High-density lipoprotein and C-reactive protein, friend and foe in cardiovascular disease
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Letters to the Editor: In vivo effects of C-reactive protein (CRP)-infusion into humans

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Dear Editor,

We have diligently read the research commentary by Pepys et al.\(^1\), which amongst others addresses the in vivo effects of C-reactive protein (CRP)-infusion into humans, as recently reported by us in a previous issue of this journal \(^2\). We would like to clarify some misconceptions surrounding our study, as generated in their report. Pepys et al. observed, using recombinant human (rh)CRP that still contained large quantities of endotoxin, that this rhCRP-solution induced an inflammatory reaction both in vitro as well as in mice, whereas CRP from human resources had no such effect. Pepys et al. then extrapolate these findings to our study in humans and conclude that the in vivo effects of rhCRP we observed must have been caused by contaminants rather than rhCRP itself. In fact, these authors arrive at the wrong conclusion and they fail to acknowledge several shortcomings in their own experiments.

First, their dialysed commercial rhCRP displayed residual endotoxin activity of 46.6 endotoxin units (EU) per mg of rhCRP and would for that sake never have been allowed for human use. In comparison, endotoxin activity of our rhCRP was 30-times lower (< 1.5 EU/mL), which resulted in less than 1.6 EU per mg of rhCRP (at a CRP-concentration of 0.91 mg/ml). Interestingly, the trace amounts in our rhCRP are very similar to those reported by Pepys et al. in their natural human (nh)CRP solution, i.e. 0.9 EU per mg of nhCRP. Needless to state that extrapolation of their findings, using highly contaminated rhCRP, to our study results does not make any sense.

Second, Pepys et al. argued that contaminants, rather than CRP, are responsible for the inflammatory effects in humans and tested this hypothesis in a mouse model. Using rhCRP with very high endotoxin activity, they observe a substantial acute phase response in accord with previous reports on the clinical sequelae of endotoxin\(^3\). Nevertheless, Pepys et al. fail to acknowledge that the residual endotoxin levels in our rhCRP have been proven insufficient to cause any bio-activity in vivo \(^4\) and in vitro. Notably, data on nhCRP now provided by Pepys et al., containing comparable amounts of endotoxin as our rhCRP, further corroborate this observation. To even further substantiate this, we infused lipopolysaccharide (LPS) (E.coli lipopolysaccharide, lot G2B274, United States Pharmacopeial Convention Inc, Rockville, USA) into two healthy volunteers at a dose (1.5 EU/kg) that equaled the mean co-infused dose during the CRP-infusion experiments. Compared to the reference values of higher dose LPS infusion (10 EU/kg) (n=4) and rhCRP infusion (n=4) groups, none of the subjects receiving the low dose LPS infusion showed any change in TNF-α, as assayed by cytometric bead array analysis (BD Biosciences, San Jose, CA, USA)(figure 1). Despite the heterogeneity in potency among endotoxins of different sources, these data demonstrate that the trace amounts of endotoxin present in our rhCRP-solution can not have contributed to the inflammatory reactions we observed in our human study subjects.

Third, it should be noted that assessing CRP biology in a mouse model has been criticized. As emphasized by Reifenberg et al., the mouse is not a suitable model for assessment of the consequences of human CRP on atherogenesis \(^5\). The latter is most markedly illustrated by the complete absence in this rodent model of one of CRP’s main biological functions, i.e. lipopro-
Figure 1. Effects of intravenous injection of 1.5 EU/kg LPS, 10 EU/kg LPS and 1.25 mg/kg rhCRP on temperature, haematological responses and systemic inflammation in humans. Mean (± SE) values of temperature, leukocyte numbers, percentage monocytes, and levels of TNF-α are depicted. Compared to reference values of high-dose LPS (n=4) and rhCRP (n=4) groups, none of the subjects of the low-dose LPS group (n=2) developed an increase in body temperature. No early leukopenia with monocyte depletion was observed. Further, antigen levels of TNF-α remained unaltered during the experiments.

tein-dependent complement activation. In line, recent observations in genetically engineered mice that regard the effect of human CRP on atherosclerosis are rather contradictory. Finally, the statement by Pepys et al that the massive SAA-response to rhCRP-infusion may even increase risk of AA amyloidosis is completely misplaced. In reality, the SAA levels observed in our study are comparable to SAA levels seen in adults infected with uncomplicated influenza.
In conclusion, we concur with Pepys et al. that data on the downstream pro-inflammatory consequences of CRP must be interpreted with caution. However, the data currently generated by Pepys et al. bear no relevance with respect to our findings in humans. This is due, amongst others, to the 30-fold higher endotoxin activity in their solution as well as to the use of a rodent model in evaluating the effects of human CRP. Hence, we still whole-heartedly support Pepys in his conclusion that CRP represents an attractive target for cardiovascular prevention.

Reference List
