

Perplexity of monocyte responses to C-reactive protein (CRP)

János G. Filep

Research Center, Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, Quebec, Canada

C-reactive protein (CRP), a member of the pentraxin family, is one of the prototypic plasma biomarkers of inflammation (1, 2). The physiological roles of CRP in the innate immune response have, however, remained elusive as it possesses both pro- and anti-inflammatory actions (1, 2). Inflammation is pivotal in all phases of atherosclerosis from the nascent lesion through progression to the culmination in acute coronary syndromes (3). To date, over two dozen large-scale prospective clinical studies have reported that moderate elevations in base-line CRP levels predict, albeit to varying degrees, future cardiovascular disease and death across all levels of low-density lipoprotein (LDL) cholesterol and of the Framingham Risk Score (4, 5). CRP has been implicated in multiple aspects of atherogenesis and plaque vulnerability; however, whether CRP plays a direct causal role in these events remains controversial (reviewed in [6]).

For example, transgenic expression of human CRP in apolipoprotein E-deficient mice has been reported to accelerate atherosclerosis development (7, 8), to show no effect (9) or even to slow atherosclerosis development (10). *In vitro*, CRP induces release of pro-inflammatory interleukins IL-1, IL-6, IL-8 and tumor necrosis factor- α NF (11, 12) from human monocytes; promotes monocyte adhesion to endothelial cells through upregulation of endothelial adhesion molecules (13, 14) and enhances phagocytosis of oxidized LDL (6). These observations are consistent with the role of monocytes in the initiation and progression of atherosclerosis (3). However, monocytes may have a more complex role in atherogenesis than previously thought. In this issue of *Thrombosis and Haemostasis*, Hanriot et al. (15) show that CRP modulates expression of genes known to mediate both pro- and anti-inflammatory responses. These novel observations raise the intriguing possibility that CRP could activate yet unidentified pathways in human monocytes.

Using a custom-made 250 oligonucleotide microarray for screening CRP regulated genes in monocytes isolated from apparently healthy men and women (aged 25 to 35 years), Hanriot et al. detected changes in approximately 10% of the genes represented on the microarray. The authors confirm previous studies on CRP induction of pro-inflammatory cytokine gene expression with the exception of TNF, which was unaffected by

CRP, and show increased mRNA expression of monocyte chemotactic protein-1 (MCP-1), Gro- β , the chemokine receptors CCR-8 and CXCR-4, and plasminogen activator inhibitor type 2 (PAI-2). In concert with IL-8, MCP-1 and Gro- β facilitate monocyte recruitment and adhesion to endothelial cells, whereas CCR-8 and CXCR-4 mediate monocyte (and macrophage) chemotaxis. The function of PAI-2 appears to be more complex. PAI-2 promotes fibrin deposition in the arterial wall, thereby contributing to progression of the initial lesion, whereas it may stabilize more advanced plaques by inhibiting activation of matrix metalloproteinases, enzymes overexpressed in human atheromata (3).

While the concept of CRP's anti-inflammatory role is not new (reviewed in [1, 2]), Hanriot et al. also report CRP-induced changes in monocyte gene expression that are consistent with suppression of inflammation. Thus, the authors show that CRP attenuates expression of the monocyte/lymphocyte chemotactic proteins MIP-1 α and MIP-1 β and α 2-macroglobulin, which antagonize the actions of the anti-inflammatory cytokine transforming growth factor- β . Protection of mice overexpressing human CRP from allergic encephalomyelitis is mediated through reduction of MIP-1 (16). The most striking finding is that CRP increases expression of the nuclear receptor liver X receptor- α (LXR- α) and one of its target genes the ATP-binding cassette transporter A1 (ABCA1). ABCA1 promotes reverse cholesterol transport from macrophages in atherosclerotic plaques to high density lipoprotein (HDL) and suppresses genes involved in macrophage inflammatory signaling and apoptosis, thereby inhibiting atherosclerosis (17). CRP induction of LXR- α expression and activation is mediated through the high affinity IgG receptor Fc γ R1 (CD64) and, to lesser a degree, the low affinity IgG receptor Fc γ R2 (CD32), and involves activation of both p42/44 MAPK and phosphatidylinositol 3-kinase signaling pathways.

A critical issue whether CRP could effectively facilitate reverse cholesterol transport in monocytes (and macrophages) remains to be elucidated. The complexity of LXR- α responses makes it difficult to extrapolate the beneficial or deleterious effects of a target gene in isolation to human pathology when target genes are likely being modulated in concert. For example, an-

Correspondence to:

János G. Filep

Research Center, Maisonneuve-Rosemont Hospital

University of Montreal

5415 boulevard de l'Assomption

Montreal, QC, Canada H1T 2M4

Tel.: +1 514 252 3400 ext. 3388, Fax: +1 514 252 3430

E-mail: janos.g.filep@umontreal.ca

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other LXR- α target gene, sterol regulatory element binding protein-1c is involved in lipogenesis, leading to enhanced production of triglycerides, which are pro-atherogenic.

How would CRP then induce opposing actions? CRP-induced activation of NF- κ B and LXR- α may signal for expression of pro- and anti-inflammatory genes, respectively. The authors provide limited information on the receptor(s) mediating NF- κ B activation. CRP's pro-inflammatory actions on monocytes may be mediated through different receptors as reported for human neutrophils (18), an issue not addressed in this study.

Although Hanriot et al. present convincing control experiments to exclude the potentially confounding effects of sodium azide and LPS contamination present in commercial and recombinant CRP preparations, some concerns remain. In particular, it is still not known why recombinant CRP was somewhat more potent than *de novo* purified CRP. CRP affects LXR- α expression at clinically relevant concentrations, in particular at 2.5 μ g/ml, which predict moderate risk (4). While vascular endothelial cells and smooth muscle cells may express CRP under certain circumstances (19, 20) it is uncertain whether CRP levels could be 10-times higher in the vessel wall than in the plasma as suggested by some investigators.

Hanriot et al. show similarities as well as striking gender differences in CRP-induced gene expression patterns in monocytes

from men and women. In general, the number of genes affected by CRP is higher and the increases in PAI-2 and IL-6 mRNA expression appear to be more pronounced in women than men. These observations are interesting and can likely be attributed to the influence of endogenous estrogens, even though their biological significance is not known at the present time.

The sophisticated analysis presented by Hanriot et al. may reflect the complexity of monocyte responses to CRP. The plethora of changes raises the intriguing possibility that CRP modulation of monocyte gene expression may depend on the lesion type or different regions within a given atherosclerotic lesion, an issue that requires additional studies. Perhaps more global assessment of monocyte gene expression in patients with cardiovascular disease would identify individuals whose monocytes express a predominantly pro- or anti-inflammatory response to CRP. Furthermore, it remains to be investigated whether there is a molecular switch that directs pro-inflammatory versus anti-inflammatory actions of CRP and how this translates into the human pathology. These concerns notwithstanding, the provocative findings of Hanriot et al. highlight the complexity of monocyte gene expression in response to CRP and have potential significance in defining the role of modest plasma CRP elevations in cardiovascular disease.

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