Lipopolysaccharide (LPS) Contamination Plays the Real Role in C-Reactive Protein–Induced IL-6 Secretion From Human Endothelial Cells In Vitro

To the Editor,

In a recent article, Taylor et al.1 have shown that C-Reactive Protein (CRP), per se, does not activate endothelial cells; rather, lipopolysaccharide (LPS) and azide were responsible for the observed cellular activation. We would like to provide our data to further support the conclusion by Taylor and colleagues.

We have tested >10 batches of commercial human CRP preparations from 3 companies (Calbiochem, TriChem, and Sigma). Human saphenous vein endothelial cells (HUVECs, passages 3 to 5) were grown in MCDB-131 medium supplemented with 10% FBS. The cells were incubated with CRP for 24 hours, and the culture supernatants were assessed for IL-6 using the IL-6 ELISA kit (R&D). It was reported previously that CRP induced IL-6 production from HUVECs.2 Our main observations are summarized as follows.

All recombinant human CRP (rCRP, Escherichia coli–derived) that we tested stimulated HUVECs to secrete IL-6 significantly, and all of these CRP preparations contained endotoxin (10 to 100 EU/mL) as measured by the LAL gel clot assay (Associates of Cape Cod).3 The CRP-induced IL-6 secretion was correlated with the level of endotoxin contamination. Moreover, all these CRP preparations lost their ability to activate HUVECs after endotoxin was removed using a detoxi-gel column (Pierce). Furthermore, reintroducing LPS to CRP to a level equivalent to that presented before purification re-established the ability of CRP to stimulate HUVECs (Figure). The CRP preparation purified from human plasma (Sigma) had low endotoxin levels (<0.31 EU/mL) and did not stimulate HUVECs (up to 100 µg/mL of CRP). However, the CRP purified from human plasma and purchased from TriChem contained high levels of endotoxin (160 EU/mL) and caused a robust production of IL-6 (up to 5 ng/mL). Analysis by size exclusion chromatography and SDS-PAGE suggest that removal of LPS by detoxi-gel column did not result in change in CRP structurally. Taken together, endotoxin contamination plays a confounding role in the interpretation of biologic actions previously attributed to CRP.

Sandhya S. Nerurkar
Patrick J. McDevitt
Gilbert F. Scott
Kyung O. Johanson
Robert N. Willette
Tian-Li Yue

Departments of Investigative and Cardiac Biology (S.S.N., R.N.W., T.-L.Y) and Protein Chemistry (P.J.M., G.F.S., K.O.J.) GlaxoSmithKline Pharmaceuticals, King of Prussia, Penn