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Invited commentary

Is Toll-like receptor responsiveness a marker and predictor of coronary artery disease?

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The responsiveness of the innate immune system to microbial components depends on the expression of specific pattern recognition receptors such as the Toll-like receptors (TLRs) [1]. The TLR family of receptors has evolved to recognize pathogen associated molecular patterns such as lipopolysaccharide (LPS) on the surface of Gram negative bacteria (TLR4) and lipopeptides on Gram positive bacteria (TLR2), as well as viral nucleic acids such as such as double- and single-stranded RNA (TLR3, TLR7 and TLR8). The TLRs can also recognize so called damage associated molecular patterns generated during tissue injury such as heat-shock proteins and extra-cellular matrix components including extra domain A of fibronectin [2]. Upon activation, the TLRs activate signals resulting in release of cytokines that promote and coordinate inflammation. Due to their central role in regulating inflammation the TLRs have been implicated in the pathogenesis of many diseases including cardiovascular disease. The bacterial lipid sensing TLR2 and TLR4 receptors have attracted much attention in atherosclerosis research. Targeted deletion in mouse models of experimental atherosclerosis have shown that TLR2, TLR4, and the intracellular adaptor molecule MyD88, all promote atherosclerosis development [3–7]. TLR2 and TLR4 are expressed in human atherosclerotic lesions [8]. Polymorphisms in the TLR4 gene have also been suggested to be associated with cardiovascular disease [2]. Surface expression of TLR2 and TLR4 on circulating monocytes has been shown to be increased in acute

myocardial infarction and unstable angina patients compared to healthy control patients [9–11]. Given that TLR expression has been implicated atherosclerosis progression, it is likely that the magnitude of TLR induced release of inflammatory cytokines, i.e. the TLR responsiveness, could be used to monitor cardiovascular disease burden and severity. Also, because repetitive stimulation of TLRs attenuates the induced inflammatory response, it has been proposed that TLR responsiveness could be altered during atherosclerosis development due to a chronic exposure to endogenous TLR ligands [12]. Furthermore, the relative ease by which whole blood stimulations and measurements of cytokine release are performed also fulfills important criteria required of a useful biomarker.

The hypothesis that TLR-induced cytokine release could be a marker of atherosclerotic disease burden or severity is tested by Elsenberg *et al* in this issue of Atherosclerosis by evaluating TNFα, IL-6 and IL-8 release from whole blood after LPS or Pam_3CSK_4 (P3C; a synthetic lipohexapeptide analog of bacterial lipoprotein) stimulation ofTLR4 and TLR2, respectively, in 260 patients with stable angina [13]. The authors chose to only include patients with stable angina to minimize potential confounding effects of inflammation associated with acute myocardial ischemia. For the same reason patients with active inflammatory conditions or patients taking immunosuppressive drugs were excluded. Elsenberg

et al. employed three measures of disease burden or severity: number of diseased coronary vessels, the largest stenosis irrespective of number of affected vessels and finally the occurrence of secondary events during a 9-month follow-up period. The number of diseased vessels and occurrence of secondary events were not significantly associated with TLR4 or TLR2 responsiveness, whereas patients with >90% maximum stenosis were less responsive to TLR4 stimulation than patients with <90% maximum stenosis. The finding of an association between a high degree of stenosis and reduced TLR4 responsiveness is in contrast to a previous study from the same group where they reported an increased TLR responsiveness in patient with >90% stenosis in a smaller cohort of 70 patients [14]. The degree of stenosis may not, however, reflect plaque vulnerability or disease burden and the results should be interpreted in the light of this uncertainty. Disappointingly, no consistent associations were found between traditional risk factors and TLR responsiveness, with the exception of that smokers were hyper-responsive to TLR4 stimulation.

In addition to the studies in the patient cohort with stable coronary artery disease, Elsenberg *et al*. compares the TLR responsiveness of a subgroup of the patients (n=146) to that of healthy controls (n=15) [13]. TLR4 mediated release of TNFα and IL-6, but not IL-8, was significantly higher in coronary artery disease patients

than controls. Interestingly, non-stimulated (buffer addition only) release of TNFα and IL-6 was also higher in patients compared to controls. Although the healthy control group was small these data seem to indicate that TLR responsiveness could be different between diseased and non-diseased individuals. This notion is backed up by other reports showing that TLR4 mediated release of pro-inflammatory cytokines is higher in unstable angina or acute myocardial infarction patients than in patients with stable angina or healthy controls [9,11,15]. Responsiveness to TLR ligands has been assumed to be correlated with surface expression of the corresponding receptor. The expression of TLR4 on monocytes has been shown to correlate to TNFα and IL-6 release after LPS stimulation [11]. Also, surface expression of TLR2 and TLR4 on circulating monocytes has been shown to be increased in acute myocardial infarction and unstable angina patients compared to healthy control patients [9,10]. The predictive power of TLR surface expression, however, could be questioned [16].

Taken together the emerging picture is that TLR expression and responsiveness is different between healthy controls, stable angina, unstable angina and acute myocardial infarction patient groups. So far, however, it is unclear if TLR responsiveness can be used to predict outcome, or to stratify or re-classify patients. Still, larger and more in depth studies to evaluate the usefulness of TLR

responsiveness as a biomarker are justified. Prospective studies in particular are needed and employing more nuanced read-outs after TLR stimulation, including anti-inflammatory or regulatory mediators, and not only pro-inflammatory cytokines, will be useful when evaluating TLR responsiveness and its association to coronary artery disease.

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