

Background and objectives C-reactive protein (CRP) is an acute-phase protein whose serum concentration increases massively in response to tissue injury or inflammation. Beside membranes of bacteria CRP is known to bind calcium dependent to altered mammalian membranes (as occurring in cell death) via phosphocholine, the polar headgroup of phosphatidylcholine and its derivatives. CRP also opsonises intracellular components leaking from necrotic cells. This study was performed to analyse whether CRP differentially binds to primary and secondary necrotic cells. Primary necrosis is defined as immediate cell death appearing after severe death stimuli, whereas secondary necrosis follows after an apoptotic stadium.

Materials and methods We prepared directly FITC-labelled CRP to determine the binding of CRP to primary and secondary necrotic cells by flow cytometry and confocal microscopy. The release of a CRP target from dying cells was detected in the supernatant by inhibition assays. The released CRP antagonist was identified by enzymatic cleavage and by mass spectrometry.

Results The binding of CRP to primary necrotic cells was significantly stronger compared to secondary necrotic ones. We detected a CRP antagonistic activity in supernatants of secondary necrotic cells, which was identified as glycerophosphocholine (GlyceroPC). Actually, CRP binding to necrotic cells was blocked by synthetic GlyceroPC. The release of the CRP antagonist was significantly reduced by inhibitors of Phospholipase A2 and Caspases.

Conclusions Secondary necrotic cells show a weaker binding of CRP resulting from the release of the CRP target during late apoptosis. That may have implications for opsonisation with CRP of dying and dead cells and for CRP-mediated effects in clearance. Secondary necrotic cells, for example in SLE patients, may release high amounts of the CRP antagonist with the following consequences (1) missing CRP binding targets on the dead cells and (2) neutralising of circulating CRP, both possibly contributing to clearance failure.

A19 CRP DISCRIMINATES PRIMARY FROM SECONDARY NECROSIS

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