Role of pattern-recognition receptors in cardiovascular health and disease

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Abstract

A role for PRRs (pattern-recognition receptors) in immune cell function is now well established. In macrophages and other immune cells, activation of TLRs (Toll-like receptors) and cytosolic NLRs [NOD (nucleotide oligomerization domain) proteins containing a leucine-rich repeat] results in the induction of genes and release of immunoregulator hormones including cytokines and NO (nitric oxide). In addition to immune cells, structural cells of the cardiovascular system including endothelial cells, vascular smooth muscle and cardiac myocytes express functional PRRs and sense PAMPs (pathogen-associated molecular patterns). Furthermore, bacteria and PAMPs activate the coagulation system and platelets. TLRs are now implicated in a range of cardiovascular diseases and syndromes including atherosclerosis and sepsis. Our group is working on the hypotheses that differences exist in how tissues of the cardiovascular system, including vessels, endothelium, heart and blood, sense pathogens compared with immune cells (principally macrophages) and that identifying such differences will reveal new therapeutic targets for the treatment of cardiovascular disease. We have identified examples of similarities and differences in how cardiovascular tissues and macrophages sense PAMPs. These findings will be discussed together with our interpretation of how this information may lead to new treatments.

Involvement of the cardiovascular system in innate immunity

Blood vessels

Blood vessels innervate every organ of the body. Their function is to carry blood, oxygen and nutrients and remove waste products from actively respiring cells and tissues. Blood vessels are multicellular structures made up of bundles of vascular smooth-muscle cells arranged into the main tube of the vessel. Nerves innervate the smooth-muscle component and, in larger vessels, a separate blood supply, the vas vasorum, is also present to serve the vessel wall. The luminal surface of all blood vessels is lined by the endothelium, which covers the surface in a monolayer just one cell thick (Figure 1). In healthy vessels, the endothelium is a highly active endocrine organ that synthesizes vasoactive hormones, most of which act locally. Endothelial-derived hormones include NO (nitric oxide) and prostacyclin, which act on the luminal side to keep the blood fluid and on the abluminal side of the vascular smooth-muscle component to regulate flow. The vascular smooth-muscle component is essentially metabolically inert and serves to hold form and respond to hormones released by the endothelium to drive and deliver blood to organs in need.

In mammals, the maintenance of vascular homoeostasis is essential to well-being. In addition to blood vessels, the lung, heart and blood tissues participate in a highly organized ‘cardiovascular system’, which works in separate compartments as well as in a unit to ensure cardiovascular homoeostasis. In addition to the normal functions of the body, the cardiovascular system is exposed to invading pathogens either at the local levels, involving blood, capillaries and small vessels, or in the event of sepsis, at the systemic level involving all tissues of the cardiovascular system. Structures of the cardiovascular system respond to pathogens and to danger signals, which makes them important components of the innate immune system.

Endothelial cells are usually the first tissues of the cardiovascular system to sense pathogens. Endothelial cells are activated by PAMPs (pathogen-associated molecular patterns) including LPS (lipopolysaccharide) via TLR4 (Toll-like receptor 4) [1] to release cytokines, chemokines and dilator hormones. This response facilitates the ‘calling’ of leucocytes to the site of injury and allows for increased blood flow to flush the area of inflammation in order to facilitate resolution. In addition to endothelial cells, the underlying vascular smooth muscle in vessels can also sense pathogens via TLRs [2–4], which results in the induction of vasoactive hormones, limitation of constrictor responses and increased flow. The role of vascular smooth muscle in a useful innate immune function is not clear, but the effect of pathogens in

Key words: blood vessel, cardiac myocyte, endothelium, nucleotide oligomerization domain (NOD), pattern-recognition receptor, Toll-like receptor (TLR).

Abbreviations used: LPS, lipopolysaccharide; MD-88, myeloid differentiation factor 88; NOD1, nucleotide oligomerization domain 1; NOS, nitric oxide synthase; NOX3, NADPH oxidase 3; PAMP, pathogen-associated molecular pattern; PRR, pattern-recognition receptor; TLR, Toll-like receptor; TNF, tumour necrosis factor.

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Figure 1 | Potential effects of infection within tissues of the vessel wall

Bacterial infection activates endothelial cells and vascular smooth muscle via PRRs, leading to vascular collapse and, in severe situations, septic shock. Lower level infection localized to susceptible areas of the vessel wall act as a risk factor for the development of atherosclerosis. PRRs on endothelial cells are thought to be involved. Platelets express PRRs, but their function in platelets is not known.

### Vessel wall

- **PRRs**
- **Infection (eg. E. Coli)**
- **Risk factors**
- **Endothelium**
- **Smooth muscle**
- **Acute systemic inflammation**
- **Septic shock**
- **Platelets**
- **Latent local inflammation**
- **Atherosclerosis**

this part of the vessel clearly contributes to pathology and cardiovascular dysfunction (Figure 1) (see below).

### Cardiac myocytes

In addition to vascular cells, the main cell type of the heart, the cardiac myocytes express TLRs [5] and can also sense bacteria and PAMPs directly [6]. The function of innate immune signalling in cardiac myocytes is not known but, as is the case for vascular smooth muscle, it is thought to contribute to cardiovascular dysfunction.

### Blood and platelets: evidence for non-genomic effects of PRRs (pattern-recognition receptors)

The role of PRRs in blood is more controversial. Blood comprises nucleated leukocytes, un-nucleated platelets and red cells as well as highly active proteins. Clearly, circulating leukocytes have well-defined innate immune functions mediated by a range of PRRs, as reviewed elsewhere [7]. Activation of the coagulation system and platelets is an important early part of the innate immune response which serves to prevent further entry of pathogens to wounds. However, the role of PRRs in platelets is unclear and controversial. Nevertheless, this is a very important aspect of TLR biology for us to understand, not least because any responses seen in platelets would clearly be mediated independently of the nucleus and by non-genomic pathways.

From what we understand about PRRs, including TLRs, their effects are generally understood to be mediated by the activation of transcription factors and the induction of genes, and there is little evidence of PRRs mediating acute responses. However, platelets do express TLR proteins [8,9]. The possibility that these proteins are functionally linked and active is where the debate in this area lies. Some groups [9] including our own (F. Ali and J.A. Mitchell, unpublished work; http://www.lifesciences2007.org) have found that whole bacteria or PAMPs can activate platelets to stimulate aggregation. In our hands, the effect of bacteria (including *Staphylococcus aureus* and *Escherichia coli*) or PAMPs (including LPS, FSL-1 (fibroblast stimulating lipoprotein-1) and Pam3CSK4) occurred in isolated platelets as well as in whole blood, but required the presence of a platelet-activating molecule, such as ADP (F. Ali and J.A. Mitchell, unpublished work). Others have found, however, that while platelets express TLR2 and TLR4, PAMPs for these receptors did not activate human platelets [10]. It therefore remains unclear how pathogens and PAMPs activate the coagulation system and the role that PRRs may play in this response.

### Role of PRRs in cardiovascular health

Clearly, PRRs including TLRs are implicated in human disease. However, less well understood is the potential role of TLRs in the maintenance of health, particularly in the cardiovascular system. In a recent study, we observed that vessels and heart from ‘healthy’ TLR4-deficient mice were compromised. Specifically, we found that, despite having an intact endothelial layer, which expressed competent levels of NOS (nitric oxide synthase) (III), vessels from TLR4−/− mice did not respond to dilator hormones [11]. Similar responses in blood vessels are found from experimental animals with systemic hypertension or diabetes, which have a reduced life expectancy through cardiovascular disease. In line with this, we also found that the hearts from TLR4−/− mice were enlarged with signs of hypertrophy [11]. Interestingly, we did not find any vascular dysfunction in tissue from TLR2−/− mice. These observations suggest that TLR4, and perhaps other PRRs, sense endogenous ligands that signal physiological processes as part of vascular homeostasis. Increasingly studies suggest...
Figure 2 | Hypothesis to explain why vessels from TLR4−/− mice do not dilate when the endothelium is stimulated to make NO [11]. NOX3 is induced in tissues from TLR4−/− mice [12]. NOX3 catalyses the formation of superoxide anions (O2−), which inactivate NO, forming peroxynitrite (ONOO−) as a product. Increased levels of O2− greatly limit the biological activity of NO and thereby vasodilator responses.

that endogenous ligands can activate PRRs. In the case of endothelial cells, the sensing of physical forces such as shear stress is a vital part of homoeostasis. It is therefore tempting to speculate that TLR4 may be involved in this process.

The explanation for why endothelium-dependent dilator function is compromised in tissue from TLR4−/− mice is not clear, particularly because, as mentioned above, NOS III expression was unchanged. However, in a separate study, Zhang et al. [12] showed how the enzyme complex NOX3 (NADPH oxidase 3) was up-regulated in lung tissue from TLR4−/− mice. NOX3 catalyses the formation of superoxide anions (O2−), which bind to NO, forming peroxynitrite (ONOO−), blocking its biological activity (Figure 2). If NOX3 is similarly induced in endothelial cells of TLR4−/− mice, we would expect that endothelial-dependent dilator pathways would be compromised as a result (Figure 2). The role of NOX3 in the vascular dysfunction seen in tissues from TLR4−/− mice remains the subject of investigation.

Interestingly, in a small-scale study examining the links between polymorphisms in TLR2 [13] and coronary restenosis, results suggested that a functional TLR2 was protective, maintaining vascular homoeostasis. Clearly, this is an important area of PRRs and cardiovascular function that will no doubt continue to be expanded.

Role of PRRs in cardiovascular disease
There is now overwhelming evidence from animal models that PRRs including TLR4 and TLR2 mediate cardiovascular disease [14]. The most studied areas of cardiovascular disease/dysfunction and PRRs are atherosclerosis [15] and bacterial septic shock [3] (Figure 1). In animal models of atherosclerosis, protection is seen in TLR4, TLR2, MyD88 (myeloid differentiation factor 88) or CD14-knockout animals [16,17]. However, information from human studies is less conclusive and may be confounded by a dual action of TLRs in mediating vascular health (see above) as well as disease. In atherosclerosis, it is suggested that ‘low-level’ localized bacteria associated with plasma or within the plaque itself could be the initial inflammatory hit required to begin the disease process. Clearly, if this is the case, it is easy to see how PRRs are involved (Figure 1). However, atherosclerosis is also associated with a range of host-derived molecules that may equally activate PRRs and inflammation [18].

The strongest case for a causative role of PRRs and related pathways is in sepsis and septic shock. Sepsis occurs when the innate immune system is overwhelmed and infection spreads via the circulation (Figure 1). In this scenario, all tissues of the cardiovascular system are affected. In laboratory animals or in humans, septic shock is characterized by a profound and unresponsive decline in blood pressure, which emerges after several hours. This type of shock is very different from anaphylaxis for example, which can occur acutely. The reason why the shock is delayed in sepsis is because it is associated with the induction of genes in vascular and cardiac tissues. In anaphylaxis, by contrast, shock is caused by the release of acute and preformed mediators. In sepsis, the endothelium and vascular smooth muscle are pan-activated by bacteria, PAMPs and danger signals. In endothelial cells, the normal expression of vasoactive genes such as NOS III and COX-1 (cyclo-oxygenase-1) declines and the physical barrier that protects the underlying smooth muscle becomes damaged and lost in some areas [19]. The underlying vascular smooth muscle is then exposed and activated by pathogens such as Gram-positive or Gram-negative bacteria [4,20], resulting in the induction of inflammatory genes including NOS II [3], leading to vascular collapse. Understanding how pathogens activate vascular tissue is an important area of sepsis research. While the mechanisms by which vascular cells sense pathogens are still not fully understood, PRRs are clearly involved. In vessels, whole Gram-positive bacteria (S. aureus) are sensed by the TLR2–TLR6 heterodimer, with no apparent involvement of TLR1 (Figure 3) or TLR4. MyD88 and TNF (tumour necrosis factor) are required, leading to the induction of NOS II [4]. Gram-negative bacteria (E. coli) are sensed in vessels by TLR4, with no involvement of TLR2. MyD88 and TRIF (TIR (Toll/interleukin-1 receptor) domain-containing adaptor protein inducing interferon β) pathways are required, but TNF is not involved in the NOS II-mediated vascular dysfunction that results [4]. Vascular smooth muscle is unusual in how it senses pathogens compared with other cells such as macrophages. As mentioned above, Gram-positive bacteria are sensed via TLR2–TLR6 and not TLR2–TLR1 in vascular smooth muscle, whereas in macrophages, the TLR2–TLR1 and TLR2–TLR6 dimers seem equally important [21,22] (Figure 3). Interestingly, in cardiac myocytes, activation of the TLR2–TLR6, but not the TLR2–TLR1 heterodimer accounts for the ‘heart failure phenotype’ induced by Gram-positive bacteria [6]. A more striking difference in the role of PRRs in vascular cells compared with macrophages was seen when studying NOD1 (nucleotide oligomerization domain 1). Activation of NOD1 alone in macrophages induces no or very low level responses. However, activation of NOD1 together with TLRs results
in an amplification of responses [23] in macrophages. Work from our group has shown that the situation is different in vessels and vascular smooth muscle. Activation of NOD1 induces a profound response, resulting in the induction of NOS II, vascular dysfunction and shock [20] (Figure 3). In vessels, vascular dysfunction induced by NOD1 activation was independent of TLRs or classical TIR-domain adaptor proteins [20]. The signalling pathways and the consequence to health and disease of these observations are the subject of ongoing investigations.

Summary and conclusions

The cardiovascular system is a complex and highly organized unit consisting of blood, vessels and the heart. Tissues within the cardiovascular system are exposed to pathogens and danger signals, resulting in activation of PRRs including TLRs and NOD receptors. We have identified a putative positive role for TLR4 in the maintenance of a healthy cardiovascular system. However, when tissues of the cardiovascular system are exposed to pathogens, they become activated via PRR pathways, leading to dysfunction and disease. We have identified clear differences in how vessels or the heart sense pathogens compared with macrophages. These differences include the exclusive usage of TLR6 in response to Gram-positive bacteria and the adaptation of the NOD1 signalling pathways to act alone in vessels to induce profound dysfunction. The identification of differences in pathogen-sensing pathways in cardiovascular tissues and in essential innate immune cells (such as macrophages) will lead to new therapeutic targets, the inhibition of which could prevent cardiovascular disease, while preserving immune function.

References


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