Platelets Affect the Structure and Function of C-Reactive Protein

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C-reactive protein (CRP) is an acute-phase reactant and an active regulator of the innate immune system. Prospective clinical studies have shown that elevations in baseline CRP levels confer, albeit to varying degrees, additional prognostic value for predicting future cardiovascular events and death across all levels of the Framingham risk score. CRP has been implicated in multiple aspects of atherogenesis and plaque vulnerability; however, a direct pathogenetic role for CRP in these events is presently controversial. CRP has at least 2 conformationally distinct forms, native pentameric (pCRP) and monomeric (m)CRP. Native CRP consists of 5 identical subunits arranged in a cyclic pentamer. Loss of pentameric symmetry in pCRP, yielding mCRP, is associated with expression of distinct bioactivities. To date, little is known about the in vivo source(s) of mCRP.

Platelets represent an important interface between thrombosis, innate and adaptive immunity, and atherogenesis. Platelet-triggered inflammatory pathways contribute to atherosclerotic lesion formation and atherothrombosis. In this issue of Circulation Research, Eisenhardt et al provide new insights into the relationship of platelets, CRP, and inflammation. They show that activated platelets dissociate pCRP into mCRP and provide evidence, suggesting that mCRP, rather than pCRP, localizes monocyte-mediated inflammation to the atherosclerotic plaque.

Native CRP is thought to be very stable and is not expected to dissociate into separate subunits without denaturation. However, calcium-dependent binding of pCRP to liposomes or cell membranes results in separation into subunits. Eisenhardt et al show that binding of pCRP to lysophosphatidylcholine expressed on the surface of activated platelets and apoptotic mononcytic THP-1 cells triggers its rapid dissociation into mCRP. Formation of mCRP involves dissociation of the CRP pentameric disk, resulting in a structural change from predominantly β-sheet structure to an α-helical structure and expression of intersubunit contact residues, in particular residues 197 to 202, the predominant epitope only expressed on mCRP. mCRP-specific antigen has been detected in inflamed tissues.

Immunohistological staining of human atherosclerotic lesions consistently places CRP within the lesion, frequently along with the terminal complement complex. Because most anti-CRP antibodies, including the widely used antibody clone 8, recognize both pCRP and mCRP,10 it is uncertain whether lesions express mCRP, pCRP, or both. Eisenhardt et al show that human aortic and coronary artery atherosclerotic plaques stained with the clone 8 antibody and a specific anti-mCRP antibody but not with a specific anti-pCRP antibody, indicating accumulation of mCRP rather than pCRP in atherosclerotic lesions. mCRP-staining correlated with areas that stained for macrophages or platelets, suggesting generation of mCRP by these cells. Formal colocalization studies would lend additional support to this notion. Future studies are needed to investigate whether deposition of pCRP into lesions precedes in situ mCRP generation. Of note, CRP mRNA is also expressed (at very low levels) in blood vessel wall cells, although it is unlikely whether these cells are major sources for CRP in plaques.

Although pCRP and mCRP exhibit distinct biological activities (see the Figure), it is controversial which form mediates CRP’s proinflammatory actions. For instance, pCRP induces proinflammatory cytokine release from endothelial cells and monocytes and evokes endothelial dysfunction and monocyte adhesion to the endothelium. Other studies found mCRP to be a considerably more potent activator of endothelial cells and monocytes than pCRP.11 mCRP and pCRP exert opposing actions on neutrophil trafficking into tissues and platelet deposition and thrombus growth. Transgenic expression of human CRP protects mice against endotoxin shock and microbial pathogens. Transgenic expression of CRP in apolipoprotein E–null mice has been reported to accelerate, to show no effect, or even to slow atherosclerosis development. Such divergent results are not easily explained and may reflect inherent difficulties in interpreting results from mouse models of atherogenesis.

An unexpected observation was that mCRP-induced monocyte adhesion could be partially inhibited by blockade of CD64 (FcγRI), CD32 (FcγRIIA), or CD16 (FcγRIII). In phagocytes and endothelial cells, pCRP binds primarily to CD32 and to some extent to CD64, whereas mCRP binds to CD16. Eisenhardt et al show that disruption of lipid rafts fully inhibited monocyte activation by mCRP. Although intriguing, the data should be interpreted with caution. In monocytes, binding of pCRP to CD64 and CD32 was found to activate nuclear factor κB and the nuclear liver X receptor-α. Nuclear factor κB regulates transcription of proinflammatory cytokines. Liver X receptor-α increases expression of the ATP-binding cassette transporter A1 and suppresses...
genes involved in inflammatory signaling and apoptosis. It is tempting to speculate that an as yet unidentified molecular switch may direct proinflammatory versus antiinflammatory responses in monocytes. Furthermore, cell-specific differences in molecular mechanisms activated by pCRP and mCRP should also be considered. For instance, pCRP inhibits insulin activation of endothelial nitric oxide synthase via FcγRIIB and SHIP-1 (Src homology 2 domain-containing inositol 5'-phosphate), whereas mCRP induces cytokine release and upregulates expression of adhesion molecules on endothelial cells through CD16, lipid rafts microdomains, and p38 mitogen-activated protein kinase.

In conclusion, the article by Eisenhardt et al11 brings the concept of platelets being important in the formation of mCRP and thus localizing inflammation to atherosclerotic plaques a step closer to the clinical setting. These observations highlight that an analysis of CRP in the plasma alone may not be suitable to reflect its function and to serve as a biomarker in atherosclerosis. The benefits of therapeutically targeting CRP were recently shown in a rat model of myocardial infarction.28 Considering the pro- and antiinflammatory actions of CRP, it remains a future challenge to investigate whether therapeutic interventions aimed to selectively block its proinflammatory actions, perhaps by preventing dissociation of native CRP into mCRP, could have clinical benefits.

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None.

References
C-reactive protein (CRP) is a protein involved in the innate immune system. It is elevated in response to inflammation and is a marker for cardiovascular disease. CRP binds to a variety of receptors and can mediate biological effects. For example, CRP can activate the liver X receptor, a transcription factor involved in lipid metabolism.

CRP is also known to induce pro-inflammatory cytokines and contribute to the formation of atherosclerotic plaques. It promotes endothelial dysfunction and increases the risk of plaque rupture. CRP is also involved in the activation of platelets, which can lead to thrombus formation.

There is ongoing research into the role of CRP in atherosclerosis, including the development of CRP-targeted therapies. Understanding the mechanisms by which CRP promotes inflammation and atherosclerosis is crucial for developing effective treatments for cardiovascular disease.
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