The Role of C-Reactive Protein Activation of Nuclear Factor Kappa-B in the Pathogenesis of Unstable Angina*

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Inflammation is pivotal in all phases of atherosclerosis, from the nascent lesion to the culmination in acute coronary syndromes (ACS) (1). Nuclear factor kappa-B (NF-κB) is a master switch in the inflammatory cascade and indeed has been documented in human atheroma (2). C-reactive protein (CRP), the prototypic marker of inflammation, has been documented in human atheroma (2). C-reactive protein (CRP), the prototypic marker of inflammation, has been advanced as a risk marker for heart disease, and indeed has been identified in monocyte macrophages, smooth muscle cells (SMC), and endothelial cells (EC) in human atherosclerotic vessels but not in healthy vessels. Furthermore, plaques from patients with ACS exhibit increased expression of several NF-κB–regulated genes such as cytokines, chemokines, and so on.

The important question that arises is whether CRP activates NF-κB, as suggested by Liuzzo et al. (4), and if this has effects on vascular cells that are relevant to atherothrombosis and ACS. Indeed, although Liuzzo et al. (4) show increased NF-κB activation with carefully purified CRP on human monocytes, it is important to emphasize that this has also been documented in vascular smooth muscle cell (VSMC) and EC. However, the first demonstration that CRP activation of NF-κB induces a relevant proinflammatory effect was the observation by Devaraj et al. (5), who clearly showed that CRP induced expression of the critical chemokine IL-8, a powerful trigger of monocyte adhesion to endothelium in human aortic (HA) EC and human coronary artery (HCA) EC via up-regulation of NF-κB, using 3 different techniques: electrophoresis mobility shift assay, nuclear p65, and IκB kinase in the cytosol. Those investigators also showed, using various inhibitors of NF-κB, that the effect of CRP on IL-8 could be abrogated.

Another important effect of purified CRP that is pertinent to the paper of Liuzzo et al. (4) is the demonstration of increased plasminogen activator inhibitor 1 (PAI-1) activity in aortic endothelial cells since PAI-1 impairs fibrinolysis. Nakakuki et al. (6) carefully documented that the activation of PAI-1 by purified CRP is through Rho kinase signaling pathway via activation of NF-κB. Using various strategies, including inhibitors, they carefully showed that inhibiting Rho inhibited NF-κB and PAI-1.

Further relevance of CRP activation of NF-κB was recently reported by 3 independent laboratories with respect to monocyte endothelial cell adhesion (7–9). Kawanami et al. (7) demonstrated in bovine EC that CRP induced vascular cell adhesion molecule (VCAM) 1 expression through the NF-κB signaling pathway. In addition, they suggest that the p65 homodimer but not the p65/p50 heterodimer binds to NF-κB–binding sites of the VCAM promoter, suggesting that p65 homodimer and not p65/p50 heterodimer is an important transactivator in CRP-stimulated VCAM gene expression in vascular EC. It would have been important to demonstrate that this indeed was relevant to monocyte adhesion to EC. However, 2 subsequent papers examined the effect of CRP on monocyte adhesion. In the paper by Liang et al. (8), they show that CRP induces VCAM expression in human venous EC and this results in increased monocyte adhesion to EC. Previously, Devaraj et al. (9) delineated that purified CRP augments monocyte–EC adhesion under static conditions via the Fc receptors CD32 and CD64. In the paper by Liang et al. (8), they showed that CRP activates NF-κB in EC. Furthermore, they showed that by blocking CD32 using siRNA, they

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prevent VCAM up-regulation by CRP. They also showed that CRP induces 1kB degradation and nuclear translocation of NF-κB in human umbilical vein and HAEC and that this was inhibited by CD32. These studies were done mainly in venous EC, and they did not report on the role of CD64 in CRP-induced activation of NF-κB and adhesion.

In a continuation of their previous findings, Devaraj et al. (9) showed that in HAEC purified CRP induces intercellular adhesion molecule (ICAM), VCAM, NF-κB, and monocyte EC adhesion under static conditions. Using inhibitors and a dominant negative to 1kB, they demonstrated significantly decreased ICAM, VCAM, and subsequent monocyte-EC adhesion. Finally, by preincubating with antibodies to CD32 and CD64 and using transient transfection of CD32 siRNA, they attenuated CRP-induced NF-κB activity, ICAM, VCAM, and monocyte-EC adhesion. Furthermore, they showed that by blocking CD32 and CD64, they prevented purified CRP-activated EC-supported monocyte rolling, transmigration, and adhesion under shear flow (2 dynes/cm²).

Thus, collectively these 3 groups have independently arrived at a similar conclusion, i.e., monocyte EC adhesion and CAM up-regulation is mediated via NF-κB activation by CRP. The latter 2 groups went further and showed that this was largely through the Fc receptor.

Also, Hattori et al. (10) have shown that monocyte chemoattractant protein-1 (MCP-1) stimulation by CRP in VSMC is probably mediated via NF-κB, and Cirillo et al. (11) suggest that CRP induces tissue factor expression in EC and VSMC via NF-κB. However, those authors did not use dominant negative or other state-of-the-art inhibitors of NF-κB.

To summarize, the effects of CRP that can be ascribed to NF-κB activation include up-regulation of IL-8, MCP-1, and PAI-1, and increased ICAM, VCAM, monocyte-EC adhesion, and tissue factor (Table 1). Although these effects are important in atherogenesis, they clearly could contribute to the genesis of ACS by recruiting leukocytes and promoting thrombosis.

Thus, these in vitro findings concur with the observation of Liuzzo et al. (4) that in monocytes CRP also activates NF-κB. Disappointingly, Luizzo et al. did not explore if this activation of NF-κB resulted in the induction of IL-6 and TNF in unstable angina patients with elevated CRP levels. Liuzzo et al. (4) also found that patients with increased CRP and activated NF-κB in monocytes had at least a 2-fold greater frequency of acute coronary events. However, the sample size was small, and this important observation needs to be confirmed in larger clinical trials. Although the investigators do not report on statin use per se in the study population, it is important to point out that in addition to statins as a class of drugs lowering CRP (12), they may also have effects in ameliorating proinflammatory and prothrombotic effects of CRP on EC (13). In this regard, it is germane to point out the in the PROVE-IT (Pravastatin or Atorvastatin Evaluation and Infection Therapy) study (14) the concomitant reduction of low-density lipoproteins and CRP resulted in greatest benefit.

Finally it should be borne in mind that the relationship between CRP and NF-κB could be bidirectional. In addition to CRP mediating proinflammatory effects as outlined above, CRP mRNA and protein has been documented in human atheroma and vascular cells by many investigators (15). Interestingly, in carotid atheroma CRP appears to colocalize with NF-κB (16). Also, in hepatocytes regulation of CRP is under transcriptional control of NF-κB, STAT3, and CEBP (17). Thus, it is not unreasonable to presuppose that upon activation, vascular cells in atheroma could also be producing CRP by activation of NF-κB, which could further induce a vicious cycle culminating in ACS (18).

Table 1  Biologic Effects Mediated Through CRP Activation of NF-κB

<table>
<thead>
<tr>
<th>Effect</th>
<th>Description</th>
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<tbody>
<tr>
<td>IL-8</td>
<td>Chemokine that promotes neutrophil and monocyte EC adhesion</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Chemokine that promotes monocyte chemotaxis and recruitment</td>
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<tr>
<td>PAI-1</td>
<td>Key regulator of fibrinolysis by inhibiting tissue plasminogen activator</td>
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<tr>
<td>ICAM-1, VCAM-1</td>
<td>Soluble cell adhesion molecules that activate monocyte adhesion to endothelium</td>
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<tr>
<td>Tissue factor</td>
<td>Primary initiator of the serine protease cascade of the coagulation system promoting thrombosis</td>
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EC = endothelial cell; ICAM = intercellular adhesion molecule; IL = interleukin; MCP = monocyte chemoattractant protein; NF-κB = nuclear factor kappa-B; PAI = plasminogen activator inhibitor; VCAM = vascular cell adhesion molecule.

REFERENCES

8. Liang YJ, Shyu KG, Wang BW, Lai LP. C-reactive protein activates the nuclear factor-kappaB pathway and induces vascular cell adhesion molecule-1