Acute-Phase Response and Thrombin Generation in Predicting Coronary Risk

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An acute-phase response can be detected from the presence of elevated plasma levels of liver-derived acute-phase proteins, including C-reactive protein, fibrinogen, serum amyloid A, components of the complement system, and secretory type II phospholipase A_2 [1,2]. The acute-phase response is a host defense mechanism that helps to remove pathogens and limits excessive tissue damage. In evolutionary terms, the acute-phase response is directed against acute infections and the accompanying inflammation. However, if the inflammatory process becomes chronic, the acute-phase response also continues. Recent data indicate that prolonged mild elevation of acute-phase proteins – notably C-reactive protein, the prototype of acute-phase proteins – is associated with an increased risk of coronary artery disease. However, the molecular basis of this association has remained elusive.

The present issue of *IMAJ* contains two papers dealing with the role of acute-phase proteins in the development of CAD. The paper by Magadle et al. [3] is a review on the epidemiological aspect of the association between CRP and CAD, with a brief discussion on potential pathophysiological mechanisms. The original paper by Cavusoglu et al. [4], on the other hand, describes the associations of increased plasma levels of CRP and fibrinogen with the extent of angiographically verified coronary artery disease. Moreover, the latter work also provides evidence suggesting that CAD is associated with decreased plasma levels of antithrombin III.

The last decade witnessed an expansion of research on chronic inflammation and the risk of CAD. This novel aspect of the predictive epidemiology of CAD is exemplified by the exponential growth of publications dealing with CRP and CAD [Figure 1]. Nevertheless, the key issue, i.e., the origin of the inflammation, is still unresolved. According to current thinking, the signals leading to increased production of acute-phase proteins in the liver originate from the inflamed tissue [1]. These signals consist of specific cytokines, particularly interleukin-1 beta, IL-6, and tumor necrosis factor-alpha. Detection of a strong inflammatory component in atherosclerotic coronary lesions [5–7] has led to the intriguing speculation that in patients suffering from CAD the lesions themselves are the origin of the pro-inflammatory signals. This possibility is supported by the close association between plasma levels of CRP and the extent and severity of coronary atherosclerosis [8]. However, it is difficult to envisage how the small-sized coronary lesions can produce sufficient quantities of cytokines to induce a significant acute-phase response in the liver. An explanation may lie in the fact that coronary atherosclerosis is a component of a systemic atherosclerotic disease that also involves the aorta and the peripheral arteries. Therefore, it is likely that in patients with generalized atherosclerosis the major determinants of the plasma concentration of acute-phase proteins are the cytokines derived from the extracoronary lesions [9].

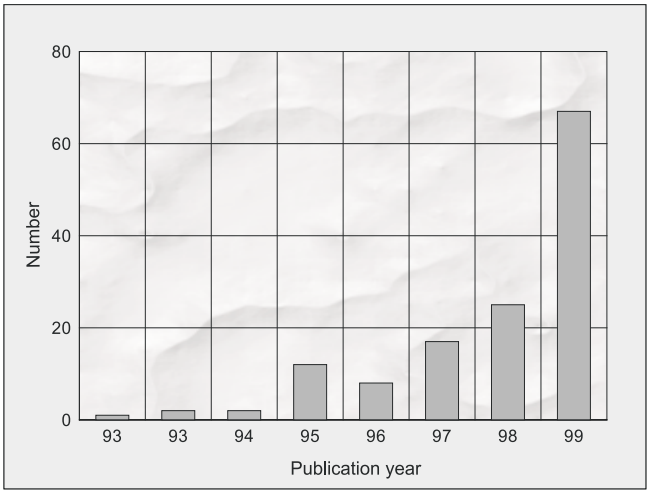

Figure 1. The exponential growth of publications relating C-reactive protein to coronary artery disease

Another group of inflammatory markers are the adhesion molecules, such as intercellular adhesion molecule 1, vascular cell adhesion molecule, E-selectin, and P-selectin [10]. They are

CAD = coronary artery disease
CRP = C-reactive protein

IL = interleukin

expressed in the atherosclerotic lesions as a response to local inflammation. Thus, oxidized low density lipoproteins in the lesions may serve as a pro-inflammatory stimulus causing the endothelial cells of the lesions to express ICAM-1 and VCAM-1 [11]. A fraction of these adhesion molecules are proteolytically cleaved from the endothelial surface and released into the circulation. Accordingly, their plasma levels can be measured and used as markers of inflammation. Indeed, a predictive association has been found between soluble adhesion molecules and CAD [12]. Regarding their origin in the arterial tree, the same uncertainty applies as for the atheroma-derived pro-inflammatory cytokines. Thus, at present, we know of no coronary-specific plasma markers of inflammation.

What other sources of pro-inflammatory cytokines, besides atherosclerotic lesions, might exist in CAD patients with elevated plasma concentrations of acute-phase proteins? Epidemiological studies have demonstrated associations between CAD and chronic bacterial and viral infections, especially dental and pulmonary infections [13–15], suggesting that these tissue sites are potential candidates for cytokine production.

A third potential source of pro-inflammatory cytokines is the adipose tissue. Adipocytes have been shown to produce TNF-α [16], and obesity has been associated with low grade inflammation reflected by increasing CRP levels [17]. Recent data also relate the insulin resistance syndrome to increased levels of the proteins associated with acute-phase response [18]. These data suggest that one of the metabolic pathways leading from obesity to CAD is mediated by acute-phase proteins.

Irrespective of the cellular source of the cytokines that will stimulate the production of acute-phase proteins, their serum levels are also influenced by lifestyle and genetic factors. An illustrative example is the regulation of fibrinogen by smoking and by the polymorphism of the fibrinogen gene, both of which affect its plasma protein level. The recently observed polymorphic regulation of the IL-6 level [19,20] provides an initial clue, suggesting that the plasma concentration of CRP and other acute-phase reactants are also genetically regulated.

Little is known about the actual mechanisms linking elevated plasma levels of acute-phase proteins with CAD. Most published early studies have dealt with the coagulation factor fibrinogen. Even moderate (20–30%) increases in its plasma level are associated with a significant increase in the risk of CAD [21]. This may result from the fact that fibrin(ogen) can contribute to CAD by a variety of different mechanisms [22]. Fibrinogen and its degradation products, the fibrinopeptides, are found in atherosclerotic lesions [23]. Both fibrin and the fibrinopeptides may stimulate the proliferation of smooth muscle cells in the lesions, and fibrin will contribute to plaque growth when deposited in the plaque. However, it is likely that fibrinogen makes its greatest contribution to CAD via its participation in the atherothrombotic events. Even moderately increased levels of fibrinogen inhibit fibrinolysis and tend to promote thrombus growth [24], thus increasing the likelihood of an occlusive thrombus.

Recently, studies on the possible mechanistic links between elevated CRP and CAD have also been published. An important observation was that CRP is present in atherosclerotic lesions [25]. In the lesions, CRP may bind to modified low density lipoprotein particles, enhance complement activation [26,27], and so contribute to plaque rupture. In light of the above considerations, fibrinogen and CRP are associated with plaque growth and/or with plaque rupture and the ensuing thrombotic response. Thus, these two substances may contribute to the development of coronary atherosclerosis both in the early sub-clinical and in the late thrombotic phase.

Antithrombin III, unlike fibrinogen, the other coagulation factor studied by Cavusoglu et al., is not an established coronary risk factor. The present findings by Cavusolgu and co-workers on a possible association between low antithrombin III and CAD, although not statistically significant, are suggestive and, from a biological standpoint, highly relevant. In advanced atherosclerotic lesions the endothelium has lost some of its antithrombotic properties and may serve as a local site for thrombin generation [28]. Upon thrombin formation, antithrombin III forms a complex with thrombin, and this complex is rapidly removed from the circulation, thereby tending to decrease the concentration of antithrombin III. In patients with advanced and widespread peripheral atherosclerosis, markers of thrombin generation – such as thrombin-antithrombin III complex and prothrombin fragment (F 1+2), and also those of fibrin generation and degradation – are elevated [29]. The fact that Cavusolgu et al. were unable to reach statistical significance for the observed low antithrombin III level in patients with CAD may reflect a low degree of concomitant peripheral atherosclerosis in the study population. Moreover, since thrombin generation is a rapid and highly dynamic process, it is necessary in future studies to include in the analysis additional markers of thrombin generation. In any event, the study of Cavusolgu et al. suggests that an increased risk of CAD may be associated not only with impaired fibrinolytic activity [30] but also with an overactive fibrin generation.

What are the clinical implications of elevated levels of acute-phase proteins? At present, clinical data indicate that the predictive accuracy of some classical CAD risk factors (low HDL-cholesterol, smoking) is increased when the CRP level is simultaneously high [31,32]. In addition, in hypercholesterolemic CAD patients, statin treatment reduce an elevated CRP level [33]. We speculate that the mechanism at work here is the anti-inflammatory effect of statins, which reduces the generation of IL-6 by the inflammatory cells in atherosclerotic lesions, and hence also the production of CRP by the liver. Moreover, CRP may play a direct role in promoting the inflammatory component of atherosclerosis [34]. Thus, a high CRP level would be an additional indication for statin treatment.

ICAM-1 = intercellular adhesion molecule 1
VCAM-1 = vascular adhesion molecule 1
TNF-α = tumor necrosis factor-alpha

HDL = high density lipoprotein

References

1. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448–54.
2. Kovanen PT, Pentikäinen MO. Secretory group II phospholipase A2: a newly recognized acute-phase reactant with a role in atherogenesis. *Circ Res* 2000;86:610–12.
3. Magadle R, Weiner P, Yanay N-B, Magadle P. C-reactive protein and coronary disease. *IMAJ* 2001;3:50-2.
4. Cavusoglu Y, Gorenek B, Alpsoy S, Unalir A, Ata N, Timuralp B. Evaluation of C-reactive protein, fibrinogen and antithrombin-III as risk factors for coronary artery disease. *IMAJ* 2001;3:13-16.
5. Libby P, Hansson GK. Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions. *Lab Invest* 1991;64:5–15.
6. Kaartinen M, Penttilä A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. *Circulation* 1994;90:1669–78.
7. Van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994;89:36–44.
8. Heinrich J, Schulte H, Schönfeld R, Köhler E, Assmann G. Association of variables of coagulation, fibrinolysis and acute-phase with atherosclerosis in coronary and peripheral arteries and those arteries supplying the brain. *Thromb Haemost* 1995;73:374–8.
9. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentrations of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425–8.
10. Repo H, Harlan JM. Mechanisms and consequences of phagocyte adhesion to endothelium. *Ann Med* 1999;31:156–65.
11. Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest* 1992;90:1138–44.
12. Jang Y, Lincoff AM, Plow EF, Topol EJ. Cell adhesion molecules in coronary artery disease. *J Am Coll Cardiol* 1994;24:1591–601.
13. Mattila KJ, Nieminen MS, Valtonen VV, Rasi VP, Kesäniemi YAK, Syrjälä SL, Jungell PS, Isoluoma M, Hietaniemi K, Jokinen MJ, Huttunen JK. Association between dental health and acute myocardial infarction. *Br Med J* 1989;298:779–81.
14. Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Mäkelä PH, Huttunen JK, Valtonen V. Serological evidence of an association of a novel *Chlamydia*, TWAR, with chronic coronary heart disease and acute myocardial infaction. *Lancet* 1988;ii:983–6.
15. Jousilahti P, Vartiainen E, Tuomilehto J, Puska P. Symptoms of chronic bronchitis and the risk of coronary disease. *Lancet* 1996;348:567–72.
16. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995;95:2409–15.
17. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *Br Med J* 1996;312:1061–5.
18. Festa A, DAgostino R Jr, Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as a part of the insulin resistance syndrome. The insulin resistance atherosclerosis study (IRAS). *Circulation* 2000;102:42–7.
19. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998;102:1369–76.
20. Woods A, Brull DJ, Humphries SE, Montgomery HE. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. *Eur Heart J* 2000;21:1575–83.
21. Ernst E. Plasma fibrinogen – an independent cardiovascular risk factor. *J Intern Med* 1990;227:365–72.
22. Cook NS, Ubben D. Fibrinogen as a major risk factor in cardiovascular disease. *Trends Pharmacol Sci* 1990;11:444–51.
23. Bini A, Fenoglio J Jr, Mesa-Tejada R, Kudryk B, Kaplan KL. Identification and distribution of fibrinogen, fibrin and fibrin(ogen) degradation products in atherosclerosis: use of monoclonal antibodies. *Arteriosclerosis* 1989;9:109–21.
24. Blombäck B. Fibrinogen and fibrin – proteins with complex roles in hemostasis and thrombosis. *Thromb Res* 1996;83:1–75.
25. Zhang YX, Cliff WJ, Schoefl GI, Higgins G. Coronary C-reactive protein distribution: its relation to development of atherosclerosis. *Atherosclerosis* 1999;145:375–9.
26. Bhadki S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol* 1999;19:2348–54.
27. Lagrand WK, Visser CA, Hermens WT, Niessen HWM, Verheught FWA, Wolbink G-J, Hack E. C-reactive protein as a cardiovascular risk factor. More than an epiphenomenon? *Circulation* 1999;100:96–102.
28. Pearson JD. Endothelial cell function and thrombosis. *Baillieres Clin Haematol* 1999;12:329–41.
29. Lassila R, Peltonen S, Lepäntalo M, Saarinen O, Kauhanen P, Manninen V. Severity of peripheral atherosclerosis is associated with fibrinogen and degradation of cross-linked fibrin. *Arterioscler Thromb* 1993;13:1738–42.
30. Meade TW, Ruddock V, Stirling Y, Chakrabarti T, Miller GJ. Fibrinolytic activity, clotting factors and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet* 1993;342:1076 –9.
31. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of the total and HDL cholesterol in determining risk of first myocardial infaction. *Circulation* 1998;97:2007–11.
32. Roivainen M, Viik-Kajander M, Palosuo T, Toivanen P, Leinonen M, Saikku P, Tenkanen L, Manninen V, Hovi T, Mänttäri M. Infections, inflammation, and risk of coronary heart disease. *Circulation* 2000; 101:252–7.
33. Strandberg TE, Vanhanen H, Tikkanen MJ. Effects of statins on C-reactive protein in patients with coronary artery disease. *Lancet* 1999;353:118–19.
34. Pasceri V, Willerson JT, Yeh ETH. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000;102:2165–8.

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