Protective Role of Uncoupling Protein 2 in Atherosclerosis

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Background—Uncoupling protein 2 (UCP2) regulates the production of reactive oxygen species in macrophages. However, its role in atherosclerosis is unknown.

Methods and Results—Irradiated low-density lipoprotein receptor deficient mice (LDLR-/-) were transplanted with bone marrow from either UCP2 deficient mice (Ucp2/-/-) or wild type mice (Ucp2+/+). Mice were fed an atherogenic diet for 7 weeks. Engraftment of bone marrow cells was confirmed by the presence of UCP2 protein expression in spleen cell mitochondria of Ucp2+/+ transplanted mice and its absence in Ucp2-/- transplanted mice. Leukocyte counts and plasma cholesterol levels were comparable in both groups. We found a marked increase in atherosclerotic lesion size in the thoracic aorta of Ucp2-/- transplanted mice compared with control Ucp2+/+ transplanted mice (8.3±0.9% versus 4.3±0.4%, respectively; P<0.005), as well as in the aortic sinus (150 066±12 388 μm² versus 105 689±9 727 μm², respectively; P<0.05). This was associated with increased nitrotyrosine staining, which suggests enhanced oxidative stress. Analysis of plaque composition revealed a significant increase in macrophage accumulation (P<0.05) and apoptosis (P<0.05), along with a decrease in collagen content (P<0.05), suggesting a potentially more vulnerable phenotype.

Conclusion—These results suggest a protective role for UCP2 against atherosclerosis. (Circulation. 2003;107:388-390.)

Key Words: atherosclerosis ■ free radicals ■ inflammation

Atherosclerosis is a chronic arterial inflammatory disease characterized by the accumulation of lipids, inflammatory cells, smooth muscle cells, and extracellular matrix in the arterial intima. Numerous studies suggest that reactive oxygen species (ROS) are involved in plaque formation. Oxidation of low-density lipoprotein seems to be one of the first steps in atherosogenesis, allowing endothelium activation and leading to the recruitment of macrophages and lymphocytes within the arterial wall. Once assembled, all plaque cellular components may respond to and be damaged by ROS, which may contribute to plaque progression. Indeed, symptomatic human plaques seem to undergo more oxidative phenomena than asymptomatic ones.

Given the strong evidence in favor of a role for oxidative stress in atherosclerosis, recent studies have been conducted to identify the source(s) of ROS production in the plaque and their respective roles in plaque development. A significant role for the NADPH oxidase and lipo-oxygenase enzymes in this process has been reported. We investigated whether mitochondrial uncoupling protein 2 (UCP2)-dependent ROS production can also influence the development of atherosclerosis. UCP2 is a newly discovered member of the mitochondrial anion carrier family and shares 80% identity with UCP3 (expressed only in muscle) and 60% sequenced identity with the well known thermogenic UCP1 from brown adipose tissue. UCP2 is expressed in the lung, spleen, intestine, white adipose tissue, and macrophages. The protein is strongly induced by lipopolysaccharide treatment. In contrast to UCP1, the uncoupling activity of UCP2 and UCP3 is still matter of debate. It has been suggested, however, and later established that UCP2 and UCP3 play a major role in the regulation of ROS production. The role of mitochondrial ROS in macrophages has been recently shown by the intriguing phenotype of the Ucp2-/- mice, which survived infection by Toxoplasma gondii, an intracellular parasite. Isolated macrophages from Ucp2-/- mice exhibited a higher toxoplasmaacidal activity linked to a higher level of ROS.

To address the specific role of UCP2 in atherosclerosis, we performed bone marrow transplantation (BMT) into irradiated low-density lipoprotein receptor-deficient (LDLR-/-) mice with either wild type or Ucp2-/- donors. Cholesterol levels, atherosclerotic lesion size, and composition were compared between both groups after an atherogenic diet.
Methods

Mice

Ucp2-/- mice from our own colony (Animal Resource Center, CNRS UPR9078, Meudon, France) were transferred onto a C57BL/6J genetic background (98.4% C57BL/6J, 1.6%129SvJ). Littermates were used as controls. Female C57BL/6J LDLR-/- mice were 8 weeks old.

Bone Marrow Transplantation and Atherogenic Diet

Medullar aplasia was induced by 9.5 Gray total body irradiation. Mice were reconstituted intravenously with bone marrow cells (1.2x10^6) extracted from the femur and tibia of either Ucp2 +/- or Ucp2-/- mice. Mice recovered for 4 weeks and were then subjected for 7 weeks to a diet containing 15% fat, 1.25% cholesterol, 0% cholate.

Since spleen cells are very sensitive to irradiation. BMT success was assessed by determining UCP2 protein expression in isolated spleen cell mitochondria using Western blot as described. Plasma levels of total and high-density lipoprotein (HDL) cholesterol were measured using Infinity Cholesterol Reagents (Sigma).

Morphometric Analyses

The basal half of the ventricles, aortic sinus, and spleen were frozen. Serial sections of the aortic sinus were assayed for lipid deposition and collagen detection (with Oil Red and Sirius Red, respectively). Immunohistochemistry was performed as previously described by using a monoclonal rat anti-mouse macrophage monoclonal antibody, clone MOMA-2 (Biosource International), a polyclonal goat anti-CD 3 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif), a monoclonal anti α-smooth muscle actin, clone 1A4 (Sigma), and a rabbit polyclonal anti-nitrotyrosine antibody (Upstate Biotechnology). Apoptosis assay was performed using the ApopDETEK kit (DAKO) based on the TUNEL method (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling). Oil Red staining and lesion quantification in the thoracic aorta were performed as previously described.

Statistical Analysis

Data are expressed as mean±SEM. Comparisons between groups were made by one-way ANOVA. A P<0.05 was considered statistically significant.

Results

Engraftment of bone marrow cells was confirmed by Western blot analysis showing UCP2 protein expression in spleen cell mitochondria in Ucp2 +/- transplanted mice and its absence in Ucp2-/- transplanted mice (Figure 1). Animal weights and plasma cholesterol levels were comparable between the 2 groups (Table). Leukocyte counts were similar in both groups (data not shown).

Atherosclerotic Plaque Size

The mean size of the atherosclerotic plaques in the aortic sinus of Ucp2-/- transplanted mice revealed a significant 42% increase compared with Ucp2 +/- transplanted mice (Table). Likewise, the mean percentage of plaque surface in the thoracic aorta showed a marked 93% increase in Ucp2-/- transplanted mice compared with controls (Table).

Figure 2. Representative examples of MOMA2 immunostaining in red (a and b), TUNEL staining in red (arrows in c and d), Sirius red (arrows in e and f), and nitrotyrosine staining in dark brown (g and h) in Ucp2 +/- (a, c, e, and g) and Ucp2-/- (b, d, f, and h) transplanted mice. m indicates media; L, lumen.
Figure 2d and 2e). Despite the larger size of the lesions in Ucp2-/- mice, there was a 30% decrease in collagen content (10.9 ± 1.1% in Ucp2-/- mice versus 15.5 ± 2.0% in Ucp2+/+ mice, P < 0.05; Figure 2f and 2e, respectively).

There were no significant differences either in CD3 positivity or in α-actin staining between groups (86.6 ± 23.3 T lymphocytes/mm² in Ucp2-/- mice versus 69.1 ± 10.4 in controls, and 2.5 ± 0.4% α-actin staining in Ucp2-/- mice versus 3.5 ± 0.8% in controls).

To confirm increased ROS production in plaques of Ucp2-/- mice, we evaluated the presence of nitrotyrosine as an indirect marker of peroxynitrite generation that results from the reaction between nitric oxide and superoxide. Nitrotyrosine staining was barely detectable in Ucp2+/+ mice (Figure 2g) but was strongly positive in Ucp2-/- mice (Figure 2h).

**Discussion**

Oxidative stress has long been thought to be involved in the process of atherogenesis. Thus, different approaches to stop ROS production aiming to alter disease progression have been used. Apolipoprotein E (apoE)-/- mice lacking the p47phox subunit of the NADPH oxidase develop smaller thoracic aorta plaques, 4 as do atherosclerosis-prone mice lacking 12-15-lipoxygenase. 5, 6 In contrast, apoE-/-/glycoprotein 91-/- mice exhibit no reduction in lesion size. 20

The production of ROS by the macrophage, the main cellular component of the plaque, has been shown to be in part regulated by UCP2 under various stress conditions. 15, 21 Therefore, we examined the specific role of UCP2 deficiency in blood cells on the development and composition of early atherosclerotic lesions. Ucp2-/- transplanted mice had significantly larger aortic sinus and aorta lesions than their controls despite similar cholesterol levels, indicating a protective role for blood cell-derived UCP2 in atherosclerosis.

Plaque composition was also altered toward a potentially more vulnerable phenotype. The percentage of lesion cross-sectional area occupied by macrophages was significantly increased in Ucp2-/- transplanted mice compared with Ucp2+/+ mice, suggesting an enhanced inflammatory response. Moreover, plaques of Ucp2-/- transplanted mice contained less collagen with no decrease in smooth muscle cell content, suggesting increased collagen degradation rather than decreased production. These observations are consistent with matrix metalloproteinase activation in the context of increased ROS generation, 22 which is suggested by the positive nitrotyrosine staining in plaques of Ucp2-/- mice. Furthermore, we found increased levels of apoptotic cell death in the plaques of Ucp2-/- transplanted mice, which could contribute to enhanced lipid core size and thrombogenicity. 23

In conclusion, the lack of UCP2 in blood cells accelerated atherosclerotic plaque development and induced a macrophage-rich but collagen-poor plaque phenotype, which suggests a protective role for blood cell-derived UCP2 in the early stages of atherosclerosis. Finally, this BMT experiment cannot exclude a protective role of vascular cell-UCP2 in atherosclerosis.

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