NADPH oxidases and atherosclerosis: unraveling the details

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NADPH OXIDASE (NOX)-mediated generation of reactive oxygen species (ROS) was first described in phagocytic cells, and for many years it was assumed that the primary role of NADPH oxidase activation was bacteriocidal; phagocytes generated a burst of ROS following activation. More recently, it has become clear that not only do multiple homologous NADPH oxidase subunits exist but that the expression of these and the constitution of functional NADPH oxidases vary under different conditions and in different cell types and tissues. The ever-growing family of NADPH oxidases is comprised of a mix of homologous subunits that not only vary by cell type but also depend on the physiological setting. In non-phagocytic cells, multi-subunit NADPH oxidases provide for much more subtle alterations in cellular oxidative stress, resulting in the initiation of intracellular signals that regulate processes including cell migration, growth, and physiological responses to stress. The study of Judkins et al. (6), reported in this issue of the American Journal of Physiology-Heart and Circulatory Physiology, is the most recent of a number of reports focused on the role of NADPH oxidase in vascular dysfunction and atherosclerosis.

The principal finding of their study is that deficiency of the catalytic component of NADPH oxidase, Nox2 (previously called gp91phox), results in reduced atherosclerosis in apolipoprotein E-null (ApoE−/−) mice. To put this observation into context, it is useful to restate the enormous growth in our understanding of the composition and function of NADPH oxidases in recent years.

The phagocytic NADPH oxidase was the first to be characterized biochemically with the conclusion that gp91phox was a unique and required component for oxidase activity. The discovery of Nox1 in 1999, a homologue of gp91phox, provided the first unambiguous hint that the subunit composition of NADPH oxidases might vary in different cells (11). Since that time, a total of seven Nox homologues have been identified, along with numerous homologues of regulatory NADPH oxidase subunits.

At least four Nox homologues—Nox1, Nox2, Nox4, and, most recently, Nox5—are expressed in cultured vascular cells and in intact blood vessels, all with subtly different expression patterns and functions. Nox1 is expressed primarily in vascular smooth muscle cells (VSMCs) and is inactive under basal conditions. Nox2 is present in endothelial cells, adventitial fibroblasts, and in circulating macrophages. Like Nox1, Nox2 is upregulated under pathophysiological conditions. The functional Nox2-NADPH oxidase consists of, in addition to Nox2, membrane-bound p22phox and cytosolic regulatory subunits, p47phox, p67phox, and Rac. Nox4 is highly expressed in all vascular cells—endothelial cells, VSMCs, and adventitial cells—and appears to be constitutively active. While the precise characterization of subunits of Nox1- and Nox4-NADPH oxidases remains elusive, it is clear that in Nox1-NADPH oxidase, p67phox, is likely replaced by NoxA1 (1, 9). Recent reports suggest that polymerase delta-interacting protein 2 is a novel cytosolic and nuclear regulator of the activity of Nox4, which had previously been thought to solely rely on its association with p22phox for activity (8).

Taken together, these data indicate that an ever-increasing level of complexity is involved in NADPH oxidase function. These data are, however, consistent with the notion that at least one mechanism for NADPH oxidase-mediated ROS production is regulated expression of specific Nox isoforms.

In their sophisticated series of experiments, Judkins et al. (6) demonstrated that Nox2 deficiency in ApoE−/− mice results in significantly less atherosclerosis from arch to the iliac bifurcation than in ApoE−/− mice with normal levels of Nox2 expression. This decrease in atherosclerosis is associated with decreased aortic ROS production and increased nitric oxide bioavailability. While not fitting the generally accepted dogma in vascular pathology, the finding that Nox2-NADPH oxidase is important in early atherosclerosis in ApoE−/− mice is not entirely unexpected. Several years ago we reported that ApoE−/− mice lacking p47phox (a requisite component of Nox2-NADPH oxidase) had a marked reduction of atherosclerosis in the descending aorta (3). Although the diversity of Nox homologs was not known at the time, further support for a role of Nox2-NADPH oxidase in athogenesis came from our subsequent finding that the protective effect of p47phox deficiency in an ApoE−/− background was due to both a decrease in Nox2-NADPH oxidase activity in vessel wall cells as well as in circulating macrophages (12).

Several lines of evidence suggest that multiple NADPH oxidases are likely involved in athogenesis. The fact that p47phox is also required for Nox1-NADPH oxidase activity (9) is consistent with a role for Nox1. Reports that a deficiency of Nox1 protects mice from an angiotensin II-induced increase in blood pressure (4) and injury-induced neo-intima formation (7) support a role for Nox1-NADPH oxidase. It may well be that ROS produced by the Nox4-NADPH oxidase present in vascular cells contribute to athogenesis. Relevant to this, Judkins et al. (6) found that in contrast to Nox2, the expression of which is increased in the aortas of ApoE−/− (proatherosclerotic) mice, Nox4 levels did not significantly vary between ApoE−/− versus wild-type mice. Nox4-NADPH oxidase regulates adipogenesis, a risk factor for atherosclerosis, by mediating preadipocyte differentiation in response to insulin treatment (10), and cytosolic regulatory factors such as polymerase delta-interacting protein 2 activate Nox4 and modulate VSMC migration (8), which provide support for the theme that a diversity of NADPH oxidases is important in the complex vascular phenotype of atherosclerosis.

An additional, important finding of Judkins et al. (6) has to do with the subtleties of atherosclerosis in the ApoE−/− mouse model. Judkins et al. (6) found, as we have (3), an important effect of ROS generation throughout the aorta, with the excep-
tion of the dense atherosclerosis that occurs just above the aortic valve. Atherosclerotic lesions develop rapidly in this region—in fact, out of proportion to the development of atherosclerosis elsewhere. Their data add to the now increasing evidence in the literature that atherosclerosis at the level of the aortic valve in ApoE−/− mice may involve multiple processes and is not representative of inflammatory processes that lead to atherosclerosis in humans. In fact, the progression of atherosclerosis at the level of the aortic valve is very rapid, so much so that studies on factors that may impact atherogenesis cannot be adequately assessed in these very proximal lesions.

Lastly, it is important to consider whether ROS generation and NADPH oxidase activity vary with aging. Age itself is an important risk factor for atherosclerosis. Adults over age 65 yr are four times more likely to suffer from coronary heart disease than those in the 40–49-yr age group. Using the same mouse models of atherosclerosis, numerous groups have demonstrated an effect of aging. Our own studies indicate that it is possible to recapitulate the effect of aging on atherosclerosis in mouse models. NADPH oxidase activity is important in these models. It also appears that mitochondrial ROS production is also of importance. We previously demonstrated that ApoE−/− mice deficient in manganese superoxide dismutase, a mitochondrial antioxidant enzyme, exhibited increased early atherosclerosis, associated with early increases in markers of oxidative stress (2). Our unpublished data indicate that atherosclerosis in these mice is exacerbated with aging and that the protective effect of decreased Nox2-NADPH oxidase activity (by knockout of p47phox) becomes less important in these aged mice. An important but unanswered question is whether NADPH oxidase-mediated and mitochondrial-mediated ROS generation both are important in atherogenesis with aging.

One must not forget, however, that to date, clinical studies on antioxidant therapies have been disappointingly negative. Given the results of Judkins et al. (6) and others (3), it is attractive to speculate that selective and specific small molecule inhibitors of various Nox will have a salutary effect on at least premature atherosclerosis. Such inhibitors are currently being investigated (5). Whether or not these inhibitors are clinically useful, however, they will be immensely useful in characterizing the downstream molecular and biochemical pathways stimulated by Nox activation in the aorta and small blood vessels during atherogenesis.

Over the past fifteen years, much has been learned about the role of NADPH oxidases in many areas, including in atherosclerosis. The report of Judkins et al. (6) published here represents an important advance in our knowledge in this field. With the development of ever more sophisticated animal models of atherosclerosis and the advent of specific NADPH oxidase inhibitors, the future offers opportunities in fundamental biology and potential therapy directed at ROS generation and modulation in the vasculature.

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No conflicts of interest are declared by the author(s)

REFERENCES