S ubendothelial macrophages are a major cellular component of atherosclerotic lesions. In response to subendothelial retention of modified lipoproteins, blood-borne monocytes enter the subendothelial space, differentiate into macrophages, and accumulate large amounts of lipoprotein-derived cholesterol (foam cells). Specific consequences of macrophage foam-cell formation include both physical effects, such as intimal thickening, and biological effects, such as internalization of lipoproteins and secretion of biologically active molecules. Importantly, atherosclerosis is significantly attenuated in knockout mice with deficient lesional macrophages attributable to dysfunctional macrophage colony–stimulating factor or decreased monocyte chemotaxis.

Remarkably, subpopulations of lesional macrophages show signs of proliferation, and other subpopulations are noted to have morphological characteristics as well as biochemical and cellular markers of apoptosis. Cell culture studies have shown that low levels of oxidized LDL can cause macrophages to proliferate, whereas higher levels can result in macrophage death. Both cell culture and in vivo studies have also suggested that intracellular accumulation of excess unesterified cholesterol may be another important cause of lesional macrophage death. Regarding consequences of these cellular events, one might speculate that macrophage proliferation is harmful in view of the aforementioned studies with macrophage-depleted mice. Despite these mouse studies, however, there is another school of thought that suggests that macrophages may initially have a protective role by ridding the subendothelium of potentially damaging lipoproteins. This hypothesis would predict that macrophage proliferation could, under certain circumstances, be protective.

In terms of lesional macrophage death, safe apoptotic death (ie, cellular condensation followed by the phagocytosis and disposal of apoptotic bodies) may simply limit the steady-state number of lesional cells and thus have opposite effects from those mentioned above for proliferation. However, apoptosis can precede what has often been referred to as necrosis and does not always prevent release of cellular contents from dying cells. In addition, the phagocytosis of apoptotic bodies may be inhibited by the presence of oxidized lipoproteins and lipids in atherosclerotic lesions or by the cholesterol-loaded state of the phagocyte, as has been demonstrated in cell-culture studies. Finally, even if the initial response were the engulfment of foam-cell apoptotic bodies by neighboring macrophages, these phagocytes would now be engorged with apoptotic foam-cell remnants, including abundant lipids. Thus, macrophage apoptosis, particularly in advanced lesions, may contribute to lesion pathology, particularly lesional necrosis, by leading to the release of harmful molecules.

To address these hypotheses in vivo, investigators have begun to genetically manipulate specific molecules involved in cellular proliferation or apoptosis in induced mutant mouse models. Not surprisingly, p53 has been one of the first molecules examined in this light. p53 is a tumor-suppressor protein that has both antiproliferative and proapoptotic actions. Cellular stress, such as that caused by DNA damage, hypoxia, or oxidized lipoproteins, activates p53 primarily through inhibition of p53 degradation. Under normal conditions, p53 is both inactivated and rapidly turned over via its interaction with MDM2, which blocks the transcriptional activation domain of p53 and, importantly, promotes the ubiquitination and proteasome-mediated degradation of p53. Elevated levels of p53 lead to antiproliferative and proapoptotic responses by a combination of gene activation (eg, p21WAF1 and Bax), gene repression (eg, IGF-II and bcl-2), and direct protein-protein interaction (eg, helicases and caspases). Of interest, two other members of the p53 family, p73 and p63, are both proapoptotic proteins. In atherosclerotic lesions, p53 colocalizes with nonproliferating or apoptotic macrophages, and one study observed that p53-positive apoptotic macrophages had decreased staining for MDM2 (see above). Finally, treatment of cultured macrophages with the apoptosis-inducer nitric oxide was associated with decreased proteasome-mediated degradation of p53 (above), whereas incubation with aggregated LDL, an important form of modified LDL in lesions, protected macrophages from apoptosis by inducing a ubiquitin-conjugating enzyme that led to proteasome-mediated degradation of p53.

The first in vivo study exploring the role of p53 in atherosclerosis was conducted by Guevara et al. These authors crossed a p53 knockout mouse with the atherosclerosis-susceptible apolipoprotein E (apoE) knockout mouse. Previously, p53 knockout mice were shown to be developmentally normal but susceptible to spontaneous neoplasms, and embryonic fibroblasts from these mice have higher proliferative rates compared with cells from wild-type mice. At 7 weeks of age, the p53-positive and -negative apoE knockout mice were fed a high-fat Western-type diet. Plasma cholesterol and triglyceride levels were similar between the two groups of mice at up to 10 weeks of fat feeding, although by 15 weeks of the diet, cholesterol levels were ~40% less in...
the double-knockout mice. En face analysis of aortic lesion area showed that the double-knockout mice had a statistically significant 50% to 100% increase in lesions at 6, 10, and 15 weeks of fat feeding, and morphological analysis of aortic cross sections demonstrated the presence of bulky, hypercellular lesions in the p53-negative mice, although the ratio of intimal cells to lesion area was not quantified. Using an in situ DNA end-extension (TUNEL) assay for detecting apoptotic cells, the authors found a very low frequency of positive cells, but, unfortunately, there was a trend toward slightly increased apoptotic cells in the lesions of the double-knockout mice (2.48% versus 1.15%, P = 0.066). To measure cellular proliferation, bromodeoxyuridine (BrDU) incorporation studies were conducted and revealed a slightly higher percentage of labeled cells in the lesions of double-knockout versus apoE knockout mice (3.61% versus 1.31%, P = 0.001). The authors mentioned preliminary studies that showed that most, although not all, of the BrDU-positive cells were macrophages. From these data, the authors concluded that increased proliferation, and not decreased apoptosis, contributed to the larger and more hypercellular lesions in the double-knockout mice.

Despite the aforementioned preliminary studies regarding the identity of the BrDU-positive cells, the issue of which lesional cell types were most important in the atherosclerosis effect in the double-knockout mice remained an important unanswered question in view of the possible role of p53 in lesional smooth muscle and endothelial cells. For example, p53 and one of its downstream effectors, p21WAF1, have been found in nonproliferating lesional smooth muscle cells and endothelial cells, and the role of p53 in smooth muscle cell proliferation has been a particularly popular topic in the literature. In this context, van Vlijmen et al.,29 whose study appears in this issue of Circulation Research, created mice whose lesions contained only bone marrow–derived p53-negative cells (eg, mostly macrophages and T cells). This goal was accomplished by transplanting apoE*3-Leiden mice, a variant form of the apoE knockout mouse, with bone marrow from p53 knockout mice. In this study, the mice were fed a high-fat diet for 12 weeks starting at 4 weeks after transplantation. Plasma cholesterol levels, lipoprotein profiles, body weight, and percentage of monocytes and T cells in the blood and spleen were similar between the p53−/−→apoE*3-Leiden mice and the control p53+/−→apoE*3-Leiden mice. Cross-sectional aortic area in the proximal aorta was 2.3-fold higher in the p53−/−→apoE*3-Leiden mice (P = 0.04). The lesions in these mice also had more macrophages, T cells, and necrotic areas, but these parameters seemed to be directly related to the increase in lesion size.

BrDU studies showed that most of the proliferating cells were macrophages, but they could detect no significant increase in these BrDU-positive cells in the lesions of p53−/−→apoE*3-Leiden mice (4.8% of total lesional cells and 3.4% of macrophages were BrDU-positive in the experimental mice versus 3.7% of total cells and 2.8% of macrophages in the control mice). The percentage of TUNEL-positive nuclei in the lesions of control mice was very low (0.42%), but there was a non–statistically significant trend toward an even lower value in the lesions of p53−/−→apoE*3-Leiden mice (0.14%, P = 0.071).

What conclusions can we take away from these two important in vivo studies? Both studies agree that p53 deficiency is associated with increased atherosclerotic lesion size, and the present work clearly implicates bone marrow–derived cells, likely macrophages or T cells. What is lacking, however, is a clear mechanism, and different observations regarding cellular proliferation and apoptosis in the two studies additionally cloud this issue. The two studies differ in some important aspects, not the least of which is the cell-specific approach of the present study. Two other potentially important differences include the lack of functional apoE secretion by macrophages in the first study30 and the use of an inflammation-inducing, cholate-containing diet in the second study.31 In the study by Guevara et al.,26 but not that by van Vlijmen et al.,29 there was an increase in BrDU-positive lesional cells. Although this finding is consistent with the known effects of p53 deficiency,28 there are inherent flaws with this static technique of assessing cellular proliferation.32 Moreover, the percentages of labeled cells were very small, and the determination as to whether the increased number of positive cells is a cause or a consequence of increased lesion size is lacking. Similarly, the effect of p53 on lesional cell apoptosis seemed to differ between the two studies, but conclusions from either study are difficult to draw. As with the BrDU experiments, the TUNEL method is prone to artifacts,11 and the steady-state level of TUNEL-positive cells was very low in both studies. In summary, therefore, the proatherogenic effects of p53 deficiency cannot yet be definitively explained by either an increase in cellular proliferation or a decrease in apoptosis.

Thus, what we know is that p53 deficiency in both the whole animal and in bone marrow–derived cells promotes atherogenesis in two murine models in which atherosclerosis is induced by remnant-like lipoproteins with absent or dysfunctional apoE. In this context, future studies will be required to reproduce these effects in models of LDL-dependent atherosclerosis, such as the LDL receptor knockout mouse. Nonetheless, bolstered by these interesting yet enigmatic in vivo data, the time is now ripe to go back into cell culture. Through the use of powerful biochemical, genomic, and proteonomic techniques, researchers can explore possible proatherogenic properties of p53-deficient cells, particularly macrophages. Such investigation may reveal a previously unknown role of p53 in the uptake or metabolism of atherogenic lipoproteins or in the secretion of proatherogenic molecules by arterial-wall cells.

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p53 and Atherosclerosis
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