High-density lipoprotein and C-reactive protein, friend and foe in cardiovascular disease
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Letters to the Editor: reply to van den Berg, CW and Taylor, KE

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Dear Editor:

We appreciate the interest of van den Berg and Taylor in our article on the direct actions of CRP in humans. In the context of numerous *in vitro* studies demonstrating that CRP elicits pro-atherogenic changes, our study was designed to examine the bioactivities of CRP in a human setting.

Van den Berg states that filtration methods are not very rigorous in removing endotoxin. However, the currently used purification process was successful in lowering the endotoxin burden by more than 97.5% within the recombinant human CRP (rhCRP) preparation to levels insufficient to cause any bio-activity *in vivo*, as demonstrated by standard methods. The final purified product was >97% pure by high-pressure size-exclusion chromatography (SEC-HPLC) and reverse-phase HPLC, whereas Time-of-Flight mass spectrometry provided supporting data for high purity, showing no other protein fractions besides the rhCRP.

Second, van den Berg poses that the kinetics of cytokine production observed upon CRP-infusion fit the cytokine profile of endotoxin contamination. However, upon comparison to endotoxin activities in humans, we show a clear disparity in cytokine profiles between that mediated by CRP and endotoxin. Administration of endotoxin to animals as well as humans is invariably associated with rapid induction of TNFα, a central and obligatory mediator that orchestrates a consistent sequence of events regarding cytokine production and coagulation activation. Markedly, throughout all human CRP protocols, we were unable to detect any change in TNFα expression, either at leukocyte mRNA levels or circulating plasma levels. Furthermore, heat-inactivation of the CRP preparation significantly diminished the capacity of CRP to evoke an inflammatory response in whole blood stimulation assays (Bisoendial, data on file). This finding, that is consistent with data from other investigators, demonstrates again that CRP itself is largely responsible for the pro-inflammatory effects rather than potential contaminants in this highly purified rhCRP preparation.

Finally, van den Berg questions the kinetics of CRP levels which appears to be inconsistent with levels of IL-6 and IL-8 that decrease from 4 hour onwards. Apart from the disparity in half-life between CRP and measured cytokines, several aspects of the inflammatory response merit further clarification. Thus, CRP induces the secretion of cytokines from arterial endothelium and monocyte macrophages. Cumulated evidence suggests that these effects are receptor-mediated. Deveraj et al. recently demonstrated that CRP exerts its biological activities in human aortic endothelial cells (HAEC) via binding and internalisation through Fcy receptors, CD32 and CD64. Similarly, biological activity upon ligand-receptor engagement has been shown by other investigators in monocytes. In this regard, saturable binding and/or uptake of CRP in HAECs as well as leukocytes may have blocked further cytokine production in our study. An alternative explanation for the temporary cytokine response despite persistently high CRP levels involve activation of potent counter-regulatory systems, including IL-10 and induction of A20 (data on file). Moreover, CRP has been shown to regulate soluble IL-6 receptor (sIL-6R) shedding in human neutrophils and markedly increase sIL-6R/IL-6 complex formation. Apart from the biological responses that IL-6 may elicit via its receptor, the IL-6/sIL-6R complex may perpetuate its own biological activity by binding the signal-transducing
component of the IL-6R complex. Infusion of rhIL-6 was recently shown to be associated with a delayed increase in plasma CRP in humans, which was sustained for more than 16 hours. Thus, self-perpetuating mechanisms in view of inflammatory responses are not uncommon, and the IL-6 increase may conceivably have resulted in the second rise in CRP levels after 24 hours.

In conclusion, we concur with van den Berg that caution is mandatory with respect to the use of purified, commercial rhCRP in experimental studies. However, the currently available data provide compelling evidence that CRP itself mediates pro-inflammatory and pro-thrombotic effects in vivo in humans. Within the next few years, novel strategies, directed at specifically targeting CRP-activity, probably may enable us to further elucidate the biological relevance of these actions in humans.

Reference List