

Commentary

C-Reactive Protein and Atherogenesis

New Insights from Established Animal Models

Jan Torzewski

*From the Department of Internal Medicine II-Cardiology,
University of Ulm, Ulm, Germany*

In this issue of *The American Journal of Pathology*, Sun and colleagues¹ publish a potential key article in the field of C-reactive protein (CRP) and atherosclerosis, which is currently a lively topic in cardiovascular research. The reasons for this wide-reaching interest are briefly summarized as follows. First, CRP has been identified as a powerful cardiovascular risk marker.^{2,3} Second, CRP may not only be a cardiovascular risk marker but also a risk factor, ie, CRP may be causally involved in atherogenesis.^{4,5} Third, if CRP is indeed a cardiovascular risk factor, the molecule may well be a target for drug development, and CRP inhibition might be a strategy for primary or secondary prevention of cardiovascular disease.⁶ The medical and economical impact of these possibilities is evident. In analogy to other active areas in research, the role of CRP in cardiovascular disease is controversially discussed. Such controversial discussion relates to each of the three points raised above.

The role of CRP as a cardiovascular risk marker is almost generally accepted, and evidence is based on several large and well-controlled trials.^{2,3} The American Heart Association has already included CRP measurement into the guidelines for cardiovascular risk prediction.⁷ Few populations, however, have shown less strong correlation of CRP plasma levels with cardiovascular events,⁸ and consequently, some researchers doubt that CRP measurement adds useful information to the Framingham risk score.

Concerning the second point, ie, the potential role of CRP as a cardiovascular risk factor, there is definitely no international consensus. CRP was first hypothesized to be involved in atherogenesis in 1982 when researchers demonstrated that CRP interacts with low-density lipoprotein (LDL),⁹ a finding that has recently been confirmed by others using various modified and nonmodified LDL molecules.^{10–12} Scientific interest waned, however, when the same group consecutively published that CRP is present

neither in human nor in rabbit arterial lesions.¹³ Two smaller publications on CRP deposition in human lesions were primarily ignored.^{14,15} Interest returned with the results from epidemiology and the identification of CRP as a cardiovascular risk marker.^{2,3} In search for complement-activating molecules in human atherosclerotic lesions, it was demonstrated in 1998 that CRP is indeed ubiquitously present in all stages of human atherosclerosis and that it co-localizes with activated complement fragments.¹⁶ This finding also has been confirmed by several groups,¹⁷ and since then various researchers have investigated a potential active contribution of CRP to atherosclerosis, suggesting that CRP activates monocytes/macrophages,^{18,19} endothelial cells,^{20,21} and vascular smooth muscle cells.²² A critical point in all these studies is the use of commercially available CRP preparations that may contain contaminants such as lipopolysaccharide and azide. Indeed, it has recently been demonstrated that almost all of the published effects of CRP on endothelial cells are due to contamination of CRP preparations with azide and lipopolysaccharide.^{23,24} In this context it should be noted that CRP-mediated activation of endothelial cells and smooth muscle cells may not be biologically plausible and may lack a convincing molecular explanation.

To understand a molecule's role in disease, the best approach might be to reconsider its role in physiology. CRP is an ancient immune molecule that shares many functional properties with antibodies: CRP binds to a variety of ligands (including LDL or modified LDL), activates complement,²⁵ opsonizes biological particles,²⁶ and binds to and signals via Fc γ -receptors.^{27,28} CRP and complement appeared very early in the evolution of the immune system and so did monocytes/macrophages. It is thus likely that the target cell for CRP in vascular biology and atherosclerosis is the monocyte/macrophage and not the endothelial cell or smooth muscle cell, especially in view of the fact that CRP co-localizes with

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Address reprint requests to Jan Torzewski, M.D., M.Phil., University of Ulm, Department of Internal Medicine II-Cardiology, 89081 Ulm, Germany. E-mail: jan.torzewski@medizin.uni-ulm.de.

monocytes/macrophages and foam cells in the human atherosclerotic lesion.^{16–18} In addition CRP and complement fragments are chemotactic for monocytes/macrophages,^{18,29} which express Fc γ receptors and complement receptors at high levels. Fc γ receptors and complement seem to be involved in CRP-mediated opsonization of LDL.¹⁹ Therefore, it might be hypothesized that LDL, CRP, complement, and macrophages orchestrate an inflammatory process in the arterial wall that promotes atherogenesis.

In vitro studies have been supplemented by *in vivo* investigations, with contradictory results. It is important to note here that the most broadly available animal model, ie, the mouse, is considered useless regarding the study of CRP functions because CRP is not an acute phase reactant in mice. To overcome this problem, a transgenic mouse that overexpresses human CRP was generated, and this model has been used to study the role of CRP in cardiovascular disease.^{30–33} Whereas two initial publications suggested an active contribution of CRP to atherosclerosis and acute cardiovascular events in this model,^{30,31} two more recent studies have detected no effect.^{32,33} It is difficult to determine which of the reports is right or wrong, valid or invalid, because the model itself encounters several problems. Human CRP is a foreign antigen in the mouse with many uncertainties concerning its functional role in the immune system of these animals. The situation becomes even more complicated when these mice are crossed with ApoE-deficient mice that obviously lack a fully functional complement system.³⁴ To cut a long discussion short, it may be appropriate to say that it was worth generating these model systems but hardly possible to answer definitively whether CRP actively contributes to human atherogenesis or not.

In this context the article by Sun and colleagues¹ published in this issue of *The American Journal of Pathology* may be of considerable interest. The authors use well established animal atherosclerosis models, ie, both cholesterol-fed and Watanabe heritable hyperlipidemic (WHHL) rabbits, as models to study the role of CRP in atherogenesis. Interestingly, it is the rabbit again, and thus the same species that stopped scientific interest in the matter more than 20 years ago,¹³ that attracts our attention today. Being experts in working with this animal model, the authors elaborate three major results. First, CRP levels are significantly elevated in hypercholesterolemic rabbits. Second, elevated CRP levels strongly correlate with the extent of atherosclerosis in these animals. Third, CRP is ubiquitously present in atherosclerotic lesions in rabbits, and this lesional CRP is derived from the circulation rather than being synthesized locally in the arterial wall. These results are similar in both cholesterol-fed and WHHL rabbits, and each point is well established through analysis of a large number of animals. The article certainly does not prove a causal involvement of CRP in atherogenesis, and even though CRP is an acute-phase reactant in rabbits, many questions concerning CRP functions in these animals remain to be resolved.¹

The article does, however, describe models that may help address important issues that hint to the third point

we raised in the introduction to this commentary: is CRP inhibition with specific drugs a modality to treat atherosclerosis? Four major strategies of CRP inhibition are feasible: 1) transcriptional inhibition of hepatic CRP synthesis,⁶ 2) anti-sense strategies,³⁵ 3) blockage of CRP-mediated complement activation, and 4) blockage of CRP receptor(s). Pharmaceutical companies are currently examining these strategies. The article by Sun and colleagues¹ in this issue of *The American Journal of Pathology* provides *in vivo* models that may be appropriate to test future CRP inhibitors. If these compounds do not, in parallel, affect LDL levels, discrimination between LDL and CRP effects on atherogenesis may be possible. Finally, these rabbit models might be appropriate for examining bioavailability, specificity, and toxicology of various treatments.

In awareness of the controversial discussion and the controversial data on CRP and atherosclerosis, I would like to finish with a personal opinion from a cardiologist's perspective. We should try to inhibit CRP for the treatment of atherosclerosis; otherwise, we might miss a chance to help our patients suffering from cardiovascular disease.

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