

## The Toll way to hypertension: role of the innate immune response

Gisele Facholi Bomfim<sup>1,2\*</sup>, Theodora Szasz<sup>1</sup>, Maria Helena C. Carvalho<sup>2</sup> and R. Clinton Webb<sup>1</sup>

<sup>1</sup>Department of Physiology, Georgia Health Sciences University, Augusta, GA, USA

<sup>2</sup>Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil

### Abstract

The involvement of pro-inflammatory processes in cardiovascular disease such as hypertension is a well accepted concept. Human hypertension is associated with increased levels of circulating cytokines and their participation in the associated target organ damage has clearly been demonstrated. Recent evidence suggests that at least in animal models of hypertension, components of the adaptive immune system such as T cells are activated and infiltrate target organs, promoting inflammation and thus participating in the maintenance of increased blood pressure. However there is a paucity of information on the role of the innate immune response in hypertension. Moreover, the identity of the original stimuli responsible for the immune system activation as well as the chronology of these events during hypertension pathogenesis is unknown. The current paradigm is that the immune system is activated by danger signals originating from stressed or injured cells and tissues which release molecules (damage-associated molecular patterns or DAMPs) recognized by pattern recognition receptors, such as Toll like receptors, on antigen-presenting cells (APCs). APCs in turn activate the adaptive immune response and both kinds of response will lead to release of pro-inflammatory factors, such as cytokines and chemokines. During hypertension, cytokine/chemokine signaling in the kidney and vasculature may cause further injury and release of DAMPs continuously, resulting in a sustained chronic inflammatory process. In the present review, we describe recent research supporting the hypothesis of the immune system activation as a causative factor of hypertension, with a special focus on the involvement of the innate immune response in the initiation of hypertension and the hypertensive vascular and renal dysfunction.

**Keywords:** Hypertension; Toll-Like receptor; Innate immune response

### Introduction

Hypertension is a chronic disease characterized by systemic high blood pressure and is the most common and important risk factor for the development of cardiovascular diseases. Hypertension affects more than a quarter of the world's adult population and plays a major pathogenetic role in the development of cerebrovascular disease, ischemic heart disease, cardiac and renal failure. The etiology of hypertension is heterogeneous and remains elusive. In the last decade, low grade systemic inflammation has been described as an important feature of hypertension. Recent investigations suggest that the innate immune response may be activated by host-derived molecules or DAMPs (damage-associated molecular pattern) leading in turn to activation of the adaptive immune response. The participation of different lymphocyte populations, effectors of the adaptive immune response, has been demonstrated for angiotensin II-induced hypertension [1]. Nevertheless, few authors have discussed the participation of the innate immune response in hypertension, which is responsible for the recognition of the DAMPs and activation of the adaptive response. In this article we will discuss the inflammatory process in hypertension and the contribution of the immune system to this disease state. We will focus on recent data on the participation of innate immune response in hypertension.

### Hypertension as a pro-inflammatory disease

Many studies have described an inflammatory process in hypertension. This process can contribute in many ways to the maintenance of high blood pressure and organ damage. Epidemiological and clinical studies have shown strong and consistent relationships between markers of inflammation and hypertension.

The association between inflammatory markers and high blood pressure has been analyzed in animal models and in humans. Some cross-sectional studies showed that, compared to normotensive individuals, the plasma levels of inflammatory markers, such as CRP (C-reactive protein); cytokines (TNF- $\alpha$  - tumor necrosis factor- $\alpha$  and

IL-6); chemokines, (MCP-1 - monocyte chemoattractant protein) and adhesion molecules (P-selectin and sICAM-1) are increased in patients with essential hypertension and no evidence of cardiovascular disease [2,3]. Moreover, the levels of specific inflammatory markers such as CRP effectively predict future hypertension, [4,5] suggesting that inflammation may precede and not just follow blood pressure elevation.

A major class of inflammatory molecules present in hypertension that we will discuss is the cytokines. They play an important role in the renal damage and vascular resistance associated with hypertension, and some have clinically been associated with increased arterial stiffness, [6] which independently predicts cardiovascular events in hypertensive patients. They regulate the expression and function of several proteins, including adhesion molecules, mitogen-activated protein kinases (MAPKs), extracellular matrix components and growth factors, which are important in vessel hypertrophy and vascular dysfunction described in hypertension. Cytokines also seem to influence the balance between vasoconstrictor and vasodilator factors, as well as regional differences in the release and responsiveness to these factors, contributing to the increased responsiveness within a specific vascular bed in hypertension. In perivascular fat and kidney, the cytokines affect adjacent vascular beds and tubular epithelium [7,8]. Here we will describe the main cytokines involved in hypertension.

TNF- $\alpha$  is a pro-inflammatory cytokine generally involved in

**\*Corresponding author:** Gisele Facholi Bomfim, 1120 15th St Physiology CA-3101 Augusta, GA 30912, USA, Tel: 706-721-3547; Fax: 706-721-7299; E-mail: [gifacholi@yahoo.com.br](mailto:gifacholi@yahoo.com.br)

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neutrophil activation and increased vascular permeability. Besides these acute effects, TNF- $\alpha$  stimulates growth of vascular smooth muscle cells (VSMC) and increases migration. Additionally, it increases the expression of adhesion molecules, other cytokines and metalloproteinases. Studies have shown that the TNF- $\alpha$  antagonist etanercept reduces the hypertension caused by fructose feeding in rats prevents vascular dysfunction and prevents the hypertension caused by angiotensin II infusion [9,10]. Etanercept also reduced blood pressure and attenuated renal damage in a mouse model of systemic lupus erythematosus [11]. In mineralocorticoid and salt-sensitive hypertension models, TNF- $\alpha$  antagonism can also prevent end-organ damage of the kidney, without lowering blood pressure [12,13].

IL-6 is a pro-inflammatory cytokine that is released from numerous cell types, including endothelial cells, VSMCs, and macrophages. IL-6 stimulates the synthesis of many acute-phase reaction proteins, including C-reactive protein, serum amyloid A and fibrinogen [14]. IL-6 also promotes VSMC proliferation, a hallmark of hypertension and atherosclerosis. Previous work from Lee and colleagues showed that hypertension caused by high-salt diet and angiotensin II was blunted in mice lacking a functional gene for IL-6. Part of this IL-6 effect is explained by increased sodium and water reabsorption as a result of high AT1 receptor density in proximal tubules [15]. Recently, it was also demonstrated that IL-6 levels are increased in kidneys of patients with chronic kidney disease and hypertension [16].

TGF- $\beta$ 1 is a polypeptide that controls the proliferation and differentiation of some cell types, as lymphocytes. Studies have measured TGF- $\beta$ 1 mRNA levels in hypertensive rat models and in patients with hypertension. Left ventricular TGF- $\beta$ 1 mRNA levels are increased in stroke prone SHR and post-myocardial infarcted rat and also in renal cortex of DOCA-salt rats. In essential hypertensive patients, TGF- $\beta$ 1 mRNA and protein are overexpressed [17]. The Arg(25) and +869T/C polymorphisms in the TGF- $\beta$ 1 gene are associated with high blood pressure in the human population [18].

Recently, it was described that the novel proinflammatory cytokine IL-17 contributes to hypertension, since IL17 $^{-/-}$  mice do not sustain hypertension induced by angiotensin II to the same level as wild type mice [19]. This cytokine is secreted by Th-17 lymphocytes and stimulates the release of other cytokines, ICAM expression and T-cell proliferation.

Vanhala *et al* observed that high levels of IL-1 $\beta$ , a cytokine involved in monocyte activation and a risk factor for atherogenesis, precede future changes in systolic blood pressure [20]. LPS-stimulated IL-1 $\beta$  secretion is increased in neutrophils from hypertensive compared to normotensive patients and certain anti-hypertensive agents attenuate this effect [21].

Some laboratories have investigated the effect of anti-inflammatory cytokines in hypertension. For example, recently, Nonaka-Sarukawa and colleagues showed that the anti-inflammatory cytokine IL-10 has a significant antihypertensive effect. They employed adeno-associated virus type 1-based vector in order to sustain serum levels of IL-10 in Dahl salt-sensitive rats and stroke prone SHR and observed that IL-10 has protective actions on the vascular and renal dysfunction [22].

In humans, treatment with immunosuppressives would not be indicated in patients with essential hypertension alone; however, treatment with the immunosuppressive mycophenolate mofetil decreased blood pressure in patients with psoriasis and rheumatoid arthritis and associated hypertension [23].

Another molecule that has been extensively investigated in

hypertension is the nuclear factor  $\kappa$ B (NF $\kappa$ B). The activation of NF $\kappa$ B results in the upregulation of adhesion molecules, cytokines and chemokines by vascular endothelial cells and many other cell types. In most cases, cells from infected or injured tissue initiate the inflammatory pathways through NF $\kappa$ B in response to inflammatory stimuli. NF $\kappa$ B plays an important role in the pathogenesis of cardiovascular diseases. McAllister-Lucas *et al* showed that in vascular smooth muscle cells and endothelial cells, angiotensin II activates NF $\kappa$ B [24]. Rodriguez-Iturbe *et al* showed that in SHR (spontaneously hypertensive rat) the NF $\kappa$ B activation and immune cell infiltration in the kidney occurred as early as 3 weeks-old when compared to Wistar-Kyoto rats (WKY). Conversely, long-term inhibition of NF $\kappa$ B using pyrrolidone dicarbamate can result in attenuation of renal interstitial inflammation and in prevention of hypertension in this model [24]. Wu & Schmid-Schonbein also demonstrated the increased activation of NF $\kappa$ B in kidney and brain hypothalamus, but not in myocardium and cerebral cortex of SHR compared to WKY. They showed that NF $\kappa$ B expression is involved in the enhanced MMP-2 and MMP-9 activity in SHR [26]. Activation of NF $\kappa$ B and IKK- $\beta$  in the normal mouse hypothalamus increased blood pressure via sympathetic-mediated hemodynamic changes and activation of POMC (pro-opiomelanocortin) neurons [27].

Clearly, many studies have described a pro-inflammatory process in hypertension. However many questions still need to be answered, such as how this inflammatory process starts, how the immune system is activated, and what are the possible molecules or signaling events/pathways involved in this response.

## Immune response

The main function of the immune system is defending a host against pathogen invasion. For this function the immune response is a complex system that has two main arms: innate immunity, which mediates the early reactions, and adaptive immunity, which is a late and more specific response.

**Innate immune response:** The innate immune response is the first line of defence against antigens. This kind of response is able to recognize different antigens, process them and present molecules from this antigen to cells of the adaptive immune response, which will develop a specific response against this molecule. In addition to the defensive function of this system, it must also be able to distinguish between infection and tissue damage and know how to respond appropriately to these two scenarios.

In the 1950, Frank Burnet postulated the concept of self-non-self of immunity, where the immune system can discriminate between self and non-self, and lymphocytes specific to self antigens are eliminated to prevent immune reactions against one's own antigens. Together with his colleague Peter Medawar, Burnet won the Nobel Prize in Medicine in 1960 for this theory.

Subsequently, a major modification to this concept was proposed by Charles Janeway, Jr, in the late 80s, when he introduced the "infectious non-self model". In this theory, he introduced the antigen-presenting cells (APCs) and the co-stimulatory molecules into the model. The APCs are activated via encoded pattern recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs) on bacteria. On activation, APCs up-regulate costimulatory signals (MHC, B7), process the antigens, and present them to passing T cells. In his view, the PRRs allow APCs to discriminate between "infectious-nonsel" and "noninfectious-self". All these concepts were instrumental in understanding many aspects of the immune response; however, they failed to explain other immunological phenomena, such as the inflammatory process in autoimmune diseases.

In the 90s, Polly Matzinger introduced a novel idea known as the danger model. Briefly, this model hypothesizes that the cells of the innate immune response are activated not by the infections non-self, but by danger or alarm signals generated from injured host cells, damaged tissues or metabolic stress. Thus, the primary function of the immune system is to detect and protect the host against danger and the initiation of the immune response is not the foreignness or strange microbes (non-self), but its function is to detect alarm signals generated from injured or damaged cells and tissues. As Matzinger highlighted in an excellent review [28], alarm signals can be constitutive or inducible, intracellular or secreted, or even a part of the extracellular matrix. Because cells dying by normal programmed processes are usually scavenged before they disintegrate, they do not generate danger signals. However cells that die from necrosis release their contents and therefore any intracellular product could potentially be a danger signal. Inducible alarm signals can include any substance made, or modified, by distressed or injured cells. To conform to the term “pathogen associated molecular pattern” (PAMP), the term “damage associated molecular pattern” (DAMP) was subsequently coined for these endogenous danger signals. The identification of these stimuli and putative receptors has provided significant insight into the initiation and regulation of innate immune response.

The main APCs present in the body are macrophages/monocytes, dendritic cells, B lymphocytes, vascular endothelial cells as well as epithelial and mesenchymal cells. These cells recognize PAMPs and DAMPs by pattern recognition receptors (PRRs). These receptors can be classified into cell-associated (TLR, NLR (NOD-like receptors), RLR, Dectin) and secreted forms (C1q and MBP, mannose binding protein). Some of these cell-associated receptors are found either on the plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6) or in association with the membranes of endosomes, the endoplasmic reticulum or endolysosomes (TLR3, TLR7, TLR9). There are also cell-associated PRR that are present intracellularly in the cytosol (RLR, NLR). Generally, following interaction with appropriate ligands, the PRRs activate certain transcription factors which then translocate to the nucleus to turn on and upregulate genes involved in host defense and inflammation (e.g., proinflammatory cytokines). A constant and excessive activation of PRR contribute to the development of autoimmunity and the induction of sepsis [29].

The most extensively described PRR that can recognize and be activated by DAMPs are the TLR. For this reason, in the following section we will review the TLR signaling and the studies that describe the role of these receptors in hypertension.

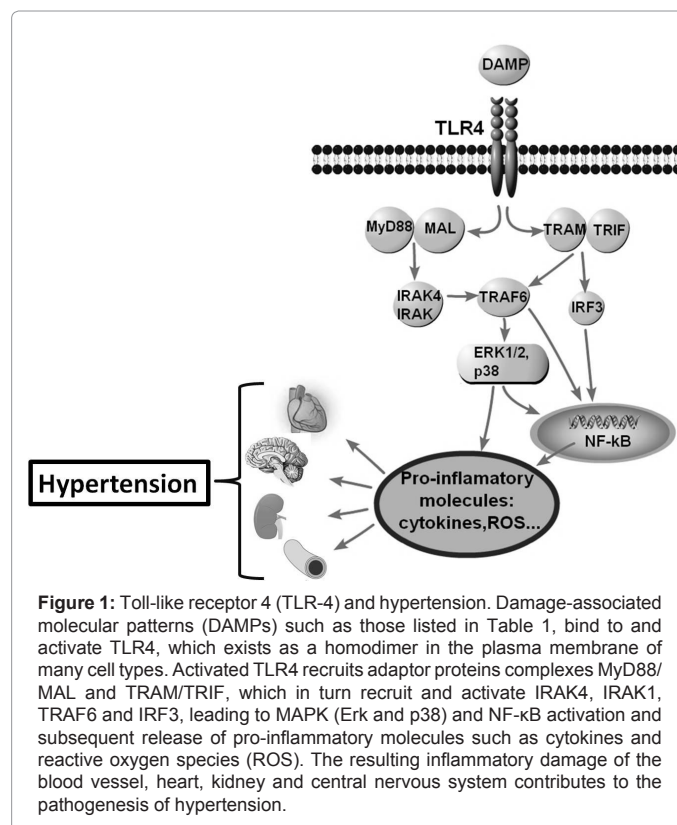
### Toll like receptors

The TLR receptor family was the first pattern recognition receptor to be identified. TLRs are type I transmembrane proteins of the interleukin-1 receptor family that possess an amino-terminal leucine-rich repeat (LRR) domain for ligand binding, a single transmembrane domain and a carboxy-terminal intracellular signaling domain. These intracellular domains are able to trigger signaling pathways known to activate the nuclear factor kappa B (NF- $\kappa$ B), which in turn leads to the secretion of proinflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-8. TLRs are widely expressed by many cell types. TLR ligands are varied, consisting of bacterial cell wall components; bacterial genomic DNA; viral, fungal and parasitic products; and synthetic analogs of natural products. However, TLRs can also bind autologous (self) molecules such as heat shock proteins (HSPs), intercellular matrix products, mitochondrial DNA and mammalian genomic DNA, revealing that the TLR immune system is concerned with damage signals from injured tissue. It is suggested that TLRs recognize not only PAMPs, but also

stress- or damage-associated molecular patterns. However how TLRs are able to discriminate between ligands is still poorly understood.

The first TLR pathway described requires two adaptor proteins, MyD88 (myeloid differentiation factor 88) and Mal (MyD88 adaptor-like). These adaptors recruit IRAK4 and IRAK1 (serine/threonine kinases) to trigger the activation of TRAF6 and IKK complex, resulting in quick or early activation of the transcription factor NF- $\kappa$ B. The second pathway requires TRIF (TIR domain-containing adaptor inducing interferon- $\beta$ ) and TRAM (TRIF-related adaptor molecule). This pathway induces late NF- $\kappa$ B activation and LPS-mediated phosphorylation, as well as dimerization of the transcription factor IRF (interferon regulatory factors), resulting in IFN- $\alpha$  and IFN- $\beta$  production and activation of acquired immunity. Both MyD88/TRAM and TRAM/TRIF complexes can activate TRAF6, which induces MAPK activation. Recently, two more adaptor proteins were described: TRAM (Toll-receptor-associated molecule) and SARM (sterile alpha and HEAT/Armadillo motif protein). TRAM transduces signals from all of the TIR (Toll/interleukin-1 receptor homologous region) domains, activating protein kinases and then transcription factors that cause inflammatory effects. The function of SARM has yet to be defined, but it was described as a negative adaptor. The adaptor protein MyD88, which is recruited by the Mal adaptor protein, is utilized by all kinds of TLR receptors, but not by TLR3 (Figure 1) [30].

TLR signaling pathways induced by endogenous molecules in different cell types are poorly investigated, but recent studies report usage of distinct signaling pathways downstream of TLRs when stimulated by exogenous or endogenous molecules. Activation of TLR4 by LPS can induce both TRIF and MyD88-dependent pathways. In contrast, studies have shown that the endogenous TLR4 agonist tenascin-C signals via MyD88. Similarly, biglycan has been shown to signal through TLR2 and TLR4 in a MyD88-dependent manner [31].



**Figure 1:** Toll-like receptor 4 (TLR-4) and hypertension. Damage-associated molecular patterns (DAMPs) such as those listed in Table 1, bind to and activate TLR4, which exists as a homodimer in the plasma membrane of many cell types. Activated TLR4 recruits adaptor proteins complexes MyD88/MAL and TRAM/TRIF, which in turn recruit and activate IRAK4, IRAK1, TRAF6 and IRF3, leading to MAPK (Erk and p38) and NF- $\kappa$ B activation and subsequent release of pro-inflammatory molecules such as cytokines and reactive oxygen species (ROS). The resulting inflammatory damage of the blood vessel, heart, kidney and central nervous system contributes to the pathogenesis of hypertension.

In view that TLRs are expressed in many tissues, including the cardiovascular system and kidney and that these receptors are activated by endogenous ligands, many authors have been focusing on the role of TLR in cardiovascular diseases [32].

The most studied area of cardiovascular disease and TLRs is atherosclerosis. Studies using knockout mice for TLR4, CD14 and MyD88 have demonstrated that TLR4 has an important role in the development of atherosclerosis. Double homozygous progeny of TLR4 knockout mice crossed with the atherosclerosis-prone apolipoprotein E (ApoE) knockout mice exhibited reduced atherosclerosis when compared with ApoE knockout controls [33]. The same were observed in MyD88-ApoE and CD14-ApoE double-knockout mice [34] confirming the proatherogenic effect to TLR4.

In addition to atherosclerosis, the involvement of TLR4 has also been shown in angiogenesis and in diseases such heart failure, ischemic injury and septic cardiomyopathy [32]. However few works have studied the beginning of the story, that is, when and how the TLR4 start to be activated and contribute to the development of all these diseases. One important question that must be answered is whether the risk factors for the development of cardiovascular disease can activate this receptor and initiate the inflammatory process in these conditions. One of the main and most studied risk factor is hypertension. Nevertheless the role of TLR4 in hypertension is still uncertain.

One of the first studies that started to associate high blood pressure with TLR4 was one published by Ha *et al* [35] in 2005. They showed that TLR4-deficient mice subjected to aortic banding for 2 weeks and with pressure overload had reduced cardiac hypertrophy when compared to wild type mice [35].

Another study conducted by Eissler *et al* [36] showed that TLR4 expression is increased in hearts from adolescent and adult SHR compared to those from WKY rats, suggesting that a brief period of elevated blood pressure was needed to induce this receptor. They also showed an enhancement in TLR4 expression in WKY rats treated with L-NAME, another hypertensive model. Treatment with ramipril (angiotensin converting enzyme inhibitor) decreased TLR4 expression in these models, showing that the expression of TLR4 is modulated by blood pressure [36].

Sales *et al* found that the polymorphism TLR4 variant Asp299Gly and TLR6 variant Ser249Pro is associated with lower left ventricular wall thickness and left ventricular mass in hypertensive women, but the same association was not found in men. In the same work, they described that monocytes from hypertensive women carrying TLR6 polymorphism exhibited diminished zymosan-induced cytokine release (TNF- $\alpha$  and IL-6) compared with sex-matched cells carrying the Pro allele. They also showed that heterozygous monocytes from hypertensive subjects with TLR4 polymorphism of both genders exhibited a lower production of IL-6 after been stimulated with LPS, compared to hypertensive individual without TLR4 polymorphism [37,38].

We recently observed increased TLR4 mRNA expression not only in the heart but also in the kidney and mesenteric resistance arteries from SHR of 15 weeks of age compared with Wistar and SHR of 5 weeks-old (normotensive). We also treated SHR with antibody anti-TLR4, to block the activation of this receptor, and observed that this treatment decreased blood pressure and inflammatory markers (IL-6, TNF- $\alpha$  and NF $\kappa$ B) and ameliorated the abnormal contractile function of mesenteric resistance arteries.

In summary, these studies describe, in part, the participation of

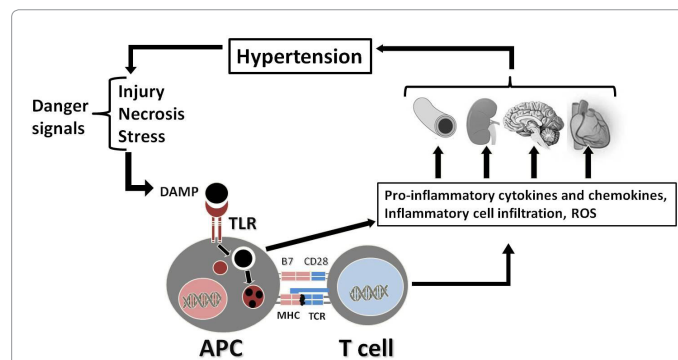
TLR4 in hypertension. However many questions must be answered about the activation of this receptor in the kidney, central nervous system and vessels that are important organ which contribute to blood pressure regulation. Another question is what is the ligand(s) responsible for TLR4 activation in hypertension, and whether more types of TLR are involved in this condition.

### DAMPs in hypertension

The innate immune response is effective and necessary when fighting limited pathological situations. Cellular PRRs are also crucial to mounting an effective inflammatory response. However, if the stimuli persist and inflammation becomes chronic, the immune response can produce complex results which, although initially protective, can eventually lead to tissue damage and cause localized or even systemic disease. Hypertension is an example of chronic inflammatory disease, and many DAMPs are present in this condition contributing to persistent inflammation (Figure 2).

Recognition of host-derived molecules by TLRs and subsequent TLR activation may be an important link between hypertension and activation of the immune system. Many studies have clearly shown that TLR4 is responsible for inflammation induced by endogenous ligands, such as C-reactive protein and heat shock proteins (HSP60 and HSP70) in smooth muscle cells and many other cell types. In hypertension, the circulating levels of these molecules are increased and they could act as long-term TLR4 activators, resulting in augmented expression of several pro-inflammatory cytokines in cardiovascular tissues. In table 1 we list several examples of potential TLR activators in hypertension and what type of TLR and cell it was described to be activating. This list is by no means exhaustive, as numerous other yet unknown molecules may fulfill our inclusion criteria of being TLR ligands that are augmented during hypertension.

**HSP60 and HSP70:** The heat shock proteins are molecular



**Figure 2:** Hypothesis of immune system activation in hypertension. Increases in products from damaged, stressed or necrotic cells and tissues have been described in hypertension. These products, acting as damage-associated molecular patterns (DAMPs), can be recognized by pattern recognizing receptors, such as Toll-like receptors (TLRs) on antigen-presenting cells (APCs). APCs will process the DAMPs and present them on major histocompatibility complex (MHC) molecules to T cells. In this context, interaction between MHC/antigen and the T-cell receptor (TCR), co-stimulated by the binding of B7 to CD28 will activate the T cell. Activated T cells, as well as APCs, will release cytokines and chemokines, causing inflammatory cell infiltration and increases in ROS. These events will cause damage of various targets, such as the blood vessels, heart, kidney and central nervous system, all of which are main regulators of blood pressure. The resulting blood pressure increase itself will lead to further damage and stress which will increase DAMP levels, generating a positive feedback loop where increasing tissue damage perpetuates pro-inflammatory responses leading to chronic inflammation, resulting in vascular dysfunction and renal damage which may cause and contribute to the maintenance of hypertension.

DAMPs	TLR	Cell type	Reference
HSP 60	2, 4	cardiomyocytes	Kim et al, 2009
HSP 70	2, 4	macrophage, cardiomyocytes	Asea, A et al, 2002; Mathur S. et al, 2011
Angiotensin II	4	VSMC	Ji, Y et al, 2009
C-reactive protein	4	VSMC	Liu, N et al, 2010; Li, N et al, 2011
Oxidized LDL	4	macrophage	Miller, YI et al, 2003
HMGB-1	2, 4	macrophage	Park, J et al, 2004
Fibrinogen	4	cardiomyocyte, monocytes	Li, T et al, 2009; Kuhns D, et al 2007
Uric acid	2, 4	macrophage	Liu-Bryan, R. et al, 2005
ADMA	4	adipocyte	Yang, Z.C. et al, 2009

VSMC: vascular smooth muscle cells; HSP: heat shock protein; HMGB: high mobility group protein  
 LDL: low density lipoprotein; ADMA: asymmetric dimethylarginine

**Table 1:** Damage-associated molecular patterns (DAMPs) that are present in higher levels in hypertension and are known to activate Toll-like receptors (TLR).

chaperones involved in folding and unfolding of other proteins. Their expression is increased when cells are exposed to high temperatures or other stress conditions such as inflammation and infection. Elevated blood pressure is a mechanical stress to the endothelium – theoretically it may be a factor inducing enhanced expression of HSP on the endothelial cell surface. In patients with hypertension, it was described that HSP70 is increased in kidney and in lymphocytes [39]. Hypertensive humans also exhibit increased plasma levels of HSP60 compared to normotensive individuals [3]. Animal models have also revealed a relationship between elevated blood pressure and increased expression of HSP [40].

Many fragments from HSP70 and HSP60 are immunogenic and it was demonstrated that these proteins can stimulate macrophages to release TNF- $\alpha$  and synthesize NO in a TLR4-dependent manner [41,42]. Mathur et al. showed that HSP70 induces an inflammatory signal via TLR2 and MyD88, using primary cardiomyocytes isolated from TLR2 and MyD88 knockout mice. Another group has demonstrated that HSP60-induced apoptosis in cardiac myocytes is TLR4 dependent [43,44].

**Angiotensin II:** Angiotensin II (Ang II) is a main peptide hormone of the renin-angiotensin system. Ang II causes endothelial dysfunction, VSMC proliferation and migration, and stimulates inflammatory processes in many cells of the body, including vascular tissue and kidney. Furthermore, Ang II is able to stimulate vascular cells to produce cytokines (e.g. CRP, IL-6, VCAM-1, MCP-1) and growth factors (e.g. PDGF and VEGF) via receptor activation and subsequent activation of multiple intracellular signaling pathways, which contribute to vascular inflammation and cardiovascular disorders.

Ji et al [45] demonstrated for the first time that Ang II induces inflammatory responses partly via TLR4-dependent mechanisms in VSMCs. They showed that Ang II activates TLR4 through AT1 receptor and subsequent ERK1/2 activation in VSMCs [45].

Another study demonstrated that Ang II blockade via losartan administration decreases the innate immune response caused by cyclosporine-induced renal injury, by decreasing TLR2 and MHC class II protein expression and the number of infiltrated and matured dendritic cells in the rat kidney [46].

**C-Reactive Protein (CRP):** CRP is an acute phase reactant produced by hepatocytes. The concentration of CRP increases 4 to 6 hours after acute tissue injury or inflammation and declines rapidly with resolution of the injurious process. CRP is considered an independent predictor

of cardiovascular disease and has been associated with increased risk for development of hypertension [47]. CRP induces pro-inflammatory cytokine release from human monocytes and up-regulates CCR-2, NADPH oxidase and NF $\kappa$ B activity. Higher levels of CRP may increase blood pressure by reducing nitric oxide production in endothelial cells, resulting in vasoconstriction and increased production of endothelin-1 [48]. CRP may also function as a proatherosclerotic factor by up-regulating AT1 receptor expression [4].

A study demonstrated that CRP is able to stimulate IL-6 and TNF- $\alpha$  production in vascular smooth muscle cells via NF $\kappa$ B and MyD88-independent TLR4 signaling pathway [49]. The same group also showed that the stimulation of TLR4 by CRP increases the expression of VEGF-A (vascular endothelial growth factor A) and iNOS (inducible nitric oxide synthase) [50].

**Fibrinogen:** Fibrinogen is a soluble protein that is mainly secreted by hepatic cells and is involved in the coagulation cascade. In conditions such as inflammation, tissue injury and neoplasia, circulating levels of fibrinogen are elevated and stimulate its extracellular deposition. At sites of inflammation, fibrinogen mediates the adhesion of leukocytes to vascular endothelium through intercellular adhesion molecule 1 (ICAM-1), stimulates macrophage chemokines secretion and fibroblast replication, and potentiates endothelial cell proliferation stimulated by fibroblast growth factor-2. Plasma fibrinogen has been considered as a risk factor for cardiovascular disease and is associated with cardiovascular abnormalities, such as left ventricular hypertrophy [51]. Shear-stress-induced deposition of fibrinogen promotes atherosclerosis [52]. Many studies have demonstrated an increase in fibrinogen levels in hypertension, mainly in the presence of target organ damage [3].

*In vitro* studies showed that fibrinogen stimulation induces a hypertrophic response of neonatal cardiomyocytes and activates the TLR4/MyD88-dependent NF $\kappa$ B signaling pathway [53]. Other work showed that human monocytes and murine and human macrophages stimulated with fibrinogen increase the production of pro-inflammatory cytokines via the TLR4 pathway [54].

**High mobility group box-1 (HMGB1):** HMGB1 is a highly conserved, ubiquitous protein in the nucleus and cytoplasm of almost all cells. HMGB-1 can be found in plasma, generally derived from necrotic cells. After binding to its receptors, the receptor for advanced glycation end products (RAGE) or TLR4 [55]. HMGB1 activates macrophages/monocytes and vascular endothelial cells which express proinflammatory cytokines, chemokines and adhesion molecules. Thus, HMGB1 may act as a critical mediator of inflammation and promote tissue repair and regeneration [56]. Many recent studies demonstrated that HMGB1 plays a pivotal role in cardiovascular diseases, such as atherosclerosis, myocardial ischemia/reperfusion injury, heart failure and myocardial infarction [57].

**Oxidized low density lipoprotein (ox-LDL):** Ox-LDL originates mainly from mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase. These molecules contribute to endothelial damage and induce atherosclerosis by stimulating monocyte infiltration and VSMC migration and proliferation. Increased LDL oxidation levels were found in young men with borderline hypertension and decreased arterial elasticity. Another study in humans showed that ox-LDL has a negative correlation with protective prostacyclin in hypertensive patients [58].

An interesting review published by Miller Y. et al [59] describes how ox-LDL is recognized by receptors of innate immunity. The authors discuss that minimally modified LDL and oxidized phospholipids

have been found to bind CD14 or activate TLR4 on macrophages [59]. In turn, various biological processes are induced, including the stimulation of cytoskeletal rearrangement that alters phagocytic activity and the stimulation of cytokine secretion.

**Uric acid:** Uric acid contributes to pathogenesis of hypertension in a rat model through the activation of the renin-angiotensin system, down regulation of nitric oxide, and induction of endothelial dysfunction and vascular smooth muscle proliferation [60]. In 2010, a meta-analysis of published prospective studies showed that hyperuricemia is associated with an increased risk of incident hypertension, independent of other risk factors. This risk appears more pronounced in younger individuals and women [61].

Uric acid has been described as the main immunogenic molecule released by necrotic cells. Shi *et al* [62] isolated major danger signals from necrotic cells by HPLC and identified uric acid as a primary component of dendritic cell activation fraction [62]. Another group demonstrated that TLR4, TLR2 and MyD88 null macrophages had decreased responses to uric acid exposure, in terms of production of IL-1 $\beta$ , TNF- $\alpha$ , CXCL1 and TGF $\beta$ 1, suggesting that TLR2 and TLR4 are receptors for uric acid [63].

**ADMA (asymmetric dimethylarginine):** ADMA is an endogenous nitric oxide synthase inhibitor which has potent pressor/vasoconstrictor effects. Studies demonstrated a close association between plasma ADMA levels and carotid intima-media thickness, one of the markers of atherosclerosis in the general population [64]. In Dahl-salt rats fed high salt diet the urinary ADMA excretion is increased and it is positively correlated with arterial pressure. This increase in ADMA was not found in SHR [65]. Recently, Yang *et al* showed that ADMA and oxidants activate TLR4 in adipocytes, however no work has described the activation of TLR4 by ADMA in other cell types [66].

**Shear stress:** Shear stress may first modify the properties of the plasma membrane and then affect ion channels, receptors, adhesion proteins, and the phosphorylation status of signalling molecules. Mechanical stress itself is also considered an important stimulus for the activation of cells of the innate immune system. As discussed above, the immune system recognizes endogenous and exogenous molecules. However, additionally, the danger model proposes that the immune system may be activated also by degradation products such as heparan sulfate and polysaccharide fragments of hyaluronan, which indicate tissue injury, infection, and tissue remodelling [67]. Recent reports have also shown that hyaluronic acid, a glycosaminoglycan found in the extracellular matrix, can also activate dendritic cells by engaging TLR2. Thus stimulation by host-derived extracellular matrix factors may serve as an intrinsic mechanism for initiating an inflammatory response after tissue injury.

### Adaptive immune response and hypertension

During the immune response, after cells from the innate immune response recognize the antigen by the PRRs, they will migrate and present molecules from this antigen to cells of the adaptive immune response. The adaptive immune response is initiated by the recognition of foreign antigens by specific lymphocytes. Lymphocytes respond by proliferating and by differentiating into effectors cells, whose function is to eliminate the antigen, and into memory cells, which show enhanced responses upon subsequent encounters with the antigen.

The two major cell types of the adaptive immune response are B and T lymphocytes. The participation of T lymphocytes to the pathogenesis

of hypertension was demonstrated by Guzik *et al.* [1] who in 2007 found that mice lacking T-lymphocytes are resistant to the development of both angiotensin II and DOCA-salt induced hypertension. Adoptive transfer of T, but not B cells restored hypertension in these animals [1]. Another group showed that SCID mice, which lack both T and B lymphocytes, have a blunted blood pressure response and reduced sodium retention during angiotensin II-induced hypertension [68].

The same group also reported that IL-17 produced by Th17 lymphocytes plays a critical role in maintenance of Ang II-induced hypertension and vascular dysfunction. IL-17 is increased in T cells from Ang II-infused mice and IL-17 knockout mice infused with Ang II had less blood pressure elevation than control mice [19].

In an abstract presentation by Thabet *et al*, the authors showed that mice lacking CD8 lymphocytes are resistant to Ang-II induced hypertension when compared to wild type C57LB/6J mice [70]. In contrast, Ang-II-induced hypertension is increased in knockout mice to CD4 lymphocytes, compared to CD8 knockout and wild type C57BL/6J mice. This work suggests an important contribution of CD8 lymphocytes and not CD4 lymphocytes to hypertension induced by Ang II.

Another type of T cell which has a role in hypertension is the T regulatory cell (Treg). These cells suppress innate and adaptive responses. Recently, Barhoumi *et al* demonstrated that Treg adoptive transfer prevented Ang II-induced hypertension, vascular damage and immune cell infiltration [69].

Although several studies have described the importance of lymphocytes in hypertension, the question still remains as to how exactly the adaptive immune response is activated in the first place in these models of hypertension. Another point is what are the antigens that activate the innate immune response, and which PRRs are responsible for this response.

### Conclusion

In this review, we described recent evidence supporting the role of the innate immune system in hypertension. We propose that in hypertension, elevated levels of DAMPs activate TLRs such as TLR4 on APCs which then signal directly or through cells of the adaptive immune response to elicit an inflammatory process in organs and systems that regulate blood pressure. One important question that still remains unanswered is whether DAMP activation of TLR occurs before the initial blood pressure increase (cause), as a consequence of high blood pressure damage of target organs (effect), or both (positive feedback loop). The vast majority of studies linking the immune response and hypertension were performed in animal models and definitive proof in humans is still lacking. However, understanding these interactions may allow for manipulation of the immune system as a therapeutic tool in resistant hypertension and/or hypertensive target organ damage.

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