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EDITORIAL COMMENT

ADMAring Endothelial Progenitor Cells

Accident, Association, or Antecedent*

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Despite embryonic stem cell research capturing the imagination and $3 billion in California, adult progenitor cell research continues to generate interest. Why? It bypasses the ethical issues and consequent regulatory hurdles of embryonic stem cell research while maintaining much of the novelty and promise. During the last five years, attention has focussed on the contribution of adult progenitors, and in particular circulating endothelial progenitor cells (EPCs), to vascular health and disease. The EPCs, initially identified as circulating in the peripheral blood of adults (1), are now known to originate from the bone marrow and maintain a greater proliferative capacity (2) than mature endothelial cells within the vasculature.

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Functionally, these sentinels play a role in repairing ongoing vascular damage (3), promoting vascular growth (4), and in endothelialization (5). However, to perform their function, EPCs produced by the bone marrow must be competent and mobilized specifically to areas of injury or growth before differentiating into mature endothelial and, perhaps other, cell types.

CONTROL OF MOBILIZATION AND DIFFERENTIATION

A population of EPCs can be found circulating in the peripheral blood of healthy individuals. Studies show that the population can increase dramatically at times of acute pathophysiologic stress, such as myocardial infarction (6), and physiological stress such as exercise (7). In addition to these intra-individual variations by circumstance, considerable interindividual variation also exists. The cause for variation between subjects is not fully understood but may in part relate to ongoing vascular injury because those with diabetes (8) and coronary artery disease (9) have a smaller circulating population of EPCs with diminished function. Furthermore, in patients with risk factors but without overt vascular disease, the size of the EPC population is inversely related to the cardiovascular risk score and capacity for endothelium-dependent vasodilation (3). These observations can be interpreted variously. The most obvious possibility is that the number and reparative capacity of EPCs is impaired by the factors that predispose to atherosclerosis. Given the sentinel function of EPCs, it is then tempting to speculate that subclinical or overt atherosclerosis is a consequence of the EPC deficit rather than vice versa. The scenario of EPC deficiency causing, rather than being a manifestation of, vascular disease has fuelled research into the mechanisms responsible for EPC differentiation and mobilization in the hope they can be recapitulated to improve vascular health.

A principal stimulus behind EPC mobilization is ischemia (10), which results in an increase in the concentration and gradient of cytokines responsible for release and maturation. The levels of cytokines have been shown to increase with age, perhaps reflecting amplification of a paracrine loop as the bone marrow becomes less able to release functional EPCs (11). The cause for down-regulation of function is largely unknown, although recent studies suggest activation of an intracellular signaling pathway involving p38 mitogen-activated protein kinase, which impairs EPC as well as myocyte proliferation and differentiation (12,13). Another factor influencing mobilization is endothelial nitric oxide synthase (eNOS) activity, which is demonstrated most elegantly by the restoration of neovascularization in the $eNOS^{-/-}$ mouse by the intravenous infusion of wild-type progenitor cells (14). Because eNOS activity is influenced by various factors, is there evidence that these thereby influence EPC function?

ASYMMETRIC DIMETHYARGININE (ADMA)

In 1992, ADMA was first recognized as an endogenous inhibitor of eNOS by Patrick Vallance (15). It is formed by the methylation of arginine residues within proteins and is released during subsequent proteolysis to produce the free false substrate. When administered systemically, it increases blood pressure and decreases cardiac output and heart rate (16). Concentrations of ADMA have been shown to increase with renal impairment (15), heart failure (17), diabetes (18), and other cardiovascular risk factors (19). Thus ADMA levels increase in many of the circumstances associated with a decrease in EPCs. In common with EPC deficiency, an excess of ADMA has been proposed to cause, rather than be an index of, cardiovascular disease.

ADMA AND EPCS

Thum et al. (20) in this issue of the *Journal* tie together these two potential mediators of cardiovascular disease. They note that plasma concentrations of ADMA and the severity of coronary artery disease are inversely correlated with measures of EPC number by expression of surface markers and number/function by endothelial cell outgrowth colonies. The potential

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mechanism for this relationship is indicated by complementary in vitro findings in which ADMA, in a concentration-dependent manner, represses EPC proliferation, differentiation, NO production, and tube formation. These effects, most apparent at an ADMA concentration of 10 μmol/l, are reversed by rosuvastatin. The measured plasma ADMA concentration varied from 0.47 μmol/l in control patients to 0.58 μmol/l in those with triple vessel disease.

The issues raised by the current article are complicated by the circumstantial nature of the evidence linking ADMA and vascular disease (21). In common with Thum et al. (20), most investigators find the concentrations of ADMA required to see biological effects in vitro are far higher than those circulating (22). A further difficulty is that ADMA concentrations may merely reflect active vascular remodeling and/or decreased renal clearance, both of which are likely to occur in patients at risk of, or with established, atherosclerosis.

ATHEROSCLEROSIS, ADMA, AND EPCS: DIFFERENTIATING ASSOCIATION FROM CAUSATION

Currently a wealth of clinical studies exist showing an association between EPCs and vascular health. Inherent to most is the issue of co-correlation. This problem is exemplified by our observation of a relationship between myocardial collateral flow index (CFI) and EPCs (23). However, we also found patients with high CFIs had more severe coronary stenoses and likely more severe myocardial ischemia. It is thus difficult to be certain whether EPCs relate to CFI or ischemia (23). Similar issues complicate the interpretation of the study by Thum et al. (20). Both ADMA concentration and EPC number/function may be perturbed by the atherosclerotic burden, which causes the co-correlation between these factors. Put more simply, the atherosclerotic burden is the "horse" pulling along the "carts" of EPCs and ADMA. Thum et al. (20) are to be congratulated in their attempts to differentiate cart from horse. Despite the caveats of the concentration disparity, it seems possible increases in ADMA concentration are the antecedent causing damage to EPCs, which in turn are unable to maintain vascular integrity leading to atherosclerosis. It is within this framework the authors emphasize the unique ADMA-lowering abilities of rosuvastatin. However, it is unlikely such properties are unique to this statin because atorvastatin, (24) simvastatin (25), mevastatin (26), and almost certainly others are known to increase EPC number and function. Nonetheless, this study and the in vitro observations within it help address the scarcity of mechanistic detail in this exciting field.

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