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Immune regulatory cells: circulating biomarker factories in cardiovascular disease.

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Abstract

Toll Like receptors are the first line of defence of the innate immune system. These receptors recognise not only exogenous but also endogenous ligands that are expressed following arterial injury and atherosclerotic disease. Expression of the Toll like receptors 2 and 4 is observed in macrophages and different vascular cells and is increased in atherosclerotic disease. It is suggested that Toll Like receptor expression and functional responsiveness following ligation could serve as biomarkers for atherosclerotic disease progression. Recently published research papers support the concept that the Toll Like receptors are fast responders upon arterial injury. In the current issue of Clinical Science new evidence emerges that point to the potential prognostic properties of the Toll like Receptors.

The search for biomarkers that reflect progression of atherosclerotic disease has resulted in a large number of systemically expressed candidates that are mostly thought to mirror a vascular inflammatory response. How expression of these surrogate markers for vessel wall disease is regulated and influenced by the atherosclerotic process is unknown. Systemically expressed markers may have been secreted by cells in the vasculature but may also originate from circulating cells. The concept of white blood cells as the ultimate circulating sensors for vascular disease is intriguing. In this issue of Clinical Science, Ishikawa and co-workers studied the expression of Toll Like Receptor 4 (TLR4) on circulating monocytes in blood samples that were obtained locally near the culprit lesion as well as more systemically near the aortic arch. They observed that blood samples that were obtained near the culprit lesion revealed higher TLR4 expression levels (1).

Toll-like receptors (TLRs) serve as pattern recognition receptors within the innate immune system and recognize exogenous ligands in response to infection. Among these receptors, TLR4 is activated by bacterial lipopolysaccharide (LPS) and is therefore known as the LPS receptor. During inflammation and oxidative stress, TLRs can also be activated in response to endogenous ligands, such as heat shock proteins and the alternatively spliced extra domain A (EDA) of fibronectin (2), resulting in the release of proinflammatory factors.

The last couple of years, TLR2 and TLR4 have been associated with the inflammatory responses that play a role in the pathogenesis of atherosclerotic plaques destabilisation and intimal hyperplasia after arterial injury (3,4). In addition, it is generally appreciated that these TLRs also play a dominant role in myocardial tissue remodelling following ischemic myocardial events (5).

TLR2 and TLR4 are expressed by the circulating immunologically active cells that play a role in innate immunity. In addition, these receptors are also expressed by cells that are generally present in the vasculature. Endothelial cells, smooth muscle cells and fibroblasts may all show increased expression of TLRs in arterial injury models or when arteries are triggered to undergo physiological remodelling.

An interesting observation reported in the paper by Ishikawa et al. was the finding that in a retrospective study TLR expression was associated with disease manifestation and that also prospectively expression

levels were related with cardiovascular endpoints. Finally, the cells expressing TLRs also demonstrated enhanced levels of responsiveness in patients who suffered from an acute coronary syndrome (AMI) following cell stimulation. This resulted increase of TLR2 and TLR4 on local and systemic monocytes in AMI patients compared to SA patients and controls and showed higher TNF- α supernatant levels in AMI patients than in SA patients and controls. Although the authors previously showed that circulating monocytes release HSP70 (6) after MI it should be realized that the role of HSP70 as the endogenous TLR2 and 4 ligand has not been established. The patient population was small which is another limitation that merits careful consideration.

The challenging concept that the cellular responsiveness of TLRs upon ligand stimulation could serve as a biomarker for manifestations of atherosclerotic disease progression is supported by previous observations.

First, association studies have shown that the clinical manifestation of atherosclerotic disease correlates with cytokine release following TLR4 stimulation. Luzzio et al. (7) demonstrated that cytokine release following LPS stimulation and TLR4 expression is significantly increased in patients suffering from recurrent unstable angina compared to patients with stable angina and healthy controls. In another study, circulating TLR4+/CD14+ monocytes were reported to be approximately 2.5-fold increased above controls and patients with stable angina compared with patients suffering from unstable angina or myocardial infarction (8). The latter was paralleled by enhanced transcript levels of TLR4 and Myd88 in patients with acute manifestations of coronary artery disease.

Secondly, strong changes in cellular TLR responsiveness are found to be induced by local vascular injury. In general, the human body is not overwhelmed by a general damaging immune response following any exogenous or endogenous stimulus. A second stimulation of cells by TLR ligands results in an attenuated release of pro-inflammatory cytokines in comparison to the first exposure, a situation referred to as tolerance. Moreover, cross-tolerance of TLR2 for TLR4 ligands (and vice versa) has been reported indicating a waning of the immune response upon ligand exposure. A state mimicking TLR ligand tolerance is observed upon the infliction of surgical trauma (9).

We recently demonstrated a comparable effect after inducing minimal vascular trauma in a human population. Following a coronary stenting procedure, the white blood cells appeared to show a strong attenuated cytokine release following TLR2 and TLR4 ligation (10). White blood cells in the peripheral circulation were found to become less sensitive to excitation when a local vascular coronary injury was applied. This effect was induced within several minutes suggesting that the immune system is a very sensitive sensor for vascular trauma.

Thirdly, the same study (10) revealed that baseline fractional flow reserve, a functional measure for myocardial ischemia, was positively associated with the cytokine release following whole blood TLR stimulation. This observation strengthens the idea that the TLR response by the circulating white blood cell could act as a measure for myocardial ischemia.

The mechanisms for these findings are not fully understood. The attenuation of the pro-inflammatory response may serve as a protection against an excess of cytokine release after an ischemic episode or vascular injury. Thus, a decreased cytokine release after trauma could be functional in that it prevents additional damage inflicted by the immunological response. This hypothesis is supported by observations of Kariko et al. (11) who described a comparable role for TLR inhibition and cytokine signaling following cerebral ischemia. An acute ischemic episode leads to the production of pro-inflammatory cytokines, which increase vascular permeability leading to secondary ischemia and accumulation of immune cells in the affected region. To prevent an excess of pro-inflammatory cytokines being released in a subsequent ischemic period, a negative regulation of TLR response occurs. This so-called pre-conditioning might also take place following acute balloon induced myocardial ischemia or vascular trauma.

But what could be the functional role of increased TLR expression and receptor responsiveness in chronic ischemia? It is well established that collateral formation is an inflammatory process requiring chemokines and macrophage infiltration in the ischemic region. It could be suggested that enhanced TLR expression and ligand responsiveness is a response upon chronic haemodynamic significant ischemia that is needed to stimulate collateral formation.

In summary, the observations described in this issue of Clinical science by Ishikawa et al. support the concept that the TLRs on circulating white blood cell could act as sensors and messengers reflecting the state of atherosclerotic disease. Future larger prospective studies are required to examine the prognostic value of TLR expression and function in coronary artery disease.

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