Arginine/Arginase NO NO NO

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Arginine is a semi-essential amino acid that plays a critical role in several metabolic pathways. The best known of these is as the immediate precursor of urea in the liver and to a lesser extent in the intestine. The enzyme responsible for the cleavage of arginine to produce urea is arginase, which is the only enzyme in the urea cycle that comes in two forms, arginase I, a cytosolic enzyme, and arginase II, a mitochondrial enzyme. These enzymes exist not only in the liver and intestine where urea is generated but are also widely distributed in the body. Ornithine, the other product of the cleavage of arginine by arginase, is a precursor of proline and polyamines such as spermine, spermidine, and putrescine. Arginine is also the precursor of nitric oxide (NO).

In an important article authored by Teupser and colleagues, arginase in macrophages has been identified as a candidate gene influencing atheroresponsiveness. They created and studied two strains of rabbits with genetically determined high (HAR) and low (LAR) atherosclerosis susceptibility. Subtractive suppression hybridization was used to screen for genes that were more highly expressed in macrophages from LAR rabbits. Among the 42 clones identified were four differentially expressed genes with potential relevance to atherogenesis. These were OC2 protein, fibronectin, ferritin H-chain, and arginase I. Despite their identification in the screen, the first three genes were not differentially expressed in macrophages between HAR and LAR animals when checked by quantitative RT-PCR. In contrast, arginase I was more highly expressed in LAR than HAR macrophages, and its expression level was correlated with higher arginase enzymatic activity. They further identified a length polymorphism of the mRNA for arginase I that was attributable to the presence (long variant) or absence (short variant) of a 413-bp C repeat in the 3’ untranslated region. The short variant was expressed highly in the LAR rabbit macrophages and was associated with significantly higher arginase I mRNA levels and arginase activity. It was also shown that the long transcript variant was less stable than the short variant. Homozygosity for the long variant was found only among the HAR rabbits, whereas homozygosity for the short variant was primarily found in LAR rabbits. Though arginase promoter variants were also noted, these were not related to arginase expression levels. Although plausible, the notion that variation in arginase I activity accounts for the difference in atherosclerosis susceptibility between HAR and LAR rabbits has yet to be fully proven. This will likely be done by creating arginase I genetic variants in a uniform genetic background, which will allow for a more rigorous quantitative assessment of the role of arginase activity in contributing to atherosusceptibility.

The plausibility of arginase as a candidate gene for atherosusceptibility rests on suggestive evidence in the literature. Arginine administration has been shown to improve vascular reactivity and to be atheroprotective. In addition to being an important amino acid for protein synthesis and as the terminal intermediate in ureogenesis, arginase is also the precursor for the formation of NO by nitric oxide synthases (NOS) (see Figure). The generation of NO in the endothelium results in vasorelaxation and is generally regarded as atheroprotective, reducing adhesion molecule expression. Vascular responsiveness to NO is reduced in the presence of such atherosclerosis risk factors as hypercholesterolemia, hypertension, and diabetes, and this can be reversed by administration of arginine. Endothelial nitric oxide synthase (eNOS) is a highly regulated enzymatic activity, located primarily in the caveolae of the endothelial cell. It is regulated by shear stress and is more highly expressed in the endothelium underlying laminar flow of blood. It is also regulated by HDL in an SR-BI dependent fashion and by estrogen. NO can interact with superoxide to form the oxidant peroxynitrite (ONOO−). This happens in the endothelium particularly when tetrahydrobiopterin, the cofactor for eNOS, is limiting, resulting in the so called uncoupling of eNOS.

The work of Teupser and colleagues focused on gene expression in the macrophage because it is a central cell involved in atherogenesis. Arginase was identified in human atherosclerotic plaques mainly colocalized with the macrophages around necrotic cores. In macrophages the major NOS is the inducible nitric oxide synthase (iNOS) which generates NO and peroxynitrite in the presence of superoxide. Thus in contrast to eNOS, the formation of NO by iNOS is mainly proinflammatory and hence proatherogenic. If higher arginase activity reduced the available arginine substrate for NO production by iNOS and consequent peroxynitrite, this could be one mechanism for an atheroprotective effect of high macrophage arginase activity. It is of interest that the antiinflammatory cytokines interleukin (IL)-4, IL-10, and IL-13 are capable of inducing arginase I synthesis, with IL-4 and IL-10 acting cooperatively. This could be one mechanism by which the Th2 T cells and regulatory T cells through the production of their prototypic cytokines may exert their atheroprotective action. Interestingly, these cytokines influence macrophage arginase I expression, but not arginase II.
arginase expression by each of these cell types can only be considered after NO production by iNOS. The contribution of increased arginase I activity in smooth muscle cells may lead to decreased production of peroxynitrite (ONOO\textsuperscript{-}). Increased arginase I activity in macrophages may be antithrombogenic by reducing NO production leading to reduced generation of peroxynitrite (ONOO\textsuperscript{-}). Reduced arginine levels, although still above the Km for NOS, decreases NO generation probably by reduced translation of NOS or reduced competitive displacement of the competitive inhibitor ADMA (asymmetrical dimethyl arginine). This is known as the arginine paradox. On the other hand, increased arginase I activity in endothelial cells could be proatherogenic because of decreased endothelial cell NO production, and in smooth muscle cells this could result in increased production of collagen and enhanced cell proliferation attributable to the metabolism of ornithine into proline and polyamines, respectively.

Endothelial cells and macrophages are not the only cells of the atherosclerotic plaque involved in the arginase–NO network. The smooth muscle cell also produces arginase I. Ornithine, one of the products of arginase activity, is the precursor of proline, glutamate, and polyamines. Proline is a major substrate for collagen synthesis. The polyamines promote cell cycle progression and therefore cell proliferation.\textsuperscript{11,12} Both of these processes could influence the nature and composition of the atherosclerotic plaque.

Variation in arginase type and activity may also be expressed in endothelial cells and smooth muscle cells as well as in macrophages, though this is yet to be explicitly shown. Alterations in the expression of arginase I in these vascular wall cells may have complex effects on atherogenesis. One might predict that increased arginase I expression in endothelial cells would not be atheroprotective because of decreased NO production and decreased vasorelaxation, whereas expression in smooth muscle cells could influence the cellular matrix composition of an atherosclerotic plaque as a result of increased generation of proline and polyamines. On the other hand, increased expression in macrophages and smooth muscle cells may lead to decreased production of peroxynitrite after NO production by iNOS. The contribution of arginase expression by each of these cell types can only be resolved by experiments with the selective overexpression of arginase in endothelial cells, macrophages, or smooth muscle cells. The phenotypes of tissue specific arginase transgenic mice crossed with a standard murine atherosclerosis model, eg, apoE-deficient mouse, would be of interest.

The long and short variant arginase transcripts in rabbit depends on the presence or absence of the C-type repeat in the 3’ untranslated region of the transcript, affecting message stability. The C-type repeat belongs to a family of short interspersed nucleotide elements or SINES.\textsuperscript{13,14} This may be unique to the rabbit and perhaps other lagomorphs because other species have different types of SINES. For example, SINES belonging to B1 and B2 subfamilies are found in mice.\textsuperscript{15,16} Thus, there is no current basis for anticipating the variation in arginase I transcripts will be duplicated in the mouse.

What is particularly important and attractive about the work on rabbit arginase is that it shows that quantitative variation in macrophage arginase activity correlates with different atherosclerosis responses. However, the role of arginase in competing for arginine substrate otherwise available for NOS is more complex than might be apparent. The arginine Km for NOS is much lower than the intracellular concentration of arginine in most cells.\textsuperscript{17} The Km for arginase is much higher, though its Vmax is much higher than that of iNOS. With this in mind, it is not straightforward that modifying the availability of arginine, either by providing or removing exogenous arginine or by degrading intracellular arginine as a result of increased expression of arginase, would affect iNOS activity. This has been designated the “arginine paradox.” That arginine availability may influence NO generation is clearly illustrated by the phenotype of rare genetic arginine deficiency in humans.\textsuperscript{18} Two explanations for the arginine paradox have been offered. Decreased availability of arginine influences the synthesis of iNOS not by direct competition for substrate but by interfering with iNOS mRNA translation. Both arginine and leucine are required for initiation of protein synthesis and the level of arginine influences the phosphorylation and activity of eukaryotic initiation factor.\textsuperscript{19} Another possible explanation is that arginine competes with asymmetrical dimethyl arginine (ADMA), an inhibitor of iNOS and eNOS.\textsuperscript{20,21} ADMA is generated by the proteolysis of ADMA containing proteins.

In conclusion, the work of Teupser and colleagues highlights the potential importance of arginine in vascular inflammation and atherosclerosis via iNOS, and in vascular response and remodeling. A great deal of work remains to be done to unravel all the mechanisms at work, especially in relation to the effect of arginine metabolism in the participant cells during atherogenesis. Although depletion of arginine by arginase is here implicated in atherogenesis, it may also influence other pathologies, eg, immune function and asthma.\textsuperscript{22} Because quantitative variation in arginase activity appears to impact on atherosusceptibility, it will be of interest to ascertain whether mice wild type for arginase or heterozygous for arginase deficiency exhibit differences in atherosusceptibility.

References


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Arterioscler Thromb Vasc Biol. 2006;26:237-239
doi: 10.1161/01.ATV.0000202014.54609.9d
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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