Arginase and Arginase
Endothelial NO Synthase Double Crossed?

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In a remarkable article published in this issue of Circulation Research, Ryoo et al. propose endothelial arginase II as a novel target for the treatment of atherosclerosis. Actually, they already had done so 2 years ago, based on work mainly on cultured human aortic endothelial cells, which indeed appeared to provide an unexpected way to explain endothelial dysfunction in terms of NO production. This new road to endothelial dysfunction proposed that oxidized low-density lipoproteins (LDLs), which we all agree are a main culprit in the initiation of the atherosclerotic process, increase the activity of arginase II, which, in turn, decreases NO production presumably by shunting the common substrate arginine away from endothelial NO synthase (eNOS) (Figure).

The present study demonstrates that the activity of endothelial arginase II is augmented in apolipoprotein E–null (ApoE/−/−) mice, as well as in wild-type mice, fed a high cholesterol diet. Inhibitors of arginases restored endothelial function to normal in the former and genetic deletion of arginase II did so in the latter. The study covers a wide array of convincing experiments, both in vitro and in vivo, performed by a large group of investigators (actually, there are almost as many authors on the report as mice reported in the results section) with established expertise in the field of arginases and endothelial function.

One can always question the selectivity of pharmacological agents, and the authors recognize that part of the effect of the drugs used in their in vivo studies may be attributable to inhibition of arginase in the vascular smooth muscle cells. However, the combination of the effect of the inhibitors in ApoE/−/− mice and the reduction of the impact of high cholesterol in Arg/−/− animals is particularly convincing. Thus, one can only bow to the evidence and, after reading the article, accept the conclusion of the authors that arginase II plays a critical role in the pathophysiology of cholesterol-mediated endothelial dysfunction, at least in the mouse.

This is not the first (or second) time that an increased arginase II activity has been involved in atherosclerotic endothelial dysfunction in the ApoE/−/− mouse and that the enzyme has been proposed as a new therapeutic possibility for atherosclerosis, which raises the question: how novel is novel? Interestingly, this earlier and the present studies agree that the protein level of the enzyme is not increased in the ApoE/−/− endothelium. Thus, it is all a matter of activity and access to eNOS. Ming et al. propose that the augmented activity is attributable to an augmented RhoA protein level. Ryoo and colleagues propose that oxidized (Ox)LDL increases arginase II activity by a sequence of regulatory events that involve decreased association with microtubules and a later increase in transcription. The dissociation of the enzyme from the microtubule cytoskeleton facilitates its cytosolic localization and thus its homing to the eNOS sites where the 2 enzymes can compete for the common substrate, arginine. Obviously, those 2 mechanisms could reinforce each other.

Arginases are present in 2 isoforms, I and II. Arginase I is the hepatic isoform, whereas arginase II has a mainly extrahepatic, mitochondrial localization. Both isoforms catalyze the hydrolysis of L-arginine to L-ornithine and urea, the final step of the urea cycle (see elsewhere), and the enzymes have been involved in a number of pathologies. With regard to atherosclerosis specifically, although arginase I can contribute to endothelial dysfunction in aging and diabetic rats, cultured human endothelial cells do not appear to express this isoform, and it is not induced by stimuli that augment the activity of arginase II. Thus, if arginase were involved in human endothelial dysfunction, the II isoform would be the likely culprit, which reinforces the interest of the studies by Ming et al. and Ryoo colleagues. However, before jumping to the conclusion of such involvement, a few specific and general questions need to be answered.

First, specifically, how does this competition work? Shared substrate (L-arginine) availability for 1 enzyme (eNOS) may be influenced by the activity of the other (arginase II). Thus, it is proposed that increased arginase activity and L-arginine utilization limits the access of the common substrate to NO synthase, leading to uncoupling with reduced production of NO and augmented production of superoxide anions. In support of this interpretation are the observations that OxLDL reduce the production of NO metabolites and augment that of oxygen-derived free radicals (reactive oxygen species [ROS]) and that arginase inhibitors have an opposite effect. A nonspecific antioxidant effect of the inhibitors supposedly was ruled out by showing no reduction of the ROS signal generated in vitro by the xanthine-xanthine oxidase reaction. However, in the rodent endothelium, the major enzymes generating free radicals contributing to endothelial dysfunction are NADPH oxidase and cyclooxygenase, and one can but wonder how apocynin and indomethacin would affect the results. Also, the mere presence of an augmented...
arginase activity may well interfere substantially with the accurate measurement of NO and its metabolites.15

The affinity of L-arginine for eNOS is considerably greater than that for arginases.3,8,16 The substrate is present not only in the cytosol of the endothelial cells but also in the plasma and thus in the caveolae. Having lived for the last 20 years with the conviction that the availability of L-arginine is not really a limiting step for the function of eNOS in vivo, as it is for inducible NOS (iNOS), one finds it hard to envisage how a homing cytosolic enzyme, even if its greater activity leads to similar rates of substrate utilization8,16 could lead to local deprivation of L-arginine to the extent of causing uncoupling of eNOS. This suspicion is supported by the comparison of the reported NO production and the expression of eNOS,1,2 which seems to indicate that the former parallels the presence of the latter, rather than true uncoupling.

Could it be that something else is going on following arginase activation? Clearly, if this enzyme preferentially swaps up L-arginine, this will not only possibly lead to less production of NO, but will also increase the endothelial levels of both ornithine and urea. Is it of those responsible for the downregulation of eNOS and thus the decreased bioavailability of NO? Increased production of ornithine has been associated with vascular lesions.8 As always in science, new findings lead to more questions. These do not take away from the elegance of the report by Ryoo et al.1

From a general perspective, the authors equate endothelial dysfunction to lesser release of NO. There is no doubt that indeed the impaired production of this endothelial mediator plays a key role in vascular disease,17,18 but endothelial function and dysfunction go way beyond normal/abnormal production of NO, as endothelium-dependent hyperpolarizations (endothelium-dependent hyperpolarizing factor [EDHF]-mediated responses)19 and vasoconstrictor prostanoids (endothelium-derived contracting factor [EDCF])20 contribute in particular in human arteries. This may not be the case for EDHF-mediated responses in the mouse aorta21 but certainly is for EDCF.22 How do the used inhibitors of arginases affect cyclooxygenase? An inhibitory effect on endothelial cyclooxygenase-1 may explain why in the aorta of ApoE−/− mice treated with arginase inhibitors the relaxation to acetylcholine tended to be larger than that of wild type animals.1

Another general comment is as old as the ApoE−/− mouse itself: how relevant a model is it for human disease? Human atherosclerosis is a lengthy process that spans over years, and one is always wondering whether or not 6 to 8 weeks of high cholesterol feeding in the mouse truly reflects the human situation. In cell cultures derived from areas of pig coronary arteries covered with regenerated endothelium after balloon denudation, a model advocated to be close to human disease,17,18,23 all signs of increased ROS production are present,24–26 but the expression of arginase II is downregulated.26

Because we do not treat cultured cells or genetically modified animals but, instead, people, only human research will tell whether or not arginase II is a major player in vascular disease. If this were the case, would arginase II be a valid target for drug discovery? For sure, one would not want to perturb too much the urea cycle in vivo, and thus absolute specificity for the II isoform of the enzyme would be required because absence of arginase I activity leads to severe hyperammonemna and death shortly after birth.8,27,28 Even a specific arginase II inhibitor could lead to a host of unwanted side effects, in view of the ubiquity of the mitochondrial enzyme,8 except if it were targeted specifically to endothelial cells. It may be easier to prevent the production of ROS and the activation of arginase II by available therapeutic agents, for example, statins.9

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