The Modern Art of Atherosclerosis
A Picture of Colorful Plants, Cholesterol, and Inflammation

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Atherosclerosis is a disease of large arteries that accounts for more than 50% of all deaths in the developed countries and, according to the World Health Organization prediction, it will gain a status of the major morbidity cause worldwide by the year 2010. Atherosclerosis is widely viewed as an inflammatory disease with hypercholesterolemia being a dominant underlying risk factor. It is believed to be initiated by retention of LDL particles in the lesion-prone areas, which is followed by monocyte recruitment and their differentiation into cholesterol-laden macrophage foam cells. Excessive cholesterol accumulation in macrophages exaggerates innate immune response that is manifested by upregulated production and secretion of inflammatory cytokines and chemokines, thus dramatically amplifying initial signal originated from the injured artery.

Despite the overall consensus on the causative roles of excessive cholesterol build-up and inflammation in the development and progression of atherosclerosis, the relationship between aberrant cholesterol metabolism and exaggerated innate immune response has not been totally established. Recent reports showing reduced inflammation in association with the cholesterol-lowering statin therapy provided indirect evidence linking cholesterol metabolism with regulation of immune response. However, this class of cholesterol lowering drugs not only inhibits cholesterol biosynthesis but also suppresses the synthesis of isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Thus, inhibition of prenylation pathways such as those required for activation of Ras and Rho GTPase family proteins may be responsible for some of the cardiovascular benefits of statin therapy independent of its cholesterol-lowering effects. Among the most studied and understood mechanisms showing a direct linkage between cholesterol metabolism and inflammatory response are those involving exogenous bacterial endotoxin and endogenous CD40 ligand (CD40L). It has been established that the basal innate immune response to the circulating endotoxin could be greatly amplified by hypercholesterolemia through dramatic activation of the Toll-like receptor (TLR) signaling. The other mechanism includes hyperactivation of the CD40 receptor on its ligation by CD40L. In both cases, the activation of the TLR and CD40 receptor is regulated by either hetero- (TLR) or homo- (CD40) oligomerization, the processes that are strongly dependent on the cholesterol domain (raft) structure of the cell plasma membrane.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Xia et al reported a novel mechanism implicating plasma membrane cholesterol level as one of the major regulators of the CD40 receptor signaling. Following their previous observations that the ubiquitous plant pigments, anthocyanins, attenuated atherosclerotic plaque formation in apolipoprotein E (ApoE)-deficient mice, possibly through the upregulation of the ABCA1-mediated cholesterol efflux in macrophages, as well as taking a careful note of the recent reports that described the CD40 receptor adaptor protein TRAF-2 translocation to the raft fraction of the plasma membrane on B-cell stimulation with CD40L, these authors demonstrated that anthocyanins Cy-3-g and Pn-3-g significantly attenuated CD40L-induced proinflammatory response in endothelial cells (ECs) as measured by reduction in the inflammatory cytokine production, such as interleukin (IL)-6, IL-8, and MCP-1. The extent of the above reduction was very similar to that of CD40L blocking antibody. The authors proceeded to show a strong correlation between the anthocyanin-mediated suppression of inflammatory response and inhibition of the NF-κB activation by using ECs transfected with CD40 and monitoring dose-dependent inhibition of NF-κB reporter activity by anthocyanins. In the same system, Cy-3-g and Pn-3-g pigments also reduced NF-κB binding to DNA in response to soluble CD40L.

The next step was to evaluate a role TRAF-2 in mediation of the CD40 receptor signaling. To that end, Xia et al silenced TRAF-2 expression in ECs by using specific siRNA. As the result, production of the cell inflammatory markers in response to CD40 activation was reduced by 30% to 40%. Overexpression of TRAF-2 significantly increased NF-κB activation, whereas EC transfection with the dominant-negative TRAF-2 almost completely eliminated cell response to CD40 activation. Altogether, these data provided strong evidence regarding an important role of TRAF-2 in mediation of the CD40 receptor signaling.

To further delineate a mechanism by which TRAF-2 positively regulates CD40 receptor signaling, the authors demonstrated CD40–TRAF-2 association in response to the receptor stimulation by immunoprecipitating the respective complex. In the same setting, they also demonstrated TRAF-2 recruitment to the detergent (Triton X-100) insoluble plasma membrane fraction. Additionally, they demonstrated that
TRAF-2 translocated to the detergent insoluble plasma membrane fraction (rafts) and form a complex with CD40.

Xia et al. examined a role of membrane cholesterol domains as a platform for CD40 signaling by lowering cell cholesterol content through treatment with β-cyclodextrin (β-CD). As the result, such treatment almost completely abrogated TRAF-2 and CD40 association by blocking TRAF-2 translocation to lipid rafts. Lastly, the authors addressed a mechanism of antiinflammatory effect of anthocyanins by probing CD40–TRAF-2 association in lipid rafts as well as by measuring cholesterol content in the detergent soluble and insoluble membrane fractions before and after cell incubation with these pigments. As it turned out, pre-treatment of ECs with Cy-3-g and Pn-3-g before their stimulation with sCD40L resulted in the efficient and dose-dependent prevention of TRAF-2 translocation to the lipid rafts and its association with CD40. This was correlated with significant cholesterol depletion of detergent-resistant, but not detergent-sensitive plasma membrane fractions.

Altogether, the results reported by Xia et al led to a model that would link cell cholesterol metabolism to regulation of innate immune response (Figure). According to this model, upregulation of cell cholesterol efflux by anthocyanins could lead to the selective cholesterol lowering in the detergent-insoluble membrane fraction, thus blocking TRAF-2 translocation to lipid rafts and its subsequent association with CD40 receptor in response to CD40L stimulation. The significantly impaired CD40 activity would result in the diminished activation of NF-κB and, consequently, in the reduced production of inflammatory cytokines and muted chronic inflammation.

Overall, anthocyanins could represent a novel class of therapeutics for the treatment of atherosclerosis. However, such perspective must be viewed with caution, particularly with regard to possible treatment of patients with Familial Hypercholesterolemia. Indeed, anthocyanins appear to mediate their cell cholesterol lowering and antiinflammatory effects through activation of the liver orphan receptor (LXR).
and cholesterol transporter, ABCA1. Therefore, a systemic treatment of those patients with anthocyanins could lead to the development of the fatty liver as well as an increase in circulating proatherogenic lipoproteins as it has been recently demonstrated in two LDL receptor–deficient animal models. Whether anthocyanins can effectively reduce atherosclerotic plaque progression in animal models and in humans with normal LDL receptor activity remains to be determined.

**Disclosures**

None.

**References**

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