Reactive Oxygen Species and Death

Oxidative DNA Damage in Atherosclerosis

Martin R. Bennett

Reactive oxygen species (ROS) (eg, superoxide, peroxide, and hydroxyl radicals) and reactive nitrogen species (eg, peroxynitrite) are generated in both atherogenesis and advanced atherosclerosis, particularly by macrophages. ROS have many actions, including oxidative modification of LDL and oxidative damage of DNA.

Oxidative Modification of LDL

Although LDL is essential to deliver cholesterol to tissues, increased LDL cholesterol is associated with increased risk of cardiovascular disease. Oxidative modification of LDL promotes recruitment and retention of monocytes with formation of fatty streaks, the earliest lesions in atherosclerosis. Both macrophages and vascular smooth muscle cells (VSMCs) bind oxidized LDL via specific scavenger receptors, forming foam cells. Macrophage foam cells contain potent oxidant-generating systems that target lipids, including myeloperoxidase, nitric oxide (NO) synthase, and 15-lipoxygenase, allowing increased recognition and uptake by macrophages, creating a positive feedback loop.

Oxidative Damage to DNA

ROS also induce oxidative damage of DNA, including strand breaks and base and nucleotide modifications, particularly in sequences with high guanosine content. Oxidative modification induces a robust repair response, characterized by excision of modified bases and nucleotides. Double-stranded DNA breaks also activate DNA repair enzymes, including ATM (mutated in ataxia telangiectasia) and ATR (ATM-related kinase). Both ATM and ATR directly phosphorylate and activate specific checkpoint kinases, such as chk2 and hCDS1, with subsequent phosphorylation of the tumor suppressor gene p53.

p53 is the commonest mutation in human cancer and has a major role in genomic surveillance. p53 stimulates base excision repair but also coordinates the cell’s response to damage. p53 phosphorylation stabilizes the protein and increases its transcriptional activity, inducing both growth arrest and apoptosis. Thus, ROS-induced DNA damage leads to p53 activation, and growth arrest and apoptosis after DNA damage depend partly on p53.

Although the presence of ROS within the atherosclerotic plaque is not disputed, the major target cells of ROS in vivo are unclear. In particular, ROS induce toxicity in many vascular cell types in culture. In view of this, the study by Martinet et al in this issue of Circulation Research provides important insights into the role of ROS in atherogenesis and plaque stability. These investigators demonstrated that cholesterol feeding of rabbits induces oxidative damage in plaques, manifested by expression of 8-oxo-G, an oxidative modification of guanine residues in DNA, DNA strand breaks, and apoptosis. A graded response was seen, the percentage of cells expressing markers being 8-oxo-G>strand breaks>apoptosis, consistent with fewer cells demonstrating increasing degrees of oxidative damage. These changes were associated with expression of DNA repair enzymes and p53. Importantly, these markers of oxidative damage were rapidly reversible with cholesterol lowering.

The study by Martinet et al follows a body evidence implicating p53 as a regulator of macrophage number in atherosclerosis. Macrophages seem to be the major dividing cell in advanced atherosclerosis, particularly after plaque rupture. p53 is expressed in inflammatory cell–rich areas of the plaque, and plaques in p53-null animals are more extensive and show increased cell proliferation compared with control animals, suggesting that p53 limits macrophage cell number. In addition, Martinet et al found a negative correlation between markers of cell proliferation and DNA damage or p53 expression.

Importantly, this study also demonstrates that macrophages are targets for ROS-induced DNA damage, themselves a major source of ROS. Indeed, previous studies have found that apoptotic macrophages in human atherosclerotic plaques are activated, express antigen, and are oxidatively stressed. Thus, local ROS generation may induce both macrophage growth arrest and apoptosis. The recent view of macrophages in atherosclerosis hypothesizes that activated macrophages promote plaque instability by secretion of metalloproteinases that degrade matrix and collagen. In addition, macrophage products containing NO can induce VSMC apoptosis, with consequent reduction in collagen synthesis. Thus, plaque stability has been considered to be a balance of negative effects of macrophages and positive effects of VSMCs; reduction in macrophage number by inhibiting proliferation or apoptosis would therefore be considered beneficial. Indeed, cholesterol lowering and treatment with HMGCoA

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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Circulation Research is available at http://www.circresaha.org

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reductase inhibitors, therapies with proven clinical benefit, reduce macrophage content of human and animal lesions.

In contrast, recent studies showing that macrophage apoptosis colocalizes with plaque rupture in humans suggest that this viewpoint may misrepresent the complex plaque microenvironment. One explanation for colocalization is that macrophage apoptosis directly promotes necrotic core formation within advanced lesions. Thus, most apoptosis in advanced lesions occurs in macrophages located around the necrotic core. The necrotic core has the major procoagulant activity of the lesion, being partly attributable to microparticles formed by apoptosis of inflammatory cells. Colocalization of apoptotic macrophages with plaque rupture may therefore reflect apoptotic macrophages promoting core formation; rupture of plaques with more extensive cores may cause more extensive coagulation, vessel occlusion, and cardiac death. However, Kolodgie et al. found that macrophage infiltration and apoptosis in the fibrous cap were associated with plaque rupture. This argues strongly that macrophage apoptosis, or processes that ultimately lead to apoptosis, promotes both atherogenesis (via core formation) and plaque instability, possibly by a separate effect in the fibrous cap. Thus, oxidative damage to macrophages may directly promote plaque rupture in addition to promoting necrotic core formation.

Rates of VSMC apoptosis are also increased in advanced atherosclerotic plaques versus normal vessels and in unstable versus stable lesions, suggesting that VSMC apoptosis promotes plaque rupture. VSMC apoptosis also promotes thrombosis and monocyte recruitment via expression of monocyte chemoattractants. Thus, VSMC apoptosis in advanced atherosclerosis seems to be uniformly detrimental. Although the triggers for plaque VSMC apoptosis are unknown, VSMC apoptosis occurs in areas of high macrophage content, macrophage cytokines and NO induce p53, VSMC apoptosis colocalizes with p53 expression, and p53 induces human plaque VSMC apoptosis. This suggests the attractive hypothesis that ROS-activated macrophages induce VSMC apoptosis through cytokine or NO induction of p53. Against this hypothesis, Martinet et al. found that VSMC apoptosis occurred in cell-poor areas not associated with DNA synthesis or repair.

The findings by Martinet et al. also help determine macrophage fate within plaques during cholesterol reduction. Many studies have demonstrated that macrophages disappear from lesions on cholesterol reduction. Clearly, cells may disappear by either cell death or emigration. Martinet et al. found that oxidative damage, DNA strand breaks, and apoptosis are reduced during lesion regression, suggesting that macrophage numbers reduce by emigration. If macrophage apoptosis is detrimental to plaque stability, how macrophages leave plaques (apoptosis versus emigration) may determine clinical outcome. How monocytes migrate into the vessel wall has been intensively studied. In contrast, little is known about mechanisms of macrophage emigration. If macrophage reduction does determine plaque stability, then such mechanisms should also be an intensive area of study.

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References


Key Words: apoptosis ■ p53 ■ reactive oxygen species ■ DNA repair
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Circ Res. 2001;88:648-650
doi: 10.1161/01.RES.0000041554.31305.1B

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:
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