

Nitric Oxide Prevents Aortic Neointimal Hyperplasia by Controlling Macrophage Polarization

Begoña Lavin, Monica Gómez, Oscar M. Pello, Borja Castejon, Maria J. Piedras, Marta Saura, Carlos Zaragoza

Objective—Nitric oxide synthase 3 (NOS3) prevents neointima hyperplasia by still unknown mechanisms. To demonstrate the significance of endothelial nitric oxide in the polarization of infiltrated macrophages through the expression of matrix metalloproteinase (MMP)-13 in neointima formation.

Approach and Results—After aortic endothelial denudation, NOS3 null mice show elevated neointima formation, detecting increased mobilization of LSK (lineage-negative [Lin]-stem-cell antigen 1 [SCA1]+KIT+) progenitor cells, and high ratios of M1 (proinflammatory) to M2 (resolving) macrophages, accompanied by high expression of interleukin-5, interleukin-6, MCP-1 (monocyte chemoattractant protein), VEGF (vascular endothelial growth factor), GM-CSF (granulocyte-macrophage colony stimulating factor), interleukin-1 β , and interferon- γ . In conditional c-Myc knockout mice, in which M2 polarization is defective, denuded aortas showed extensive wall thickening as well. Conditioned medium from NOS3-deficient endothelium induced extensive repolarization of M2 macrophages to an M1 phenotype, and vascular smooth muscle cells proliferated and migrated faster in conditioned medium from M1 macrophages. Among the different proteins participating in cell migration, MMP-13 was preferentially expressed by M1 macrophages. M1-mediated vascular smooth muscle cell migration was inhibited when macrophages were isolated from MMP-13-deficient mice, whereas exogenous administration of MMP-13 to vascular smooth muscle cell fully restored migration. Excess vessel wall thickening in mice lacking NOS3 was partially reversed by simultaneous deletion of MMP-13, indicating that NOS3 prevents neointimal hyperplasia by preventing MMP-13 activity. An excess of M1-polarized macrophages that coexpress MMP-13 was also detected in human carotid samples from endarterectomized patients.

Conclusions—These findings indicate that at least M1 macrophage-mediated expression of MMP-13 in NOS3 null mice induces neointima formation after vascular injury, suggesting that MMP-13 may represent a new promising target in vascular disease. (*Arterioscler Thromb Vasc Biol.* 2014;34:1739-1746.)

Key Word: angioplasty ■ endothelial nitric oxide synthase 3 ■ macrophage ■ matrix metalloproteinase-13 ■ neointima

Vascular stents and percutaneous balloon angioplasty, despite their undoubted benefits, often have adverse effects, including restenosis of the vessel wall.^{1,2} Arterial wall restenosis is caused by mechanical disruption of the vascular endothelium, which, by unknown mechanisms, induces extensive smooth muscle and endothelial cell migration and proliferation, resulting in increased arterial wall thickness and a narrowing of the lumen.³⁻⁶

The increased cell migration, cell proliferation, and platelet aggregation that lead to neointima formation are triggered by nitric oxide (NO)-dependent endothelial dysfunction.⁷ The ability of endothelial NO synthase 3 (NOS3) to limit these events was demonstrated by viral and nonviral transfection in rabbits with NOS3, which significantly reduced neointima

formation and endothelial regeneration, and by pharmacological inhibition in rats.⁸⁻¹¹

The early response to arterial injury involves the recruitment, infiltration, and activation of M1 macrophages, which produce proinflammatory cytokines, chemokines, and basement membrane-degrading extracellular matrix metalloproteinases (MMPs), resulting in increased cell migration and proliferation. Polarization to M2 (resolving) macrophages induces the expression of several genes involved in wound healing and re-endothelization. Differentiated macrophages are thought to be able to switch between the M1 and M2 phenotypes, a phenomenon known as macrophage plasticity. This would imply that cells that initially promote an inflammatory response can later have anti-inflammatory properties and

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From the Fundación Centro Nacional de Investigaciones Cardiovasculares CNIC Melchor Fernandez Almagro 3, Madrid, Spain (B.L., M.G., O.M.P., B.C., C.Z.); Departamento de Fisiología, Facultad de Medicina, Universidad de Alcalá, Ctra Madrid-Barcelona, Alcalá de Henares, Spain (M.S.); and Facultad de Medicina, Universidad Francisco de Vitoria, Pozuelo de Alarcón, Madrid, Spain (M.J.P., C.Z.).

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Correspondence to Carlos Zaragoza, PhD, Facultad de Medicina, Universidad Francisco de Vitoria, Ctra Pozuelo-Majadahonda Km 1.800, 28223, Pozuelo de Alarcón, Madrid, Spain. E-mails cpatitos@gmail.com; c.zaragoza.prof@ufv.es

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Nonstandard Abbreviations and Acronyms	
IL-6	interleukin-6
MMP	matrix metalloproteinase
NOS	nitric oxide synthase
VSMC	vascular smooth muscle cell
WT	wild type

suggest that controlling the signals that trigger macrophage polarization presents potential for the therapeutic regulation of neointima formation in injured vessels.¹²⁻¹⁵

Macrophage inducible NOS (NOS2) drives the expression of several proinflammatory genes in M1 macrophages.¹⁶ Nitric oxide induces the expression and activity of collagenase MMP-13,¹⁷⁻²³ which regulates proliferation and migration of several cell types. The effect of NO-mediated MMP expression on neointima formation is largely unknown. Here we describe the contribution of macrophage polarization through MMP-13-mediated expression on neointimal formation in the absence of NOS3.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Lack of NOS3 Promotes Neointima Formation

To explore the role of intimal derived endothelial NOS3 on neointimal formation, we used a nonwire surgical denudation procedure, to prevent destruction of smooth muscle cells from tunica media, and a wire denudation injury for validation purposes in aortas from wild-type (WT) and NOS3 null mice. For a 30-day period after endothelial denudation, aortas lacking NOS3 showed significant neointimal formation compared with WT aortas in both procedures (Figure 1A).

We calculated the intima, intima/media, lumen, total vessel areas, and the amount of smooth muscle cells at the time points indicated finding significant differences between WT and NOS3 null aortas at 15 and 30 days after denudation, revealing an impaired remodeling of NOS3 knockout mice. Endothelial regeneration was also defective in these mice

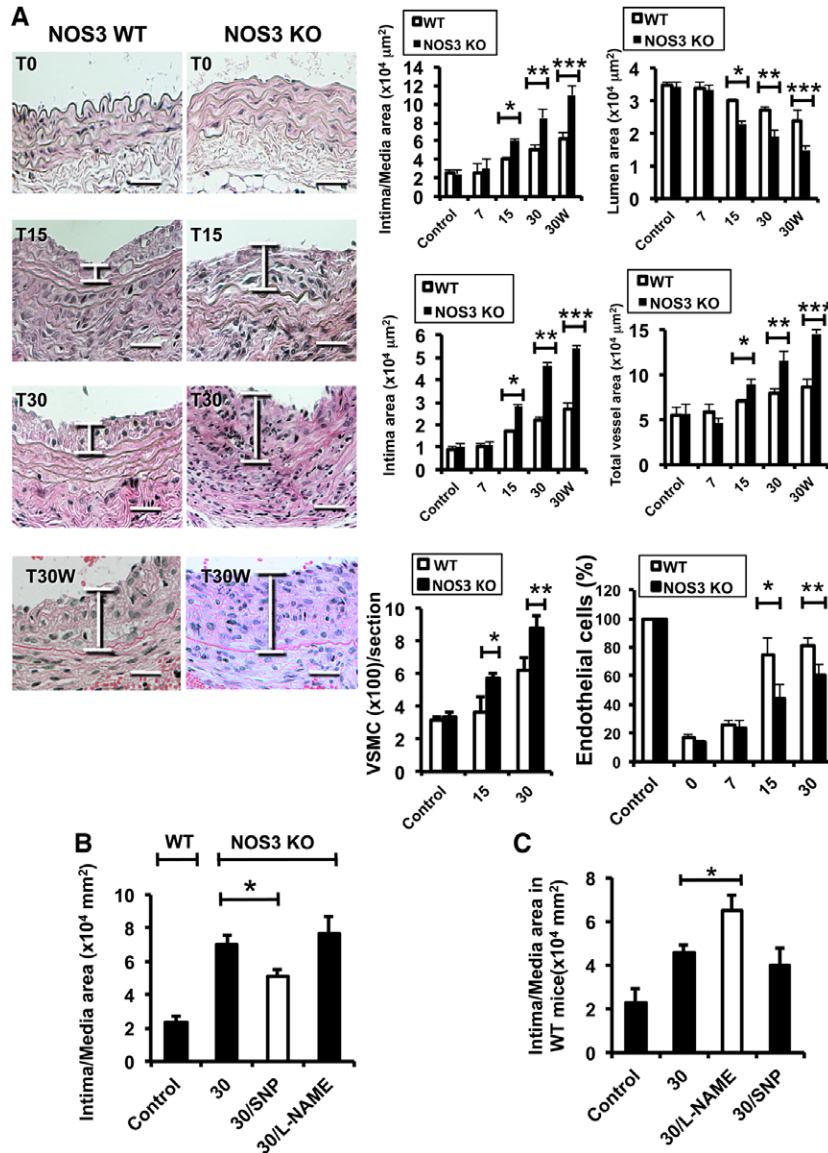


Figure 1. Lack of nitric oxide synthase 3 (NOS3) promotes neointimal formation and impairs endothelial regeneration in denuded abdominal aortas. **A**, Hematoxylin-eosin staining of aortic rings from wild-type (WT) and NOS3 knockout (KO) mice after endothelial denudation (0, 15, and 30 days). **Bottom**, WT and NOS3 aortic rings 30 days after wire injury [30W]. **Right**, Intima/media, lumen, intima, total vessel areas, endothelial cell, and vascular smooth muscle cell counts of the same aortas (n=6 mice/group per day by triplicate; mean±SD; *P<0.05 NOS3 KO 15 vs WT 15; **P<0.05 NOS3 KO 30 vs WT 30; ***P<0.05 NOS3 KO 30W vs WT 30). Scale bars, 50 μm. **B**, Intima/media area of denuded aortas from WT, NOS3 KO, and NOS3 KO mice weekly injected with 0.25 mg/kg per day of the NO donor sodium nitroprusside (SNP) or 50 mg/kg per day L-N^G-nitroarginine methyl ester (L-NAME) as indicated (n=6 mice/group per day by triplicate; mean±SD; *P<0.05 NOS3 KO 30 vs NOS3 KO 30+SNP; **P<0.05 NOS3 KO 30 vs NOS3 KO 30+L-NAME). **C**, Intima/media thickness of denuded aortas from WT mice injected with 50 mg/kg per day L-NAME or 0.25 mg/kg per day SNP as indicated (n=6 mice/group per day by triplicate; mean±SD; *P<0.05 WT 30 vs NOS3 KO 30; **P<0.05 WT 30 vs WT 30+L-NAME).

(Figure 1A, right). The protective effect of NOS3 was further verified in this model by intraperitoneal injection of the NO donor sodium nitroprusside into NOS3 null mice, showing significant regression of the neointimal layer (Figure 1B), whereas in WT mice injection of the NOS inhibitor L-NAME (L-N^G-nitroarginine methyl ester) increased intima/media thickness, mimicking the effect of NOS3 gene deletion (Figure 1C). Sodium nitroprusside and L-NAME had no effect in endothelial denuded WT and NOS3 null mice, respectively (Figure 1B and 1C).

Transplantation of NOS3 bone marrow into irradiated WT animals had no effect on neointima formation (Figure 1B in the online-only Data Supplement), whereas transplantation of WT bone marrow into NOS3-deficient mice significantly reduced wall thickness compared with NOS3 null mice transplanted with NOS3 null bone marrow and with non irradiated/bone marrow-transplanted NOS3 null mice (Figure 1C in the online-only Data Supplement), indicating that bone marrow from NOS3-expressing mice may participate in the prevention of neointimal formation in response to endothelial injury.

To explain why bone marrow from WT mice had such impact on NOS3 denuded aortas, we analyzed bone marrow LSK (lineage-negative [Lin]-stem-cell antigen 1 [SCA1]+KIT+) (multipotent hematopoietic progenitor cells) and myeloid progenitor cells from WT and NOS3 null animals, finding no differences in cell content between strains in basal conditions (Figure 1D in the online-only Data Supplement). However, in response to injury, at 7 days after endothelial denudation, NOS3 knockout mice contain twice LSK progenitor cells with respect to their WT counterparts in circulating blood (Figure 1E in the online-only Data Supplement, left), indicating that in the absence of NOS3 more progenitor cells are mobilized, and thereby NOS3 may prevent neointimal formation by controlling the migration of bone marrow-derived cells in response to endothelial denudation (Figure 1; Figure 1C in the online-only Data Supplement). Nevertheless, no differences were detected in myeloid progenitor cell mobilization between mice (Figure 1E in the online-only Data Supplement, right), suggesting a key role of lymphoid cell mobilization in NOS3 null mice.

Lack of NOS3 Induces Accumulation of M1 Proinflammatory Macrophages After Denudation

Aortic denudation provoked an inflammatory response characterized by leukocyte infiltration into the vessels. No differences in CD68+ cell content was observed in both strains, although in the absence of NOS3, there is an imbalance M1>M2 of proinflammatory macrophages per lesional area (CD68+/inducible NOS+), whereas in WT aortas extensive M2 infiltrated macrophages at 14 days after injury (CD68+/MRC1+ [mannose receptor, C type]) were detected, thus contributing to the shortening of inflammation (Figure 2A; Figure IIA in the online-only Data Supplement).

This was also evidenced in mice conditionally deficient for macrophage specific c-Myc expression (Figure 2B), which blocks their polarization to the M2 phenotype,²⁴ and in which extensive aortic wall thickening and M1 macrophage accumulation was also detected compared with NOS3-WT mice (Figure 2C; Figure IIB in the online-only Data Supplement).

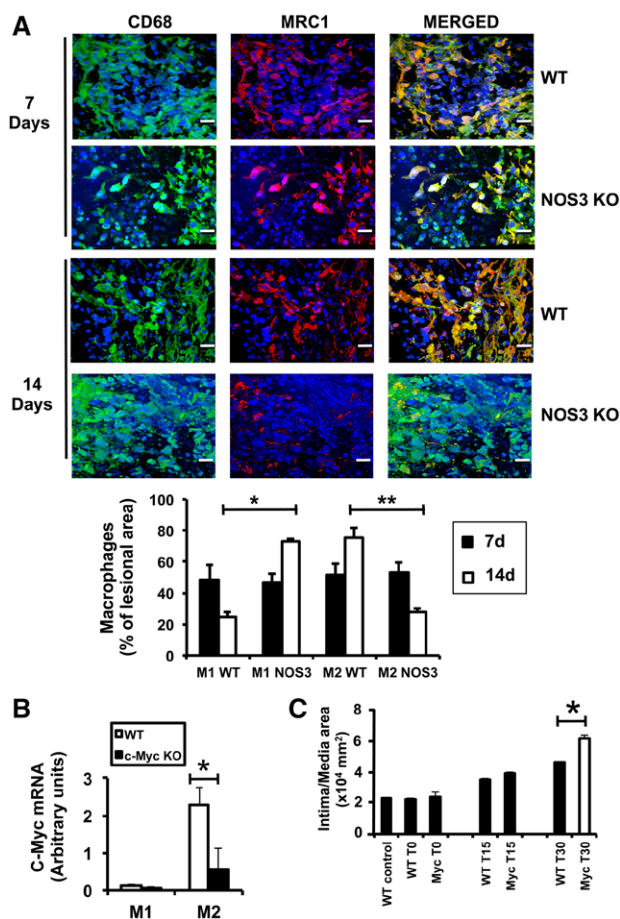


Figure 2. Mice lacking nitric oxide synthase 3 (NOS3) show increased levels of proinflammatory M1 macrophages after aortic endothelial denudation. **A**, Wild-type (WT) and NOS3 knockout (KO) aortic CD68+/MRC1- (mannose receptor, C type) (M1) and CD68+/MRC1+ (M2) infiltrated macrophages at 7 and 15 days after endothelial denudation. Representative images show en face confocal microscopic detection of CD68+ (fluorescein isothiocyanate, green), MRC1+ (Cy3, red), and nuclei (Hoecht, blue). Data were graphically represented as percentage of lesional area, (n=6 mice/group per day by triplicate; mean±SD, *P<0.05 M1 WT 14 vs M1 NOS3 14, **P<0.05 M2 WT 14 vs M2 NOS3 14. **B**, Bone marrow macrophages from WT and c-Myc conditional null mice were polarized to M1 and M2 macrophages, and the levels of c-myc mRNA were evaluated by quantitative reverse transcription polymerase chain reaction (n=6 mice/group by triplicate; mean±SD *P<0.05 M2 WT vs M2 c-Myc KO). **C**, Intima/media area of aortas collected from WT and c-Myc conditional null mice at 0, 15, and 30 days after endothelial denudation (n=6 mice/group by triplicate; mean±SD; *P<0.05 WT 30 vs Myc 30).

Lack of NOS3 Increases the Levels of Cytokines and Chemokines That Promote Polarization and Accumulation of Proinflammatory M1 Macrophages

The data shown below explain why NOS3 prevents abnormal remodeling of the vessel wall by dampening accumulation of proinflammatory versus resolving macrophages at the injured area. To investigate the role of NOS3 deficiency in the accumulation proinflammatory macrophages, we first measured 20 cytokines in plasma collected from NOS3-WT and NOS3 null mice after aortic denudation at the times indicated (Figure 3A). Increased levels of interleukin-5, interleukin-6 (IL-6), and monocyte chemoattractant protein

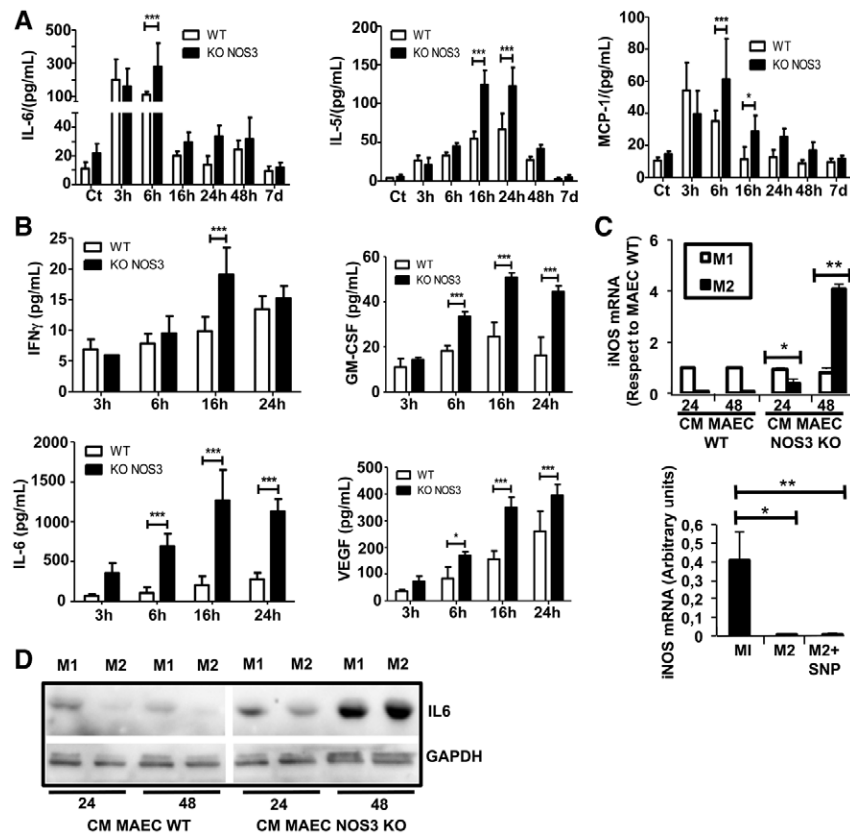


Figure 3. Vascular endothelium regulates macrophage polarization. **A**, Plasma levels of proinflammatory cytokines isolated from wild-type (WT) and nitric oxide synthase 3 (NOS3) null mice after aortic endothelial denudation at the times indicated ($n=3$ mice/group per day by triplicate; mean \pm SD; *** $P<0.05$ WT vs NOS3 knock-out [KO]). **B**, Multiplex analysis of secreted cytokines from injured WT and NOS3 null endothelial cell monolayers and collected at the times indicated ($n=3$ by triplicate; mean \pm SD; *** $P<0.05$ WT vs NOS3 KO). **C**, **Top**, Expression of inducible NOS (iNOS) by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in polarized M1 and M2 macrophages and incubated for 24 and 48 hours with conditioned medium from murine aortic endothelial cell (MAEC) WT and NOS3 KO ($n=3$ by triplicate; mean \pm SD; * $P<0.05$ M1 24 hours vs M2 24 hours; ** $P<0.05$ M1 48 hours vs M2 48 hours). **Bottom**, qRT-PCR expression of iNOS from bone marrow macrophages 24 hours after polarization into M1, M2, and M2-treated macrophages with the NO donor sodium nitroprusside (SNP; $n=3$ by triplicate; mean \pm SD; * $P<0.05$ M1 vs M2; ** $P<0.05$ M1 vs M2+SNP). **D**, Expression of interleukin-6 (IL-6) from M1- and M2-polarized macrophages and incubated for 24 or 48 hours with conditional medium from WT or NOS3 KO MAEC as indicated. Expression of GAPDH was assayed as control ($n=3$ by triplicate).

(MCP)-1 were detected in the first 24 hours after denudation. By contrast, the levels of monocyte chemoattractant macrophage colony stimulating factor (MCSF) and C-C motif ligand (CCL)5 proteins remained unchanged (Figure III in the online-only Data Supplement). To address whether vascular endothelial cells are responsible of such profile, we analyzed cell culture supernatants collected from wounded NOS3-WT and NOS3 null murine aortic endothelial cell monolayers. In these conditions, we also observed significant levels of proinflammatory cytokines and chemokines including IL-6, GM-CSF (granulocyte-macrophage colony stimulating factor), VEGF (vascular endothelial growth factor), interferon- γ , and interleukin-1 β in NOS3 null murine aortic endothelial cell (Figure 3B).

To assess whether NOS3 deficiency may also induce M2 repolarization to an M1 phenotype, we cultured WT M1- and M2-polarized bone marrow-derived macrophages in conditioned medium from endothelial cells isolated from WT or NOS3 null mouse aortas. Conditioned medium from NOS3 null murine aortic endothelial cell induced WT M2-polarized macrophages to express high levels of inducible NOS (Figure 3C, top), a classical marker of M1 proinflammatory activated macrophages (Figure 3C, bottom), together with marked levels of proinflammatory cytokine IL-6, compared with the levels found in M2 macrophages incubated with conditioned medium from WT murine aortic endothelial cell, as detected by immunoblot (Figure 3D).

Taken together, lack of NOS3 induces proinflammatory macrophage accumulation by inducing M1 polarization and repolarization of M2 macrophages to the M1 phenotype.

M2-Polarized Macrophages Slow Vascular Smooth Muscle Cell Proliferation and Migration in the Absence of NOS3

Vascular smooth muscle cell (VSMC) proliferation and migration are key steps during neointimal formation. To test whether proinflammatory or resolving macrophages may have an effect on these processes, we incubated VSMC with conditioned medium from M1- and M2-polarized macrophages, finding that cells proliferate more and migrate faster with conditioned medium from M1 respect to M2 macrophages (Figure 4A and 4B), and confirmed by exogenous administration the NO donor sodium nitroprusside to M2-polarized macrophages (Figure 4C). Interestingly, M1 and exogenous M2 macrophages incubated with sodium nitroprusside express high levels of MMP-13 mRNA and secreted MMP-13, a protein implicated the migration of different cell types, compared with polarized M2 macrophages (Figure 5A). To test whether macrophage-mediated MMP-13 may regulate VSMC migration, we found no significant differences when macrophages were isolated from MMP-13 null mice (Figure 5B), whereas exogenous administration of MMP-13 restored the migratory phenotype (Figure 5C). Taken together, M1 macrophage-mediated MMP-13 expression and activity induce VSMC migration in the absence of NOS3, shedding light about the mechanism by which NOS3 prevents neointimal formation.

MMP-13 Expression Regulates Neointima Hyperplasia in NOS3-Injured Vessels

To test whether MMP-13 plays a role in the absence of NOS3, we generated a double NOS3/MMP-13 null mouse strain

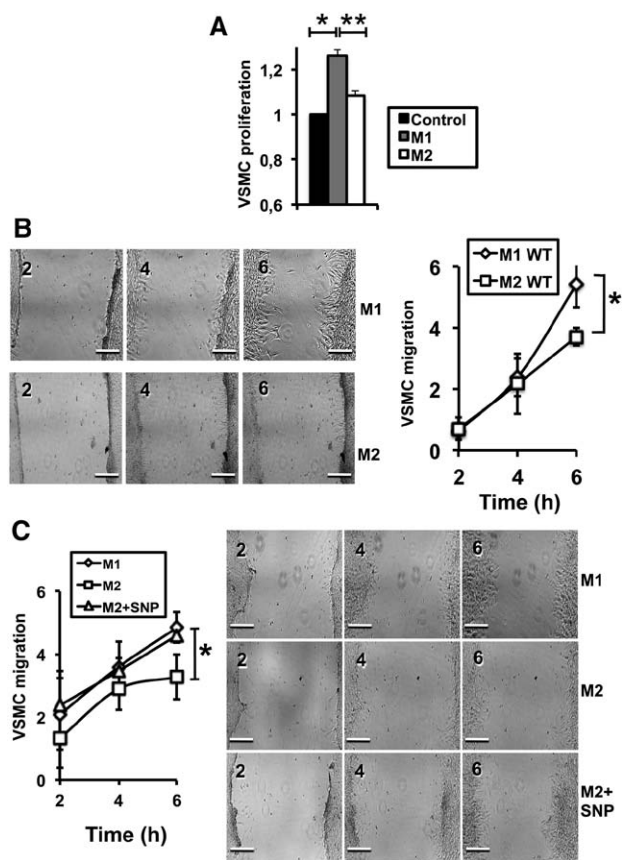


Figure 4. M1 macrophages regulate vascular smooth muscle cell (VSMC) proliferation. **A**, Proliferation of VSMC after 24 hours of incubation with conditioned medium from M1- and M2-polarized macrophages ($n=3$ by triplicate; mean \pm SD; * $P<0.05$ control vs M1; ** $P<0.05$ M1 vs M2). **B**, VSMC monolayers were injured, and migration of cells was evaluated at the times indicated ($n=3$ by triplicate; mean \pm SD; * $P<0.05$ M1 vs M2). Representative micrographs of migrating wounded cells taken at the times indicated are depicted. **C**, VSMC migration as in **B**, in which the contribution of exogenous nitric oxide administration (sodium nitropruside [SNP]) to M2-polarized macrophages was evaluated ($n=3$; mean \pm SD; * $P<0.05$ M2 vs M2+SNP). Scale bars, 100 μ m.

(Figure 6A). Although the absence of MMP-13 in NOS3-expressing vessels (MMP-13 null mice) has no effect on neointima formation, lacking of MMP-13 in the NOS3 null background (double NOS3/MMP-13 null mice) prevented the aortic wall thickening seen in mice singly deficient for NOS3 30 days after endothelial aortic denudation (Figure 6B).

Transplantation of MMP-13-deficient bone marrow (either from MMP-13 null mice or double MMP-13/NOS3 knockout mice) into irradiated NOS3 null animals significantly reduced wall thickness compared with NOS3 null denuded aortas from nonirradiated mice (Figure 6C), indicating that MMP-13-expressing bone marrow-derived cells may regulate neointimal formation in NOS3 null mice.

Consistent with these findings, we detected high levels of MMP-13 in cross sections of human aortic endarterectomies, together with high numbers of M1 (CD68+ inducible NOS+)-polarized macrophages, as detected by confocal microscopy, and extensive neointimal hyperplasia, whereas in healthy human arteries, MMP-13 was barely detected (Figure 6D). A

proposed mechanism is shown in Figure IV in the online-only Data Supplement.

Discussion

We show for the first time a MMP as a target of NOS3 in the prevention of arterial neointimal hyperplasia. In the absence of NOS3, aortic endothelial disruption increases neointimal formation by promoting inflammation through an imbalance of M1 proinflammatory versus M2 resolving macrophage infiltrates, as result of significant accumulation of several proinflammatory cytokines in plasma and in endothelial cells from NOS3-deficient mice. M1 macrophages expressing MMP-13 induce VSMC migration and proliferation, which are inhibited by M1 macrophages from MMP-13 null mice and reversed by exogenous replacement of the missing protease. Furthermore, in double NOS3/MMP-13-deficient animals, lack of MMP-13 was sufficient to prevent NOS3 null-dependent neointimal formation. These data, together with the detection of extensive MMP-13 and M1 macrophage colocalization in human carotid endarterectomies, suggest MMP-13 as a new target to prevent human neointima hyperplasia.

NOS3 prevents neointimal hyperplasia by limiting smooth muscle cell proliferation through as-yet undefined mechanisms.²⁵ Arterial wall thickness is reduced in animal models of vascular injury by experimental approaches, including NO delivery by exogenous systemic or local administration of NO donors, or by viral-mediated transfection with NOS3.^{26,27} However, no preclinical findings have been successfully implemented to date. Recent contributions elegantly describe the effect of NO in the ubiquitin-proteasome system^{28–30} and the inhibition of the PDGF-surviving pathway during flow-dependent vascular remodeling.³¹ Our work is the first to describe the role of NOS3 in the resolution of the immune response elicited in the vessel as result of endothelial denudation, identifying MMP-13 as a new target.

After endothelial denudation, monocytes infiltrate in response to chemokines produced by a variety of cells, including immune and endothelial cells. MCSF induces recruitment and differentiation of circulating monocytes into macrophages.¹³ The relevance of NOS3 to macrophage polarization has not been described to date. We found high levels of MCP-1 but not MCSF or CCL5 in plasma from NOS3 null mice, which may explain why monocyte recruitment is not affected by the absence of NOS3 (Figure 2A; total WT versus total knockout). However, the extensive expression of GM-CSF and interferon- γ in the absence of NOS3 may explain the increased M1 proinflammatory macrophage polarization in these mice.

The levels of IL-6 detected in vascular endothelium and plasma from NOS3-deficient denuded aortas are of significant interest. During the past decade, a large number of studies have demonstrated the importance of IL-6 in the progression of several inflammatory cardiovascular complications including unstable angina,³² coronary artery disease,³³ and myocardial infarction.³⁴ Coronary artery atherosclerosis is characterized by infiltration of activated macrophages, which generate high levels of IL-6. IL-6 is associated with increased mortality and also it serves to identify patients who may benefit most from early invasive strategies.^{35,36} Furthermore, heart transplanted patients treated with simvastatin have low levels of IL-6 and

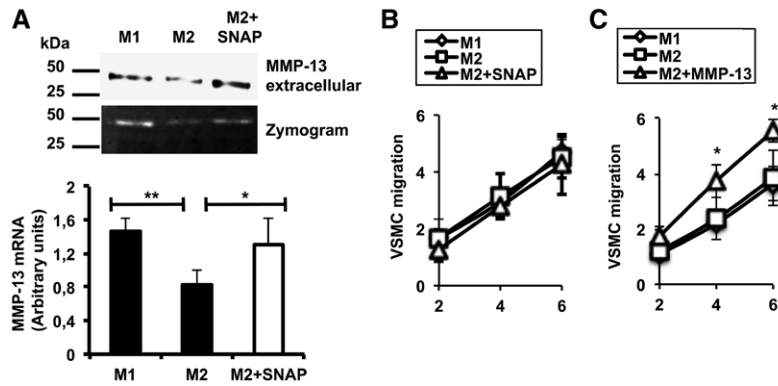


Figure 5. M1-mediated vascular smooth muscle cell (VSMC) migration depends on macrophage-dependent matrix metalloproteinase (MMP)-13 expression. **A**, Expression of MMP-13 in polarized macrophages. Immunoblot detection of MMP-13 (top), gelatin zymography (middle) from macrophage cell culture supernatants (top), and macrophage MMP-13 mRNA detection (lower graph) 24 hours after polarization into M1, M2, or into M2 macrophages and incubated with the nitric oxide donor sodium nitroprusside (SNP; n=3 by triplicate; mean±SD; *P<0.05 M2 vs M2+SNP; **P<0.05 M1 vs M2). **B**, VSMC migration in the presence of polarized M1, M2, and M2 +SNP macrophage supernatants isolated from MMP-13 null mice (n=3 by triplicate; mean±SD). **C**, VSMC migration as in **B**, in which cell monolayers were also incubated with exogenous recombinant active MMP-13 (n=3 by triplicate; mean±SD; *P<0.05 MMP-13 KO M2 4 hours vs MMP-13 KO M2+MMP-13 4 hours; **P<0.05 MMP-13 KO M2 6 hours vs MMP-13 KO M2+MMP-13 6 hours).

improved levels of endothelial function.³⁷ In this regard, inhibition of NO-mediated endothelial cell-driven relaxation is also strongly associated with increased levels of IL-6.^{38,39} The levels of IL-6 found in denuded aortas from NOS3 null mice further support the importance of IL-6 in the progression of neointimal formation.

MMPs play an important role in the proliferation and migration of several cell types,^{18,21} but the impact of NOS3-mediated MMP regulation of neointimal hyperplasia is yet unknown. Here, we found that MMP-13 expression is increased in M1 and NO-treated M2 macrophages and induces migration of VSMC. MMP-13 null M1 macrophages by contrast had no

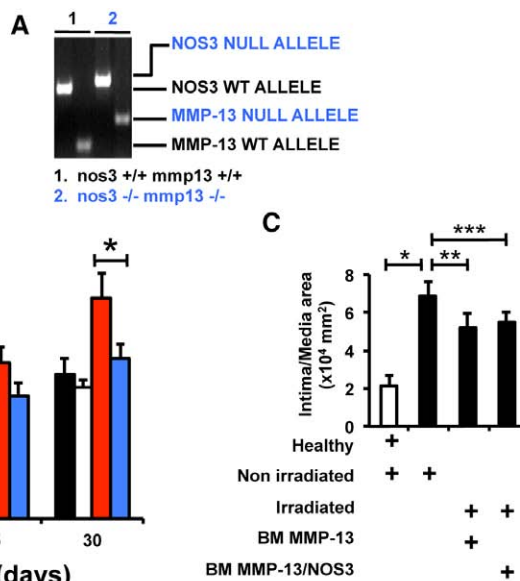


Figure 6. Lack of matrix metalloproteinase (MMP)-13 prevents neointimal thickening of nitric oxide synthase 3 (NOS3) null mice. **A**, DNA electrophoresis from wild-type (WT) and double NOS3- and MMP-13-deficient mice, showing the different allelic combinations as indicated. **B**, Aortic intima/media thickness from WT, MMP-13 null mice, NOS3 null mice, and NOS3/MMP-13 null mice 15 and 30 days after endothelial denudation (n=10 mice/group per day by triplicate; mean±SD; *P<0.05 NOS3 null 30 vs NOS3/MMP-13 null 30). **C**, Aortic intima/media thickness from a 30-day injured and transplanted NOS3-deficient mice with bone marrow from MMP-13 null or double MMP-13/NOS3 null mice (n=6 mice/group per day by triplicate; mean±SD *P<0.05 NOS3 healthy control [open bar] vs NOS3 knockout [KO] denuded 30 days; **P<0.05 NOS3 KO denuded 30 days vs NOS3 KO irradiated/BM MMP-13 KO; ***P<0.05 NOS3 KO denuded 30 days vs NOS3 KO irradiated/BM MMP-13/NOS3 KO). **D**, Immunohistochemical detection of MMP-13 in cross sections of carotid samples from endarterectomized patients and healthy mammary arteries. The white box corresponds to the carotid magnified region as also shown. Scale bars, 50 μm. **Bottom**, Confocal microscopy detection of CD68 (green, fluorescein isothiocyanate) and NOS2 (red Cy3) in the same carotid section as above. Colocalization of both signals is represented in yellow (merged). Arrows indicate colocalization of macrophage infiltrates and MMP-13.

effect on cell migration, unless exogenous MMP-13 is administered, pointing MMP-13 as a target of M1 macrophage-derived VSMC migration.

Macrophage expression of MMP-13 can be stimulated by proinflammatory cytokines, including endothelial IL-6, which is strongly upregulated in the absence of NOS3, thereby promoting proinflammatory macrophage accumulation and increased macrophage-mediated MMP-13 expression, as detected, and as described in osteoblasts,⁴⁰ fibroblasts,⁴¹ and cancer cells. High levels of MMP-13 were detected in carotid M1 infiltrated macrophages from patients undergoing carotid endarterectomy, with extensive neointimal hyperplasia. To this regard, in patients undergoing percutaneous transluminal coronary angioplasty, neointima hyperplasia might be reduced by promoting M1 proinflammatory macrophage polarization to M2 resolving macrophages, in which expression of MMP-13 may play a pivotal role.

Lack of MMP-13 neither induce neointimal hyperplasia in mice expressing NOS3 nor promotes M1 macrophage-mediated VSMC migration, whereas increased aortic thickness shown in the absence of NOS3 is significantly reduced in double NOS3/MMP-13-deficient mice. Taking all these findings together, we propose that NO may prevent neointimal formation in mice by at least targeting MMP-13.

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Disclosures

None.

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Significance

Arterial wall restenosis may be caused by mechanical disruption of the vascular endothelium, inducing extensive smooth muscle and endothelial cell migration and proliferation, resulting in increased arterial wall thickness and a narrowing of the lumen. We show the impact that arterial endothelium expressing nitric oxide has in the modulation of the inflammatory response, elicited as result of endothelial disruption, in which endothelial nitric oxide synthase 3 plays a critical role. Nitric oxide synthase 3 regulates macrophage polarization by preventing accumulation of proinflammatory macrophages, thereby contributing to the resolution of inflammation, and limiting restenosis. The significance of this finding is based in the effect on proinflammatory macrophage-derived matrix metalloproteinase-13 stimulation of smooth muscle cell proliferation and migration in the absence of nitric oxide synthase 3, proposing matrix metalloproteinase-13 as a novel target in the prevention of restenosis.