C-Reactive Protein and Reendothelialization

NO Involvement

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Disruption of the endothelium and its subsequent dysfunction appears to set the stage for atherogenesis. The maintenance of vascular homeostasis depends in part on a balance between endothelium-derived relaxing and contracting factors. Alterations in this balance lead to inflammation and the formation of fatty streaks and fibrous plaques. The diminished production or availability of nitric oxide (NO) appears paramount to setting the stage for inflammation and vascular injury.

In addition to its vasodilatory effect on the vasculature, endothelium-derived NO promotes endothelial cell growth, survival and migration, stimulates angiogenesis and neovascularization in part by endothelial progenitor cell (EPC) recruitment, inhibits leukocyte adhesion to the endothelium, maintains vascular smooth muscle in a nonproliferative state, and limits platelet aggregation and thrombosis. Maintenance of an intact and functional endothelium protects against vascular disease while its disruption is detrimental. The study by Schwartz et al, in this issue of Circulation Research, adds to our understanding of how C-reactive protein (CRP) works to promote vascular pathology by inhibiting NO synthesis.

CRP, long regarded as an acute phase protein and an active participant in the innate immune system, has more recently been linked to the development of cardiovascular disease. On the basis of several prospective epidemiologic studies, circulating high sensitivity CRP has emerged as an independent predictor of cardiovascular disease risk in various participant populations. In vitro studies have supported CRP’s role as a direct mediator of atherogenesis at various stages of disease development, including the earliest stage marked by endothelial dysfunction. First and foremost, CRP potently downregu-

lates endothelial nitric oxide synthase (eNOS) protein expression and destabilizes eNOS mRNA, resulting in the decreased release of basal and stimulated NO and enhanced inflammatory cell adhesion. CRP further disrupts the integrity of the endothelium by its detrimental effect on EPC biology, interfering with EPC differentiation, function and survival, in part by impairing antioxidative processes, increasing the production of reactive oxygen species and promoting apoptosis. Finally, CRP has a direct pro-inflammatory effect on endothelial cells, inducing expression of ICAM-1 and VCAM-1.

Criticism surrounding the use of commercial CRP preparations for in vitro studies, namely that some of the findings may be explained by the presence of sodium azide (NaN₃) and endotoxin, have largely been dismissed with demonstration that azide and LPS free CRP elicit similar effects on endothelial activation as previously reported. Professor Jialal’s group recently used Toll-like receptor 4 (TLR4) knockout endothelial cells to specifically answer this question. Human aortic endothelial cells (HAECs) transfected with prevalidated TLR4 small interfering RNA (siRNA) and scrambled siRNA controls were challenged with pleural fluid-derived CRP or LPS for 12 to 16 hour. Secreted interleukin-6 (IL-6), IL-1β, IL-8, and plasminogen activator inhibitor-1 (PAI-1) levels and eNOS activity were determined. TLR4 knockdown in HAECs significantly decreased LPS-induced IL-1β, IL-6, and IL-8, whereas the stimulatory effects of CRP were similar in both scrambled control and TLR4 knockdown cells. Furthermore, CRP significantly stimulated PAI-1 levels in both control and TLR4-transfected cells and inhibited eNOS activity, whereas LPS effects were negated in TLR4-transfected cells, clearly supporting a direct biological effect of native CRP.

To investigate the in vivo actions of CRP on the endothelium and in particular, on reendothelialization, Schwartz and colleagues used mice expressing rabbit CRP under the regulation of the phosphoenolpyruvate carboxykinase promoter, which allows for the manipulation of CRP levels by changing the amount of carbohydrate intake. The same mouse model was used previously to demonstrate that modest elevations in CRP were sufficient to increase systolic blood pressure via the downregulation of vascular angiotensin type 2 receptor expression. The observed effect could be in part reversed by an NO donor, suggesting NO deficiency was involved in CRP-mediated systolic hypertension. Thus, the effect of CRP on vascular eNOS expression was further examined with the current set of experiments. Using a carotid injury model, modest elevations in serum CRP levels (9 μg/mL) impaired reendothelialization after 5 days, an effect which was related to a decline in vascular eNOS expression, eNOS...
mRNA and NOS enzymatic activity. The resulting decrease in bioavailable NO impaired endothelial cell growth and migration. The in vivo findings were consistent with results obtained with ex vivo incubations of mouse carotid arteries with recombinant human CRP, as well as with the earlier in vitro work previously described. Of note, the authors used heat inactivation of the recombinant CRP to further demonstrate that the ex vivo observations were because of the actions of CRP and not of contaminants within the CRP preparations. The authors further show that CRP-responsive elements reside within the eNOS promoter, providing a potential mechanism through which CRP may directly alter eNOS gene expression.

The current in vivo findings linking CRP to impaired endothelium-derived NO production support the observation that elevated CRP levels may result in a greater risk of hypertension because of impaired vasorelaxation and exaggerated vascular responses to stress. Had the authors extended their period of observation beyond 5 days post injury, additional structural changes to the vasculature, and not simply a lack of reendothelialization, may have been observed. It would be interesting to note if chronic exposure to elevated CRP levels in the carotid injury model would result in enhanced neointimal hyperplasia. In fact, our group, using a human CRP transgenic mouse model, has recently shown that chronic CRP elevation in vivo promoted endothelial dysfunction with resultant changes in vascular structure and endothelial responses to injury. Macrophage infiltration into the arterial wall and the aortic expression of the chemokine MCP-1 as well as the adhesion protein VCAM-1 were increased in mice with elevated CRP levels. Thus, by promoting endothelial dysfunction in vivo, CRP sets the stage for atherogenesis.

Previous experiments performed in human CRP transgenic mice, demonstrated that human CRP creates a prothrombotic phenotype, as evidenced by higher rates of thrombotic occlusion following arterial injury, likely a consequence of reduced NO bioavailability. Furthermore, by crossing CRP transgenic mice with the atherosclerosis prone apoE knockout mice, demonstrated that human CRP creates a prothrombotic phenotype, as evidenced by higher rates of thrombotic occlusion following arterial injury, likely a consequence of reduced NO bioavailability. In addition, increased NOS enzymatic activity was associated with increased complement deposition and elevated expression of AT1-R, VCAM-1 and collagen within the lesions. However, subsequent studies did not find similar results, highlighting the difficulties with using a murine model to examine human disease states.

Using the aforementioned human CRP transgenic mice, Grad et al. recently demonstrated a similar effect of CRP on NO biology in vivo. Using a femoral artery wire injury model, the authors demonstrated that eNOS protein and mRNA expression were significantly suppressed in the injured arteries of CRP transgenic mice, a change which translated into a measurable reduction in NO release post injury. Thus, reports from two independent groups, using two different CRP transgenic mouse models, reach the same conclusion. CRP modulates eNOS expression and NO metabolism in vivo, paralleling prior investigations in cultured endothelial cells.

The current work by Schwartz et al. clearly demonstrates in an in vivo transgenic mouse model that CRP impairs reendothelialization following vascular injury in part by decreasing eNOS gene transcription and activity. The demonstration that CRP-responsive elements reside within the eNOS promoter, provide a potential mechanism through which CRP may directly alter eNOS gene expression. Confirming in vitro findings with in vivo results will no doubt further elucidate the pathogenic role of CRP in vascular disease. It will be interesting to see whether subsequent studies using these in vivo CRP models will confirm other in vitro findings. For example, the lack of reendothelialization mediated by CRP observed in the study by Schwartz et al may have been influenced by impaired EPC recruitment to the area of vascular denudation. As our understanding of how CRP works to promote vascular pathology increases, strategies that combat CRP and promote vascular healing and reendothelialization following invasive procedures may become part of our clinical armamentarium in the treatment of cardiovascular disease. The recent synthesis and characterization of 1,6-bis(phosphocholine)-hexane as a specific small-molecule inhibitor of CRP, with potent cardiovascular protective effects, is one big step in this direction.

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References


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