

## LETTER TO THE EDITOR

### PLATELET ACTIVATION STIMULATED BY THE TOXIN OF THE BROWN RECLUSE SPIDER REQUIRES SERUM AMYLOID P COMPONENT, NOT C-REACTIVE PROTEIN

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PREVIOUS studies in our laboratory, published in this journal (REES *et al.*, 1988) implicated C-reactive protein (CRP) as a co-factor required for platelet activation induced by the toxin of the brown recluse spider. Isolated human platelets free from plasma proteins were activated to aggregate and secrete serotonin when incubated with the toxin, calcium and adult plasma. However, if umbilical cord plasma was substituted for adult plasma no detectable activation of platelets was observed. Furthermore, we reported that the addition of supraphysiologic concentrations of CRP enabled cord plasma to support the toxin's effects on platelets.

More recent data from our laboratory establishes that serum amyloid P component (SAP), not CRP, is most likely the protein required by the toxin to activate human platelets *in vitro*, since SAP could be used at physiological concentrations. The results described above for supraphysiologic concentrations of CRP were probably attributable to contaminating SAP. It is well recognized that SAP is a common contaminant in CRP purifications (PEPYS *et al.*, 1977). This possibility could not be directly confirmed for the CRP preparation used in our initial study, however, since the supplier (Difco, Detroit, MI) had discontinued its production.

To our surprise, CRP obtained from other suppliers (Pierce, Rockford, IL, and Calbiochem-Behring, San Diego, CA) was unable to support platelet activation over a wide range of concentrations. No SAP contamination was observed in these CRP preps when analyzed by sodium dodecyl sulfate-polyacrylamide electrophoresis; moreover, the SAP prep that supported platelet activation was also found to be pure.

Others have shown that CRP and SAP are deficient in umbilical cord plasma compared to adult plasma (PEPYS *et al.*, 1978). The cord plasma samples used in both our initial and all subsequent experiments were analyzed by a modified electroimmunoassay technique (LAURELL, 1972) and found to contain low levels of SAP ( $6.1 \pm 5.0 \mu\text{g/ml}$  compared to  $50 \pm 18 \mu\text{g/ml}$  for adult plasma). When SAP was added to cord plasma to levels equivalent to that found in adult plasma, it then supported platelet aggregation and serotonin secretion as well as did adult plasma. Highly purified CRP, even at supraphysiologic levels, could not substitute for SAP in mediating toxin-induced platelet activation in cord plasma.

In conclusion, we acknowledge and hope to have now clarified the apparent discrepancy in the data from our laboratory. Other investigators as discussed by DE BEER and PEPYS (1982) have experienced similar difficulties, in different experimental systems, when

attempting to reproduce results obtained with certain CRP preparations. We hope that the data described here are convincing evidence that SAP rather than CRP is the plasma component required for the brown recluse spider toxin's *in vitro* effects on platelets.

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