C-reactive protein and atherothrombosis

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Circulating concentrations of C-reactive protein (CRP), the classical acute phase protein and sensitive systemic marker of inflammation, significantly predict atherothrombotic events and outcome after acute myocardial infarction, demonstrating the key role of inflammation in atherosclerosis and its complications. The binding specificity of CRP for low density lipoproteins, for modified low density lipoproteins, and for damaged and dead cells, coupled with the capacity of bound CRP to activate complement, and with the presence of CRP in atheroma and acute myocardial infarction lesions, all suggest a possible pathogenetic role of CRP. Development of drugs to block binding of CRP to its various ligands *in vivo* will enable this hypothesis to be tested.

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Inflammation is a major feature of the arterial lesions of atherosclerosis[1], and there is substantial evidence of an association between antecedent or concurrent systemic inflammatory activity and the occurrence of atherothrombotic events, especially myocardial infarction[2]. C-reactive protein (CRP), the classical acute phase protein, is an exquisitely sensitive systemic marker of inflammation and tissue damage[3], and there is therefore nothing inherently surprising about the recent torrent of reports demonstrating a powerful predictive relationship between increased CRP production, even within the range previously considered to be normal, and atherothrombotic events[4-13]. Indeed circulating CRP values correlate closely with other diverse markers of inflammation, some of which show similar, albeit generally less significant, predictive associations[14,15]. However CRP itself is particularly interesting because not only does it bind selectively to low density lipoproteins (LDL)[16], especially oxidised and enzyme modified LDL as found in atheromatous plaques[17], but it is actually deposited in the majority of such plaques[18,19] and it has a range of pro-inflammatory properties that could potentially contribute to the pathogenesis, progression and complications of atheroma[20-22]. Furthermore, CRP is invariably produced in large amounts in response to myocardial necrosis[23,24], the peak values of circulating CRP powerfully predict outcome after myocardial infarction[24-27], CRP is deposited within all acute myocardial infarcts[28,29], and there is compelling evidence that CRP contributes to the severity of ischaemic myocardial injury[30].

The production of CRP following myocardial necrosis is the typical acute phase response to cell death and inflammation, mediated by the action on the liver of the cytokine cascade, especially interleukin-6, triggered by such events. However the stimuli that trigger the very low grade up-regulation of CRP production that predicts coronary events in general populations[10,11,13], or the more substantial CRP values associated with poor prognosis in severe unstable angina[5,31,32] or after angioplasty[33], have not been clearly identified. They may arise from inflammation within the atheromatous lesions themselves, and thus reflect their extent and/or severity or instability. Alternatively they may reflect inflammation elsewhere in the body, although there is no strong correlation with serological evidence of the various chronic microbial infections, such as *Chlamydia pneumoniae* and *Helicobacter pylori*, that have been putatively linked with coronary heart disease[13]. Indeed, within what was until recently accepted as the reference range for circulating CRP concentration, up to 5 or 10 mg/l[34], higher values have now been found to be strongly associated with increased body mass index[35] and, more specifically, with many features of the insulin resistance or metabolic syndrome[36], up to and including frank diabetes mellitus[37]. This may reflect in part the fact that adipocytes are the source of a substantial portion of base-

line interleukin-6 production[38], but in general it suggests that some or even most of the inflammatory marker profile associated with increased atherothrombotic risk in the population at large, may not be triggered by inflammation or tissue damage in the classical sense. Rather it may be a sign of a particular metabolic state which happens also to be pro-atherogenic, or at least predisposing to atherothrombotic events. Interestingly oral contraceptive use[39] and post-menopausal hormone replacement therapy[40-42] are also associated with significantly raised baseline CRP concentrations without any sign of tissue-damaging inflammation. A further intriguing association exists between endothelial dysfunction, a marker of atherosclerosis related to coronary events, and systemic inflammation indicated by increased CRP production[43,44].

At the other pole of interpretation of these important new observations is the possibility that CRP itself may have a significant pathogenetic role in atherogenesis, plaque destabilisation and atherothrombosis. The binding of CRP to lipids, especially lecithin, and to plasma lipoproteins, especially what was formerly called β-lipoprotein, has been known for over 60 years, and we first suggested a possible relationship to atherosclerosis when we showed that aggregated, but not native, non-aggregated, CRP selectively bound just LDL from whole serum[16,45]. We looked hard for CRP in various arterial lesions but failed to detect it convincingly by immunofluorescence techniques[46]. Subsequently there were reports of the ubiquitous deposition of CRP in all human atherosclerotic plaques[47,48], but we were unable to confirm the immunological specificity of the reagents and procedures used. However recent studies with more sensitive immunoperoxidase methods[18] and with appropriately rigorous controls for specificity of CRP staining[19] have convincingly shown that CRP is indeed present in most plaques, and we have confirmed this in our own laboratory.

CRP thus binds LDL, the major lipoprotein class deposited in the arterial wall in atheroma, and binds especially well to modified LDL of the type found in lesions[17]; furthermore CRP is present in the actual plaques. What effects could it have there? First, the capacity of aggregated and ligand-complexed human CRP to activate the classical complement pathway has long been known[49,50]. Although there is discussion about the efficiency of such activation in generating the lytic terminal complement complex[51], the potent activation of C3 is undisputed and can thus unleash the major opsonic and chemotactic functions of the complement system. Second, bound CRP may be recognised by a subset of cellular Fc(γ) receptors and thus directly activate phagocytic cells[52-54]. Third, CRP has been reported to stimulate tissue factor production by peripheral blood monocytes and could thereby have important pro-coagulant effects[55,56]. However this latter action of CRP has not been well defined or robustly controlled; for example, all the published work has been done with commercially sourced CRP of incompletely defined provenance, purity, etc., and there have been few robust specificity controls. Nevertheless if the phenomenon is reproducible it provides a possible direct link between increased CRP production and atherothrombotic events.

Once arterial occlusion has occurred and there is ischaemic tissue damage with cellular necrosis and ensuing local inflammation, the possible pathogenetic contribution of CRP has lately become much more clear. Apart from the epidemiological association between higher peak CRP values and poor prognosis, there is robust immunohistochemical evidence of CRP deposition within all acute myocardial infarcts, co-localised with activated complement components[29]. Although this suggested that CRP might have deleterious effects, it is not yet possible to investigate such mechanisms in man. We therefore utilised the rat model of myocardial infarction, produced by ligation of the coronary artery, to take advantage of our original finding that rat CRP does not activate rat complement[57] and cannot therefore engage the pro-inflammatory effects available to human CRP. In contrast human CRP potently activates rat as well, of course, as human complement[57]. When rats undergoing coronary artery ligation received daily injections of pure human CRP, they became sicker than similarly operated rats receiving buffer alone or the closely related human protein, serum amyloid P component (SAP), that does not activate complement[30]. Injection of human CRP into un-operated rats had no adverse effects. Some of the coronary artery ligated rats treated with human CRP died, and those surviving to day 5, when all animals were killed, had infarcts 40% larger than buffer or SAP-treated controls[30]. This dramatic enhancement of infarct size by human CRP was completely abrogated by *in vivo* complement depletion of the rats using cobra venom factor, and was thus absolutely complement dependent[30]. Indeed, it has long been known that *in vivo* complement depletion markedly reduces inflammation and infarct size in this and similar animal models[58-61]. We thus conclude that a substantial proportion of final myocardial infarction size following acute coronary occlusion is determined by complement mediated inflammation, and that human CRP, both in our rat model and very likely also in the clinical situation, is responsible for at least some of this complement activation.

The development of drugs to block CRP binding *in vivo* is now a high priority, to which we are giving energetic attention[62]. In addition to affording potential cardioprotective therapy in acute myocardial infarction, such drugs will provide the means to investigate whether CRP contributes to pathogenesis of atheroma and/or atherothrombosis. We have solved the three-dimensional structure of CRP and its ligand complex with phosphocholine at atomic resolution[63], have developed high throughput screening assays for inhibitors of CRP binding to pathophysiological ligands, including enzyme modified LDL, and already have extensive expe-

rience in identification and evaluation of drugs that inhibit pentraxin binding, through our successful development of the SAP inhibitor, (R)-1-[6-(R)-2-carboxypyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (Patent EP-A-915088). We are therefore optimistic that new reagents with which to investigate these exciting questions about CRP will become available reasonably soon.

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