**Thioredoxin-1 Promotes Anti-Inflammatory Macrophages of the M2 Phenotype and Antagonizes Atherosclerosis**

Khadija El Hadri, Dler Faieeq Darweesh Mahmood, Dominique Couchie, Imene Jguirim-Souissi, Felicitas Genze, Vimala Diderot, Tatiana Syrovets, Oleg Lunov, Thomas Simmet, Mustapha Rouis

**Objective**—Oxidative stress is believed to play a key role in cardiovascular disorders. Thioredoxin (Trx) is an oxidative stress-limiting protein with anti-inflammatory and antiapoptotic properties. Here, we analyzed whether Trx-1 might exert atheroprotective effects by promoting macrophage differentiation into the M2 anti-inflammatory phenotype.

**Methods and Results**—Trx-1 at 1 μg/mL induced downregulation of p16INK4a and significantly promoted the polarization of anti-inflammatory M2 macrophages in macrophages exposed to interleukin (IL)-4 at 15 ng/mL or IL-4/IL-13 (10 ng/mL each) in vitro, as evidenced by the expression of the CD206 and IL-10 markers. In addition, Trx-1 induced downregulation of nuclear translocation of activator protein-1 and Ref-1, and significantly reduced the lipopolysaccharide-induced differentiation of inflammatory M1 macrophages, as indicated by the decreased expression of the M1 cytokines, tumor necrosis factor-α and monocyte chemoattractant protein-1. Consistently, Trx-1 administered to hyperlipoproteinemic ApoE2.Ki mice at 30 μg/30 g body weight challenged either with lipopolysaccharide at 30 μg/30 g body weight or with IL-4 at 500 ng/30 g body weight significantly induced the M2 phenotype while inhibiting differentiation of macrophages into the M1 phenotype in liver and thymus. ApoE2.Ki mice challenged once weekly with lipopolysaccharide for 5 weeks developed severe atherosclerotic lesions enriched with macrophages expressing predominantly M1 over M2 markers. In contrast, however, daily injections of Trx-1 shifted the phenotype pattern of lesional macrophages in these animals to predominantly M2 over M1, and the aortic lesion area was significantly reduced (from 100%±18% to 62.8%±9.8%; n=8; P<0.01). Consistently, Trx-1 colocalized with M2 but not with M1 macrophage markers in human atherosclerotic vessel specimens.

**Conclusions**—The ability of Trx-1 to promote differentiation of macrophages into an alternative, anti-inflammatory phenotype may explain its protective effects in cardiovascular diseases. These data provide novel insight into the link between oxidative stress and cardiovascular diseases. (Arterioscler Thromb Vasc Biol. 2012;32:00-00.)

**Key Words:** atherosclerosis | inflammation | macrophage | thioredoxin-1

A crucial step in atherogenesis/atherosclerosis is the infiltration of monocytes into the subendothelial space of large arteries and their subsequent differentiation into tissue macrophages.1 Macrophages residing within atherosclerotic lesions represent a heterogeneous cell population whose activation and function are influenced by various cytokines and microbial products. Thus, interleukin-1β (IL-1β), interferon-γ, and endotoxin lipopolysaccharide (LPS) increase the classical or inflammatory activation profile yielding the so-called M1 macrophages,2 which produce proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-6, and IL-12 as well as reactive oxygen species (ROS) and nitrogen intermediates.3,4 Consistently, M1 macrophages are associated with inflammation and tissue damage. In contrast, IL-4, IL-13,5,6 peroxisome proliferator–activated receptor-γ (PPAR-γ) activators,7 and adiponectin8,9 promote polarization of macrophages into the anti-inflammatory, alternative M2 type. M2 macrophages secrete the anti-inflammatory cytokine IL-10, transforming growth factor-β, and IL-1 receptor antagonist, and upregulate scavenger receptors, the mannose receptor CD206, and arginase-1. Accumulation of M2 macrophages leads to reduction of inflammation.

Increasing evidence suggests that risk factors for cardiovascular disease can lead to dramatic increases in the concentration of ROS in the vascular wall. When the rate of ROS formation exceeds the capacity of the antioxidant defense system, oxidative stress occurs, which markedly contributes to atherosclerosis.10 Various antioxidants, including dietary antioxidants protect against oxidative stress. However, their protective mechanisms are still not well defined.11

Received on: 24 April 2011; final version accepted on: March 22, 2012.
From the Unité de Recherche, UR-04, Vieillissement, Stress et Inflammation, Université Pierre et Marie Curie, Paris, France (K.E.H., D.F.D.M., D.C., IJ.S., V.D., M.R.); and Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, Helmholtzstr. 20, D-89081 Ulm, Germany (F.G., T.S., O.L., T.S.).
K.E. Hadri and D.F.D. Mahmood contributed equally to this work.

The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.112.249334/-/DC1. Correspondence to Mustapha Rouis, UR-04 Vieillissement, Stress et Inflammation, Université Pierre et Marie Curie-Paris 6, Bât. A. 5e étage/Case courrier 256, 7, Quai St-Bernard, 75252 Paris Cedex 5, France. E-mail mustapha.rouis@smv.jussieu.fr
© 2012 American Heart Association, Inc.
Thioredoxin-1 (Trx-1), a 12-kDa highly conserved protein, has recently been recognized as a critical protection system against oxidative stress.10,12 Trx-1 is important for keeping intracellular cysteine residues in the reduced state.13-15 It functions by the reversible oxidation of 2 Trx-specific redox-active cysteine residues (Cys-32 and Cys-35) to form a disulfide bond that, in turn, can be reduced by the action of Trx reductase and nicotinamide adenine dinucleotide phosphate. Trx-1 exerts most of its antioxidant ROS scavenging properties through the activity of Trx peroxidase.16 Trx-1 reduces the oxidized form of Trx peroxidase, and the reduced Trx peroxidase scavenges ROS17 not only in the cytosol but also in the nucleus. Because ROS can modify the activity of transcription factors, Trx-1 by limiting nuclear ROS levels also affects gene transcription.18-22 In addition, Trx-1 interacts with and inhibits the activity of the apoptosis signal–regulating kinase 113,14,18,23 as well as the tumor suppressor gene phosphatase and tensin homolog deleted on chromosome ten (PTEN).24 Through these mechanisms, Trx-1 inhibits apoptosis.

Trx-1 is negatively regulated by interaction with a specific inhibitor called thioredoxin-interacting protein (TXNIP),19,26 which exerts its activity in the cytosol as well as in the nucleus.27 Trx-1 is ubiquitously distributed and, despite its lack of a signal peptide, it can be secreted by several cell types through an unknown pathway.28,29 Indeed, Trx-1 is actively secreted by a variety of normal and transformed cells and neither brefeldin A nor dinitrophenol, 2 drugs that block the exocytic pathway and inhibit secretion of Trx, indicating that the latter does not follow the classical ER-Golgi route.30 It appears that the secretory mechanism for Trx-1 shares several features with the alternative pathway described for IL-1β.31 Nevertheless, translocation of Trx-1 to the membrane requires TXNIP and Trx-1 binding,32 indicating that this association might be instrumental for the secretion process. Because it is a ubiquitous protein, the precise origin of Trx-1 found in plasma is not known, but every tissue could contribute to its plasma level. The mechanism of cellular uptake remains undefined as well. However, in preliminary experiments with fluorescent-labeled Trx-1 we observed a specific and saturable binding on human macrophages, suggesting the presence of specific cellular receptors or binding sites for Trx-1 (unpublished data).

Apart from its intracellular function, Trx-1 may be released by cells and can act as a cytokine.29,31-34 We have previously shown in vitro that human recombinant Trx-1 downregulates the expression of a number of inflammatory genes such as IL-1β, TNF-α, IL-6, and IL-8 in human macrophages.35 It has also been shown that homocysteine (Hcy) can induce Trx-1 expression in human monocytes.36 and secreted Trx-1 was found to inhibit Hcy-induced ROS and monocyte chemotactant protein-1 (MCP-1) production in human monocytes.37 In addition, Trx-1 specifically cross-desensitizes monocytes to MCP-1.38 Moreover, a direct association of Trx-1 with macrophage migration inhibitory factor was reported, suggesting that Trx-1 on the cell surface serves as 1 of the migration inhibitory factor binding molecules or migration inhibitory factor receptor components, and it inhibits migration inhibitory factor–mediated inflammatory signals.39 Furthermore, overexpression of human Trx-1 in transgenic mice attenuated focal ischemic brain damage40 and increased the resistance to various oxidative stresses leading to longer survival compared with that of control mice.41

Although these in vitro and in vivo studies document anti-inflammatory properties of Trx-1, the effects of Trx-1 on the macrophage phenotype have not been addressed yet. We hypothesized that Trx-1 might promote polarization of human and murine macrophages toward an anti-inflammatory M2 phenotype. As an in vivo atherosclerosis model, we have chosen ApoE2.Ki mice expressing human ApoE2 (2/2), which virtually exhibits all characteristics of type III hyperlipoproteinemias in humans. Accordingly, their plasma cholesterol and triglyceride levels are 2× to 3× those of normolipidemic mice.42 These animals are markedly defective in clearing β-migrating very-low-density lipoprotein particles, and spontaneously develop atherosclerotic plaques, even on a regular diet. In apoE2.Ki mice on an atherogenic diet43 or exposed to LPS, an exacerbation of atherosclerosis is observed.42,44

**Methods**

For in vitro studies, both murine peritoneal and human macrophages were used. Cells were left untreated or treated with LPS, IL-4 or IL-4 + IL-13, Trx-1, or a combination thereof. In some samples, a specific antibody against full-length human Trx-1 was added. To study the binding of Trx-1, recombinant Trx-1 was fluorescently labeled and incubated with macrophages, and fluorescence images were taken using Zeiss LSM710 confocal scanning microscope. The expression of macrophage phenotype markers, CD206, MCP-1, IL-10, and TNF-α, and other involved genes, p16 INK4a, activator protein (AP)-1 and Rel-1, were investigated at both transcription and protein levels using real-time polymerase chain reaction and immunoblotting or ELISA, respectively. In vivo study was performed on C57Bl/6. ApoE2.Ki mice. Heart, liver, spleen, thymus, pancreas, and kidneys were excised, fixed, and immunohistochemical studies were performed. Serial paraaffin-embedded sections on proximal aorta sections were stained and the mean lesion area per animal was quantified. Human atherosclerotic vessel specimens from patients undergoing vascular surgery for atherosclerotic complications were fixed and human recombinant Trx-1 (5 mg/mL) for 24 hours. Data are presented as mean ± standard error of the mean (SEM). *P < 0.05, **P < 0.01 as indicated. One representative Western blot is shown in Figure 1A.
Thioredoxin (Trx)-1 promotes an anti-inflammatory phenotype in freshly isolated murine peritoneal macrophages. A, mRNA levels of CD206 after treatment with recombinant Trx-1 (1 µg/mL), interleukin (IL)-4 (15 ng/mL), and specific antibody against full-length human Trx-1 (5 µg/mL) for 24 hours. mRNA levels of CD206 were quantified with real-time polymerase chain reaction (qPCR) and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). B, Quantification of Western blots of CD206 and β-actin after treatment with recombinant Trx-1 (1 µg/mL), IL-4 (15 ng/mL), and specific antibody against full-length human Trx-1 (5 µg/mL) for 24 hours. Data are presented as mean±SE of 3 independent experiments performed in triplicate. **P<0.01 as indicated. One representative Western immunoblot was inserted.

Thus, in IL-4-treated cells, Trx-1 significantly increased the expression of CD206 mRNA from 2.8±0.6 a.u. to 4±0.2 a.u., P<0.05 (Figure 1A) and CD206 protein levels from 2.8±1.2 a.u. to 6±1.2 a.u., P<0.05 (Figure 1B). The effect of Trx-1 on macrophage polarization was specific, because it could be virtually abolished by an anti-Trx-1 antibody (Figure 1A and 1B).

Similarly, IL-4 induced the expression of IL-10 mRNA, 3.2±0.3 a.u. versus control cells 1.0±0.2 a.u., P<0.01 (Figure 2A) as well as protein, 33±7 pg/mL versus control cells 6.5±3.4 pg/mL, P<0.01 (Figure 2B), and this expression was amplified by Trx-1 from 3.2±0.3 a.u. to 4.8±0.2 a.u., P<0.01 (for mRNA) and from 33±7 pg/mL to 70±5 pg/mL, P<0.05 (for protein) (Figure 2A and 2B). The effect of Trx-1 on macrophage polarization was specific, because again it could be neutralized by an anti-Trx-1 antibody (Figure 2A and 2B). Analogous upregulation of CD206 and IL-10 expression by Trx-1 was observed in macrophages treated with IL-13 alone or in combination with IL-4 (data not shown). Moreover, a similar increase in M2 markers was also observed in human monocyte–derived macrophages treated with Trx-1 (Figure III in the online-only Data Supplement). Of note, we used Trx-1 at 1 µg/mL, because in pilot experiments this concentration exerted the maximum effect on macrophage polarization in vitro (data not shown).

**Trx-1 Attenuates the Expression of Murine M1 Macrophages Markers**

To determine whether Trx-1 affects the expression of the M1 macrophage markers, peritoneal macrophages were treated with LPS in the absence or presence of recombinant human Trx-1. LPS significantly induced the mRNA of the M1 macrophage marker TNF-α, 5.7±0.5 a.u. versus control cells 1.0±0.2 a.u., P<0.01 (Figure 3A) as well as protein, 3.0±0.5 ng/mL versus control cells 0.6±0.2 ng/mL, P<0.01 (Figure 3B). Trx-1 significantly reduced the expression of TNF-α in LPS-treated cells. Thus, Trx-1 reduced the LPS-induced expression of TNF-α mRNA from 5.7±0.5 a.u. to 3.0±0.4 a.u., P<0.01 as well as its protein from 3.0±0.5 ng/mL to 1.3±0.5 ng/mL, P<0.01 (Figure 3A and 3B). Moreover, this effect was also almost completely abolished by an anti-Trx-1 antibody (Figure 3A and 3B).

LPS also induced significant expression of another M1 marker, MCP-1. LPS increased MCP-1 mRNA from 1.0±0.3 a.u. (controls) to 7.6±0.6 a.u., P<0.001 (Figure 4A) and for...
Trx-1 Induces M2 Macrophage Polarization Through Downregulation of p16INK4a and Reduces M1 Polarization Through Downregulation of AP-1 and Ref-1

To determine the mechanism of macrophage polarization into the M2/M1 phenotype, we analyzed the effect of Trx-1 on the expression of the cyclin-dependent kinase inhibitor p16INK4a. Only recently, it has been shown that p16 INK4a deficiency (p16−/−) modulates the macrophage phenotype. Thus, p16−/− bone marrow–derived macrophages display a decreased response to classical polarizing by interferon-γ and LPS but an increased sensitivity to alternative polarization by IL-4. Moreover, Trx-1 downregulates MCP-1 expression and secretion in oxidized low-density lipoprotein–stimulated human endothelial cells by suppressing nuclear translocation of AP-1 and redox factor-1. Therefore, we hypothesized that, on the one hand, Trx-1 might induce macrophage polarization into the M2 phenotype through downregulation of p16INK4a and on the other hand, it might reduce macrophage polarization into the M1 phenotype through downregulation of AP-1 and Ref-1 expression. Indeed, our results confirmed that Trx-1 downregulated the expression of p16INK4a at the protein level in IL-4–stimulated macrophages (Figure 5A) and the expression of AP-1 and Ref-1 in LPS-induced macrophages (Figure 5B and SC).

Short-Term Effects of Trx-1 on M1/M2 Macrophages in Tissues of ApoE2.Ki Mice

Macrophages are highly plastic cells that enter target tissues and gain the phenotypic and functional attributes of their tissue of residence. To address such phenotypic changes, we analyzed the effects of Trx-1 administration on the expression of M1/M2 markers in murine tissues. Basal plasma level of Trx-1 in 7 mice (C57BL/6) is approximately 9 ng/mL (Mahmood et al, personal communication). For the in vivo study, we injected recombinant human Trx-1 at 30 μg/g of mouse body weight because in a pilot study, mice injected with various doses of Trx-1 showed the maximum effect at 30 μg/g of mouse body weight. The results showed no changes in the expression of M1 and M2 macrophage markers in spleen, pancreas, and kidneys (data not shown). However, in agreement
with the data obtained in vitro with mouse peritoneal and human macrophages, Trx-1 increased the number of CD206+ M2 and decreased the TNF-α+ M1 macrophages in thymus (Figure VA in the online-only Data Supplement). Moreover, it decreased the LPS-induced M1 marker TNF-α to an even greater extent than IL-4 and antagonized the LPS-induced decrease of CD206+ thymus macrophages (Figure VA in the online-only Data Supplement).

Trx-1 exhibited comparable effects on liver macrophages, where it potentiated the IL-4–induced expression of CD206 (Figure VA in the online-only Data Supplement) and inhibited the LPS-induced expression of TNF-α (Figure VB in the online-only Data Supplement). Trx-1 also reversed the LPS-induced decrease of CD206 (Figure VA in the online-only Data Supplement). Thus, Trx-1 promotes M2 macrophage markers and inhibits the M1 phenotype in tissue macrophages.

**Chronic Treatment With Trx-1 Decreases the Number of Lesional Macrophages and Reduces the Size of Atherosclerotic Lesions**

ApoE2.Ki hyperlipoproteinemic mice challenged repeatedly with LPS exhibit a significant increase in lesion size compared with control animals, as we have shown previously. Lesional macrophages in these mice express high levels of TNF-α and low levels of CD206 exhibiting predominantly the proinflammatory M1 phenotype. However, when LPS-injected ApoE2.Ki mice were additionally treated with human recombinant Trx-1, the amount of TNF-α+ M1 macrophages was significantly reduced, and the number of CD206+ M2 macrophages was significantly increased (Figure 6A). Moreover, Trx-1 significantly reduced the size of aortic atherosclerotic lesions in ApoE2.Ki hyperlipoproteinemic mice challenged with LPS, 100±18% (LPS-treated) versus 62.8±9.8% (Trx-1-treated LPS-challenged mice), n=8, P<0.01 (Figure 6B). Similarly, liver tissue of mice chronically treated with Trx-1 exhibited a reduced expression of M1 macrophage marker TNF-α but an enhanced expression of the M2 marker CD206 (Figure VB in the online-only Data Supplement).

**Trx-1 Colocalizes With M2 Macrophages in Human Atherosclerotic Lesions**

Previous studies have shown the presence of M1 and M2 macrophages in atherosclerotic lesions. However, it was unknown whether Trx-1 would colocalize with M1 or M2 markers in complicated atherosclerotic lesions. Not unexpected, the staining pattern of CD206 in serial sections of human atherosclerotic plaques confirmed that Trx-1-expressing cells colocalize with the M2 macrophage marker CD206. In contrast, Trx-1 does not colocalize with TNF-α, a marker of inflammatory M1 macrophages (Figure VI in the online-only Data Supplement), indicating that Trx-1 could promote the M2 differentiation in human atherosclerotic vessels as well.

**Discussion**

Macrophages possess a remarkable diversity, which depends on the type, duration, and concentration or intensity of environmental signals such as bacterial products and cytokines. Macrophages are heterogeneous, and subpopulations exhibit differential gene expression, release different cytokines and secondary mediators, and express different cell surface antigens. According to their phenotype, macrophages can be divided into 2 major populations: those which promote inflammation and those which limit inflammation and support tissue remodeling and angiogenesis. At variance to lymphocytes, which cannot be reprogrammed, macrophages retain plasticity, meaning that alteration of environmental signals could tip the balance between M1 and M2 populations. Changing the composition of macrophage population in tissues would affect the pathogenesis, evolution, and complication of many diseases including atherosclerosis. Therefore, identification of agents that might favorably modulate the balance of macrophage polarization is of great importance.

In the present study, we show that human Trx-1, an oxidative stress-limiting protein, potentiates the expression of pro-inflammatory M2 macrophages as evidenced by the characteristic markers CD206 and IL-10 through downregulation of p16(INK4a) expression. In the same time, Trx-1 blunted the expression of proinflammatory M1 markers such as TNF-α and MCP-1 in human monocyte-derived macrophages and in murine peritoneal macrophages through downregulation of AP-1 and Ref-1 transcription factor expression. The exact correlation between human macrophage subpopulations and their murine counterparts, particularly in atheroma, is still unknown. Nevertheless, we found similar effects in human as well as in murine macrophages. In addition, we complemented our in vitro experiments with in vivo studies using a hyperlipoproteinemic transgenic mouse model.
Our in vitro data showing promotion of the M2 over the M1 phenotype by Trx-1 were supported by in vivo data in this model. Trx-1, when administered intravenously to ApoE2-KI mice for 5 days or for 5 weeks, increased the expression of the M2 anti-inflammatory macrophage marker CD206 in thymus and liver, as well as in the atherosclerotic lesions. In contrast, the high expression of the macrophage M1 marker TNF-α observed in mice injected with LPS was significantly decreased by the Trx-1 treatment. Accordingly, the surface area of atherosclerotic lesions was significantly reduced in mice treated with Trx-1 compared with mice treated with LPS alone. Moreover, in human atherosclerotic plaques, we observed colocalization of Trx-1 with CD206, a marker of anti-inflammatory M2 macrophages, but not with TNF-α, a marker of inflammatory M1 macrophages.

Taken together, these studies indicate that Trx-1 functions as a regulator of macrophage phenotype differentiation tipping the balance toward the anti-inflammatory M2 state. As a consequence, atherosclerotic plaques would become smaller and more stable. Of note, plasma cholesterol and triglycerides levels in LPS-treated mice versus LPS- and Trx-1-treated mice were not significantly different (Table II in the online-only Data Supplement). However, whether the stability of atheroma plaques attributed to M2 phenotype could be explained by a difference in lipid metabolism and lipid accumulation in M1 in comparison with M2 macrophages was unknown. Therefore, we conducted additional experiments on murine and human macrophages to determine the level of ATP-binding cassette transporter A1 expression in the M1 or M2 phenotype. These results showed a decrease of ATP-binding cassette transporter A1 expression in M2 macrophages in comparison with proinflammatory M1 macrophages (data not shown), suggesting that M2 macrophages may have reduced reverse cholesterol efflux capacities. Of note, similar results have just been published by Chinetti-Gbaguidi et al. This group also observed that, indeed, ApoA-I- and HDL-mediated cholesterol efflux was significantly lower in [H]cholesterol-AcLDL–loaded M2 macrophages compared with M1 macrophages. The consequence of a defective cholesterol efflux in M2 macrophages is an increase of cholesterol esterification by inducing lysosomal acid lipase and acetyl-CoA acetyltransferase-1 gene expression. This is a protective mechanism against the toxicity of excess free cholesterol. Moreover, expression of both scavenger receptors SR-A and CD36 was similar in M2 and M1 macrophages. In contrast, M2 macrophages displayed a lower gene expression level of LOX-1 and caveolin-1 compared with M1 macrophages. M2 macrophages accumulate less oxidized and native LDL, an effect that is expected to lower the chance of foam cell formation. In our experimental conditions, Trx-1 enhances macrophage polarization into M2 phenotype, which might reduce the number of foam cells.

One well-established pathway by which Trx-1 might control the inflammatory response is by changing NF-κB and Nrf2 redox-sensitive signaling pathways. In addition, TXNIP, the endogenous inhibitor of Trx-1, can activate the Nlrp3 inflammasome leading to caspase-1-dependent IL-1β maturation. It is known that through autocrine and paracrine action secreted IL-1β could mediate β cell death and dysfunction as well as arterial inflammation. Thus, enhanced concentrations of Trx-1 could neutralize the TXNIP and therefore prevent the Nlrp3 inflammasome activation and IL-1β production.

Moreover, in a recent publication, PPAR-γ, a ligand-activated nuclear receptor with potent anti-inflammatory properties, was found to prime human monocytes into alternative M2 macrophages with anti-inflammatory properties. However, at variance to Trx-1, PPAR-γ activation does not influence the expression of M2 markers in resting or M1 macrophages, nor does treatment with a PPAR-γ agonist influence the expression of M2 markers in atherosclerotic lesions, indicating that only native monocytes can be primed by PPAR-γ activation shifting them toward the M2 phenotype. Because we have previously shown that PPAR-γ activation significantly downregulates the expression of the Trx-1 gene and upregulates the expression of Trx-1 inhibitor TXNIP in human macrophages, PPAR-γ could not account for the effects of Trx-1 and vice versa. In addition, different to PPAR-γ, Trx-1 induces M2 differentiation in atherosclerotic lesions and in tissue macrophages in vivo, and it is also able of reverting the LPS-induced macrophage M1 polarization. Therefore, Trx-1 seems to be a more versatile inducer of M2 differentiation compared with PPAR-γ.

Indeed, a number of reports indicate that Trx-1 has beneficial effects on ischemic reperfusion injury, LPS-induced neutrophil chemotaxis in the mouse air pouch model, as well as on focal ischemic brain damage and the resistance to oxidative stress. These pathological conditions all involve the activation of an acute or chronic inflammatory response. Thus, the ability of Trx-1 to change the balance between M1 and M2 macrophages, to promote an alternative, anti-inflammatory macrophage phenotype provides an attractive mechanism explaining its protective effects under these diverse pathological conditions.

Sources of Funding
This work was supported by La Fondation Coeur et Arteres, la Fondation de France, and the German Research Foundation (DFG). Part of the flow cytometry experiments were performed at the IFR8 (Université Pierre et Marie Curie, Paris, France).

Disclosures
None.

References
6. Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clear immunologic macrophage activation significantly downregulates the expression of the Trx-1 gene and upregulates the expression of Trx-1 inhibitor TXNIP in human macrophages, PPAR-γ could not account for the effects of Trx-1 and vice versa. In addition, different to PPAR-γ, Trx-1 induces M2 differentiation in atherosclerotic lesions and in tissue macrophages in vivo, and it is also able of reverting the LPS-induced macrophage M1 polarization. Therefore, Trx-1 seems to be a more versatile inducer of M2 differentiation compared with PPAR-γ.

Indeed, a number of reports indicate that Trx-1 has beneficial effects on ischemic reperfusion injury, LPS-induced neutrophil chemotaxis in the mouse air pouch model, as well as on focal ischemic brain damage and the resistance to oxidative stress. These pathological conditions all involve the activation of an acute or chronic inflammatory response. Thus, the ability of Trx-1 to change the balance between M1 and M2 macrophages, to promote an alternative, anti-inflammatory macrophage phenotype provides an attractive mechanism explaining its protective effects under these diverse pathological conditions.
in atherosclerotic lesions and in tissue macrophages in vivo, in addition, different to PPAR-
- could not account for the effects of Trx-1 and vice versa. In

A number of reports indicate that Trx-1 has been shown that PPAR-


Thioredoxin-1 Promotes Anti-Inflammatory Macrophages of the M2 Phenotype and Antagonizes Atherosclerosis
Khadija El Hadri, Dler Faieeq Darweesh Mahmood, Dominique Couchie, Imene Jguirim-Souissi, Felicitas Genze, Vimala Diderot, Tatiana Syrovets, Oleg Lunov, Thomas Simmet and Mustapha Rouis

Arterioscler Thromb Vasc Biol. published online April 19, 2012;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2012/04/19/ATVBAHA.112.249334

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2012/04/19/ATVBAHA.112.249334.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/
### Supplemental Table I. Sequences of primers used for qPCR analysis

<table>
<thead>
<tr>
<th>Species</th>
<th>Genes</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-10</td>
<td>Forwards 5'-GATCCAGTTTTACCTGGAGGAG-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-CCTGAGGGTCTTCAGGTTC-3’</td>
</tr>
<tr>
<td></td>
<td>CD206</td>
<td>Forwards 5'-CGAGGAAGAGGTTTCAGGTT-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-GCAATCCCGGTTCATGGA-3’</td>
</tr>
<tr>
<td></td>
<td>TNF-a</td>
<td>Forwards 5'-CAGAGGGCCTGTACCTCATC-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-GGAAGACCCCTCCACAGTAG-3’</td>
</tr>
<tr>
<td></td>
<td>MCP-1</td>
<td>Forwards 5'-CCCAGTCACCTGCTTGTTAT-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-TGGAATCCTGAACACCACCT-3’</td>
</tr>
<tr>
<td></td>
<td>Cyclophilin</td>
<td>Forwards 5'-GCATACGGGTCTGGCATTC-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-ATGGTGATCTTTCTGCAGGT-3’</td>
</tr>
<tr>
<td>Mouse</td>
<td>IL-10</td>
<td>Forwards 5'-AACATACGTGACTACCAGACCTCCT-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-CTGCCTTGCTTTATTTTCACA-3’</td>
</tr>
<tr>
<td></td>
<td>CD206</td>
<td>Forwards 5'-CACCGGTTGGCACGGAGA-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-CAGCTTGATGGACTCTGGTAAG-3’</td>
</tr>
<tr>
<td></td>
<td>TNF-a</td>
<td>Forwards 5'-TCACCCACACCCGTCAAGGC-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-CAACCCATTCCCACAGGCAA-3’</td>
</tr>
<tr>
<td></td>
<td>MCP-1</td>
<td>Forwards 5'-CGACATCCCTGGAACTGCCCT-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-CAGCCAGATGCAGTTAACAAG-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-CACGTGCCCTGTCGTTCAC-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GCCTACTGATGGGATCATCTTG-3’</td>
</tr>
<tr>
<td></td>
<td>GAPDH</td>
<td>Forwards 5'-TCAACGGCAGCAGTCAAGG-3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse 5'-ACTCCACGACATACTCAGC-3’</td>
</tr>
</tbody>
</table>