CALL FOR PAPERS | Pathophysiology of Hypertension

Toll-like receptors and damage-associated molecular patterns: novel links between inflammation and hypertension

Cameron G. McCarthy,1 Styliani Goulopoulou,1 Camilla F. Wenceslau,1 Kathryn Spitler,1 Takayuki Matsumoto,2 and R. Clinton Webb1

1Department of Physiology, Georgia Regents University, Augusta, Georgia; and 2Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Tokyo, Japan

Submitted 15 April 2013; accepted in final form 17 October 2013

McCarthy CG, Goulopoulou S, Wenceslau CF, Spitler K, Matsumoto T, Webb RC. Toll-like receptors and damage-associated molecular patterns: novel links between inflammation and hypertension. Am J Physiol Heart Circ Physiol 306: H184–H196, 2014. First published October 25, 2013; doi:10.1152/ajpheart.00328.2013.—Low-grade systemic inflammation is a common manifestation of hypertension; however, the exact mechanisms that initiate this pathophysiological response, thereby contributing to further increases in blood pressure, are not well understood. Aberrant vascular inflammation and reactivity via activation of the innate immune system may be the first step in the pathogenesis of hypertension. One of the functions of the innate immune system is to recognize and respond to danger. Danger signals can arise from not only pathogenic stimuli but also endogenous molecules released following cell injury and/or death [damage-associated molecular patterns (DAMPs)]. In the short-term, activation of the innate immune system is beneficial in the vasculature by providing cytoprotective mechanisms and facilitating tissue repair following injury or infection. However, sustained or excessive immune system activation, such as in autoimmune diseases, may be deleterious and can lead to maladaptive, irreversible changes to vascular structure and function. An initial source of DAMPs that enter the circulation to activate the innate immune system could arise from modest elevations in peripheral vascular resistance. These stimuli could subsequently lead to ischemic- or pressure-induced events aggravating further cell injury and/or death, providing more DAMPs for innate immune system activation. This review will address and critically evaluate the current literature on the role of the innate immune system in hypertension pathogenesis. The role of Toll-like receptor activation on somatic cells of the vasculature in response to the release of DAMPs and the consequences of this activation on inflammation, vasoreactivity, and vascular remodeling will be specifically discussed.

innate immunity; vascular dysfunction; vascular remodeling

This article is part of a collection on Pathophysiology of Hypertension. Other articles appearing in this collection, as well as a full archive of all collections, can be found online at http://ajpheart.physiology.org/.

Introduction

Hypertension is a chronic condition characterized by elevated systemic blood pressure and is the most common and important risk factor preceding the development of other cardiovascular diseases (102, 121). Normal systemic blood pressure is defined as systolic blood pressure <120 mmHg and diastolic blood pressure <80 mmHg. Prehypertension and hypertension are systemic pressures equal to and above these values (25). Hypertension can be classified as either primary/essential, in which the disease is idiopathic, or secondary, in which the cause of the disease is known or “secondary” to another identifiable cause (e.g., renal disease), respectively. Despite the elusive nature of essential hypertension, it is known that its etiology is heterogeneous and multifactorial.

Although there is substantial debate regarding the relative contribution of kidneys, autonomic nervous system, and vasculature to the development and maintenance of hypertension, it has been established that the immune system and its aberrant activation also play a role. Low-grade systemic inflammation in these organs leads to the commencement or acceleration of pathological processes and worsening of cardiovascular risks (1, 46, 136). However, the initiation of this inflammatory response, thereby contributing to further increases in blood pressure, has not been elucidated (34).
Inflammation is one of the first responses of the immune system to danger. Clinically, inflammation can be described as dolor (pain), calor (heat), rubor (redness), tumor (swelling), or function laesa (loss of function) (122). More specifically, inflammation is the increased production of pro-inflammatory cytokines whose expression pattern guides the adaptive immune response (e.g., IL-6, TNF-α, and IFN-γ). Additionally, inflammation can be characterized as increased chemokines (chemotactic cytokines) that guide the migration of immune cells to target tissues [e.g., monocyte chemoattractant protein-1 (MCP-1)] and cell adhesion molecules that promote the binding, rolling, and infiltration of immune cells into the vascular wall and translocation to end organs (e.g., ICAM-1 and VCAM-1) (86).

The danger that stimulates inflammation can occur from not only pathogens [pathogen-associated molecular patterns (PAMPs)] but also host-derived endogenous molecules that arise due to cell death and/or injury [damage-associated molecular patterns (DAMPs)] (95, 96). This initial inflammatory response by the innate immune system subsequently signals for the adaptive immune system to elicit a more robust defense. The participation of the adaptive immune system and different lymphocyte populations in the development of hypertension has been supported for various animal models of hypertension, such as spontaneously hypertensive rats (SHRs), DOCA-salt induced hypertension, and ANG II-induced hypertension (8, 47, 64, 89, 93). However, the role of the innate immune response to DAMPs, and its subsequent effects on the activation of the adaptive immune system in the context of the pathogenesis of hypertension, is currently unknown.

Many DAMPs are present in hypertension due to chronic cell injury and death, contributing to persistent inflammation (16). Recognition of these host-derived molecules by innate immune receptors such as Toll-like receptors (TLRs), and subsequent TLR activation, may be an important link between hypertension and activation of the adaptive immune system. Accordingly, the focus of the following review will be on the emerging role of TLRs in the etiology of vascular dysfunction, vascular remodeling, and hypertension. The current literature on TLRs in cardiovascular disease will be summarized and gaps in our current knowledge regarding the potential role of TLRs in hypertension will be identified.

**Vascular Dysfunction and Vascular Remodeling: Physiological Adaptations With Pathophysiologial Consequences**

Vascular remodeling of both conduit and resistance vessels is a hallmark of hypertension that generally precedes disease development and exacerbates the disease phenotype (37, 109, 129). Although initial remodeling of the vascular wall occurs as an adaptation to normalize exacerbated shear stress, chronic shear stress results in maladaptive changes in vessel structure (43). Pressure-dependent remodeling of large artery composition is characterized by vessel stiffening (decreased compliance and elasticity) and outward hypertrophic remodeling (105, 125, 126), whereas small artery pressure-dependent remodeling is defined as either inward eutrophic remodeling or inward hypertrophic remodeling, depending on whether the media cross-sectional area is enlarged (51, 110, 130).

Compliant conduit vessels, such as the aorta, provide important buffering actions for each ventricular contraction that ameliorates pulse pressure. Augmented pulsatile hemodynamics that occur with stiffening contribute to increased cardiac afterload, decreased diastolic blood flow to tissues, and further detrimental vascular remodeling of resistance vessels and organ damage (20, 104). Remodeling of resistance vessels can lead to decreased tissue perfusion and organ damage (129).

Vascular dysfunction is another deleterious characteristic of hypertension and prehypertension (31, 136). Vascular dysfunction has multiple characteristics including increased contractility and/or decreased relaxation (via modified production and responsiveness to vasoconstrictors and vasodilators), increased expression of pro-inflammatory and/or decreased anti-inflammatory cytokines, cell death (apoptosis, necrosis, aberrant autophagy), adhesion of immune cells to the endothelium (and subsequent infiltration into the vascular wall), increased production of reactive oxygen species (ROS), and vascular smooth muscle cell (VSMC) hypertrophy, proliferation, and migration (136). Many of these manifestations of vascular dysfunction occur in parallel, and elucidation of the initial insult on the vasculature is usually difficult and multifactorial in nature.

Vascular remodeling is the summation of dysfunctional vasomotor events that ultimately reorganize the structural composition of the vessel. In other words, vascular remodeling is thought to be initiated in vessels with chronic dysfunction, either as exacerbated vasoconstriction and/or attenuated vasorelaxation (75). There is evidence that such abnormal vaso-reactivity works in conjunction with renal dysfunction and sympathetic overactivity to drive the vascular remodeling process (31). A role for augmented vasoconstrictors and/or attenuated vasodilators in the genesis and maintenance of vascular remodeling is supported by the fact that many of these vaso-active agents [e.g., ANG II, endothelin-1 (ET-1), vasopressin, prostaglandins, and nitric oxide (NO)] are pleiotropic in their effects. As a result, these vasoactive factors can also contribute to vascular remodeling through other mechanisms including VSMC proliferation, inflammation, apoptosis, and fibrosis (55).

Vascular remodeling and the progression of hypertension subsequently becomes the result of a positive feedback loop of augmented shear stress-induced changes via exacerbated vasoconstriction and attenuated vasorelaxation. Under these conditions, the maintenance of tissue perfusion through chronically constricted vessels results in increased force to the vessel wall. This force subsequently leads to remodeling of the vessels as a mechanism of normalizing this high shear stress. Eventually, a new steady state of tissue blood flow is sustained at elevated pressures by structural remodeling, rather than vaso-reactivity mechanisms. Temporally, altered vascular structure in response to vascular dysfunction has been shown to occur rapidly in resistance arteries. However, these hemodynamic changes only reach pathophysiological significance ~2 to 3 days after initiation of increased pressure, and need at least a week to be fully completed (87, 92).

A novel and potentially significant focus of research recently has been the observation that TLRs are able to modulate vascular function (15, 19, 82), and genomic analysis has revealed that different vascular beds exhibit distinctive TLR profiles (120). Specifically, TLR2 and TLR4 were ubiquitously present within the vasculature, whereas TLR7 and TLR9 were sparse, and TLR1, TLR3, TLR5, TLR6, and TLR8 were...
expressed in selective patterns. However, to the best of our knowledge, there are only two investigations illustrating a role of TLRs mediating vascular dysfunction and low-grade inflammation, subsequently contributing to hypertension (15, 82), as well as one investigation on a potential involvement of TLRs (via MyD88 adaptor protein) in vascular remodeling (142). Therefore, TLRs could be significant contributors to the etiology of vascular dysfunction, and consequently, vascular remodeling and hypertension.

The Innate Immune System: The First Line of Defense Against Danger

The innate immune system is the early warning system of the body that rapidly detects danger and damage. This consequently allows time for the adaptive immune system to mount an antigen-specific response. The pattern of inflammatory cytokine response after activation of the innate immune receptors diverts the adaptive immune system toward either the cell-mediated T helper 1 (Th1) response or the humoral/antibody T helper 2 (Th2) response.

Components of the innate immune system include antimicrobial chemicals on epithelial surfaces (skin and mucosa), phagocytes (macrophages and neutrophils), natural killer cells, and polymorphonuclear leukocytes, which include neutrophils, eosinophils, basophils, and mast cells. Additionally, the complement system is known to be an essential component of the innate immune system for the destruction of pathogens and clearing of cellular debris. Finally, TLRs and NOD-like receptors (nucleotide-binding oligomerization domain receptors) are sentinel pattern recognition receptors of the innate immune system that help initiate the inflammatory response. Although short-term inflammation is necessary for tissue defense, chronic and/or excessive activation of the innate immune system results in deleterious maladaptations (e.g., autoimmune diseases), and the salutary effects of this evolutionarily conserved system are negated (Fig. 1).

Currently, there is limited knowledge of the role of the innate immune system in the pathogenesis of hypertension and how the inflammation associated with this condition is initiated. As a result, various components of the innate immune system, including TLRs, are becoming a significant research focus in the field of hypertension (15, 49).

The Adaptive Immune System In Hypertension

Upon initiation of the innate immune response by pattern recognition receptors, antigen-presenting cells migrate to lymph nodes and present antigenic molecules to cells of the adaptive immune system. The adaptive immune response is then initiated by the recognition of antigens by T and B lymphocytes. Proliferation and differentiation of naive lymphocytes into effector cells is necessary for elimination of the antigen and production of memory cells, which protect the host upon subsequent encounters with the antigen (3).

T and B lymphocytes are the two major cell types of the adaptive immune system. The participation of T lymphocytes in the pathogenesis of experimental hypertension was demonstrated by Guzik et al. (47). This investigation found that mice lacking T lymphocytes are resistant to the development of both ANG II and DOCA-salt-induced hypertension. Adoptive transfer of T lymphocytes, but not B lymphocytes, restored hypertension in these animals (47). In corroboration of these findings, mice with severe combined immunodeficiency and lacking both T and B lymphocytes have a blunted blood pressure response and reduced sodium retention during ANG II-induced hypertension (29). However, it has also been demonstrated that T lymphocyte activation occurs due to a blood pressure elevation, since anti-hypertensive drug hydralazine was able to prevent activation of T lymphocytes in an ANG II model of hypertension (93). Therefore, if aberrant inflammation is involved in the initiation of hypertension, in contrast with its progression, elucidation of this mechanism has not been demonstrated.

Given that T lymphocytes play a critical role in hypertension, the subset of T lymphocytes that contributes to this disease has been a growing area of investigation. T-helper cells have been cited as a possibility since these cells differentiate into distinct subsets generally classified as either the cell-mediated “Th1” response or the humoral/antibody “Th2” response. These T-helper subsets perform different functions and elicit unique patterns of cytokine secretion (107). As such, imbalances between these subsets are implicated in a variety of autoimmune (74) and cardiovascular pathologies (98, 132). T-helper 17 cells are a subset of T-helper cells that produce the pro-inflammatory cytokine IL-17. The profound effects of IL-17 on the etiology of hypertension were demonstrated by Madhur et al. (89). It was revealed that ANG II increased IL-17 production from T lymphocytes and that ANG II-induced elevations in blood pressure were not sustained in IL-17−/− mice. Moreover, IL-17−/− mice showed improved vascular reactivity, decreased superoxide anion production, and less T-lymphocyte infiltration into the vascular wall (89).
Another type of T lymphocyte revealed to play a role in hypertension is T-regulatory cells (Treg). Tregs suppress innate and adaptive immune responses, as opposed to exacerbating them. In fact, adoptive transfer of Tregs prevented ANG II-induced blood pressure elevation, vascular stiffness, vascular inflammation and oxidative stress, endothelial dysfunction, and immune cell infiltration (11). The vasculoprotective effects of Tregs have also been observed in aldosterone-induced vascular dysfunction and hypertension (64).

Although the importance of the adaptive immune system in hypertension is not in question, how exactly the adaptive immune response is first activated is not well understood. TLRs are known to be expressed on immune cells such as T and B lymphocytes (54, 62) and antigen-presenting cells. Furthermore, TLRs are expressed by somatic cells of the vasculature [e.g., VSMCs and endothelial cells (ECs)]. Therefore, TLRs may be molecular links between DAMPs, chronic vascular inflammation, and hypertension (Fig. 2).

**TLRs and DAMPs: A New Source of Danger**

Researchers studying the development of the fruit fly *Drosophila melanogaster* first discovered the Toll receptor when they found that a mutation in the Toll gene resulted in abnormal development (6). The mutated flies were termed Toll (German for "wow") after embryos carrying the mutation were remarkably dissimilar to wild-type flies. A more closely related human homologue to *Drosophila* Toll was subsequently identified (101), and the human Toll was then renamed TLR4 because it was "Toll-like."

At least 13 TLRs have been reported in mammals (1–10 in humans and 11–13 in mice) (91). TLRs that primarily recognize bacterial and fungal components are localized on the cell surface (TLR1, TLR2, TLR4, TLR5, and TLR6), whereas TLRs that primarily recognize viral or microbial nucleic acids are localized to intracellular membranes such as endosomes or phagosomes (TLR3, TLR7, TLR8, and TLR9) (52). Recently, human TLR10 has been discovered (26); however, its function and specific ligand have yet to be determined. Moreover, the TLR11 gene is known to be encoded in humans; however, it contains at least one stop codon, and the protein is not expressed (156).

TLRs are responsible for recognizing and initiating an inflammatory response to dangerous molecules (95, 96). As such, potential PAMPs and DAMPs are varied and numerous and can include pathogen-derived cell wall components (e.g., LPS), DNA, and metabolic byproducts. Endogenous (host-derived) molecules that arise from injured and dying cells and activate TLRs include extracellular matrix components (e.g., hyaluronan), plasma membrane, nuclear, and cytosolic proteins (e.g., high-mobility group box protein 1), and elements of damaged/fragmented organelles [e.g., mitochondrial DNA (mtDNA)].

Table 1 provides several examples of DAMPs specific to hypertension and their corresponding TLRs [updated from reference (16)]. This list is by no means exhaustive, since numerous other yet unknown molecules may fulfill our inclusion criteria of being TLR ligands that are elevated in hypertension.

Another possibility for aberrant TLR activation, inflammation, and the development of cardiovascular pathologies (5) could be genetic abnormalities of innate immune system components (39, 112) and endogenous molecules (thus converting them to DAMPs). These anomalies may include an irregular expression of various TLR, polymorphisms and mutations, the aberrant gene expression of various cytokines, and distribution of immune cell populations. Also, TLR-induced epigenetic and chromatin modifications may be important (38). As such, these genetic components of TLRs and DAMPs may also contribute to the pro-inflammatory state seen in hypertension.

![Fig. 2. The collective contribution of the innate and adaptive immune systems to vascular dysfunction, vascular remodeling, and hypertension.](image_url)

**Table 1. Damage associated molecular patterns that are able to activate toll-like receptors on cell types pertinent to vascular function, potentially contributing to hypertension [updated from Ref. 16].**

<table>
<thead>
<tr>
<th>Damage Associated Molecular Patterns</th>
<th>Toll-like Receptors</th>
<th>Cell Type (Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetric dimethylarginine</td>
<td>4</td>
<td>Adipocytes (154)</td>
</tr>
<tr>
<td>ANG II</td>
<td>4</td>
<td>VSMCs (58, 59)</td>
</tr>
<tr>
<td>Biglycan</td>
<td>2</td>
<td>Aortic valve interstitial cells (134)</td>
</tr>
<tr>
<td>CpG DNA/mitochondrial DNA</td>
<td>9</td>
<td>Plasma (149), VSMCs (44)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>4</td>
<td>VSMCs (83), (84)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4</td>
<td>Cardiomyocytes (81), monocytes (73)</td>
</tr>
<tr>
<td>HDL (modified)</td>
<td>2</td>
<td>ECs (135)</td>
</tr>
<tr>
<td>High mobility group box-1</td>
<td>2, 4</td>
<td>ECs (70), macrophages (117)</td>
</tr>
<tr>
<td>Heat shock protein 60</td>
<td>2, 4</td>
<td>Cardiomyocytes (69), VSMCs (33)</td>
</tr>
<tr>
<td>Heat shock protein 70</td>
<td>2, 4</td>
<td>Cardiomyocytes (94), monocytes (7)</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>2, 4</td>
<td>ECs (143), macrophages (128), (61)</td>
</tr>
<tr>
<td>IL-1α</td>
<td>4</td>
<td>VSMCs (131)</td>
</tr>
<tr>
<td>Oxidized LDL</td>
<td>4</td>
<td>ECs (138), macrophages (103)</td>
</tr>
<tr>
<td>Uric acid</td>
<td>2, 4</td>
<td>Macrophages (85)</td>
</tr>
</tbody>
</table>

EC, endothelial cells; VSMC, vascular smooth muscle cells.
TLR Signaling: Interactions With Known Vascular Signaling

In addition to being expressed in immune cells, TLRs are expressed in other tissues, such as those of the cardiovascular system (40, 91). Expression of TLRs in the cardiovascular system, as well as immune cells, is consistent with the theory that tissues tailor their own immune responses (97). TLRs are type I transmembrane proteins that possess an amino-terminal leucine-rich repeat domain for ligand binding, a single transmembrane domain, and a carboxyl-terminal intracellular signaling domain that is similar to IL-1. This common intracellular signaling motif is termed the “Toll-IL-1 receptor” (TIR) homology domain.

A number of different signaling pathways are activated by TLRs, and some of these are unique to particular TLRs. This differential signaling is dependent on which adaptor molecules are present to associate with the respective TLR. These adaptors, all of which contain TIR domains, include myeloid differentiation primary response protein (MyD88), TIR domain-containing adaptor protein (TIRAP), TIR-domain-containing adaptor inducing interferon-β (TRIF), and TRIF-related adaptor molecule (TRAM) (Fig. 3). TLR signaling has been extensively reviewed previously [(40) and (91)] and will not be extensively discussed here. These signaling events result in the upregulation of pro-inflammatory mediators (cytokines, chemokines, and adhesion molecules) either through a MyD88-dependent pathway (TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9) or a MyD88-independent/TRIF-dependent pathway (TLR3 and TLR4). The MyD88-dependent pathway involves induction of the NF-κB gene, stimulation of the transcription factor activator protein 1 (AP-1) by MAPKs, or activation of interferon regulatory factor (IRF). The MyD88-independent pathway involves IRF3 activation, as well as NF-κB signaling (4, 65). The unique signaling cascades for each TLR allow for the induction of specific responses. This specificity may be attributed to the type of cell where the TLR is expressed (32) and/or the defense needed for that particular tissue (97). The ability of TLRs to discriminate particular stimuli expanded the defensive repertoire of the innate immune system.

As stated above, NF-κB activation is downstream of MyD88-dependent signaling. The NF-κB family is composed of homo- and heterodimers of Rel proteins [NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, and c-Rel (Rel)]. The NF-κB (p50/p65) transcription factor is a ubiquitous, constitutive, and inducible heterodimer with domains for dimerization and DNA binding, a nuclear translocation signal, and a binding site for the inhibitor of NF-κB (IκB) (17, 23). Along with regulating pro-inflammatory cytokines, NF-κB activation mediates the expression of inducible NO synthase (iNOS), cyclooxygenase 2, growth factors, inhibitors of apoptosis, and effector enzymes in response to ligation of TLRs and other receptors involved in immunity, including T lymphocyte receptors and B lymphocyte receptors (17).

Synthesis of NF-κB is not de novo; therefore, its transcriptional activity is silenced by interactions with inhibitory IκB proteins present in the cytoplasm. Downstream of specific TLR signaling, the inhibitor IκB complex phosphorylates IκB, which leads to its ubiquitylation and subsequent degradation by the proteasome. As a result, the inhibitory influence on NF-κB is alleviated and NF-κB is then able to translocate from the cytoplasm to the nucleus. MAPK regulation of pro-inflammatory mediators is also MyD88 dependent. The MAPK module contains at least 3 protein kinases in series that culminate in the activation of a multifunctional MAPK (ERK1/2, JNK/SAPK, and p38). These MAPKs subsequently result in the activation of the transcription factor activator protein (AP-1), which then translocates to the nucleus. Interferon regulatory factor (IRF) is also MyD88 dependent, but is only found downstream of TLR9. Phosphorylation and dimerization of IRF7 activate its translocation to the nucleus. The MyD88-independent/TRIF-dependent pathway downstream of TLR3 and TLR4 involves IRF3, as well as NF-κB activation. Like IRF7, IRF3 undergoes phosphorylation and dimerization for activation and translocation to the nucleus, dsRNA, double-stranded RNA; HSP, heat shock protein; mHDL, (pathophysiologically) modified HDL; ssRNA, single stranded RNA.

Fig. 3. TLR ligands/DAMPs, cellular location, and signaling in the vasculature. Evolutionarily conserved similarities between TLRs on immune cells have been extended to somatic cells of the vasculature (i.e., VSMCs and ECs). TLR1, TLR2, TLR4, TLR5, and TLR6 are expressed on the plasma membrane, and TLR3, TLR7, TLR8, and TLR9 are expressed on endosomal vacuoles. Activation of these receptors by DAMPs and PAMPs leads to complex cellular signaling cascades mediated by myeloid differentiation primary response protein (MyD88), Toll-IL-1 receptor (TIR)-domain-containing adaptor inducing interferon β (TRIF), TIR domain-containing adaptor protein (TIRAP), and TRIF-related adaptor molecule (TRAM). These adaptor molecules signal via MyD88-dependent or TRIF-dependent pathways that result in the upregulation of pro-inflammatory mediators (cytokines, chemokines, and adhesion molecules). The MyD88-dependent pathway includes NF-κB translocation to the nucleus to regulate inflammatory gene expression. TLR signaling activates the endogenous NF-κB inhibitor IκB, which phosphorylates IκB and leads to its ubiquitylation and degradation by the proteasome. IκB degradation relieves the inhibitory influence on NF-κB, and NF-κB is then able to translocate from the cytoplasm into the nucleus. MAPK regulation of pro-inflammatory mediators is also MyD88 dependent. The MAPK module contains at least 3 protein kinases in series that culminate in the activation of a multifunctional MAPK (ERK1/2, JNK/SAPK, and p38). These MAPKs subsequently result in the activation of the transcription factor activator protein (AP-1), which then translocates to the nucleus. Interferon regulatory factor (IRF) is also MyD88 dependent, but is only found downstream of TLR9. Phosphorylation and dimerization of IRF7 activate its translocation to the nucleus. The MyD88-independent/TRIF-dependent pathway downstream of TLR3 and TLR4 involves IRF3, as well as NF-κB activation. Like IRF7, IRF3 undergoes phosphorylation and dimerization for activation and translocation to the nucleus, dsRNA, double-stranded RNA; HSP, heat shock protein; mHDL, (pathophysiologically) modified HDL; ssRNA, single stranded RNA.
cytoplasm into the nucleus to regulate gene expression (17, 23) (Fig. 3).

In VSMCs and ECs, ANG II activates NF-kB (99, 140, 148), and sustained inhibition of NF-kB with pyrrolidine dithiocarbamate prevents inflammation and hypertension (108, 123). Because NF-kB has been established in the development of inflammation and hypertension, investigation of the pro-inflammatory process upstream of NF-kB activation is warranted; thus TLRs represent an ideal candidate.

Another mechanism of MyD88-dependent signaling is MAPK activation of AP-1 transcription factor. Mitogen-activated protein kinases are serine/threonine-specific protein kinases involved in directing cellular responses to a diverse array of stimuli. Mitogen-activated protein kinase expression and signaling is initiated through a MAPK module, which functions to prolong transient cell signals into sustained ones that can be relayed downstream to the nucleus to alter the pattern of gene expression. This module contains at least three protein kinases in series that culminate in the activation of a multifunctional MAPK (Fig. 3). Once activated, the MAPK relays the signal downstream by phosphorylating various proteins within the cell, including gene regulatory proteins and other protein kinases. Mitogen-activated protein kinases are major components of pathways controlling embryogenesis, cell differentiation, cell proliferation, and cell death (119). After TLR activation, MAPK signaling leads to translocation of AP-1 transcription factor to the nucleus and upregulation of the expression of pro-inflammatory mediators (66) (Fig. 3).

Prominent MAPKs include ERK1 and ERK2, JNK/SAPK, and p38 MAPK pathways. The ERK1/2 phosphorylation cascade involves MEK1/2 (MAP/ERK kinase), whereas the signaling processes leading to JNK/SAPK and p38 MAPK activation involve MEK4/7 and MEK3/6, respectively (119). Further upstream signaling of MAPKs, within the MAPK module, is recognized to be primarily dependent on the nonreceptor tyrosine kinase c-Src (56, 88, 147). However, c-Src-independent regulators of MEK, such as the Ras-Raf pathway, have also been reported (72, 155).

In VSMCs and ECs, MAPKs are involved in a multitude of physiological and pathophysiological actions (e.g., contraction, migration, adhesion, collagen deposition, growth, differentiation, and survival) (68, 119, 136). Inhibition of ERK1/2 abolishes sustained contraction and normalizes the Ang II effects on mesenteric VSMCs from SHR (145), and rapid tyrosine phosphorylation of ERKs occurs in human VSMCs treated with Ang II (146). The hypertension-associated effects of ERK are not limited to Ang II-dependent hypertension. Basal aortic tone of DOCA-salt hypertensive rats was significantly attenuated by ERK inhibition (67), and DOCA treatment resulted in augmented aortic contractile responses mediated by increased phosphorylation of ERK1/2 and decreased expression of mitogen-activated protein kinase phosphatase 1 (42).

In addition to ERK, JNK/SAPK and p38 MAPKs also contribute to vascular dysfunction and the hypertensive phenotype. The role of p38 is unequivocal, with its inhibition improving indexes of inflammation, oxidative stress, endothelial function, vascular reactivity, cardiac hypertrophy, renal function, and blood pressure, as well as morbidity/mortality (10, 13, 80, 111, 115). In addition, inhibition of JNK/SAPK signaling attenuates contractile responses in isolated aortic segments (79, 113, 158), suggesting a role of JNK/SAPK in vasoconstriction mechanisms. These findings collectively indicate a role of MAPKs in the etiology of vascular dysfunction and hypertension. Therefore, novel upstream mediators of MAPK signaling, such as TLRs, should be investigated.

The final MyD88-dependent signaling transcription factor is IRF7. IRF7 activation is specific to TLR9 (65), and it requires phosphorylation and dimerization to trigger its translocation to the nucleus and induce type I IFNs and IFN-inducible genes (Fig. 3). Currently, knowledge of the contribution of IRF7 on vascular function and hypertension is not known. However, in one investigation that used human ECs infected with bacteria Chlamydia pneumonia, IRF7-dependent signaling was activated, as well as IRF3-dependent and mitochondrial antiviral signaling. This signaling induced type I IFN expression that successfully inhibited bacterial growth. We subsequently hypothesized that type I IFNs, produced via IRF signaling, may contribute to the control of infectious-vascular lesions in atherosclerosis (18).

MyD88-independent signaling is associated only with TLR3 and TLR4 and results in the induction of type I IFNs and IFN-inducible genes (4, 65). Because this signaling involves the recruitment of the adaptor proteins TRIF and TRAM instead of MyD88, it is also known as TRIF-dependent signaling. MyD88-independent signaling results in the phosphorylation and dimerization of IRF3, as well as activation of NF-kB signaling (Fig. 3).

Research into the contribution of MyD88-independent signaling to vascular dysfunction and hypertension is limited. However, it has been demonstrated in VSMCs that C-reactive protein stimulates IL-6 production and inhibits peroxisome proliferator-activated receptor-γ expression via TLR4-MyD88-independent signaling (84). Additionally, using an in vivo model of hindlimb ischemia-reperfusion, TLR4 on myocytes mediated pro-inflammatory responses through MyD88, whereas regenerative processes occurred through TLR4 and TRIF (124). Although, these investigations observed a roll of TLR-MyD88-independent signaling in vascular dysfunction, more are still required to establish its roll in hypertension pathogenesis.

**TLRs in Cardiovascular Disease Etiology**

Unlike other TLRs, which are functionally active as homomers, TLR2 has developed the unique ability to signal via heterodimers with TLR1 or TLR6. In fact, signaling does not appear to occur downstream of TLR2 homodimers (116). TLR2 is required for functional recognition of gram-positive, gram-negative, fungi, viral, and mycoplasma lipoproteins, and peptidoglycan, and heterodimerization of TLR2 with TLR1 and TLR6 allows it to attain specificity for a diverse range of ligands (141). In addition to microbial factors, TLR2 can bind pathophysiologically modified HDL (135), as well as endogenous heat shock proteins (e.g., heat shock proteins 60 and 70) (7, 33).

Illustrating its potential for cardiovascular disease pathogenesis, TLR2 activation induces a dedifferentiated migratory and proliferative phenotype in VSMCs (33, 78). In ECs, “abnormal” HDL from patients with chronic kidney disease acted upon TLR2 (via a TLR1- or TLR6-coreceptor-independent pathway) and reduced NO bioavailability, leading to impaired endothelial repair and enhancing endothelial pro-inflammatory activation (135). These data in cells provide evidence as to why
TLR2 activation has been associated with the development of atherosclerosis (30). Nonetheless, the contribution of TLR2 to vascular dysfunction in hypertension remains to be clarified.

TLR3 recognizes double-stranded RNA, and TLR7 and TLR8 recognize single-stranded RNA released from viruses (or retroviruses) or necrotic cells. TLR3 has been shown to have a protective effect on the vascular wall after mechanical and hypercholesterolemia-induced arterial injury (27). This protective effect may stem from the ability of TLR3 to induce the expression of cytoprotective and anti-inflammatory glycoprotein clusterin/apolipoprotein J to counteract the progression of atherosclerosis (9). On the other hand, maternal hypertension (from here on referred to as preeclampsia) was developed in rodents treated with agonists of TLR3, -7, and -7/8 (combined) (21, 22, 144). In pulmonary artery VSMCs, TLR3 induction was found to release IL-8, IFN-γ-induced protein 10 (also known as CXCL10), and ET-1 (41). Yang et al. (153) identified that TLR3 activation induced the expression of chemokine MCP-1 and pro-inflammatory cytokine IL-6 in human coronary VSMCs. Finally, activation of TLR7/8 with its agonist (Clo97) exacerbated the pro-inflammatory effects exerted by ANG II and nicotine in SHR splenocytes (49). These effects were replicated in vivo and may have important implications for ANG II and nicotinic modulation of the autonomic nervous system and sympathetic drive in hypertension (1, 93). Accordingly, these data illustrate that TLR3, -7, and -8, and their endogenous ligand (RNA), could contribute to the development of hypertension and other hypertensive disorders (e.g., preeclampsia).

TLR4 has been well characterized in various cardiovascular diseases (40), and it has been observed to induce a pro-inflammatory and proliferative phenotype to endogenous molecules in VSMCs (33, 131, 152). Endogenous agonists of TLR4 include vasoactive molecules heat shock protein 60 and ANG II (36).

TLR4 was the first TLR to be implicated in the etiology of vascular dysfunction and hypertension (15). Specifically, Bomfim et al. (15) showed that TLR4 is elevated in SHRs and that treatment with anti-TLR4 antibody attenuated aortic contractility, serum IL-6 levels, and mean arterial pressure. This ability occurs due to the evolutionarily conserved similarities between mitochondria and saprophytic bacteria, which at one point during evolution entered the eukaryotic cell and became an intracellular organelle (127). It has been demonstrated that pressure-overload released mtDNA that escapes autophagic degradation, leads to TLR9-mediated inflammatory responses in cardiomyocytes, inducing myocarditis and dilated cardiomyopathy (114). Additionally, activation of TLR9 with its synthetic agonist (ODN 2395) exacerbated the pro-inflammatory effects exerted by ANG II and nicotine in SHR splenocytes (49). Nonetheless, the role of TLR9 on vascular function, vascular remodeling, and hypertension remains to be elucidated, and this hypothesis will be proposed later in this review.

Interestingly, it has been shown the TLR adapter molecule MyD88 is necessary for vascular remodeling of carotid arteries. Specifically, inward remodeling was associated with MyD88-dependent and superoxide anion initiated cytokine and chemokine generation, as well as macrophage infiltration and activation in the vascular wall. (142). However, an association between MyD88 and a specific TLR was not deduced, since MyD88 is downstream of various TLRs (Fig. 3).

An endogenous peptide that is dysregulated during the development and establishment of hypertension is ANG II. Along with its profound effects on vasoconstriction, aldosterone and antidiuretic hormone (ADH) secretion, sympathetic activation, and fluid and ion reabsorption, ANG II is also able to influence various TLRs and their signaling. For example, ANG II upregulated TLR4 protein and mRNA expression, as well as TLR4-induced myeloperoxidase secretion in murine macrophage (RAW264.7) cells (60). Activation of TLR7/8 and TLR9 exacerbated the IL-6 secretion to ANG II in SHR splenocytes, but not WKY, and this pro-inflammatory cytokine production in SHRs following ANG II and TLR7/8 agonist administration was further replicated in vivo (49).

In human aortic VSMCs, induction of TLR4 expression by LPS increased iNOS expression and NO production. Furthermore, LPS upregulated the expression of IL-8, vascular endothelial growth factor (VEGF), ICAM-1, and VCAM-1 (53).

Despite the fact that TLR4 polymorphisms have not been shown to be a significant predictor of coronary heart disease in healthy men (106), the abovementioned investigations illustrate a significant contribution of TLR4 to the development of cardiovascular pathologies, including hypertension. As a result, the contribution of other TLRs in hypertension and cardiovascular disease development should be investigated, as well as progressing TLR4-targeted therapy.

TLR5 functions similarly to TLR2 in that it recognizes flagella (2, 133). Specifically, TLR5 recognizes flagellin, a principal component of flagella from both gram-positive and gram-negative bacteria (50). Although an endogenous molecule or DAMP specific for TLR5 has not been reported, TLR5 deficiency leads to altered gut microbiota and metabolic syndrome (150), which can encompass hypertension.

Unmethylated CpG dinucleotides activate TLR9. Although these unmethylated CpG motifs are most common of microbial DNA, unmethylated CpG motifs can also be found in mtDNA, which can be released during cell injury and/or death. Circulating mtDNA released due to trauma is then able to act on TLR9 and induce an inflammatory response (157). This ability occurs due to the evolutionarily conserved similarities between mitochondria and saprophytic bacteria, which at one point during evolution entered the eukaryotic cell and became an intracellular organelle (127). It has been demonstrated that pressure-overload released mtDNA that escapes autophagic degradation, leads to TLR9-mediated inflammatory responses in cardiomyocytes, inducing myocarditis and dilated cardiomyopathy (114). Additionally, activation of TLR9 with its synthetic agonist (ODN 2395) exacerbated the pro-inflammatory effects exerted by ANG II and nicotine in SHR splenocytes (49). Nonetheless, the role of TLR9 on vascular function, vascular remodeling, and hypertension remains to be elucidated, and this hypothesis will be proposed later in this review.
TLR9 and Mitochondrial DNA: Novel Contributors to Hypertension?

As mentioned earlier, TLR9 has affinity for unmethylated CpG dinucleotides common of bacteria and viruses but not for methylated CpG dinucleotides common of vertebrate DNA. This ability of the immune system to discriminate the methylation pattern of DNA is important for preventing TLR9-dependent autoimmunity (12, 137). However, unmethylated CpG dinucleotides are also found in mtDNA, as mitochondria evolved from saprophytic bacteria to become intracellular organelles (127). Recently, the inflammatogenic properties of mtDNA, as a result of TLR9 activation, have been demonstrated (114, 157). Although the existence of TLR-independent pathways activated by nucleic acids have been previously described (151), emerging data suggest that activation of TLR9 by mtDNA could be a novel mechanism of vascular dysfunction, vascular remodeling, and hypertension. In support of this hypothesis, previous investigations have shown that cell-free CpG DNA is increased in patients with essential hypertension (149) and preeclampsia (28).

TLR9 is expressed in various immune cells such as B lymphocytes, monocytes, macrophages, and plasmacytoid dendritic cells (52), as well as vascular tissues (44). In immune cells, immature TLR9 is localized to the endoplasmic reticulum, and upon CpG internalization via class III phosphatidylinositol 3-kinase-dependent mechanism (71), translocates to endosomal vacuoles (76). Activation of TLR9 by CpG motifs involves an intracytoplasmic signaling cascade that proceeds through MyD88, IL-1-receptor-activated kinase (IRAK), and TNF receptor -associated factor 6 (TRAF6) cascade. This signaling leads to the activation of both MAPKs and IKK complexes and culminates in the upregulation of pro-inflammatory transcription factors, including NF-κB, AP-1 via MAPKs, and IRF7, stimulating the production of a TGF response (71). Given that NF-κB and MAPKs are also involved in the pathogenesis of vascular dysfunction and hypertension, it is conceivable that TLR9 may play a role in augmented expression of pro-inflammatory cytokines, chemokines, and adhesion molecules seen in association with vascular remodeling. We have recently found that TLR9 is expressed in both conduit (aortic) and resistance (mesenteric) VSMCs, and activation of TLR9 with its synthetic ligand, ODN 2395, results in augmented contractile responses in isolated conduit (100) and resistance (45) arteries.

TLR9-mediated generation of pro-inflammatory cytokines, chemokines, and adhesion molecules have been extensively investigated in immune cells (157), and recently our laboratory (15, 44), and others (19, 82), have extended some of these findings into the vasculature. Plausibly, vascular remodeling and hypertension may be promoted via mtDNA activation of TLR9 in VSMCs, ECs, and immune cells (e.g., monocytes and macrophages) in concert. For example, TLR9 activation and the presence of mitochondrial DAMPs increase endothelial permeability (57, 139). We propose it would only take a minor insult to cause cellular injury and initiate the deleterious cascade of mtDNA release and TLR9 activation (e.g., prehypertensive stimuli such as Ang II, high salt, or chronic stress). This release of mtDNA and other DAMPs could lead to further pressure- and ischemia-induced cell injury and death as vascular remodeling ensues and blood pressure rises.

In summary, although TLR9 promotor polymorphisms have not been shown to play a role in atherogenesis (48), the inflammatogenic properties of circulating mtDNA could be the missing link between TLR9 activation, vascular dysfunction, vascular remodeling, hypertension, and further cardiovascular pathologies (Fig. 4).

Clinical Implications

It is well known that resistance arteries are the major contributors to total peripheral resistance and thus blood pressure regulation, due to large composite cross-sectional area and sympathetic innervation. As such, deleterious alterations to their structure can promote the development and progression of hypertension (129). Moreover, stiffening of conduit vessels influences blood pressure regulation through propagation of blood flow to the downstream arterial tree (e.g., turbulent or laminar blood flow and subsequent vascular adaptations to
shear stress). In fact, aortic stiffening is a precursor to the development of hypertension (63), is associated with inflammation in patients with untreated essential hypertension (90), and is a strong independent predictor of cardiovascular morbidity in hypertension (77). Therefore, investigations of the molecular mechanisms that contribute to global vascular remodeling throughout the arterial tree should be conducted.

Although the etiology of essential hypertension is heterogeneous and has not been fully elucidated, immune system activation and chronic inflammation have been recently acknowledged as a significant contributor to the hypertensive process (1, 34, 46, 55, 118, 136). The interaction between DAMPs and TLRs on vascular tissues may be the starting point for the development of vascular dysfunction and remodeling, the adaptive immune system response, and the genesis and establishment of hypertension (Fig. 2).

Perspectives

The discovery of TLRs has guided the field of immunology to an era of accelerated advancement and has created exciting therapeutic and experimental possibilities for targeting infections and noninfectious inflammatory diseases (14). The involvement of TLRs in the pathogenesis of hypertension, as described in the current review, extends these therapeutic possibilities above and beyond the current challenges in immunology. TLRs may be the missing link between host-derived “dangerous” molecules (DAMPs such as mtDNA), low-grade inflammation, vascular remodeling, and hypertension. Moreover, activation of TLRs may be a necessary precursor of adaptive immune system involvement in the development of hypertension. The etiology of essential hypertension is multifactorial, and a plethora of endogenous molecules have been previously linked to the development of this disease (Table 1), further increasing the complexity its pathophysiology. Paradoxically, TLR signaling presents a low complexity system (i.e., 10 TLRs in humans, 4 adaptor molecules, and 2 downstream inflammatory transcription factors are required for most invading and host-derived molecule recognition) (14). The low complexity of TLR signaling and the existence of specific inhibitors and antagonists for components of the TLR signaling may lead to exciting and novel therapeutic interventions for hypertension and other hypertensive disorders.

GRANTS

This study was supported in part by the American Heart Association (No. 13PRE14080019), the National Institutes of Health (R01 HL-071138 and R01 DK-083685), the Society for Women’s Health Research, the Preeclampsia Foundation (Vision Grant), the CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil, and the Naito Foundation, Japan.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


Review

H194

TOLL-LIKE RECEPTORS AND HYPERTENSION


Meredith PA, Ostergren J. From hypertension to heart failure—are there better primary prevention strategies? J Renin Angiotensin Aldosterone Syst 7: 64–73, 2006.


