Editorial

Studying mechanisms underlying shedding of endothelial membrane proteins could help patients at risk for myocardial infarction

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See article by Singh et al. [8] (pages 39–49) in this issue.

The single, most prevalent cause of premature death in the developed world is myocardial infarction, with more than 1 million occurrences each in Europe and the United States each year. For almost half of its victims, it is the first sign of coronary artery disease and, for a third, it is fatal. There is, therefore, an urgent need to define biomarkers to identify healthy individuals at high risk for myocardial infarction. These patients can then be targeted for aggressive risk factor reduction by established lifestyle and pharmacological approaches. There is also an emerging possibility of preventive coronary intervention, although this is yet to be validated in any clinical trial.

Atherosclerosis is a chronic inflammation of the vascular intima, which probably starts with endothelial dysfunction [1]. Oxidative stress impairs the action of endothelium-derived nitric oxide, antagonising its various roles as vasodilator, anti-platelet agent, and anti-inflammatory mediator [1]. Oxidative stress also upregulates a variety of transcription factors, including nuclear factor κB [2], which induce a complex of pro-inflammatory genes, including several chemokines and the endothelial leukocyte adhesion molecules, VCAM-1, ICAM-1, and P-selectin [3]. Upregulation of VCAM-1, in particular, seems to occur very early and to be a predisposing factor for atherosclerosis formation [4]. The end result is adhesion and transmigration of monocytes and T-lymphocytes across the endothelium. Macrophages take up oxidised LDL to become foam cells and secrete pro-inflammatory mediators and growth factors that promote plaque progression [3].

Most myocardial infarctions result from rupture of a lipid-rich plaque [5]. Given that ruptured plaques contain a greater proportion of inflammatory cells to vascular smooth muscle cells, inflammation is believed to favour plaque rupture as well as progression. Studies showing that inflammatory biomarkers present in patients’ blood predict myocardial infarction and other unstable coronary events support this concept [6]. Biomarkers include C-reactive protein (CRP) and its secretagogue IL-6, which are secreted from the liver and hence reflect a global, rather than specifically arterial, pro-inflammatory state [6]. Soluble CD40 ligand, possibly shed from the surface of blood platelets, is another valuable biomarker [7]. Most pertinent to endothelial dysfunction, however, are soluble forms of VCAM-1, ICAM-1, and P-selectin that are apparently shed into the blood as a result of proteolysis. Given its predisposing role, VCAM-1 may be a particularly favourable candidate as a biomarker for atherosclerosis.

In the study by Singh et al. reported in this issue [8], the authors studied shedding of VCAM-1 from a mouse endothelial cell line and primary mouse endothelial cells under baseline conditions and upon stimulation with the inflammatory cytokines TNF-α and IL-1β. They present strong evidence for the involvement of an adamalysin using the property of selective inhibition by tissue inhibitors of metalloproteinase-3 (TIMP-3) rather than TIMP-1 or TIMP-2. Moreover, shedding was greater in endothelial cells from TIMP-3 knockout compared to wild-type mice. Furthermore, they identify ADAM-17 as the responsible protease by using small interfering RNAs. These conclusions extend previous work using ADAM-17 knockout cells showing that the enzyme mediates VCAM-1 shedding in response to the nonphysiological stimulator, PMA [9]. Interestingly, cytokine stimulation [8] increased both VCAM-1 and ADAM-17 expression, which suggests that a combination...
of greater substrate availability and activation of proteolysis drives increased shedding. In the case of PMA, however, activation of proteolysis appears to be the main driver [8,9]. From inhibitor studies, protease activation appears to depend on the activation of p38 and p40/42 MAP kinases [8]. In future studies, it will be particularly important to elucidate detailed mechanisms regulating shedding because it should allow us to understand precisely what aspects of endothelial inflammation are responsible for release of the plasma biomarkers.

Limitations of the study by Singh et al. [8] are their confinement to in vitro experiments using mouse cell lines or primary cells derived from a large artery, the aorta. It will be important in the future to conduct experiments in live mice that establish the role of ADAM-17 and define the location of adhesion molecules shedding into plasma. The source of soluble VCAM-1 in human blood is more likely to be microvascular endothelium because, while the area of coronary endothelium over plaques is just a few square centimetres, the surface area of microvascular endothelium in arterioles and postcapillary venules is overwhelmingly larger. There is good evidence that microvascular as well as macrovascular endothelia are dysfunctional in atherosclerosis. For example, impaired endothelial nitric oxide production affects the resistance microvasculature of the heart and other organs in patients with atherosclerosis and its risk factors [1]. Moreover, Buffon et al. [10] showed that neutrophils become activated as they cross the coronary bed of patients with unstable angina, and neutrophil activation generally occurs by adhesive interactions with microvascular endothelium. Future studies with microvascular endothelial cells, preferably of human origin, are therefore warranted.

Future experiments are also needed to define to what extent VCAM-1 shedding represents a paradigm for other proteins. ADAM-17 has been implicated in the shedding of several other molecules, including TNF-α and its receptor, notch-1, amyloid precursor protein, and fractalkine [9]. However, other mechanisms certainly contribute. For example, shedding of CD44 can be mediated by a membrane-type matrix metalloproteinase (MMP-14) [11], while constitutive shedding of fractalkine from ECV 304 cells appears to be mediated by ADAM-10 rather than ADAM-17 [12]. Interestingly, Singh et al. [8] show that shedding of VCAM-1 from mouse endothelial cells in the absence of PMA, TNF-α, or IL-1β is not mediated by ADAM-17 and indeed seems to be independent of metalloproteinase activity altogether. A further, important, unanswered question is the physiological role, if any, of increased shedding. In the experiments of Singh et al. [8], only 8% of total VCAM-1 was shedded within 24 h, which is unlikely to have greatly affected function. It would have been interesting to see whether prolonged inflammatory stimulation eventually led to complete reversal of the increased surface expression. Furthermore, soluble VCAM-1 could, in principle, act as a ‘dominant negative’ inhibitor of leukocyte adhesion, provided that it reached appropriate concentrations.

In conclusion, shedding of endothelial surface molecules is an important research area with much potential for future development. It can give valuable new insights into the basis of endothelial activation during atherosclerosis. Moreover, it is important to understand the basis, in general, for shedding endothelial surface molecules, one of which could prove to be an ideal biomarker for predicting unstable events, in particular myocardial infarction.

References