An About-Face for C-Reactive Protein?

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Human C-reactive protein (CRP)2 is a liver-synthesized blood constituent, the production of which increases rapidly and robustly in parallel with tissue damage, infection, and trauma (1). Owing to this predictable behavior, increases in the blood concentration of CRP have long been used as a clinical gauge of inflammation (2). More recently, with the introduction of higher-sensitivity laboratory assays, it has become apparent that even a modestly increased baseline CRP concentration in healthy individuals correlates tightly with their increased future risk of cardiovascular disease (3, 4). More than 20 prospective epidemiologic studies carried out in populations of individuals with no prior history of cardiovascular disease have demonstrated that a single CRP measurement at baseline is a strong predictor of future cardiovascular events (4). The relationship between an increased baseline CRP concentration and vascular risk is apparent for both men and women and remains consistent in studies from around the world. In most cases, this relationship is independent of traditional risk factors, including age, smoking, high blood pressure, and high cholesterol concentrations (4). A high preprocedural baseline CRP value is also associated with an increased occurrence of fatal and nonfatal myocardial infarction after percutaneous coronary interventions (4, 5). Notably, the reduction in blood CRP that accompanies statin therapy improves clinical outcomes and decreases the progression of atherosclerotic coronary disease independent of cholesterol lowering, a finding that highlights the possibility of using CRP as a therapeutic target (6–8). Despite indisputable evidence that CRP is associated with inflammation and the risk of cardiovascular disease, the question of whether CRP contributes to either process remains enigmatic. If CRP does participate in the cardiovascular disease process, how does it do so? The report by Fujita et al. in this issue of Clinical Chemistry (9) provides a new clue to help answer these nagging questions, and it all has to do with how the pentraxin form of CRP relates to its function (1).

CRP is a pentameric protein composed of 5 identical subunits noncovalently bound together in radial symmetry around a central pore (1). As far as is known, the function of CRP is intimately related to this unusual structure, because the arrangement produces a disk-shaped complex with 2 opposing faces. The ligand-recognition face (the B face) contains the sites that enable binding of phosphocholine (the first CRP ligand identified) when Ca2+ is available (1, 10). Other CRP ligands relevant to cardiovascular disease—such as nuclear autoantigens, apoptotic cells, and lipoproteins—have also been identified (1), but binding to most of these ligands also requires Ca2+ and can be inhibited by phosphocholine, indicating that the single promiscuous ligand-binding site on the B face can accommodate diverse structural groups. Because each CRP subunit has a binding site, the B face of CRP associates with high avidity to surfaces that display appropriately arranged ligands, such as phosphatidylcholine on the exterior of oxidized LDL and on the surface of apoptotic cells. On the other face of CRP (the A face), the 5 protomers cooperatively form a single larger binding site that can accommodate both complement protein C1q (1, 11) and various Fc receptors (1, 12). Thus, by triggering activation of the complement system, CRP is able to opsonize targets (1). In addition, there is evidence that CRP is present in atherosclerotic lesions alongside complement proteins, and animal studies suggest that CRP activates the complement system and aggravates myocardial injury after ischemia–reperfusion in several species (4). By engaging Fc receptors, the A face of CRP also can initiate cell-signaling events; indeed, recent animal studies have indicated a requirement for certain Fcy receptors in CRP-mediated vascular disease (13). There is no evidence that the A face of CRP can bind C1q and an Fc receptor simultaneously, so what is it that controls whether CRP binds C1q or engages an Fc receptor? Here enters LOX-1.

LOX-1, lectin-type oxidized LDL receptor 1, was originally identified as a receptor for atherogenic oxidized LDL in vascular endothelial cells (14). LOX-1 shares many attributes with CRP. Like CRP, LOX-1 appears to contribute to multiple stages of cardiovascular disease, such as endothelial dysfunction, atherogenesis, myocardial ischemia–reperfusion injury, and

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2 Nonstandard abbreviations: CRP, C-reactive protein; LOX-1, lectin-type oxidized LDL receptor 1.
restenosis after balloon injury. Also like CRP, LOX-1 is produced in human atherosclerotic lesions. In recent work published in this journal, Fujita et al. reported the exciting discovery that CRP actually binds to LOX-1 in vitro, and they provided evidence that this interaction also takes place in vivo (15, 16). Thus, in a strain of rats that produces large amounts of LOX-1, intradural injection of human CRP increased vascular permeability, and the effect could be suppressed by coadministration of an anti–LOX-1 antibody. Those findings were important because they established that 2 recognized risk factors for cardiovascular disease (CRP and oxidized LDL) used the same receptor (LOX-1), which is itself linked to cardiovascular disease. In fact, LOX-1 might be the underlying reasons why both CRP and oxidized LDL are associated with cardiovascular disease. What was unknown at the time was how LOX-1 interacted with CRP and how this interaction promoted a response to vascular injury.

In the current report, Fujita et al. extend their studies of CRP and LOX-1 in 2 important ways. First, they investigated the manner by which the 2 proteins might physically interact, and, second, they sought to identify the potential mechanism(s) of CRP action that lead to increased vascular permeability in their rat model. The authors again showed that human CRP binds in a specific and saturable manner to recombinant human LOX-1 immobilized on an inert substrate (ELISA plates). Additionally, they established for the first time that this binding is Ca<sup>++</sup>-dependent and inhabitable with phosphocholine. These latter properties suggest that the interaction of CRP with the LOX-1 receptor occurs on the face of CRP that binds phosphocholine, i.e., the B face, not the face of CRP that binds to C1q and Fc receptors, i.e., the A face. If this finding is confirmed, it makes LOX-1 the only receptor known to bind CRP in this way. Another new finding was that when CRP was allowed to interact with human LOX-1 in vitro, complement was activated (as measured by the generation of the opsonin C3d). This effect was dependent on the presence of C1q. CRP-dependent deposition of C3d was achieved in the presence of LOX-1 displayed on living CHO cells that were engineered to express the receptor, as well as in the presence of recombinant LOX-1 coated on ELISA plates. Importantly, there was little complement activation under the same conditions but without LOX-1. The implication is that LOX-1–bound CRP is in an “A face up” orientation, thus presenting it to C1q for docking. In vivo experiments with rats demonstrated that intradermal injection of CRP induced complement activation (again as evidenced by the detection of C3d), and this effect was associated with an increase in the number of myeloperoxidase-positive cells (neutrophils) infiltrating the affected area. This result is entirely consistent with the generation of chemotactic complement split products, the expected outcome of CRP-triggered C1q activation (1). Importantly, however, both the CRP-mediated deposition of C3d and the infiltration of myeloperoxidase-positive cells were decreased in this animal model by injection of an anti–LOX-1 antibody. Although the authors did not demonstrate it, the anti–LOX-1 antibody presumably blocked CRP binding and thus hindered complement activation. Despite a few weaknesses, their data in sum are consistent with the authors’ hypothesis that LOX-1 might serve to localize CRP-induced complement activation to sites of inflammation—in this case to the dermal veins. This mode of action might explain the colocalization of CRP and complement observed in human hearts undergoing myocardial infarction and in blood vessels with atherosclerotic plaques.

A more detailed structural study is required to formally verify the manner by which CRP and LOX-1 interact. It would be very interesting to know the outcome of CRP administration in LOX-1–deficient and C1q-deficient animals, and it would be informative to know if the observations are reproducible in human CRP transgenic mice. In the meantime, the data presented by Fujita et al. suggest that the B face of CRP can interact not only with ligands like phosphocholine (10) but also with at least one membrane receptor (LOX-1), interactions that both require Ca<sup>++</sup>. As with the interactions of the CRP A face with C1q (11) and Fc receptors (12), the interactions of the CRP B face with phosphocholine and LOX-1 apparently cannot occur simultaneously. In my view, the most intriguing possibility raised by the data presented by Fujita et al. is that the effector pathways recruited by the A face of CRP (i.e., Fc receptor vs C1q) might be determined by what is occupying the B face (i.e., phosphocholine vs LOX-1, respectively). If so, CRP might then operate as a kind of fluid-phase adaptor protein, with each of its 2 faces capable of binding to their own unique ligand/receptor pair. Specificity would be achieved simply by the type of ligand bound and by the localization and proximity of receptors. If CRP functions as an extracellular adaptor protein, i.e., by linking certain ligands to particular receptors in a bidirectional way that targets the host response and dictates its outcome, then a variety of the seemingly paradoxical CRP-mediated effects reported in the literature could possibly be explained. This concept currently flies in the face of what is known about CRP structure and function, so it certainly warrants further investigation.

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