtome (Frigocut 2800, Reichert) and thaw-mounted on gelatin-coated slides. The sections were simultaneously treated with anti-NOS-2 antibody and monoclonal mouse antibody ED2 (diluted in PBST at 1:250 and 1:200, respectively). The ED2 antibodies that are specific for rat resident macrophages (2) were provided by Dr. C. D. Dijkstra (Free Univ., Amsterdam, The Netherlands). The secondary antibodies were antimouse IgG labeled with fluorescein isothiocyanate and antirabbit IgG labeled with CY3, both raised in the donkey (Jackson) and applied at a 1:200 dilution. The sections were mounted in Vectashield (Vector) and examined under an epifluorescence microscope equipped with an adequate filter system (Dualx Leitz). Some frozen sections were processed for immunoperoxidase detection of NOS-2, e.g., the semithin sections, and counterstained with methyl green for visualization of the NOS-2-negative cells.

NO spin trapping and electron paramagnetic resonance spectroscopy. Aortic rings (3-mm long) or corresponding adventitia or media were incubated in MEM containing L-arginine (L-Arg; 0.6 mmol/l), diethyldithiocarbamate (DETC; 5 mmol/l), ferrous sulfate (50 μmol/l), and sodium citrate (1 mmol/l) at 37°C for 1 h (17). Some experiments were performed in the presence of N^+ -nitro-L-arginine methyl ester (L-NAME; 3 mmol/l). After the incubation, tissues were frozen and then kept in liquid nitrogen. For electron paramagnetic resonance (EPR) detection of DNIC, whole thoracic aorta (30 mm long) or corresponding segments of adventitia or media were used. EPR studies were performed at 77 Kelvin (77 K) on a Bruker 300E spectrometer with a standard TE102 cavity resonator using a Dewar flask (Wilmad). EPR parameters were 10 mW of microwave power, 0.6 mT of amplitude modulation, a 9.45-GHz microwave frequency, and a 100-kHz modulation frequency. For quantification of the EPR signals, a paramagnetic solution of (NO)Fe(SO₄)₂ of known concentration was used. Comparisons were made after double integration of their EPR signals. Amounts of NO trapped by Fe-DETC were expressed either per segment of thoracic aorta (30 mm long) or per microgram of DNA. The detection limit of the absolute amount of NO-Fe-DETC in the sample was ~6 pmol. The intra-assay coefficient of variation for EPR measurement was 4% (n = 10). The interassay coefficients of variation for NO spin trapping and DNIC were 31 and 34%, respectively (n = 10 samples).

Contraction experiments. Endothelium-denuded aortic rings or rings of medial layer (3–4 mm long) were mounted under a passive tension of 2 g in organ baths (at 37°C) filled...
with Krebs solution as described previously (8, 10, 18). The effect of L-Arg was studied in rings precontracted with nor-epinephrine (NE; 1 μmol/l). To reveal the NO store, aortic rings in which adventitia was removed or not, either before or after LPS exposure, were precontracted with NE and L-NAME (3 mmol/l); when the contraction reached a steady-state level, N-acetylcysteine (NAC; 0.1–10 mmol/l) was added in a cumulative manner. The absence of functional endothelium was assessed by the lack of a relaxing effect of ACh (1 μmol/l).

**Statistical analysis.** Results are expressed as means ± SE of n experiments. Statistical comparisons were made using the nonparametric Mann-Whitney test. P values < 0.05 were considered to be statistically significant.

**RESULTS**

**Adventitia is the major site of LPS-induced NOS-2 expression.** In control aortas, Western blot analysis (Fig. 1) revealed a trace NOS-2 signal in adventitia (lane 1) and no NOS-2 signal in media (lane 2). After incubation of isolated aortas with LPS for 20 h, both aortic layers displayed a strong NOS-2 signal. However, the signal was stronger in the adventitia than in media (lanes 3 and 4, respectively). No NOS-1 signal was detected in the adventitia/media samples from either control or LPS-treated aortas (not shown). In aortas obtained from rats treated with LPS in vivo (5 h), NOS-2 signals in both layers were markedly less than in the in vitro model. Again, the NOS-2 signal was stronger in the adventitia than in media (lanes 5 and 6, respectively).

**Resident macrophages and fibroblasts are NOS-2-expressing cell types in adventitia.** No NOS-2-immunoreactive (NOS-2-ir) cells could be detected on semithin sections of control aortas (Fig. 2A). Aortas incubated with LPS for 20 h exhibited numerous NOS-2-ir cells regularly distributed in the adventitia (Fig. 2B). Some labeled endothelial and subendothelial cells could also be observed. The media was invariably NOS-2 negative. In the adventitia, most of the NOS-2-ir cells were spindle shaped, with slender and sinuous extensions, which are characteristic features of fibroblasts. Immunoperoxidase-labeled frozen sections counterstained with methyl green (to visualize all cellular elements) revealed that after 20 h incubation with LPS, the great majority of adventitial cells were NOS-2 positive (not shown). Double immunofluorescence labeling of control aortas did not reveal the presence of NOS-2-ir cells but showed the presence of ED2-immunoreactive (ED2-ir) macrophages scattered irregularly in the adventitia (Fig. 3, A and B). After 5 h of incubation with LPS, NOS-2-ir cells appeared in the adventitia. Most of...
them were ED2-ir macrophages. However, a few cells were solely NOS-2-ir or ED2-ir (Fig. 3, C and D). After 20 h of incubation with LPS, NOS-2-ir cells largely outnumbered ED2-ir macrophages (Fig. 3, E and F), indicating that cells other than resident macrophages predominantly expressed NOS-2. Aortas exposed to LPS in vivo for 5 h exhibited a double-labeling pattern comparable with that observed in the 5-h in vitro experiment (Fig. 3, G and H). As in semithin sections, NOS-2 was also undetectable in the medial layer in all frozen sections.

Adventitia is the major site of LPS-induced NO overproduction. Aortic rings preincubated with LPS for 20 h and then exposed to NO spin-trap components exhibited the EPR signal with values of EPR spectroscopic parameter g tensor equal to $g_{\perp}$ (perpendicular) 2.035 and $g_{\parallel}$ (parallel) 2.02, which are characteristic of NO-Fe-DETC (27) (Fig. 4A). The triplet hyperfine structure at $g_{\perp}$ with hyperfine splitting from the nitrogen nucleus in the NO ligand $A_{N} = 1.3$ mT was observed in the EPR signal. A decrease in EPR signals was observed in the presence of 3 mmol/l 1-NAME during spin-trapping assay (4-fold decrease; Fig. 4B) or when 10 μmol/l dexamethasone was present during the incubation period with LPS (8-fold decrease; Fig. 4C). Rubbing of intimal surface to remove the endothelium before NO assay led to a decrease in the signals of ~13% ($n = 4$; Fig. 4D). No detectable NO was found in control endothelium-denuded aortic rings ($n = 4$; not shown). NO spin-trapping studies were then performed.
in the adventitia and media separately. In the in vitro model, adventitia produced an EPR signal for NO-Fe-DETC that was twice as intensive as the corresponding segment of media (Fig. 4, E and F, and Table 1). The quantification made per amount of DNA revealed that one adventitial cell formed about six times more NO than one cell of medial layer (Table 1). In the in vivo model of exposure to LPS, EPR detection of NO in the aorta was one order less than in the in vitro model. Equivalent segments of adventitia and media gave comparable EPR signals of NO-Fe-DETC. When calculated per DNA, about three times more NO was detected in the adventitia than media (Table 1). When the medial layer was exposed to LPS in vitro (in the absence of adventitia), the rate of NO production was 210 ± 23 pmol·thoracic aorta⁻¹·h⁻¹. Thus it is unlikely that lower medial NO production compared with adventitial NO production was due to restriction of LPS accessibility to the media by the adventitia.

Crucial role of adventitia-derived NO in relaxing effect of L-Arg.

L-Arg (0.1 mmol/l) caused a profound relaxation of NE-precontracted adventitia-intact rings obtained from endotoxemic animals (Fig. 5A). Removal of the adventitia almost totally abolished the relaxing effect of L-Arg (Fig. 5B). However, the medial rings showed a profound relaxation in response to the NO donor S-nitroso-N-acetylpenicillamine (SNAP;
10 μmol/l), indicating that these preparations were responsive to NO.

**Crucial role of adventitia-derived NO in the formation of NO stores in the media.** Adventitia-intact rings incubated for 20 h with LPS and L-Arg (but not those incubated for 5 h; not shown) and then precontracted with NE plus L-NAME relaxed on addition of NAC (0.1–10 mmol/l; Fig. 6A). It has been previously shown that this relaxation was due to an interaction of NAC with a preformed NO store (18). The relaxing effect of NAC was still observed in rings subjected to adventitia removal after incubation with LPS and L-Arg (Fig. 6B). However, when adventitia removal was performed before the 20-h incubation with LPS, no relaxation to NAC was observed (Fig. 6C).

**Crucial role of adventitia-derived NO in the formation of DNIC in the media.** Preincubation of the whole thoracic aorta with LPS and L-Arg for 20 h (but not for 5 h; not shown) resulted in the appearance of a distinct axial EPR signal with $g_\perp = 2.04$ and $g_\parallel = 2.015$ (Fig. 7A), which is characteristic of DNIC bound to sulfur groups of protein(s). The concentration of DNIC was estimated to be $5.1 \pm 0.6$ nmol/g. EPR signals for DNIC were not detected when 20-h incubations were performed in the presence of L-NAME, in the absence of L-Arg, or in the absence of LPS (not shown). Removal of

![Fig. 5](image1.png)  
**Fig. 5.** Representative traces showing the effect of ACh (1 μmol/l), L-arginine (L-Arg; 100 μmol/l), and S-nitroso-N-acetylpenicillamine (SNAP; 10 μmol/l) on norepinephrine (NE; 1 μmol/l)-precontracted endothelium-denuded aortic rings with (A) or without adventitia (B) obtained from rats pretreated with LPS (40 mg/kg for 5 h). Representative traces are of 3 experiments.

![Fig. 6](image2.png)  
**Fig. 6.** Representative traces showing the effect of increasing concentrations of N-acetylcysteine (NAC; 0.1, 1.0, and 10 mmol/l) on rat aortic rings preincubated in the presence of LPS (10 μg/ml for 20 h) (A), medial rings prepared from aorta preincubated with LPS (B), and medial rings preincubated with LPS (C). The rings were precontracted with NE (1 μmol/l) plus L-NAME (3 mmol/l). Representative traces are of 3 experiments.

![Fig. 7](image3.png)  
**Fig. 7.** EPR detection of dinitrosyl iron complexes. Whole thoracic aorta was preincubated in the presence of 0.6 mmol/l L-Arg and LPS (10 μg/ml) for 20 h (A). Media (B) and adventitia (C) were separated from aorta preincubated with L-Arg and LPS. Isolated media were preincubated with L-Arg and LPS (D). EPR spectra were recorded at 77 K. Representative traces are of at least 5 experiments.
the endothelium in aortas after incubation with LPS and l-Arg did not change the amount of DNIC (4.9 ± 0.7 nmol/g). The medial layer contained about four times more DNIC than the adventitia (3.1 ± 0.5 vs. 0.8 ± 0.1 nmol/g, P < 0.01; Fig. 7, B and C). However, incubation of the media alone in the presence of LPS and l-Arg for 20 h did not result in detectable DNIC formation (Fig. 7D).

**DISCUSSION**

In this study, we demonstrate that within rat aortas exposed to LPS, both in vitro and in vivo, NOS-2 expression and NO production were the highest in the adventitia. NOS-2 was predominantly localized in adventitial resident macrophages and fibroblasts. Moreover, there was an apparent sequence of expression of NOS-2 in the adventitia, starting from macrophages and subsequently involving fibroblasts. In addition, we provide evidence that adventitia-derived NO largely accounts for l-Arg-evoked relaxation and causes generation of a releasable NO store as well as protein-bound DNIC in the medial layer.

Aortic adventitia is a connective tissue layer mainly containing fibroblasts and the much less abundant resident macrophages, mast cells, endothelial cells of vasa vasorum, and Schwann cells associated with nerve axons (20). All of these cells potentially could express NOS-2 and produce large amounts of NO in response to LPS. We found that after 5 h of exposure to LPS (both in vitro and in vivo), most of the NOS-2-expressing cells were resident ED2-ir macrophages, and, vice versa, most ED2-ir macrophages were NOS-2-ir. The similar labeling pattern in vivo and in vitro experiments pleads against the possibility of massive recruitment of circulating NOS-2-ir cells and suggests a primary role of resident adventitial macrophages at early stages. After incubation of aortas with LPS for 20 h, NOS-2-ir cells largely outnumbered ED2-ir macrophages. As judged from methyl green-counterstained, immunoperoxidase-labeled frozen sections, most adventitial cells were NOS-2-ir, suggesting that fibroblasts were predominantly involved. Convincing evidence for expression of NOS-2 in the fibroblasts might be obtained by use of double staining with NOS-2 antibodies and fibroblast-specific antibodies. However, the fibroblasts are an extremely heterogeneous population, and a general marker for rat fibroblasts is still lacking (21). Nevertheless, on semithin sections of aortas incubated with LPS for 20 h, most NOS-2-ir cells exhibited morphological features of fibroblasts.

Cell culture experiments have shown that macrophages readily express NOS-2 in response to LPS applied as a single stimulus (16). In contrast, VSMC and fibroblasts actually express NOS-2 after stimulation with proinflammatory cytokines, such as interferon-γ, tumor necrosis factor, and interleukin-1, alone and/or in synergistic pairs (4, 7, 28). Because macrophages are known to release various cytokines after contact with bacterial products (26), NOS-2 expression in adventitial fibroblasts and VSMC might be a secondary event, being under control of macrophage-derived cytokines. The relative delay of NOS-2 expression in adventitial fibroblasts is consistent with this possibility. Immunohistological methods revealed NOS-2 protein only in the adventitia and in intima but not in the media. A similar observation was recently reported by another group (30). However, NOS-2 expression and NO generation in the medial layer were clearly detected in this study by Western blot and EPR spin trapping, respectively. The apparent discrepancy of the data obtained by different methods is unknown. In the immunohistochemical study, presence of NOS-2 in the medial layer might be underestimated because of the relatively low sensitivity of the applied techniques. In addition, in Western blot and EPR spin-trapping studies, the medial NOS-2/NO might be overestimated because of the potential presence of NOS-2-expressing intimal and perhaps adventitial cells.

The EPR spin-trapping technique with Fe-DETC as the NO-trapping agent permits direct registration of NO in intact tissue (27). Recently, the expression of the neuronal isoform NOS-1 has been described in the medial and/or adventitial layer of some rat blood vessels (6, 22). However, a substantial contribution of NOS-1-derived NO in our experiments is unlikely, because neither NOS-1 protein nor NO were found in the endothelium-denuded control aorta. In these preparations, the characteristic EPR signals were detected only after exposure to LPS. Additionally, both l-NAME and dexamethasone strongly decreased the signals, suggesting the role of inducible NOS-2 in NO overproduction. It is known that NOS-2 expressed in cultured macrophages generates not only NO but also superoxide anion and peroxynitrite (29). Thus the possibility is not excluded that, under inflammatory conditions, the adventitial NOS-2 may also be an important source of peroxynitrite and other reactive species.

l-Arg-induced relaxation is an intrinsic feature of blood vessels preexposed to LPS (8, 10). Previously, we have shown that, in rat aortas incubated with LPS, this relaxation was largely dependent on the presence of adventitia (13). Here, the crucial role of adventitia in l-Arg-induced relaxation was demonstrated in aortic rings obtained from endotoxemic rats. It is possible that adventitial NO may play a role in the downregulation of vascular contractility in septic shock, as infusion of l-Arg to septic patients leads to a decrease in blood pressure (15). The expression of NOS-2 in the adventitia of omental arteries obtained from some patients with peritonitis and septic syndrome was recently described (24).

Vascular contractility can be depressed not only via NO derived from current NOS activity but also via NO derived from a preformed NO store. The formation of a NO store has been demonstrated after exposure of blood vessels to low-molecular-weight DNIC (19), peroxynitrite (1), or LPS (18). Low-molecular-weight thiols such as NAC can accelerate the release of NO from the NO store, providing a vasorelaxation (18). The fact that NAC produced a relaxing effect in LPS-treated (20...
h) aortic rings both before and after removal of the adventitia suggests the presence of an NO store in the medial layer. In contrast, NAC did not induce relaxation in medial rings incubated with LPS. These data suggest the crucial role of adventitia in LPS-induced generation of NO store in media.

There are several candidates for the NO store, including protein-bound DNIC (18, 19). Indeed, in the present study, formation of a NO store in aortas was associated with generation of DNIC. Both events were evident after long-lasting (20 h) incubation with LPS. Interestingly, DNIC were predominantly localized in the media, whereas NOS-2 activity was higher in the adventitia. Because incubation of isolated media with LPS did not result in DNIC formation, these data altogether demonstrate the key role of the adventitia in DNIC formation in the media. The potential role of DNIC in blood vessels deserves further investigation. In addition to its NO-releasing properties, DNIC can exert nitrosative (5) and antioxidant (9) activity. It has also been suggested that DNIC play a role in cytotoxic effects of NO (11).

In conclusion, the present data reveal a new role for resident adventitial cells in the response of the rat aorta to a classical inflammatory stimulus, LPS. In this situation, adventitial macrophages and fibroblasts can potentially express NOS-2 and produce NO in greater amounts than other vascular NO sources. The adventitia-derived NO (or NO-related species) can reach the underlying vascular smooth muscle and may largely account for the downregulation of contraction induced by LPS. Potentially, the large amount of adventitial NO may not only affect contraction but may also initiate many other NO-dependent mechanisms in all three tunica. On the other hand, because of the cytotoxic and cytostatic properties of NOS-2-derived NO (16), the adventitia may become an efficient barrier, preventing the entry of invasive organisms or tumor cells into the circulation. Interestingly, the adventitia occupies 10% of the vascular wall in the thoracic aorta, one-half in muscular arteries, and two-thirds in large veins, and it is absent in arterioles and venules (20). It would be intriguing to speculate that the functional importance of adventitial NOS-2/NO may be proportional to the relative thickness of the adventitia in a given segment of vascular bed.

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