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Correspondence

Sources of CRP in Atherosclerotic Lesions

To the Editor-in-Chief:

We read with great interest the recent article by Sun and colleagues[1] in which they show, in two rabbit models, that C-reactive protein (CRP) is present in the atherosclerotic lesion and indeed correlates with lesion area. Furthermore, they show in these two models a significant correlation between total cholesterol and plasma CRP, a finding that has not been observed in human patients. They provide no explanation for this correlation. Is it possible that the hypercholesterolemia in these models induces inflammation in the liver resulting in the elevated levels of CRP, which then enters the atheroma?

More importantly, however, in a sample size of eight, they demonstrate the presence of CRP in the atherosclerotic lesion, where it did not co-localize with macrophages or smooth muscle cells, and CRP was also found in the surface area of the lesion (see Sun et al,[1] Figures 6 and 7). Using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), they could not demonstrate CRP mRNA in the atherosclerotic lesion and concluded that the CRP was derived from the liver. This finding challenges a great body of data suggesting that CRP is synthesized in the atheroma.

The first observation in this regard was made by Yasojima and colleagues[2] who showed 10-fold more mRNA for CRP in atherosclerotic lesions versus normal vessels ($n = 10$ arterial samples). This finding has now been confirmed by numerous groups. Kobayashi and colleagues[3] used anti-sense riboprobe to show, in 39 directed coronary atherectomy samples, that CRP is present in the coronary atheroma. Furthermore, Jabs and colleagues[4] have shown by real-time RT-PCR that CRP is expressed in coronary artery venous bypass grafts ($n = 11$ of 15), and Vainas and colleagues[5] have documented CRP mRNA in abdominal aortic aneurysmal tissue ($n = 4$ of 16). In addition to real-time RT-PCR, some of these investigators have used other sensitive techniques to document CRP. Using Western blotting, immunohistochemistry, and real-time RT-PCR, Sattler and colleagues[6] have recently documented CRP protein and mRNA in plaques obtained from patients undergoing carotid endarterectomy ($n = 41$). Furthermore, the CRP staining was present in plaque shoulders, microvessels, or borders, mainly in foam cells and endothelial cells.

The major cells of the atherosclerotic lesion include endothelial cells, monocytes/macrophages, T lymphocytes, and smooth muscle cells. Calabro and colleagues[7] have shown by RT-PCR and enzyme-linked immunosorbent assay that vascular smooth muscle cells in culture express and secrete CRP. In a recent article in *The American Journal of Pathology*, we made the novel observation that both coronary and aortic endothelial cells express CRP mRNA and protein using a comprehensive approach of examining mRNA by RT-PCR and *in situ* hybridization and protein by Western blotting. Most importantly, we showed a 100-fold increase in secretion of CRP after incubation of aortic endothelial cells with macrophage-conditioned media.[8]

It is interesting that in the article by Sun and colleagues,[1] no mention is made about the possibility that CRP is derived from aortic endothelial cells, despite surface expression of CRP in the human atherosclerotic tissue in their study. Also, using an activated monocytic cell line, U937 cells, they fail to detect mRNA for human CRP. We have preliminary data that human monocyte-derived macrophages express CRP mRNA (unpublished data), concurring with similar findings in alveolar macrophages.[9] The authors also fail to mention other sources for CRP. In fact, CRP has now been reported in neurons of Alzheimer's brain, in renal tubular epithelial cells, and also in alveolar macrophages.[8,9] Based on our experimental experience, as well as that of others, we argue for potential paracrine and autocrine effects in microdomains in the intima resulting in potentially very high CRP levels. Although it is the general consensus that the majority of the CRP in the atheroma derives from the liver, it is not unreasonable that both vascular cells and adipose tissue contribute to some degree. Future studies will help determine their relative contributions. CRP produced in endothelial, vascular smooth muscle cells, and macrophages via autocrine and paracrine loops could exert biological effects on these cells contributing to atherothrombosis and plaque instability.[10]

In conclusion, the majority of published studies document CRP expression in atheroma and further support the notion that CRP is elaborated by cells of the vessel wall including endothelial cells, smooth muscle cells, and human monocytes/macrophages. We believe it is premature for Sun and colleagues[1] to arrive at the conclusion that CRP is derived exclusively from the liver. In fact, Ouchi and colleagues[11] have shown that the presence of CRP mRNA in adipose tissue has an inverse relationship with adiponectin. In addition, CRP secreted from adipose tissue has recently been documented.[12] Thus, we believe this is an area that clearly requires further scientific inquiry to establish the major sources of CRP in the

atheroma. We hypothesize that CRP in atheroma may derive from the liver, the atherosclerosic lesion, and adipose tissue. In this regard it is important to mention that CRP levels have been reported to be significantly higher in the coronary sinus versus the peripheral circulation,[13] arguing that at least some of the CRP is elaborated in the atheroma itself.

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References

1. Sun H, Koike T, Ichikawa T, Hatakeyama K, Shiomi M, Zhang B, Kitajima S, Morimoto M, Watanabe T, Asada Y, Chen YE, Fan J: C-reactive protein in atherosclerotic lesions: its origin and pathophysiological significance. Am J Pathol 2005, 167:1139–1148
2. Yasojima K, Schwab C, McGeer EG, McGeer PL: Generation of C-reactive protein and complement components in atherosclerotic plaques. Am J Pathol 2001, 158:1039–1051
3. Kobayashi S, Inoue N, Ohashi Y, Terashima M, Matsui K, Mori T, Fujita H, Awano K, Kobayashi K, Azumi H, Ejiri J, Hirata K, Kawashima S, Hayashi Y, Yokozaki H, Itoh H, Yokoyama M: Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. Arterioscler Thromb Vasc Biol 2003, 23:1398–1404
4. Jabs WJ, Theissing E, Nitschke M, Bechtel JF, Duchrow M, Mohamed S, Jahrbeck B, Sievers HH, Steinhoff J, Bartels C: Local generation of C-reactive protein in diseased coronary artery venous bypass grafts and normal vascular tissue. Circulation 2003, 108:1428–1431
5. Vainas T, Lubbers T, Stassen FR, Herngreen SB, van Dieijen-Visser MP, Bruggeman CA, Kitslaar PJ, Schurink GW: Serum C-reactive protein level is associated with abdominal aortic aneurysm size and may be produced by aneurysmal tissue. Circulation 2003, 107:1103–1105
6. Sattler KJ, Woodrum JE, Galili O, Olson M, Samee S, Meyer FB, Zhu XY, Lerman LO, Lerman A: Concurrent treatment with renin-angiotensin system blockers and acetylsalicylic acid reduces nuclear factor kappaB activation and C-reactive protein expression in human carotid artery plaques. Stroke 2005, 36:14–20
7. Calabro P, Willerson JT, Yeh ET: Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation 2003, 108:1930–1932
8. Venugopal SK, Devaraj S, Jialal I: Macrophage conditioned medium induces the expression of C-reactive protein in human aortic endothelial cells: potential for paracrine/autocrine effects. Am J Pathol 2005, 166:1265–1271
9. Dong Q, Wright JR: Expression of C-reactive protein by alveolar macrophages. J Immunol 1996, 156:4815–4820
10. Jialal I, Devaraj S, Singh U: C-Reactive protein and the vascular endothelium: implications for plaque instability. J Am Coll Cardiol (in press)
11. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, Matsuzawa Y: Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003, 107:671–674
12. Calabro P, Chang DW, Willerson JT, Yeh ET: Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. J Am Coll Cardiol 2005, 46:1112–1113
13. Inoue T, Kato T, Uchida T, Sakuma M, Nakajima A, Shibazaki M, Imoto Y, Saito M, Hashimoto S, Hikichi Y, Node K: Local release of C-reactive protein from vulnerable plaque or coronary arterial wall injured by stenting. J Am Coll Cardiol 2005, 46:239–245

Authors' Reply:

Dr. Jialal and his co-workers have raised several issues regarding our recently published paper.[1] Although we appreciate these comments, it seems that some concerns are related to a misunderstanding and/or personal interpretation of our results. The purpose of our study was to elucidate 1) whether elevated C-reactive protein (CRP) is associated with atherosclerosis in cholesterol-fed rabbits and Watanabe heritable hyperlipidemic (WHHL) rabbits; 2) which tissues (hepatic versus extrahepatic sources) are responsible for elevated CRP in plasma; 3) whether CRP is present in the lesions of atherosclerosis; and if so, 4) whether CRP is produced locally by vascular cells or transported from the circulation or both. In both hyperlipidemic rabbit models, we found that plasma CRP is strongly associated with atherosclerosis but weakly, if at all, associated with plasma cholesterol levels. We think that it is nearly impossible to determine whether hypercholesterolemia itself can directly raise CRP production in the liver without considering atherosclerosis (vascular inflammation) in these animals. Furthermore, compared to the relationship between lesion size and CRP, the hypercholesterolemia-CRP relationship seems to be casual, and therefore, we should not overstate its importance in terms of atherosclerosis.

The second set of issues raised by Jialal and colleagues involves whether CRP is really present in the lesions and from where such CRP is derived or whether vascular cells can directly produce CRP. Our study clearly demonstrates that the liver is the principal organ that expresses CRP mRNA in rabbits (based on Northern blotting analysis). Despite the substantial amount of CRP proteins in the arterial wall, normal aortas as well as atherosclerotic aortas expressed extremely low levels of CRP mRNA. First, CRP mRNA expression was below the detection limits of Northern blotting. Second, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) results showed that absolute values (arbitrary units) of aortic CRP expression, regardless of the presence or absence of lesions, are less than 1/100th of that from the liver. Furthermore, neither alveolar nor peritoneal macrophages showed detectable CRP expression. The same was true for human aortas (eight autopsy cases) and activated macrophages (U937 cells). Thus, our findings argue against the notion that arterial wall cells (especially macrophages) are the major sources for CRP production and responsible for the elevation of CRP in plasma. By analyzing 10 human autopsy cases by semiquantitative RT-PCR, Yasojima and colleagues[2] have shown that atherosclerotic aortas express 10.2-fold higher CRP than normal aortas and 7.2-fold higher CRP than liver. Such data have never been confirmed by other researchers in the literature, even though sporadic reports document that vascular cells indeed express extremely low levels of CRP. In this regard, CRP mRNA can be detected in saphenous veins (n = 10 of 15) and coronary artery venous bypass grafts (n = 11 of 15) but not in coronary arteries with advanced atherosclerotic lesions (n = 0 of 10),[3] abdominal aortic aneurismal tissues (n = 4 of 16),[4] carotid plaques (n = unknown of 41),[5] or coronary plaques (n = unknown of 39)[6] by various methods. It seems likely that vascular

smooth muscle cells[7] and endothelial cells,[8] along with adipocytes[9] in certain situations, may express CRP (at extremely low levels compared to those expressed by liver or HepG2 cells), although it remains unclear whether small amounts of vascular cell-derived CRP are really physiologically important in terms of atherosclerosis and plaque rupture.

Jialal and co-workers[8] have reported that treatment with macrophage-conditioned medium leads to a twofold increase of CRP production in human aortic endothelial cells and have proposed an intriguing paracrine/autocrine hypothesis. However, the relevance of these *in vitro* observations to *in vivo* situations remains unknown, and the significance of vascular cell-derived CRP *in vivo* may require more comprehensive investigations of both animal experiments and clinical studies. Furthermore, it still seems premature to conclude that vascular cell-derived CRP (compared to large amounts of circulation-derived CRP in the lesions) may actually make a major contribution to the pathogenesis of atherosclerosis and plaque stability because we do not have a clear indication of whether CRP is a "good guy" or "bad guy" in terms of lesion development.[10] Nevertheless, we are still left wondering whether CRP in the lesions is a cause, consequence, or both of atherosclerosis, because the presence of CRP at the "scene of the crime" is not necessarily evidence of guilt. CRP could be an innocent bystander, a victim, or possibly an atheroprotective force. At present, neither transgenic nor knockout mice seem useful for the study of CRP, and the development of alternative CRP experimental animals is urgently required.[11] We expect that in the next few years, with the advent of novel CRP transgenic rabbit models, we will soon find an answer to whether CRP is only a "marker" or is also a "maker"[11] of atherosclerosis and determine whether we should target CRP for the treatment of cardiovascular diseases in future.[12]

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References

1. Sun H, Koike T, Ichikawa T, Hatakeyama K, Shiomi M, Zhang B, Kitajima S, Morimoto M, Watanabe T, Asada Y, Chen YE, Fan J: C-reactive protein in atherosclerotic lesions: its origin and pathophysiological significance. Am J Pathol 2005, 167: 1139–1148
2. Yasojima K, Schwab C, McGeer EG, McGeer PL: Generation of C-reactive protein and complement components in atherosclerotic plaques. Am J Pathol 2001, 158:1039–1051
3. Jabs WJ, Theissing E, Nitschke M, Bechtel JF, Duchrow M, Mohamed S, Jahrbeck B, Sievers HH, Steinhoff J, Bartels C: Local generation of C-reactive protein in diseased coronary artery venous bypass grafts and normal vascular tissue. Circulation 2003, 108:1428–1431
4. Vainas T, Lubbers T, Stassen FR, Herngreen SB, van Dieijen-Visser MP, Bruggeman CA, Kitslaar PJ, Schurink GW: Serum C-reactive protein level is associated with abdominal aortic aneurysm size and may be produced by aneurysmal tissue. Circulation 2003, 107:1103–1105
5. Sattler KJ, Woodrum JE, Galili O, Olson M, Samee S, Meyer FB, Zhu XY, Lerman LO, Lerman A: Concurrent treatment with renin-angiotensin system blockers and acetylsalicylic acid reduces nuclear factor kappaB activation and C-reactive protein expression in human carotid artery plaques. Stroke 2005, 36:14–20
6. Kobayashi S, Inoue N, Ohashi Y, Terashima M, Matsui K, Mori T, Fujita H, Awano K, Kobayashi K, Azumi H, Ejiri J, Hirata K, Kawashima S, Hayashi Y, Yokozaki H, Itoh H, Yokoyama M: Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. Arterioscler Thromb Vasc Biol 2003, 23:1398–1404
7. Calabro P, Willerson JT, Yeh ET: Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation 2003, 108:1930–1932
8. Venugopal SK, Devaraj S, Jialal I: Macrophage conditioned medium induces the expression of C-reactive protein in human aortic endothelial cells: potential for paracrine/autocrine effects. Am J Pathol 2005, 166:1265–1271
9. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, Matsuzawa Y: Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003, 107:671–674
10. Pepys MB: CRP or not CRP? That is the question. Arterioscler Thromb Vasc Biol 2005, 25:1091–1094
11. Nilsson J: CRP—marker or maker of cardiovascular disease? Arterioscler Thromb Vasc Biol 2005, 25:1527–1528
12. Torzewski J: C-reactive protein and atherogenesis: new insights from established animal models. Am J Pathol 2005, 167:923–925