

C1q and C-reactive Protein (CRP) Modulate Platelet Activation on Adsorbed Immunoglobulin G and Albumin

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INTRODUCTION: Blood platelets have for long been recognized during hemostasis but are now also emerging as key actors during inflammation following implantation of a biomaterial, as well as in diseases with an inflammatory component such as atherosclerosis. A material in contact with blood will adsorb plasma proteins within milliseconds, e.g. immunoglobulin G (IgG) and albumin. Surprisingly, little is known regarding how protein adsorption regulates the platelet activation and their subsequent impact on other cells. C-reactive protein (CRP) is an acute phase protein which is elevated during inflammation and tissue injury, and represents a powerful predictor of coronary artery disease¹. Although the physiological role of CRP is incompletely understood, we and others have shown that CRP binds to C1q and activates the classical complement pathway, although limited to the initial stages of the complement system, i.e. no membrane attack complex is formed.

The aim of this study was to characterize the CRP, C1q and adsorbed plasma protein interaction with respect to platelet activation.

METHODS: IgG and albumin were spontaneously pre-adsorbed to methylated inorganic supports, and CRP and C1q were allowed prior humoral interaction before surface exposure. Protein adsorption was analyzed by ellipsometry, combined with polyclonal antibody detection. Surface-triggered platelet activation was investigated using a static platelet adhesion assay, where adherent platelets were stained for F-actin and visualized in a fluorescence microscope. Furthermore, platelet phosphatidylserine expression was evaluated by annexin-V-binding, and thromboxane B2 was measured as a marker for platelet secretion.

RESULTS: CRP alone did not associate with the adsorbed IgG, but when preincubated with C1q,

both C1q and CRP were detectable on the surface. Ellipsometry also confirmed that C1q bound to the adsorbed IgG, and also suggested that both C1q and CRP may bind to pre-adsorbed albumin, a protein regarded to blunt inflammation. The platelet count and the cell morphological examination showed extensively more activated platelets on IgG surfaces in reference to albumin surfaces. Interestingly, the addition of C1q or CRP reduced the adhesion to IgG, HSA and methylated matrices. Furthermore, preincubation of C1q and CRP was the most effective in reducing platelet adhesion to the adsorbed IgG. It was also observed that C1q triggered an incomprehensive platelet activation morphology, for all surfaces. The inhibitory effects of CRP and C1q were also seen with platelet phosphatidylserine expression.

DISCUSSION & CONCLUSIONS: Platelet adhesion to adsorbed plasma proteins is inhibited by complement protein C1q as well as by the acute-phase protein CRP. It is possible that C1q facilitates the binding of CRP to IgG and thereby modulate platelet activation. We suggest that this regulatory role of CRP may be important in preventing the potential harmful side effects of inflammation at artificial surfaces, e.g. extensive platelet activation.

REFERENCES: ¹ C. Sjöwall, J. Wetterö, (2007) *Clin Chim Acta* **378**: 13-23.

ACKNOWLEDGEMENTS: Agneta Askendal is acknowledged for valuable laboratory assistance. This study was supported by the strategic research area "Materials in Medicine" (Linköping University and the County Council of Östergötland), the Swedish Fund for Research without Animal Experiments, Trygg-Hansa Research Foundation, Swedish Society for Medicine and the Goljes minne and Magn Bergvall research foundations.