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Clinical Science (2005) **108**, 493–495 (Printed in Great Britain)

COMMENT

C-reactive protein and statins: IL-8 as a molecular link?

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ABSTRACT

CRP (C-reactive protein) has not only emerged as a useful biomarker for cardiovascular disease, but also as a mediator of atherosclerosis. CRP directly activates vascular endothelial cells, amplifying the inflammatory response underlying atherogenesis. The expression of IL (interleukin)-8 appears to serve as one of the downstream effects of CRP. Kibayashi and co-workers in this issue of *Clinical Science* confirm that CRP induces IL-8 production in human aortic endothelial cells *in vitro*, via the activation of MAPKs (mitogen-activated protein kinases), an effect that can be inhibited by pitavastatin.

Atherosclerosis is currently regarded as a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation [1]. Impairing vascular homoeostasis makes the vasculature susceptible to atheroma formation. CRP (C-reactive protein), formerly regarded as solely a biomarker for inflammation, is currently regarded as a prominent partaker in endothelial dysfunction and atherosclerosis [2].

With the advent of high-sensitivity assays, CRP has emerged as one of the most powerful independent predictors of cardiovascular disease, prompting the Centers for Disease Control and Prevention and the American Heart Association to issue a class IIa recommendation for its measurement as a routine part of global cardiovascular risk assessment [3]. However, as an active partaker in atherosclerosis, the molecular effects of excessive CRP action on the vasculature may serve as targets for therapeutic intervention. In this issue of *Clinical Science*, Kibayashi et al. [4] suggest that the proatherosclerotic effects of CRP upon the endothelium involve the production of IL-8, an action that is suppressed by pitavastatin.

Human recombinant CRP has been demonstrated to elicit a multitude of effects on endothelial biology favouring a proinflammatory and proatherosclerotic phenotype [1,2]. *In vitro* experiments reveal that CRP potently down-regulates eNOS [endothelial NO (nitric oxide) synthase] transcription and destabilizes eNOS mRNA, resulting in decreased basal and stimulated NO release. In a synchronous fashion, CRP stimulates ET-1 (endothelin-1) and IL (interleukin)-6 release from endothelial cells, and decreases the production of the potent vasodilator prostacyclin, shifting the balance towards endothelial dysfunction. By up-regulating the expression of adhesion molecules such as ICAM-1 (intercellular cell-adhesion molecule 1), VCAM-1 (vascular cell adhesion molecule-1) and E-selectin, and stimulating the release of MCP-1 (monocyte chemotactic protein-1), a chemokine which facilitates leucocyte transmigration, plaque progression is enhanced. Furthermore, CRP potently up-regulates NF-κB (nuclear factor κB) [5], a key nuclear factor that facilitates the transcription of numerous proatherosclerotic genes, and inhibits bone-marrow-derived EPC (endothelial progenitor cell) survival and

Key words: atherosclerosis, C-reactive protein (CRP), interleukin-8, mitogen-activated protein kinase (MAPK), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitor, statin.

Abbreviations: apoE, apolipoprotein E; AT1-R, angiotensin type 1 receptor; CRP-tg, CRP transgene; EPC, endothelial progenitor cell; ERK, extracellular signal-regulated kinase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IL, interleukin; JNK, c-Jun N-terminal kinase; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; mCRP, monomeric CRP; NO, nitric oxide; eNOS, endothelial NO synthase; VCAM-1, vascular cell adhesion molecule-1.

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differentiation [6]. EPCs have been suggested to play an important role in postnatal neovascularization, and the ability of CRP to inhibit progenitor cells may be an important mechanism inhibiting compensatory angiogenesis in chronic ischaemia [7]. CRP also has direct proatherogenic effects in vascular smooth muscle cells, directly up-regulating AT1-R (angiotensin type 1 receptor), which stimulates vascular smooth muscle migration, proliferation, neointimal formation and reactive oxygen species production [8]. Finally, CRP plays a role in the destabilization of the atheroma's fibrous cap by stimulating MMP-1 (matrix metalloproteinase-1) release [9] and impairing fibrinolysis by promoting the synthesis of PAI-1 (plasminogen activator inhibitor-1) [10]. Recent work has revealed that, in order for CRP to promote this proinflammatory phenotype, native pentametric CRP must undergo structural modification, forming active monomeric subunits [11]. Furthermore, the exact nature of the CRP receptor on endothelial cells is unknown, and may be distinct for native and mCRP (monomeric CRP), with the possible involvement of FcγRII and FcγRIII (where FcγR is Fcγ receptor) respectively [11]. Further elucidation of this molecular interaction may serve to uncover novel therapeutic targets.

These *in vitro* results were confirmed *in vivo* in human CRP transgenic mice. First, human CRP creates a prothrombotic phenotype, as evidenced by higher rates of thrombotic occlusion following arterial injury [12]. Secondly, by crossing CRP-tg (CRP transgene) mice with the atherosclerosis prone apoE (apolipoprotein E)$^{-/-}$ mice, CRP was shown to be an active player in atherogenesis *in vivo* ([13], but see [13a]). These CRP-tg/apoE$^{-/-}$ mice displayed accelerated aortic atherosclerosis, which was associated with increased complement deposition and elevated expression of AT1-R, VCAM-1 and collagen within the lesions. However, CRP also appears to up-regulate complement-inhibitory proteins and protects endothelial cells from complement-mediated cell injury [14], suggesting that a balance of proatherogenic and antiatherogenic effects of CRP on the vessel wall may be important in the development of atherosclerosis. The present study by Kibayashi et al. [4] suggests that CRP-mediated production of IL-8 may be added to the list of the detrimental vascular effects of CRP. IL-8, a member of the CXC chemokines, promotes monocyte–endothelial cell adhesion and arrest and is abundant in atherosclerotic plaques. Although CRP induced IL-8 secretion in human aortic endothelial cells has been reported previously [11,15] and the activation of p38 MAPK (mitogen-activated protein kinase) was implicated [11], Kibayashi et al. [4] show that the CRP-induced production of IL-8 was dependent on not only p38 MAPK, but also on two other signalling pathways, the ERK (extracellular signal-regulated kinase) and JNK (c-Jun N-terminal kinase) pathways. The MAPKs are signalling pathways which participate in the inflammatory response and have been implicated in the production and stabilization of cytokines and chemokines, and in cardiomyocyte death following ischaemia/reperfusion injury. CRP-induced activation of these pathways has been linked to several of the other less favourable vascular effects observed in *in vitro* studies and, probably, several additional molecular pathways will be implicated in mediating the harmful effects of CRP. Recently, p38 MAPK activation was demonstrated to impair endothelium-dependent NO-mediated vasoreactivity [16] and to have a detrimental effect on EPC number and capacity for neovascularization [17]. Nevertheless, Kibayashi et al. [4], demonstrate further that pitavastatin, an HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitor, inhibits IL-8 mRNA expression induced by CRP in human aortic endothelial cells through the inhibition of the three MAPK pathways. Thus it appears that statins may directly inhibit the cellular effects of CRP in addition to the clinically observed statin-mediated reduction in circulating CRP levels.

HMG-CoA reductase inhibitors, commonly referred to as statins, have emerged as an effective means of reducing the risk for cardiovascular disease. Statins have been demonstrated to significantly reduce cardiovascular risk in several large randomized controlled trials conducted in both primary and secondary prevention settings, beyond their effect on lipids. One of these additional non-lipid effects was believed to be an anti-inflammatory effect, involving the reduction of circulating CRP levels, which served to enhance endothelial function and subsequently impair atherogenesis.

Statin therapy was shown to reduce the level of CRP independently of its effect on lipid levels in analyses of the AFCAPS/TexCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study), the PRINCE (Pravastatin Inflammation CRP Evaluation) and the REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) trials. Furthermore, patients who have lower CRP levels after statin therapy have better clinical outcomes, including reduced rates of atherosclerosis progression, than those with higher CRP levels, regardless of the LDL (low-density lipoprotein)-cholesterol level [18,19]. This suggests that CRP lowering may be a target for intervention and that CRP should be monitored in addition to LDL-cholesterol to assess the effectiveness of statin therapy. Despite the apparent clinical data supporting the role of CRP as not only a biomarker, but also as a proatherosclerotic molecule, and the apparent reduction in CRP levels with statin therapy, no direct evidence linking the sole lowering of CRP level to overall survival currently exists. To partially address this concern, the JUPITER trial (Justification of the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin) is currently being conducted [20].

However, as the current study by Kibayashi et al. [4] suggests, the favourable pleiotropic effects of statins may

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involve more than just a reduction in circulating CRP levels, but may also negate the effects of CRP at the cellular level. Thus statins may serve a dual purpose. First, they may provide a systemic anti-inflammatory effect, and secondly, they may directly act at the tissue level inhibiting the CRP-mediated molecular pathways responsible for atherogenesis. However, it would be naïve to assume that blocking IL-8 activity alone would completely negate the detrimental effects of CRP. The inhibitory effect of pitavastatin on the MAPKs would probably extend to a promotion of NO release, favourable effects on EPC biology and a reduction in other markers of endothelial activation such as adhesion molecule expression. However, these other potential downstream consequences of CRP activity were not assessed in the present study by Kibayashi et al. [4]. Furthermore, the results may have been different had mCRP been employed. Since it appears that pentameric CRP must undergo a conformational change to mCRP to exert its activity [11], the relatively short CRP cell treatment times employed by Kibayashi et al. [4] (4 h) may not have allowed for the maximal effect of CRP to be obtained. Thus the positive results observed with pitavastatin treatment may be overestimated. Also, whether the inhibition of the MAPKs is unique to pitavastatin or represents a class effect remains to be determined. Finally, as with every *in vitro* study, will the observed results be replicated *in vivo* and, of more importance, will treatment with pitavastatin result in any noticeable clinical benefit? The present work by Kibayashi et al. [4] reveals some of the molecular links explaining the beneficial effect of statins on limiting the negative effects of CRP. However, several more molecular pieces undoubtedly remain before the entire statin–CRP story is revealed.

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Received 15 March 2005/22 March 2005; accepted 30 March 2005
Published as Immediate Publication 30 March 2005, DOI 10.1042/CS20050093

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