HHS Public Access

Author manuscript

J Mol Cell Cardiol. Author manuscript; available in PMC 2022 December 01.

Published in final edited form as:

J Mol Cell Cardiol. 2021 December ; 161: 75–85. doi:10.1016/j.yjmcc.2021.08.001.

MyD88: At the Heart of Inflammatory Signaling and Cardiovascular Disease

Abraham L. Bayer[a], **Pilar Alcaide**[a]

[a]Department of Immunology, Tufts University School of Medicine. 136 Harrison Ave, Boston MA, 02111.

Abstract

Cardiovascular disease is a leading cause of death worldwide and is associated with systemic inflammation. In depth study of the cell-specific signaling mechanisms mediating the inflammatory response is vital to improving anti-inflammatory therapies that reduce mortality and morbidity. Cellular damage in the cardiovascular system results in the release of damage associated molecular patterns (DAMPs), also known as “alarmins,” which activate myeloid cells through the adaptor protein myeloid differentiation primary response 88 (MyD88). MyD88 is broadly expressed in most cell types of the immune and cardiovascular systems, and its role often differs in a cardiovascular disease context and cell specific manner. Herein we review what is known about MyD88 in the setting of a variety of cardiovascular diseases, discussing cell specific functions and the relative contributions of MyD88-dependent vs. independent alarmin triggered inflammatory signaling. The widespread involvement of these pathways in cardiovascular disease, and their largely unexplored complexity, sets the stage for future in depth mechanistic studies that may place MyD88 in both immune and non-immune cell types as an attractive target for therapeutic intervention in cardiovascular disease.

Keywords

Cardiovascular disease; Inflammation; MyD88; Alarmin Signaling

1. Introduction:

Cardiovascular disease (CVD) is the leading cause of death worldwide, resulting in 18.6 million deaths in 2019.[1] Steadily increasing in prevalence, the top causes of cardiovascular death include myocardial infarction (MI), stroke, cardiomyopathies, and myocarditis.[2] These widely different diseases are connected through common risk factors, along with the development of chronic systemic inflammation.[3–5] Many forms of CVD lead to congestive heart failure, and early studies of these patients showed an association with

Address for Correspondence: Pilar Alcaide: Pilar.alcaide@tufts.edu 136 Harrison Ave, M&V 701, Boston MA, 02111. Tel. 6176362192. **Other Contact Information:** Abraham Bayer: Abraham.bayer@tufts.edu.

increased serum levels of the acute phase reactant C-reactive protein.[6] Since then, many different inflammatory mediators and immune cell types have been implicated in the pathogenesis and progression of CVD.[7,8] However, most anti-inflammatory therapies have failed clinical trials, with only recently promising results using inhibitors of the cytokine IL-1β.[9–12] As the “cytokine hypothesis” of CVD has yet to translate therapeutically, deeper mechanistic studies of the immune cell types, cytokines, and signaling molecules involved in disease pathogenesis will help to improve or develop new anti-inflammatory therapies.

Innate immune cells are the first to recognize cardiovascular damage, often in the form of damage associated molecular patterns (DAMPs) or alarmins, released by stressed or dying cells. Alarmins are sensed by toll-like receptors (TLRs) on innate cells such as neutrophils, monocytes, and macrophages, which initiate a rapid immune response through the production of pro-inflammatory cytokines. Alarmins also activate dendritic cells which participate in the adaptive immune response by presenting antigen to T-cells.[13] Most TLRs and several cytokine receptors signal through myeloid differentiation primary response factor 88 (MyD88), an intracellular adapter protein that coordinates pro-inflammatory signaling cascades.[14,15] Interestingly, while this pathway was widely characterized in innate cells, MyD88 and TLRs are also expressed in adaptive immune cells.[16] Further, MyD88 expression at the transcript and protein level was discovered not only in lymphoid tissues, but in multiple organs including the heart.[17,18] Profiling of TLR components uncovered mRNA expression of every TLR and all downstream signaling molecules in human cardiac tissue.[19] However, the exact function of MyD88/TLR signaling outside of innate immunity is not well understood.

MyD88 is required for full function of the innate immune response, as a result, patients with a loss of function mutation in *Myd88* have recurrent, pyogenic infections.[20] Interestingly, MyD88 knockout B and T cells have shown impaired antibody and cytokine production, and similarly, TLR agonists that signal through MyD88 can enhance T-cell activation and cytokine production.[16,21–23] As such, MyD88 deficient mice are protected in multiple autoimmune disease models, including cardiac myocarditis.[24–26] However, TLR/MyD88 signaling extends beyond immune cells, and most of the work in preclinical models have used globally deficient mice. Upregulated TLR/MyD88 signaling has been shown to alter endothelial cell function and contribute to the pathogenesis of vascular disease, expanding the importance of this pathway into other cardiovascular cell types.[27] While the role of MyD88 in the response to pathogens has been well characterized, new functions in a cell and tissue specific context are only beginning to be uncovered.

In this review, we detail the current understanding of the role of MyD88 in the development of multiple types of CVD. We focus on the tissue and cell-specific roles for MyD88 signaling, as well the contributions of MyD88-dependent vs. MyD88-independent alarmin signaling. Considering the widespread involvement of this pathway on cardiovascular function and the importance of inflammation in CVD, studying MyD88 signaling is relevant to understand the pathogenesis of CVD and to potentially develop therapeutic strategies that modulate these pathways.

2. MyD88 and TLR Signaling: The Basics

Toll-Like Receptors (TLRs) recognize signals of infection or cellular damage: pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). Ten TLRs have been discovered in humans: TLR1-TLR10, and twelve in mice: TLR1-TLR9, TLR11-TLR13 (Figure 1). Every TLR associates with and signals through MyD88 except for TLR3. Additionally, the IL-1 receptor family (IL-1R, IL-18R, and IL-33R) signals through MyD88. TLRs can be further divided into cell surface TLRs: TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 and intracellular vesicle TLRs: TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13.[14,15] Many TLRs recognize alarmins involved in CVD, such as high mobility group box 1 (HMGB1), S100 proteins, and heat shock proteins. TLR4 serves as the receptor for many alarmins, and is unique in that it can signal through MyD88-dependent and independent pathways both from the membrane and endocytosed vesicles.[13,28–30]

The Toll-IL1R (TIR) domain of MyD88 allows it to associate with TLRs and the IL-1R family (Figure 1). Upon ligand binding, MyD88 dimerizes through it's death domain (DD) and recruits IL-1R associated kinases (IRAKs), which also contain a DD. [14,15] Recruitment of IRAK4 followed by IRAK1/2 results in the assembly of a large oligomultimeric complex termed the "Myddosome," consisting of 6 MyD88s, 4 IRAK4s, and 4 IRAK1/2s. IRAK1/2 can subsequently recruit TNF receptor associated factor 6 (TRAF6), an E3 ubiquitin ligase which builds polyubiquitin chains on multiple targets to activate downstream signaling cascades.[14,15,29] MyD88-dependent activity leads to the activation of many signaling pathways, including the MAP kinase cascade, NF-κB, and interferon regulatory factor (IRF) 5/7, resulting in the transcription of pro-inflammatory cytokines and type I interferons. These pathways have been mainly characterized in macrophages to induce inflammation for pathogen clearance, but can as well propagate inflammation that contributes to CVD. [14,29]

A parallel MyD88-independent pathway exists, used by TLR3 and TLR4, that involves recruitment of an alternate adaptor protein, TIR-domain containing adaptor inducing interferon-β (TRIF), which is recruited to the TIR domain of TLR3/TLR4. This sequentially activates TRAF3, IRF3, and type I interferon production. TRIF can also activate TRAF6 through a non-canonical pathway, leading to NF-κB induction and cytokine release that contributes to cardiovascular inflammation.[15,29]. These cascades, while somewhat redundant, allow for the careful and widespread control of inflammatory mediators in response to TLR stimulation. MyD88 sits at the epicenter, and, as reviewed herein, its regulation and function is cell and context dependent, leading to pathogenic or protective roles in cardiovascular disease.

3. Cell Specific Roles of MyD88 in Cardiovascular Disease

3.1. Multicellular Involvement of MyD88 in Atherosclerotic Inflammation:

Atherosclerosis consists of arterial plaque formation, which can lead to plaque rupture and subsequent vessel blockage. Endothelial damage combined with lipid (LDL) deposition results in fatty streaks along arteries. As plaques grow, damaged endothelial cells produce

pro-inflammatory cytokines, and infiltrating macrophages phagocytose oxidized LDL forming foam cells, a hallmark of early atherosclerosis. This leads to the recruitment of helper T-cells, pronominally type 1 (Th1 cells), as well as the migration and proliferation of smooth muscle cells, resulting in an advanced atherosclerotic plaque and a complex inflammatory environment.[31,32] Characterization of both human and mouse atherosclerotic lesions showed upregulation of TLRs and MyD88.[33,34] To study MyD88 in atherosclerosis, groups have used *Myd88*$^{-/-}$ mice on the ApoE or LDL-R deficient background with or without a high fat diet, common models of atherosclerosis. Global deletion of MyD88 or TLR4 in these models resulted in smaller atherosclerotic plaques.[35–37] This effect was attributed in part to impairment of macrophage recruitment to the vascular wall, as well as to lower levels of the vasculature produced chemokines CCL2, CCL4, CCL7, CCL8, CCL12, CXCL1, CXCL10, and CXCL16. Interestingly, the cytokines IL-12 and MCP1 were lowered in serum, suggesting a role for MyD88 in T-cell recruitment and Th1 polarization, but this was not confirmed. No MyD88-dependent changes in serum lipid profile were observed, excluding this as the protective mechanism.[35–37]

Not only was macrophage recruitment decreased with MyD88 deletion, but MyD88 also affected macrophage function in plaques. Macrophages isolated from both *Tlr4*$^{-/-}$ and *Myd88*$^{-/-}$ mice showed less activation, lipid accumulation, and foam cell formation in response to ox-LDL treatment, an alarmin central to atherosclerosis. Reactive oxygen species (ROS) formation in the endothelium, a driver of ox-LDL formation, was also reduced.[38,39] Further, macrophage TLR4 expression was induced by ox-LDL treatment, directly linking TLR/MyD88 signaling to early atherosclerotic plaque formation.[40] Plaque macrophages were the source of many of the chemokines listed above, as well as IL-1β, TNFα, and IL-6. Similar effects were seen with both siRNA knockdown in macrophages *ex vivo* and in macrophages after pharmacologic inhibition of MyD88 in mice, together confirming that TLR4/MyD88 signaling is involved in macrophage phagocytosis of lipids and foam cell formation in plaques.[36,41,42]

Bone marrow transfer from *Myd88*$^{-/-}$ mice into WT mice only had a partial protective effect on plaque size compared to global knockout mice, indicating an atherogenic role for MyD88 in other types of leukocytes and non-immune cells.[37] Hosseini *et. al* showed that transfer of *Myd88*$^{-/-}$ B1a cells into mice results in less secretion of atheroprotective IgM, which deposits in lesions and blocks T-cell infiltration and IL-1β/TNFα production, slowing plaque progression. These findings support an anti-inflammatory role for MyD88 in B1a cells.[43] In line with this observed anti-inflammatory role for MyD88 in adaptive immunity, mice with a CD11c-Cre driver deletion of MyD88 exhibited worsened atherosclerosis that was attributed to impairment of T-reg recruitment to plaques by CD11c^{+} dendritic cells, which help to suppress plaque inflammation.[44] In contrast, a different study showed naive *Myd88*$^{-/-}$ CD4^{+} T-cells introduced into B and T-cell deficient mice (*Rag1*$^{-/-}$) resulted in smaller atherosclerotic lesions compared to mice given WT CD4^{+} cells. When T-cells were characterized *ex vivo* from this model, the authors showed MyD88 deletion resulted in fewer Th17 cells and lower IL-17 secretion, giving MyD88 a role in regulating T-cell effector subtypes.[45] Together, these studies show differing contributions from MyD88 in multiple immune cells towards atherosclerosis. Moreover, they highlight that in some instances, MyD88 can be anti-inflammatory, emphasizing the necessity of in depth studies

using conditional MyD88 knockout mice to help parse out in which cell types MyD88 modulation might be therapeutically beneficial.

Alteration of the vascular endothelium is an early step in atherosclerotic plaque formation. A mouse with a deletion of MyD88 using the Tie2-Cre driver demonstrated reduced levels of circulating IL-6 and CXCL1, and reduced endothelial expression of IL-1β, MCP1, ICAM1, and VCAM1. Endothelial MyD88-dependent production of GM-CSF was also shown to induce monocyte differentiation into inflammatory M1 type macrophages, making MyD88 an contributor to early atherogenic inflammation.[46] However, these effects may be attributed in part to hematopoietic cells, which lack MyD88 in the Tie2-Cre system, therefore the role of endothelial MyD88 must be confirmed using an endothelial-specific promoter. Additionally, *Liu et. al* showed that LPS treatment of aortic endothelial cells caused MyD88-dependent cytokine release that induced endothelial Proprotein convertase subtilisin/kexin type 9 (PCSK9) expression under conditions of low blood flow, a risk factor for endothelial damage and atherosclerotic initiation.[47] PCSK9 is a negative regulator of the LDL-R and known factor in promoting atherosclerosis, directly involving MyD88 in the pathogenesis of atherosclerosis through endothelial lipid processing. Vascular smooth muscle cell (VSMC) proliferation and migration also plays a significant role in late atherosclerotic plaque development.[32] Both aged and TLR4 stimulated aortic VSMCs produced higher levels of IL-6 *ex vivo*, associated with enhanced cellular migration. Isolation of VSMCs from MyD88 knockout mice, or treatment of VSMCs with a MyD88 peptide inhibitor partially abrogated this effect, demonstrating a role for VSMC MyD88 in atherogenesis.[48,49]

To add complexity to the role of alarmin sensing in atherosclerosis, studies using global $Tlr3^{-/-}$ mice to evaluate MyD88-independent signaling, showed worse atherosclerotic burden.[50,51] However, WT mice reconstituted with bone marrow from $Tlr3^{-/-}$ mice were protected from atherosclerosis, supporting that these pathways modulate vascular dependence in a cell dependent, potentially opposing manner.[52] For example, vascular smooth muscle cells from atherosclerotic plaques showed enhanced expression of TLR3, and TLR3 dependent increases in IL-6, CCL2, and CCL5.[50,53] While endothelial TLR3/TRIF has not yet been studied in the context of atherosclerosis, *Harari et. al* showed endothelial cells lacked the TRIF adapter protein TRAM, and therefore a functional MyD88-independent pathway, suggesting that alarmin signaling in endothelial cells is exclusively dependent on MyD88. Taken together, MyD88 signaling generally promotes both vascular inflammation and atherosclerotic plaque formation, with the exception of its role in B1a cells and DCs, components of the adaptive immune system. Understanding the timing and cellular components of the inflammatory and atherogenic response is critical to design pharmacologic interventions centered on MyD88.

3.2 MyD88 Promotes Inflammation and Repair Following Myocardial Infarction:

Myocardial infarction (MI) occurs when coronary arterial perfusion of the myocardium is lost after atherosclerotic plaque rupture, resulting in myocyte death and loss of cardiac function. It is well established that MI triggers an innate immune response to resolve myocardial damage. Necrotic myocytes release pro-inflammatory cytokines and chemokines

which recruit neutrophils and macrophages to clear dead tissue, followed by subsequent fibroblast activation to form a scar that promotes healing, but often comes with the consequence of chronic HF.[54] Global *Myd88*$^{-/-}$ mice exhibit smaller infarct sizes and acutely preserved cardiac function in response to experimental MI. This has been attributed in part to impaired neutrophil migration into the heart. When hearts of *Myd88*$^{-/-}$ mice post MI were studied *ex vivo*, without access for infiltrating immune cells, this resulted in restoration of infarct size, however it is unclear whether this can be attributed to neutrophil intrinsic MyD88, neutrophil recruitment, or both.[55–57] MyD88 genetic knockout or pharmacologic inhibition by ST2825, an inhibitor of MyD88 homo-dimerization, was shown to decrease the levels of P-selectin and ICAM-1 in the coronary vasculature, a mechanism for MyD88 in neutrophil trafficking[57,58] ST2825, and AS-1, a small molecule inhibitor of the MyD88/IL-1R interaction, decreased neutrophil migration, cytokine release, and infarct size.[58,59] Directly upstream of MyD88, TLR4 has been shown to be upregulated in cardiac myocytes after MI.[60] As such, TAK-242, a TLR4 inhibitor, also reduced cardiac myocyte cytokine release and subsequent damage.[61] However, these compounds showed less protection compared to genetic deletion of MyD88. Given that MyD88 has cell-specific roles in MI, broad pharmacologic approaches that lack cell specificity are expected to be less effective.

Interestingly, WT mice reconstituted with bone marrow from *Myd88*$^{-/-}$ mice and then subjected to MI exhibited similarly decreased infarct sizes as global *Myd88*$^{-/-}$ mice or pharmacologically treated mice, but no changes in cardiac cytokine or chemokine levels compared to mice reconstituted with WT bone marrow. This highlights that MyD88 in hematopoietic cells is not sufficient to mediate inflammatory responses in cardiac repair.[56] However, another group showed cardiac resident macrophages to influence both neutrophil and monocyte recruitment through chemokine production, an effect dependent on macrophage MyD88 expression.[56,57] This might be explained by the presence of existing cardiac resident WT macrophages before bone marrow transplant, which contribute to the MyD88-dependent cytokine and chemokine pool. Multiple studies have also demonstrated that MyD88 inhibition decreases the production of TNFα, IL-1α/β, IL-6, and IL-18, or the chemokines IL-8, MCP1, KC, MIP1, and CXCL10 in isolated cardiomyocytes treated with TLR agonists or at baseline in whole hearts, directly implicating MyD88 in initiation of the cardiac inflammatory response.[55,57,58,61,64] Additionally, *Lugrin et. al* showed the alarmin IL-1α from dying myocytes induced chemokine production in WT but not *Myd88*$^{-/-}$ cardiac fibroblasts.[64] This expanded the role of MyD88 to multiple cardiac resident cells in MI. *Singh et. al* farther described attenuation of ROS post MI in *Myd88*$^{-/-}$ mice, an alternative mechanism for MyD88 regulation of cardiac inflammation.[55] The balance between pro-resolution and harmful inflammation is critical in cardiac repair post MI. From the literature, we conclude that MyD88 mostly promotes harmful inflammation, given the amelioration of infarct sizes and inflammation in the absence of MyD88. However, a few studies support a role for TLR4/MyD88 in pro-resolving inflammation. For example, LPS activation of TLR4/MyD88 enhanced mesenchymal stem cell proliferation and prevented myocyte apoptosis *in vitro*, and LPS pre-treatment before infusion of mesenchymal stem cells into hearts helped recover cardiac function *ex vivo* post

MI.[65,66] Whether the observations of MyD88 deletion in cardiac repair post MI extend to chronic heart failure has yet to be studied.

MyD88-independent alarmin signaling through TLR3 has also been reported to contribute to cardiac repair, as *Tlr3*$^{-/-}$ mice also have attenuated infarct sizes. Deletion of TLR3 has been reported to decrease myocyte autophagy, increase apoptosis, modestly decrease IL-1β/TNFα production, and decrease neutrophil recruitment post MI.[67–69] Interestingly, *Trif*$^{-/-}$ mice have shown contradictory results, either with no effect or a mild decrease in infarct size.[56,67] This provides some evidence for the dual contribution of MyD88-dependent and independent signaling in the acute inflammatory response to MI. Their potential redundancy might limit the benefit of targeting MyD88 in preventing adverse cardiac damage following MI, as alarmin signaling through TLR4 might still influence cardiac pathology via TRIF. At the same time, the literature reveals novel aspects of MyD88 signaling to be studied in cardiac repair and ischemic heart failure.

3.3 MyD88 Has Tissue Specific Roles in Hypertension & AAA Formation:

Hypertension (HTN) can be classified as primary, with no identifiable cause, or secondary, caused by a specific disease such as renal failure.[70] Vascular inflammation is a hallmark of HTN, and both TLRs and MyD88 are expressed in endothelial cells. Angiotensin II (ATII) infusion, a pre-clinical model of HTN, resulted in significantly higher systolic blood pressures in *Myd88*$^{-/-}$ and *Tlr4*$^{-/-}$ mice compared to WT mice, implying a role for MyD88 in blood pressure regulation (Figure 3A).[71] ATII has also been shown to activate MyD88 in vascular smooth muscle cells (VSMCs). While the specific mechanism connecting ATII to MyD88 in HTN has yet to be discovered, *Hernanz et. al* showed TLR4 inhibition attenuated aortic remodeling, improved vascular contractility, decreased oxidative stress, and decreased aortic IL-6, TNFα, and CCL2. Using isolated VSMCs, they attributed this to decreased MyD88 signaling.[72] Additionally, TLR4 stimulation by the alarmin HMGB1 in VSMCs increased similar pro-inflammatory cytokines.[73] Taken together, this shows MyD88-dependent alarmin sensing in VSMCs contributes to ATII induced HTN, while in other, yet unknown cell types, may be protective. Other models provide further evidence of MyD88 regulating HTN. In the spontaneous hypertensive rat model (SHRM), treatment with TLR4-blocking antibodies suppressed MyD88 and resulted in decreased systolic blood pressure.[74,75] In a renovascular model of HTN, a similar dependence of blood pressure regulation on TLR4/MyD88 signaling was found in the paraventricular nucleus (PVN) of the thalamus, an area involved in autonomic homeostasis. Intracranial TAK-242 infusion lowered systolic BP, MyD88 signaling, and IL-1β/TNFα expression in the PVN.[76] Together, these studies show a complex regulatory role for MyD88 in the regulation of blood pressure that is variable based on the model used, suggesting cell and stimulus specific functions as described throughout this review.

In parsing out the TLR pathways involved, *Singh et. al* showed an increase in TLR3, TLR4, and TRIF expression in the hearts of *Myd88*$^{-/-}$ mice treated with AT-II, suggesting a compensatory upregulation of components of the MyD88-independent pathway, which might contribute to inflammation in HTN.[77] This was further confirmed in a follow up study showing *Tlr3*$^{-/-}$ and *Trif*$^{-/-}$ mice are protected from AT-II induced HTN.[71]

However, very few studies have investigated TLR3/TRIF signaling in hypertension, and the cell specificity and mechanisms involved remain completely unknown, making this an area for future study.

The implication of MyD88-dependent signaling in vascular changes associated with hypertension is further supported by studies of abdominal aortic aneurysm (AAA), for which hypertension is a significant risk factor. Whole genome sequencing of patient AAA samples showed an upregulation of the MyD88 pathway[78], consistent with studies showing *Myd88*$^{-/-}$ and *Tlr4*$^{-/-}$ mice being protected from ATII-induced AAA (Figure 3B).[37] Interestingly, when bone marrow transplants were performed from each mouse to WT mice, only MyD88-deficient bone marrow protected mice from AAA development.[37] This provides evidence that MyD88 in leukocytes promotes AAA formation, in addition to vascular inflammation discussed previously. *Trif*$^{-/-}$ mice were shown to have a similar protective effect,[79] suggesting that the MyD88-independent pathway plays some role in promotion of AAA. Further research is needed to tease out the tissue-specificity of these pathways in AAA development.

3.4 The Role of MyD88 in Adverse Cardiac Remodeling in Chronic HF:

Heart failure (HF) is a clinical syndrome resulting in the loss of cardiac function, or inability to pump blood around the body. Cardiomyocyte hypertrophy and cardiac fibrosis are hallmarks of chronic heart failure, both of which are coordinated in part by members of the immune system.[8] TLR4 up-regulation was found in the hearts of patients with non-ischemic HF on both ventricular myocytes and coronary endothelial cells,[80] in what was the earliest association described between TLR signaling and CVD. This was further supported by the observation that *Tlr4*$^{-/-}$ mice exhibit reduced cardiac hypertrophy and fibrosis in response to trans-aortic constriction (TAC), a common model of pressure overload HF.[81] TAC has been shown to induce the expression of MyD88 and its association with TLR4,[82] and *in vivo* transfections of a dominant negative MyD88 construct also showed decreased cardiac remodeling, reduced myocyte apoptosis, and improved cardiac function in response to TAC.[82,83] Surprisingly, cardiac-specific overexpression of MyD88 only slightly decreased cardiac function but did not result in cardiac hypertrophy, suggesting the need for either specific DAMP engagement of TLRs upstream of MyD88 or non-cardiac cell MyD88.[84] *In vitro* studies attempting to mimic diabetic HF by treating cardiomyocytes with RBP4, involved in insulin resistance, caused upregulation of markers of hypertrophy (*ANP, BNP, Myh7*) and pro-inflammatory cytokines (IL-1β, IL-6, TNFα) in cardiomyocytes from WT but not *Myd88*$^{-/-}$ and *Tlr4*$^{-/-}$ mice. A similar effect was seen in cardiomyocytes treated with siRNA against MyD88 in response to treatment with the thyroid hormone T3, which also induces cardiac hypertrophy and inflammation.[85,86] Comprehensive *in vivo* studies of these models using MyD88$^{-/-}$ mice have yet to be completed, however this suggests a wide variety of stimuli in addition to pressure overload can promote MyD88-dependent cardiac hypertrophy (Figure 3C).

Pharmacologic inhibition of MyD88 further supports its role in adverse cardiac remodeling. AS-1 was shown to prevent cardiac myocyte hypertrophy both *in vitro* and in response to TAC, an effect dependent on IL-1β. LM9, another pharmacologic inhibitor of MyD88,

decreased myocyte production of TNFα and IL-6, as well as macrophage production of IL-1β, TNFα, and IL-6. These two studies suggest that MyD88-dependent cytokine production by macrophages and myocytes contributes to inflammation in experimental HF. Both inhibitors reduced cardiac fibrosis in response to TAC, however the connection between MyD88 signaling and fibrosis has not been studied in non-ischemic HF.[87,88] We and others have reported the recruitment of T-cells to the heart in TAC, and that T-cell communication with cardiac fibroblasts contribute to adverse cardiac remodeling.[89,90] No study has explored the role of MyD88 in T-cells, fibroblasts, or endothelial cells, or the MyD88-independent pathways of alarmin sensing through TLR3/TRIF in experimental non-ischemic HF, despite the well established presence of alarmins in the failing heart. If the protective effect is limited to cell specific MyD88-dependent inhibition, this represents an example in which MyD88 could be a strong therapeutic candidate.

3.5 MyD88 Contributes to Cardiovascular Autoimmune Diseases:

Autoimmune myocarditis, characterized by an aberrant immune response to self-antigens causing inflammation of the heart, has been associated with abnormal TLR signaling.[24,91] In mouse models of myocarditis, *Myd88*$^{-/-}$ mice are consistently protected from disease progression, showing less cardiac hypertrophy, dilation, fibrosis, and myocardial cytokine production (Figure 3D).[24,92–94] However, the mechanisms and inflammatory mediators of this phenotype are still under debate. One study showed a partial dependence on TLR7 in response to α-myosin heavy chain peptide immunization (My-HCα) while another showed IL-1R dependence in the bone marrow compartment in response to My-HCα. Interestingly, while *Myd88*$^{-/-}$ and *Tlr7*$^{-/-}$ mice were both protected from My-HCα induced myocarditis, only *Myd88*$^{-/-}$ mice had decreased levels of auto-antibodies in serum, suggesting complex mechanistic roles for MyD88 in B vs. T cells in this model.[93,94] Another group used Biglycan, a known TLR2/4 agonist, to induce perimyocarditis in mice.[92] Therefore, different receptors mediate autoimmune pathogenesis through MyD88 in different models. Consistent with dependence on bone marrow function, *Myd88*$^{-/-}$ dendritic cells were shown to have impaired antigen presentation and T-cell activation *in vitro* as the mechanism for preventing autoimmune induction.[24,92] Overall, this supports the role of MyD88-dependent signaling in dendritic and adaptive immune cell types in promoting autoimmune myocarditis development.

Coxsackie virus is the most common infectious cause of myocarditis.[95] TLR7, TLR8, and TLR9, which respond to nucleic acids, have expectedly been shown to increase with Coxsackie virus B3 (CVB3) infection in mice and patients, as has MyD88 expression.[96–99] *Myd88*$^{-/-}$ mice, while susceptible to CVB3/CVB4 infection, display protection from cardiac virus infiltration and subsequent myocarditis. One group also reported decreased NK and T-cell induction in CVB3 infected *Myd88*$^{-/-}$ mice. Further, they found decreased level of pro-inflammatory cytokines such as IL-1β, TNFα, and IFNγ, but increased levels of type I interferons[97,98,100]. As TRIF induction of anti-viral interferons is important for the antiviral response, this suggests a compensatory flux through the MyD88-independent pathway, helping to curb viral replication and cardiac infection. The importance of the MyD88-independent pathway in this infectious model is further shown by the observation that *Tlr3*$^{-/-}$ and *Trif*$^{-/-}$ mice exhibit significantly worse CVB3/4 infection

and myocarditis. Further, WT macrophages rescued this phenotype, one effector cell type contributing to antiviral TLR3 signaling.[100,101] Therefore, in Coxsackie virus infection, MyD88-dependent signaling contributes to cardiac auto-immune inflammation, while MyD88-independent signaling contributes to the anti-viral response, although the exact effector cell types have yet to be fully uncovered.

Kawasaki disease (KD), an autoimmune vasculitis, has been shown to have a similar dependence on MyD88 signaling. *Myd88*$^{-/-}$, *Tlr2*$^{-/-}$ and *Il1*$^{-/-}$ mice are protected from KD induction by *Lactobacillus casei* cell wall injection, indicating the involvement of multiple MyD88-dependent pathways.[102,103] *Lee et. al* showed that *Myd88*$^{-/-}$ mice with a CD11c-specific MyD88 knock-in retained this protection. Surprisingly, both *Myd88*$^{-/-}$ to WT and WT to *Myd88*$^{-/-}$ bone marrow transplants were also protected from KD, therefore MyD88 signaling in and outside of bone marrow is involved in KD induction.[102] Another study narrowed their search to macrophages, showing TLR2/MyD88 signaling to mediate macrophage activation in response to KD antigen.[103] However, when considering the results of the first study, MyD88 signaling in stromal cells is likely involved too, but this has yet to be studied. Anakinra, an IL-1R antagonist, has shown efficacy in preventing myocardial dysfunction in KD, but MyD88 antagonism might prove more effective due to the involvement of TLR2.[104] Due to the importance of the adaptive immune system in autoimmune disease pathogenesis, there is likely a role for T and B-Cell MyD88 in these diseases, but this has not been studied. Additionally, considering the importance of both endosomal TLR-MyD88 and TLR3-TRIF production of anti-viral interferons, these pathways likely contribute to the immune response to COVID-19 infection. As COVID-19 patients can develop myocarditis and childhood KD-like symptoms, this remains an unexplored role for MyD88 modulation in the cardiovascular complications of COVID-19.

3.6 MyD88 is Associated With the Development of Valvular Disease:

Calcific aortic valve disease (CAVD) involves chronic inflammation and osteoblastic differentiation of aortic valvular interstitial cells (AVICs), resulting in stiff, impaired valves.[105] No pharmacologic treatments currently exist for CAVD, making the inflammatory response an attractive potential therapeutic target. Aortic valve samples from patients with calcific disease showed increased expression of TLR4 and MyD88 at the transcript and protein levels.[106] Isolated AVICs from patients with CAVD also showed increases in TLR2/TLR4 expression, IL-6, IL-8, MCP1, and ICAM-1 expression, and higher levels of the pro-calcification genes *Runx2* and *Bmp2*. This effect was diminished by siRNA knockdown of TLR2 or TLR4. This indirectly implicated MyD88-dependent signaling in osteoblastic differentiation and the development of CAVD in humans.[107] Knockdown of MyD88 in isolated AVICs decreased *in vitro* calcium deposition and the osteogenic response, confirming this connection.[108] Further, MyD88 was shown to be a point of regulation independent of TLR signaling, as miRNA-214 and IL-37 have both been shown to down-regulate MyD88-dependent cytokine stimulation in AVICs and osteoblastic differentiation.[106,109] Interestingly, multiple groups have shown an almost identical effect through TLR3/TRIF stimulation and knockdown in AVICs.[108,110,111] Together, these data support a perhaps redundant role of MyD88-dependent and independent signaling in the

development of CAVD, potentially limiting the opportunity for therapeutic intervention until the exact contributions of each are delineated.

3.7 MyD88 Signaling in Cardiac Electrical Conduction and Arrythmias:

The discovery that AV node resident macrophages can modulate cardiac electrical conduction through gap junctions has led to studying immunologic involvement in arrhythmias.[112] Macrophages have also been shown to induce arrhythmias indirectly in mouse models of diabetes. *Monnerat et. al* demonstrated *Tlr2*$^{-/-}$ mice to be protected from diabetes-associated arrhythmias, then using *in vitro* studies and macrophage depletion isolated this effect to a TLR2-stimulation of macrophage IL-1β production, inducing electrical variability in myocytes.[113] While they did not directly evaluate the role of MyD88, TLR2 only signals through the MyD88-dependent pathway. Further, knocking out MD1, a known inhibitor of TLR4 signaling, increased MyD88 expression in the cardiomyocytes of obese mice with subsequent decreases in cardiac ion channel expression leading to QT prolongation and arrhythmias.[114] Another study showed a similar TLR4-dependent effect on arrythmias that was abolished with IRF3 inhibition and not IRAK1/4 inhibition.[115] This provides evidence that in cardiac myocytes, MyD88-independent signaling influence electrical conduction while infiltrated macrophages use MyD88-dependent signaling to contribute to arrhythmias. However, these studies have focused on individual TLRs rather than MyD88 itself, therefore studies of arrythmias in global or conditional *Myd88*$^{-/-}$ mice are necessary to directly implicate MyD88.

4. Targeting MyD88 for Cardiovascular Therapeutics:

Considering the widespread involvement of MyD88 and alarmin signaling in CVD, targeting this pathway could be beneficial in both treatment and prevention. These strategies would need to increase or decrease flux through MyD88-dependent vs. independent signaling pathways, based on which is protective or harmful in each disease process. We suspect that agents targeting MyD88 directly would be more effective than agents targeting specific receptor-ligand pairs, as MyD88 is the center of many different signaling inputs that can contribute to cardiovascular inflammation. For example, IL-1β inhibition has failed clinical trials in patients with acute MI[9], yet as discussed in Section 3.2, MyD88 mediates inflammation in MI through IL-1α/β and multiple TLRs.

Effective small molecule inhibitors of MyD88 exist, which we review below and in Table 1, but none have been advanced to clinical trials thus far. Most of these inhibitors target MyD88 dimerization and receptor association.[15] AS-1 was the first inhibitor published, a small peptide mimic of the BB loop in the TIR domain of MyD88, which was shown to specifically inhibit the IL-1r/MyD88 interaction without interfering with TLR4/MyD88.[116] This compound showed efficacy in preventing cardiac hypertrophy in response to TAC as well as myocardial damage post MI when given daily to mice for 2 weeks prior.[58,88] Another peptide mimic of the BB loop, ST2825, was shown to inhibit MyD88 dimerization and subsequent cytokine production in response to both IL-1 and TLR9 stimulation, although no other TLRs were tested.[117] Pre and post-treatment with ST2825 in mouse MI models prevented the development of ventricular dilation and hypertrophy.

[59] Olson *et. al* used computational protein-protein interaction screening to be used in any *in vivo* cardiovascular studies.[118] TJ-M2010–5, yet another inhibitor of MyD88 dimerization, was shown to have anti-inflammatory properties in cancers, autoimmune disease, and preventing cardiac allograft rejection in mice given daily injections.[119–121] *Chen et. al* used TJ-M2010–5 as a backbone and created LM9, an inhibitor with a 5x lower K_d than the parent compound.[122] Subsequent studies in mice demonstrated efficacy of LM9 in preventing atherosclerosis as well as obesity-related heart failure.[41,87] The range of effective small molecules inhibiting MyD88 both *in vitro* and in animals indicate that some of these compounds might be considered for pre-clinical pharmacologic testing. Interestingly, no attempts have been made to activate MyD88 for therapies of CVD, while examples of MyD88 playing a protective role have been shown in atherosclerosis and HTN (see above).

Currently, the only approved drug that targets TLRs directly is Imiquimod, a TLR7/TLR8 agonist used in many dermatologic disorders.[123] Additionally, IL-1R blockade is a therapeutic strategy that indirectly targets MyD88. Anakinra, a recombinant form of the IL-1R antagonist, and Canakinumab, a monoclonal antibody against IL-1β, are both approved to treat inflammatory arthritis and periodic fever disorders. Interestingly, both have shown only limited potential in clinical trials of patients with HF, MI, and pericarditis.[124–126] Further, current clinical trials exist using agonists and antagonists against nearly every TLR, testing efficacy to treat cancers, infectious, autoimmune, and allergic diseases.[127,128] While many of these drugs are still at early stages or were not originally for cardiovascular purposes, future successful clinical trials will strengthen their potential for alternative uses.

A variety of other drugs already approved for other diseases have been shown to indirectly affect TLR/MyD88, opening up the possibility of repurposing existing drugs to target this pathway. Statins, which inhibit HMG-CoA Reductase to treat hyperlipidemia, were discovered to decrease TLR4 expression in whole blood from patients with HF, suppressing MyD88 signaling in monocytes.[129,130] Since then, statins have been shown to inhibit TLR signaling and attenuate atherosclerosis, cardiac remodeling, and ischemic injury.[131–133] While these drugs are already used in atherosclerotic disease, this outlines a new mechanism for their action and gives support for their use in other CVD. Additionally, Angiotensin II receptor blockers, used in HTN and HF, were shown to suppress TLR2/TLR4 mediated inflammation and protect rats from MI reperfusion injury.[134,135]

Glycosaminoglycans such as chondroitin sulfate, a dietary supplement taken by osteoarthritis patients, also inhibited MyD88-dependent inflammatory signaling in chondrocytes.[136] Chloroquine and hydroxychloroquine are anti-inflammatory drugs used in autoimmune diseases or malaria treatment, but also have been shown to decrease MyD88 levels, improve blood pressure in hypertensive rats, and reduce TLR9/MyD88 levels in patients with Sjögrens syndrome.[137,138] However, these medications have the potential to cause cardiovascular side effects like arrhythmias, therefore the full mechanisms would need to be studied before determining a potential benefit. A variety of medications in the tricyclic family, which target neurotransmitter release and uptake, have also been shown to modulate TLR activity, the effects of which were eliminated in *Myd88*$^{-/-}$ mice. The antidepressant

amitriptyline enhanced TLR2/TLR4 signaling in reporter cells while the anti-convulsant oxcarbazepine inhibited TLR4 signaling.[139] However, this family of medications also has the potential to cause hypertension and arrythmias. Finally, multiple studies have shown that opioids can modulate TLR4/MyD88 activity.[140,141] All of these drugs showed varying levels of activity on TLR/MyD88 signaling, and have varying pharmacodynamic properties, therefore could be useful for specific forms of CVD depending on the exact contributions of MyD88. However, while safety profiles have already been established, further study would need to be done to demonstrate utility in repurposing them for CVD.

5. Concluding Remarks

CVD has become intertwined with cardiac and systemic inflammation, therefore mechanistic studies are vital to improving therapies and outcomes. Cardiovascular inflammation involves complex interactions between the innate, adaptive, and non-immune cells. While MyD88 and alarmin sensors such as TLRs are considered pro-inflammatory in myeloid cells, they are broadly expressed, and their functions are continuously expanding. MyD88 signaling has complex roles, influencing cardiac immune cell recruitment, intrinsic immune cell functions, cellular proliferation or death, and directly modulating fibrosis. This complexity seems to vary between cell types as well as in the context of different inflammatory or disease states. As such, examples of MyD88 being protective or pathogenic can be found in most forms of CVD, such as MyD88 in innate cells contributing to atherosclerosis while MyD88 in adaptive cells being protective. Comprehensive studies of the exact function of MyD88 in specific cell types will determine whether MyD88 can be considered a safe therapeutic target in CVD. As conditional genetic knock-out and knock-in models are commonly available, this has become a much more feasible goal. One significant limitation of the work we have reviewed is the lack of studies in humans with CVD. Most of the literature on MyD88 signaling remains *in vitro* and in mouse models, limiting the translational benefit at this point. For example, mouse models of atherosclerosis do not exhibit plaque rupture, making study using mice of this clinically relevant feature in patients difficult. However, any already approved drugs found to indirectly modulate MyD88, such as statins, have the benefit pre-existing data from clinical trials. While we still have considerable progress before using MyD88-specific treatments in CVD, significant advances have been made in understanding the role of immune signaling in the cardiovascular system. Based on this work, we believe that MyD88 remains an important contributor to disease pathogenesis and holds potential value as a therapeutic target in many forms of CVD.

Acknowledgements:

This work was supported by HL-144477 (P.A.) and the Tufts Collaborative Cancer Biology Award (A.B.) Figures were generated with Biorender.

Abbreviations:

AAA	Abdominal aortic aneurysm
ATII	Angiotensin II
AVIC	Aortic valvular interstitial cell

CAVD	Calcific aortic valve disease
CVB	Coxsackie virus B
CVD	Cardiovascular disease
DAMP	Damage associated molecular pattern
DD	Death domain
HF	Heart failure
HMGB1	High mobility group box 1
HTN	Hypertension
IRAK	Il1R associated kinase
IRF	Interferon regulatory factor
KD	Kawasaki disease
LDL	Low density lipoprotein
MHC	Major histocompatibility complex
My-HCα	α-myosin heavy chain peptide
MD1	Myeloid differentiation protein 1
MI	Myocardial infarction
MyD88	Myeloid differentiation primary response gene 88
PAMP	Pathogen associated molecular pattern
PCSK9	Proprotein convertase subtilisin/kexin type 9
PVN	Paraventricular nucleus
RBP4	Retinol binding protein 4
ROS	Reactive oxygen species
SHRM	Spontaneous hypertensive rat model
SNP	Single nucleotide polymorphism
TAC	Trans-aortic constriction
TIR	Toll-IL1r domain
TLR	Toll-like receptor
TRAF	TNF receptor associated factor
TRAM	Translocating chain associated membrane protein 1

TRIF	TIR-domain containing adaptor inducing interferon-β
WT	Wild type

Sources:

[1]. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020 12 22;76(25):2982–3021. [PubMed: 33309175]

[2]. Virani Salim S, Alonso Alvaro, Benjamin Emelia J., Bittencourt Marcio S., Callaway Clifton W., Carson April P., et al. Heart Disease and Stroke Statistics—2020 Update: A Report From the American Heart Association. Circulation. 2020 3 3;141(9):e139–596. [PubMed: 31992061]

[3]. Philip Joseph, Darryl Leong, Martin McKee, Anand Sonia S, Schwalm Jon-David, Teo Koon, et al. Reducing the Global Burden of Cardiovascular Disease, Part 1. Circ Res. 2017 9 1;121(6):677–94. [PubMed: 28860318]

[4]. Frančula-Zaninović S, Nola IA. Management of Measurable Variable Cardiovascular Disease' Risk
Factors. Curr Cardiol Rev. 2018 8;14(3):153–63. [PubMed: 29473518]

[5]. Ruparelia N, Chai JT, Fisher EA, Choudhury RP. Inflammatory processes in cardiovascular disease: a route to targeted therapies. Nat Rev Cardiol. 2017 3;14(3):133–44. [PubMed: 27905474]

[6]. Elster SK, Braunwald E, Wood HF. A study of C-reactive protein in the serum of patients with congestive heart failure. Am Heart J. 1956 4 1;51(4):533–41. [PubMed: 13302128]

[7]. Strassheim D, Dempsey EC, Gerasimovskaya E, Stenmark K, Karoor V. Role of Inflammatory Cell Subtypes in Heart Failure. J Immunol Res [Internet] 2019 9 2;2019. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6745095/

[8]. Carrillo-Salinas FJ, Ngwenyama N, Anastasiou M, Kaur K, Alcaide P. Heart Inflammation: Immune Cell Roles and Roads to the Heart. Am J Pathol. 2019 8 1;189(8):1482–94. [PubMed: 31108102]

[9]. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017 9 21;377(12):1119–31. [PubMed: 28845751]

[10]. Abbate A, Kontos MC, Grizzard JD, Biondi-Zoccai GGL, Van Tassell BW, Robati R, et al. Interleukin-1 Blockade With Anakinra to Prevent Adverse Cardiac Remodeling After Acute Myocardial Infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot Study). Am J Cardiol. 2010 5 15;105(10):1371–1377.e1. [PubMed: 20451681]

[11]. Chung Eugene S, Packer Milton, Lo Kim Hung, Fasanmade Adedigbo A., Willerson James T. Randomized, Double-Blind, Placebo-Controlled, Pilot Trial of Infliximab, a Chimeric Monoclonal Antibody to Tumor Necrosis Factor-α, in Patients With Moderate-to-Severe Heart Failure. Circulation. 2003 7 1;107(25):3133–40. [PubMed: 12796126]

[12]. Mann DL, McMurray JJV, Packer M, Swedberg K, Borer JS, Colucci WS, et al. Targeted Anticytokine Therapy in Patients With Chronic Heart Failure. Circulation. 2004 4 6;109(13):1594–602. [PubMed: 15023878]

[13]. Yang D, Han Z, Oppenheim JJ. ALARMINS AND IMMUNITY. Immunol Rev. 2017 11;280(1):41–56. [PubMed: 29027222]

[14]. Cohen P The TLR and IL-1 signalling network at a glance. J Cell Sci. 2014 6 1;127(11):2383–90. [PubMed: 24829146]

[15]. Deguine J, Barton GM. MyD88: a central player in innate immune signaling. F1000Prime Rep [Internet]. 2014 11 4;6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4229726/

[16]. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004 10;5(10):987–95. [PubMed: 15454922]

[17]. Hardiman G, Jenkins NA, Copeland NG, Gilbert DJ, Garcia DK, Naylor SL, et al. Genetic Structure and Chromosomal Mapping of MyD88. Genomics. 1997 10 15;45(2):332–9. [PubMed: 9344657]

[18]. G H, Fl R, S B, Ra K, Jf B. Molecular Characterization and Modular Analysis of Human MyD88 [Internet]. Oncogene. 1996 [cited 2020 Jun 18]. Available from: https://pubmed.ncbi.nlm.nih.gov/8957090/

[19]. Nishimura M, Naito S. Tissue-Specific mRNA Expression Profiles of Human Toll-Like Receptors and Related Genes. Biol Pharm Bull. 2005;28(5):886–92. [PubMed: 15863899]

[20]. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku C-L, et al. Pyogenic Bacterial Infections in Humans with MyD88 Deficiency. Science. 2008 8 1;321(5889):691–6. [PubMed: 18669862]

[21]. Chaplin DD. Overview of the Immune Response. J Allergy Clin Immunol. 2010 2;125(2 Suppl 2):S3–23. [PubMed: 20176265]

[22]. Reynolds JM, Dong C. Toll-like receptor regulation of effector T lymphocyte function. Trends Immunol. 2013 10 1;34(10):511–9. [PubMed: 23886621]

[23]. Pasare C, Medzhitov R. Control of B-cell responses by Toll-like receptors. Nature. 2005 11;438(7066):364–8. [PubMed: 16292312]

[24]. Marty René R, Dirnhofer Stephan, Mauermann Nora, Schweikert Sacha, Akira Shizuo, Hunziker Lukas, et al. MyD88 Signaling Controls Autoimmune Myocarditis Induction. Circulation. 2006 1 17;113(2):258–65. [PubMed: 16401773]

[25]. Kiripolsky J, McCabe LG, Gaile DP, Kramer JM. Myd88 is required for disease development in a primary Sjögren's syndrome mouse model. J Leukoc Biol. 2017;102(6):1411–20. [PubMed: 28951424]

[26]. Androulidaki A, Wachsmuth L, Polykratis A, Pasparakis M. Differential role of MyD88 and TRIF signaling in myeloid cells in the pathogenesis of autoimmune diabetes. PLOS ONE. 2018 3 9;13(3):e0194048. [PubMed: 29522531]

[27]. Salvador B, Arranz A, Francisco S, Córdoba L, Punzón C, Llamas MÁ, et al. Modulation of endothelial function by Toll like receptors. Pharmacol Res. 2016 6 1;108:46–56. [PubMed: 27073018]

[28]. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010 5;11(5):373–84. [PubMed: 20404851]

[29]. Balka KR, De Nardo D. Understanding early TLR signaling through the Myddosome. J Leukoc Biol. 2019 2 1;105(2):339–51. [PubMed: 30256449]

[30]. Pellegrini L, Foglio E, Pontemezzo E, Germani A, Russo MA, Limana F. HMGB1 and repair: focus on the heart. Pharmacol Ther. 2019 4 1;196:160–82. [PubMed: 30529040]

[31]. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, et al. Atherosclerosis. Nat Rev Dis Primer. 2019 8 16;5(1):1–18.

[32]. Williams JW, Huang L, Randolph GJ. Cytokine Circuits in Cardiovascular Disease. Immunity. 2019 4 16;50(4):941–54. [PubMed: 30995508]

[33]. Edfeldt Kristina, Swedenborg Jesper, Hansson Göran K., Yan Zhong-qun. Expression of Toll-Like Receptors in Human Atherosclerotic Lesions. Circulation. 2002 3 12;105(10):1158–61. [PubMed: 11889007]

[34]. Cai S, Batra S, Shen L, Wakamatsu N, Jeyaseelan S. Both TRIF-Dependent and MyD88-dependent Signaling Contributes to Host Defense Against Pulmonary Klebsiella infection. J Immunol Baltim Md 1950. 2009 Nov 15;183(10):6629–38.

[35]. Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, et al. Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci U S A. 2004 7 20;101(29):10679–84. [PubMed: 15249654]

[36]. Björkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, et al. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med. 2004 4;10(4):416–21. [PubMed: 15034566]

[37]. Owens AP, Rateri DL, Howatt DA, Moore KJ, Tobias PS, Curtiss LK, et al. MyD88 Deficiency Attenuates Angiotensin II-induced Abdominal Aortic Aneurysm Formation Independent of

Signaling Through Toll-like Receptors 2 and 4. Arterioscler Thromb Vasc Biol. 2011 12;31(12):2813–9. [PubMed: 21960563]

[38]. Howell KW, Meng X, Fullerton DA, Jin C, Reece TB, Cleveland JC. Toll-like Receptor 4 Mediates Oxidized LDL-Induced Macrophage Differentiation to Foam Cells. J Surg Res. 2011 11 1;171(1):e27–31. [PubMed: 21920554]

[39]. Singh Rajesh K, Haka Abigail S, Asmal Arky, Barbosa-Lorenzi Valéria C., Grosheva Inna, Chin Harvey F., et al. TLR4 (Toll-Like Receptor 4)-Dependent Signaling Drives Extracellular Catabolism of LDL (Low-Density Lipoprotein) Aggregates. Arterioscler Thromb Vasc Biol. 2020 1 1;40(1):86–102. [PubMed: 31597445]

[40]. Xu Xiaoou Helen, Shah Prediman K., Emmanuelle Faure, Ozlem Equils, Lisa Thomas, Fishbein Michael C., et al. Toll-Like Receptor-4 Is Expressed by Macrophages in Murine and Human Lipid-Rich Atherosclerotic Plaques and Upregulated by Oxidized LDL. Circulation. 2001 12 18;104(25):3103–8. [PubMed: 11748108]

[41]. Chen T, Luo W, Wu G, Wu L, Huang S, Li J, et al. A novel MyD88 inhibitor LM9 prevents atherosclerosis by regulating inflammatory responses and oxidative stress in macrophages. Toxicol Appl Pharmacol. 2019 5 1;370:44–55. [PubMed: 30880215]

[42]. Li L-C, Varghese Z, Moorhead JF, Lee C-T, Chen J-B, Ruan XZ. Cross-talk between TLR4-MyD88-NF-κB and SCAP-SREBP2 pathways mediates macrophage foam cell formation. Am J Physiol-Heart Circ Physiol. 2013 1 18;304(6):H874–84. [PubMed: 23335792]

[43]. Hosseini H, Li Y, Kanellakis P, Tay C, Cao A, Liu E, et al. Toll-Like Receptor (TLR)4 and MyD88 are Essential for Atheroprotection by Peritoneal B1a B Cells. J Am Heart Assoc Cardiovasc Cerebrovasc Dis [Internet]. 2016 11 14;5(11). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5210362/

[44]. Subramanian M, Thorp E, Hansson GK, Tabas I. Treg-mediated suppression of atherosclerosis requires MYD88 signaling in DCs. J Clin Invest. 2013 1 2;123(1):179–88. [PubMed: 23257360]

[45]. Engelbertsen D, Rattik S, Wigren M, Vallejo J, Marinkovic G, Schiopu A, et al. IL-1R and MyD88 signalling in CD4+ T cells promote Th17 immunity and atherosclerosis. Cardiovasc Res. 2018 1 1;114(1):180–7. [PubMed: 29036304]

[46]. Yu M, Zhou H, Zhao J, Xiao N, Roychowdhury S, Schmitt D, et al. MyD88-dependent interplay between myeloid and endothelial cells in the initiation and progression of obesity-associated inflammatory diseases. J Exp Med. 2014 5 5;211(5):887–907. [PubMed: 24752299]

[47]. Liu S, Deng X, Zhang P, Wang X, Fan Y, Zhou S, et al. Blood flow patterns regulate PCSK9 secretion via MyD88-mediated pro-inflammatory cytokines. Cardiovasc Res [Internet]. 2019 [cited 2020 Jun 19]; Available from: 10.1093/cvr/cvz262/5583758

[48]. Lee G-L, Wu J-Y, Tsai C-S, Lin C-Y, Tsai Y-T, Lin C-S, et al. TLR4-Activated MAPK-IL-6 Axis Regulates Vascular Smooth Muscle Cell Function. Int J Mol Sci [Internet]. 2016 8 24;17(9). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5037674/

[49]. Song Y, Shen H, Schenten D, Shan P, Lee PJ, Goldstein DR. Aging enhances the basal production of IL-6 and CCL2 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 2012 1;32(1):103–9. [PubMed: 22034510]

[50]. Cole JE, Navin TJ, Cross AJ, Goddard ME, Alexopoulou L, Mitra AT, et al. Unexpected protective role for Toll-like receptor 3 in the arterial wall. Proc Natl Acad Sci U S A. 2011 2 8;108(6):2372–7. [PubMed: 21220319]

[51]. Richards MR, Black AS, Bonnet DJ, Barish GD, Woo CW, Tabas I, et al. The LPS2 Mutation in TRIF is Atheroprotective in Hyperlipidemic LDL Receptor Knockout Mice. Innate Immun. 2013 2;19(1):20–9. [PubMed: 22637968]

[52]. Lundberg AM, Ketelhuth DFJ, Johansson ME, Gerdes N, Liu S, Yamamoto M, et al. Toll-like receptor 3 and 4 signalling through the TRIF and TRAM adaptors in haematopoietic cells promotes atherosclerosis. Cardiovasc Res. 2013 7 15;99(2):364–73. [PubMed: 23417039]

[53]. Harari OA, Alcaide P, Ahl D, Luscinskas FW, Liao JK. Absence of TRAM Restricts Toll-Like Receptor 4 Signaling in Vascular Endothelial Cells to the MyD88 Pathway. Circ Res. 2006 5 12;98(9):1134–40. [PubMed: 16574902]

[54]. Kain V, Prabhu SD, Halade GV. Inflammation revisited: inflammation versus resolution of inflammation following myocardial infarction. Basic Res Cardiol. 2014 9 24;109(6):444. [PubMed: 25248433]

[55]. Singh MV, Swaminathan PD, Luczak ED, Kutschke W, Weiss RM, Anderson ME. MyD88 mediated inflammatory signaling leads to CaMKII oxidation, cardiac hypertrophy and death after myocardial infarction. J Mol Cell Cardiol. 2012 5;52(5):1135–44. [PubMed: 22326848]

[56]. Feng Y, Zou L, Si R, Nagasaka Y, Chao W. Bone marrow MyD88 signaling modulates neutrophil function and ischemic myocardial injury. Am J Physiol - Cell Physiol. 2010 10;299(4):C760–9. [PubMed: 20631245]

[57]. Feng Y, Zhao H, Xu X, Buys ES, Raher MJ, Bopassa JC, et al. Innate immune adaptor MyD88 mediates neutrophil recruitment and myocardial injury after ischemia-reperfusion in mice. Am J Physiol - Heart Circ Physiol. 2008 9;295(3):H1311–8. [PubMed: 18660455]

[58]. Cao Z, Hu Y, Wu W, Ha T, Kelley J, Deng C, et al. The TIR/BB-loop mimetic AS-1 protects the myocardium from ischaemia/reperfusion injury. Cardiovasc Res. 2009 12 1;84(3):442–51. [PubMed: 19586942]

[59]. Van Tassell BW, Seropian IM, Toldo S, Salloum FN, Smithson L, Varma A, et al. Pharmacologic Inhibition of Myeloid Differentiation Factor 88 (MyD88) Prevents Left Ventricular Dilation and Hypertrophy After Experimental Acute Myocardial Infarction in the Mouse. J Cardiovasc Pharmacol. 2010 4;55(4):385–90. [PubMed: 20125030]

[60]. Liu L, Wang Y, Cao Z, Wang M, Liu X, Gao T, et al. Up-regulated TLR4 in cardiomyocytes exacerbates heart failure after long-term myocardial infarction. J Cell Mol Med. 2015 12;19(12):2728–40. [PubMed: 26290459]

[61]. Su Q, Li L, Sun Y, Yang H, Ye Z, Zhao J. Effects of the TLR4/Myd88/NF-κB Signaling Pathway on NLRP3 Inflammasome in Coronary Microembolization-Induced Myocardial Injury. Cell Physiol Biochem. 2018;47(4):1497–508. [PubMed: 29940584]

[62]. Geetika Bajpai, Andrea Bredemeyer, Wenjun Li, Konstantin Zaitsev, Koenig Andrew L., Lokshina Inessa, et al. Tissue Resident CCR2− and CCR2+ Cardiac Macrophages Differentially Orchestrate Monocyte Recruitment and Fate Specification Following Myocardial Injury. Circ Res. 2019 1 18;124(2):263–78. [PubMed: 30582448]

[63]. Li W, Hsiao H-M, Higashikubo R, Saunders BT, Bharat A, Goldstein DR, et al. Heart-resident CCR2+ macrophages promote neutrophil extravasation through TLR9/MyD88/CXCL5 signaling. JCI Insight [Internet]. 2016;1(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4985028/

[64]. Lugrin J, Parapanov R, Rosenblatt-Velin N, Rignault-Clerc S, Feihl F, Waeber B, et al. Interleukin-1 alpha is a crucial danger signal triggering acute myocardial inflammation during myocardial infarction. J Immunol Baltim Md 1950. 2015 Jan 15;194(2):499–503.

[65]. Chu X, Xu B, Gao H, Li B-Y, Liu Y, Reiter JL, et al. Lipopolysaccharides Improve Mesenchymal Stem Cell-Mediated Cardioprotection by MyD88 and stat3 Signaling in a Mouse Model of Cardiac Ischemia/Reperfusion Injury. Stem Cells Dev. 2019 5 1;28(9):620–31. [PubMed: 30808255]

[66]. Zhu X, Zhao H, Graveline AR, Buys ES, Schmidt U, Bloch KD, et al. MyD88 and NOS2 are essential for Toll-like receptor 4-mediated survival effect in cardiomyocytes. Am J Physiol-Heart Circ Physiol. 2006 10 1;291(4):H1900–9. [PubMed: 16648192]

[67]. Chen C, Feng Y, Zou L, Wang L, Chen HH, Cai J, et al. Role of Extracellular RNA and TLR3-Trif Signaling in Myocardial Ischemia–Reperfusion Injury. J Am Heart Assoc Cardiovasc Cerebrovasc Dis [Internet]. 2014 2 28;3(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959703/

[68]. Gao T, Zhang S, Wang J, Liu L, Wang Y, Cao Z, et al. TLR3 contributes to persistent autophagy and heart failure in mice after myocardial infarction. J Cell Mol Med. 2018 1;22(1):395–408. [PubMed: 28945004]

[69]. Lu C, Ren D, Wang X, Ha T, Liu L, Lee EJ, et al. Toll-like receptor 3 plays a role in myocardial infarction and ischemia/reperfusion injury. Biochim Biophys Acta [Internet]. 2014 1;1842(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3879925/

[70]. Schroeder HA. Pathogenesis of hypertension. Am J Med. 1951 2 1;10(2):189–209. [PubMed: 14810725]

[71]. Singh MV, Cicha MZ, Nunez S, Meyerholz DK, Chapleau MW, Abboud FM. Angiotensin II-induced hypertension and cardiac hypertrophy are differentially mediated by TLR3- and TLR4-dependent pathways. Am J Physiol - Heart Circ Physiol. 2019 5 1;316(5):H1027–38. [PubMed: 30793936]

[72]. Hernanz R, Martínez-Revelles S, Palacios R, Martín A, Cachofeiro V, Aguado A, et al. Toll-like receptor 4 contributes to vascular remodelling and endothelial dysfunction in angiotensin II-induced hypertension. Br J Pharmacol. 2015 6;172(12):3159–76. [PubMed: 25712370]

[73]. Lai C-H, Wang K-C, Lee F-T, Tsai H-W, Ma C-Y, Cheng T-L, et al. Toll-Like Receptor 4 Is Essential in the Development of Abdominal Aortic Aneurysm. PLoS ONE [Internet]. 2016 1 7;11(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4711799/

[74]. Bomfim GF, Dos Santos RA, Oliveira MA, Giachini FR, Akamine EH, Tostes RC, et al. Toll like receptor 4 contributes to blood pressure regulation and vascular contraction in spontaneously hypertensive rat. Clin Sci Lond Engl 1979. 2012 Jun;122(11):535–43.

[75]. Bomfim GF, Echem C, Martins CB, Costa TJ, Sartoretto SM, Dos Santos RA, et al. Toll-like receptor 4 inhibition reduces vascular inflammation in spontaneously hypertensive rats. Life Sci. 2015 2 1;122:1–7. [PubMed: 25498891]

[76]. Qi J, Yu X-J, Fu L-Y, Liu K-L, Gao T-T, Tu J-W, et al. Exercise Training Attenuates Hypertension Through TLR4/MyD88/NF-κB Signaling in the Hypothalamic Paraventricular Nucleus. Front Neurosci [Internet]. 2019 10 24;13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6821652/

[77]. Singh MV, Cicha MZ, Meyerholz DK, Chapleau MW, Abboud FM. Dual Activation of TRIF and MyD88 Adaptor Proteins by Angiotensin II Evokes Opposing Effects on Pressure, Cardiac Hypertrophy and Inflammatory Gene Expression. Hypertension. 2015 9;66(3):647–56. [PubMed: 26195481]

[78]. Spin JM, Hsu M, Azuma J, Tedesco MM, Deng A, Dyer JS, et al. Transcriptional profiling and network analysis of the murine angiotensin II-induced abdominal aortic aneurysm. Physiol Genomics. 2011 9;43(17):993–1003. [PubMed: 21712436]

[79]. Vorkapic E, Lundberg AM, Mäyränpää MI, Eriksson P, Wågsäter D. TRIF adaptor signaling is important in abdominal aortic aneurysm formation. Atherosclerosis. 2015 8 1;241(2):561–8. [PubMed: 26100679]

[80]. Frantz S, Kobzik L, Kim Y-D, Fukazawa R, Medzhitov R, Lee RT, et al. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. J Clin Invest. 1999 8 1;104(3):271–80. [PubMed: 10430608]

[81]. Ha T, Li Y, Hua F, Ma J, Gao X, Kelley J, et al. Reduced cardiac hypertrophy in toll-like receptor 4-deficient mice following pressure overload. Cardiovasc Res. 2005 11 1;68(2):224–34. [PubMed: 15967420]

[82]. Li T, Wang Y, Liu C, Hu Y, Wu M, Li J, et al. MyD88-dependent nuclear factor-κB activation is involved in fibrinogen-induced hypertrophic response of cardiomyocytes. J Hypertens. 2009 5;27(5):1084–93. [PubMed: 19342961]

[83]. Ha T, Hua F, Li Y, Ma J, Gao X, Kelley J, et al. Blockade of MyD88 attenuates cardiac hypertrophy and decreases cardiac myocyte apoptosis in pressure overload-induced cardiac hypertrophy in vivo. Am J Physiol-Heart Circ Physiol. 2006 3 1;290(3):H985–94. [PubMed: 16199478]

[84]. Chen W, Huang Z, Jiang X, Li C, Gao X. Overexpression of myeloid differentiation protein 88 in mice induces mild cardiac dysfunction, but no deficit in heart morphology. Braz J Med Biol Res [Internet]. 2015 11 27;49(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4681416/

[85]. Gao W, Wang H, Zhang L, Cao Y, Bao J-Z, Liu Z-X, et al. Retinol-Binding Protein 4 Induces Cardiomyocyte Hypertrophy by Activating TLR4/MyD88 Pathway. Endocrinology. 2016 6;157(6):2282–93. [PubMed: 27100622]

[86]. Takano APC, Munhoz CD, Moriscot AS, Gupta S, Barreto-Chaves MLM. S100A8/MYD88/NF-κB: a novel pathway involved in cardiomyocyte hypertrophy driven by thyroid hormone. J Mol Med. 2017 6 1;95(6):671–82.

[87]. Zheng X, Sun C, Liu Q, Lu X, Fu L, Liang G, et al. Compound LM9, a novel MyD88 inhibitor, efficiently mitigates inflammatory responses and fibrosis in obesity-induced cardiomyopathy. Acta Pharmacol Sin. 2020 4 27;1–9.

[88]. Zhu Y, Li T, Song J, Liu C, Hu Y, Que L, et al. The TIR/BB-loop mimetic AS-1 prevents cardiac hypertrophy by inhibiting IL-1R-mediated MyD88-dependent signaling. Basic Res Cardiol. 2011 5 1;106(5):787. [PubMed: 21533832]

[89]. Tania Nevers, Salvador Ane M., Grodecki-Pena Anna, Knapp Andrew, Velázquez Francisco, Aronovitz Mark, et al. Left Ventricular T-Cell Recruitment Contributes to the Pathogenesis of Heart Failure. Circ Heart Fail. 2015 7 1;8(4):776–87. [PubMed: 26022677]

[90]. Nevers T, Salvador AM, Velazquez F, Ngwenyama N, Carrillo-Salinas FJ, Aronovitz M, et al. Th1 effector T cells selectively orchestrate cardiac fibrosis in nonischemic heart failure. J Exp Med. 2017 11 6;214(11):3311–29. [PubMed: 28970239]

[91]. Farrugia M, Baron B. The Role of Toll-Like Receptors in Autoimmune Diseases through Failure of the Self-Recognition Mechanism. Int J Inflamm [Internet]. 2017;2017. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5434307/

[92]. Popovic ZV, Wang S, Papatriantafyllou M, Kaya Z, Porubsky S, Meisner M, et al. The proteoglycan biglycan enhances antigen-specific T cell activation via MyD88 and TRIF pathways and triggers autoimmune perimyocarditis. J Immunol Baltim Md 1950. 2011 Dec 15;187(12):6217–26.

[93]. Przemyslaw Blyszczuk, Gabriela Kania, Thomas Dieterle, Marty Rene R., Valaperti Alan, Berthonneche Corinne, et al. Myeloid Differentiation Factor-88/Interleukin-1 Signaling Controls Cardiac Fibrosis and Heart Failure Progression in Inflammatory Dilated Cardiomyopathy. Circ Res. 2009 10 23;105(9):912–20. [PubMed: 19762681]

[94]. Pagni PP, Traub S, Demaria O, Chasson L, Alexopoulou L. Contribution of TLR7 and TLR9 signaling to the susceptibility of MyD88-deficient mice to myocarditis. Autoimmunity. 2010 6 1;43(4):275–87. [PubMed: 20187710]

[95]. Rose NR. Viral Myocarditis. Curr Opin Rheumatol. 2016 7;28(4):383–9. [PubMed: 27166925]

[96]. Triantafilou K, Orthopoulos G, Vakakis E, Ahmed MAE, Golenbock DT, Lepper PM, et al. Human cardiac inflammatory responses triggered by Coxsackie B viruses are mainly Toll-like receptor (TLR) 8-dependent. Cell Microbiol. 2005;7(8):1117–26. [PubMed: 16008579]

[97]. Koichi Fuse, Grace Chan, Youan Liu, Patrick Gudgeon, Mansoor Husain, Manyin Chen, et al. Myeloid Differentiation Factor-88 Plays a Crucial Role in the Pathogenesis of Coxsackievirus B3–Induced Myocarditis and Influences Type I Interferon Production. Circulation. 2005 10 11;112(15):2276–85. [PubMed: 16216974]

[98]. Riad A, Westermann D, Escher F, Becher PM, Savvatis K, Lettau O, et al. Myeloid differentiation factor-88 contributes to TLR9-mediated modulation of acute coxsackievirus B3-induced myocarditis in vivo. Am J Physiol-Heart Circ Physiol. 2010 3 12;298(6):H2024–31. [PubMed: 20228254]

[99]. Satoh M, Akatsu T, Ishikawa Y, Minami Y, Takahashi Y, Nakamura M. Association between toll-like receptor 8 expression and adverse clinical outcomes in patients with enterovirus-associated dilated cardiomyopathy. Am Heart J. 2007 9 1;154(3):581–8. [PubMed: 17719310]

[100]. Richer MJ, Lavallée DJ, Shanina I, Horwitz MS. Toll-Like Receptor 3 Signaling on Macrophages Is Required for Survival Following Coxsackievirus B4 Infection. PLoS ONE [Internet]. 2009 1 5;4(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2606033/

[101]. Abston ED, Coronado MJ, Bucek A, Bedja D, Shin J, Kim JB, et al. Th2 Regulation of Viral Myocarditis in Mice: Different Roles for TLR3 versus TRIF in Progression to Chronic Disease. Clin Dev Immunol [Internet]. 2012;2012. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3195533/

[102]. Lee Y, Wakita D, Dagvadorj J, Shimada K, Chen S, Huang G, et al. IL-1 signaling is critically required in stromal cells in Kawasaki Disease Vasculitis Mouse Model. Role of both IL-1α and IL-1β. Arterioscler Thromb Vasc Biol. 2015 12;35(12):2605–16. [PubMed: 26515418]

[103]. Rosenkranz Margalit E, Schulte Danica J, Agle Lucila MA, Wong Michelle H, Zhang Wenxuan, Ivashkiv Lionel, et al. TLR2 and MyD88 Contribute to Lactobacillus casei Extract–Induced Focal Coronary Arteritis in a Mouse Model of Kawasaki Disease. Circulation. 2005 11 8;112(19):2966–73. [PubMed: 16275884]

[104]. Gorelik M, Lee Y, Abe M, Andrews T, Davis L, Patterson J, et al. IL-1 receptor antagonist, anakinra, prevents myocardial dysfunction in a mouse model of Kawasaki disease vasculitis and myocarditis. Clin Exp Immunol. 2019 10;198(1):101–10. [PubMed: 31099056]

[105]. Mathieu P, Bouchareb R, Boulanger M-C. Innate and Adaptive Immunity in Calcific Aortic Valve Disease. J Immunol Res [Internet]. 2015;2015. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4433691/

[106]. Zheng D, Zang Y, Xu H, Wang Y, Cao X, Wang T, et al. MicroRNA-214 promotes the calcification of human aortic valve interstitial cells through the acceleration of inflammatory reactions with activated MyD88/NF-κB signaling. Clin Res Cardiol. 2019 6 1;108(6):691–702. [PubMed: 30519780]

[107]. Yang X, Fullerton DA, Su X, Ao L, Cleveland JC, Meng X. Pro-Osteogenic Phenotype of Human Aortic Valve Interstitial Cells Is Associated With Higher Levels of Toll-Like Receptors 2 and 4 and Enhanced Expression of Bone Morphogenetic Protein 2. J Am Coll Cardiol. 2009 2 10;53(6):491–500. [PubMed: 19195606]

[108]. Jarrett MJ, Yao Q, Venardos N, Weyant MJ, Reece TB, Meng X, et al. Simvastatin downregulates osteogenic response in cultured human aortic valve interstitial cells. J Thorac Cardiovasc Surg. 2021 4 1;161(4):e261–71. [PubMed: 31924353]

[109]. Zhan Q, Zeng Q, Song R, Zhai Y, Xu D, Fullerton DA, et al. IL-37 Suppresses MyD88-mediated Inflammatory Responses in Human Aortic Valve Interstitial Cells. Mol Med. 2017 3 27;23:83–91. [PubMed: 28362018]

[110]. Zhan Q, Song R, Zeng Q, Yao Q, Ao L, Xu D, et al. Activation of TLR3 Induces Osteogenic Responses in Human Aortic Valve Interstitial Cells through the NF-κB and ERK1/2 Pathways. Int J Biol Sci. 2015 3 20;11(4):482–93. [PubMed: 25798067]

[111]. Zhan Q, Song R, Li F, Ao L, Zeng Q, Xu D, et al. Double-stranded RNA upregulates the expression of inflammatory mediators in human aortic valve cells through the TLR3-TRIF-noncanonical NF-κB pathway. Am J Physiol - Cell Physiol. 2017 4 1;312(4):C407–17. [PubMed: 28052863]

[112]. Hulsmans M, Clauss S, Xiao L, Aguirre AD, King KR, Hanley A, et al. Macrophages Facilitate Electrical Conduction in the Heart. Cell. 2017 4 20;169(3):510–522.e20. [PubMed: 28431249]

[113]. Monnerat G, Alarcón ML, Vasconcellos LR, Hochman-Mendez C, Brasil G, Bassani RA, et al. Macrophage-dependent IL-1β production induces cardiac arrhythmias in diabetic mice. Nat Commun. 2016 11 24;7(1):1–15.

[114]. Shuai W, Kong B, Fu H, Shen C, Huang H. Loss of MD1 increases vulnerability to ventricular arrhythmia in diet-induced obesity mice via enhanced activation of the TLR4/MyD88/CaMKII signaling pathway. Nutr Metab Cardiovasc Dis. 2019 9 1;29(9):991–8. [PubMed: 31353205]

[115]. Monnerat-Cahli G, Alonso H, Gallego M, Alarcón ML, Bassani RA, Casis O, et al. Toll-like receptor 4 activation promotes cardiac arrhythmias by decreasing the transient outward potassium current (Ito) through an IRF3-dependent and MyD88-independent pathway. J Mol Cell Cardiol. 2014 11 1;76:116–25. [PubMed: 25169970]

[116]. Bartfai T, Behrens MM, Gaidarova S, Pemberton J, Shivanyuk A, Rebek J. A low molecular weight mimic of the Toll/IL-1 receptor/resistance domain inhibits IL-1 receptor-mediated responses. Proc Natl Acad Sci U S A. 2003 6 24;100(13):7971–6. [PubMed: 12799462]

[117]. Loiarro M, Capolunghi F, Fantò N, Gallo G, Campo S, Arseni B, et al. Pivotal Advance: Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound. J Leukoc Biol. 2007;82(4):801–10. [PubMed: 17548806]

[118]. Olson MA, Lee MS, Kissner TL, Alam S, Waugh DS, Saikh KU. Discovery of small molecule inhibitors of MyD88-dependent signaling pathways using a computational screen. Sci Rep 2015 9 18;5:14246. [PubMed: 26381092]

[119]. Xie L, Jiang F-C, Zhang L-M, He W-T, Liu J-H, Li M-Q, et al. Targeting of MyD88 Homodimerization by Novel Synthetic Inhibitor TJ-M2010–5 in Preventing Colitis-Associated Colorectal Cancer. JNCI J Natl Cancer Inst [Internet]. 2016 4 1 [cited 2020 Jun 23];108(4). Available from: https://academic.oup.com/jnci/article/108/4/djv364/2412478

[120]. Li C, Zhang L-M, Zhang X, Huang X, Liu Y, Li M-Q, et al. Short-term Pharmacological Inhibition of MyD88 Homodimerization by a Novel Inhibitor Promotes Robust Allograft Tolerance in Mouse Cardiac and Skin Transplantation. Transplantation. 2017 2;101(2):284–93. [PubMed: 27607533]

[121]. Zou Z, Du D, Miao Y, Yang Y, Xie Y, Li Z, et al. TJ-M2010–5, a novel MyD88 inhibitor, corrects R848-induced lupus-like immune disorders of B cells in vitro. Int Immunopharmacol. 2020 8 1;85:106648. [PubMed: 32504998]

[122]. Chen L, Chen H, Chen P, Zhang W, Wu C, Sun C, et al. Development of 2-amino-4-phenylthiazole analogues to disrupt myeloid differentiation factor 88 and prevent inflammatory responses in acute lung injury. Eur J Med Chem. 2019 1 1;161:22–38. [PubMed: 30342423]

[123]. Hanna E, Abadi R, Abbas O. Imiquimod in dermatology: an overview. Int J Dermatol. 2016;55(8):831–44. [PubMed: 27387373]

[124]. Dhimolea E Canakinumab. mAbs. 2010;2(1):3–13. [PubMed: 20065636]

[125]. Cavalli G, Dinarello CA. Anakinra Therapy for Non-cancer Inflammatory Diseases. Front Pharmacol [Internet]. 2018 11 6;9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6232613/

[126]. Buckley LF, Abbate A. Interleukin-1 blockade in cardiovascular diseases: a clinical update. Eur Heart J. 2018 6 7;39(22):2063–9. [PubMed: 29584915]

[127]. Hennessy EJ, Parker AE, O'Neill LAJ. Targeting Toll-like receptors: emerging therapeutics? Nat Rev Drug Discov. 2010 4;9(4):293–307. [PubMed: 20380038]

[128]. Anwar MA, Shah M, Kim J, Choi S. Recent clinical trends in Toll-like receptor targeting therapeutics. Med Res Rev. 2019;39(3):1053–90. [PubMed: 30450666]

[129]. Földes G, von Haehling S, Okonko DO, Jankowska EA, Poole-Wilson PA, Anker SD. Fluvastatin reduces increased blood monocyte Toll-like receptor 4 expression in whole blood from patients with chronic heart failure. Int J Cardiol. 2008 2 20;124(1):80–5. [PubMed: 17383753]

[130]. Kong F, Ye B, Lin L, Cai X, Huang W, Huang Z. Atorvastatin suppresses NLRP3 inflammasome activation via TLR4/MyD88/NF-κB signaling in PMA-stimulated THP-1 monocytes. Biomed Pharmacother. 2016 8 1;82:167–72. [PubMed: 27470352]

[131]. Yuan X, Deng Y, Guo X, Shang J, Zhu D, Liu H. Atorvastatin attenuates myocardial remodeling induced by chronic intermittent hypoxia in rats: Partly involvement of TLR-4/MYD88 pathway. Biochem Biophys Res Commun. 2014 3 28;446(1):292–7. [PubMed: 24582748]

[132]. Yang J, Zhang X-D, Yang J, Ding J-W, Liu Z-Q, Li S-G, et al. The cardioprotective effect of fluvastatin on ischemic injury via down-regulation of toll-like receptor 4. Mol Biol Rep. 2011 6 1;38(5):3037–44. [PubMed: 20127518]

[133]. Fang D, Yang S, Quan W, Jia H, Quan Z, Qu Z. Atorvastatin suppresses Toll-like receptor 4 expression and NF-κB activation in rabbit atherosclerotic plaques. Eur Rev Med Pharmacol Sci. 2014;18(2):242–6. [PubMed: 24488914]

[134]. Dasu MR, Riosvelasco AC, Jialal I. Candesartan inhibits Toll-like receptor expression and activity both in vitro and in vivo. Atherosclerosis. 2009 1;202(1):76–83. [PubMed: 18495130]

[135]. Yang J, Jiang H, Yang J, Ding J-W, Chen L-H, Li S, et al. Valsartan preconditioning protects against myocardial ischemia–reperfusion injury through TLR4/NF-κB signaling pathway. Mol Cell Biochem. 2009 4 16;330(1):39. [PubMed: 19370315]

[136]. Campo GM, Avenoso A, Campo S, Traina P, D'Ascola A, Calatroni A. Glycosaminoglycans reduced inflammatory response by modulating toll-like receptor-4 in LPS-stimulated chondrocytes. Arch Biochem Biophys. 2009 11 1;491(1):7–15. [PubMed: 19800307]

[137]. McCarthy CG, Wenceslau CF, Goulopoulou S, Baban B, Matsumoto T, Webb RC. Chloroquine Suppresses the Development of Hypertension in Spontaneously Hypertensive Rats. Am J Hypertens. 2017 2;30(2):173–81. [PubMed: 27623761]

[138]. Bodewes ILA, Gottenberg J-E, van Helden-Meeuwsen CG, Mariette X, Versnel MA. Hydroxychloroquine treatment downregulates systemic interferon activation in primary Sjögren's syndrome in the JOQUER randomized trial. Rheumatology. 2020 1 1;59(1):107–11. [PubMed: 31237947]

[139]. Hutchinson MR, Loram LC, Zhang Y, Shridhar M, Rezvani N, Berkelhammer D, et al. Evidence that tricyclic small molecules may possess Toll-like receptor and MD-2 activity. Neuroscience. 2010 6 30;168(2):551–63. [PubMed: 20381591]

[140]. Hutchinson MR, Northcutt AL, Hiranita T, Wang X, Lewis SS, Thomas J, et al. Opioid Activation of Toll-Like Receptor 4 Contributes to Drug Reinforcement. J Neurosci. 2012 8 15;32(33):11187–200. [PubMed: 22895704]

[141]. Hutchinson MR, Zhang Y, Shridhar M, Evans JH, Buchanan MM, Zhao TX, et al. Evidence that opioids may have toll like receptor 4 and MD-2 effects. Brain Behav Immun. 2010 1;24(1):83–95. [PubMed: 19679181]

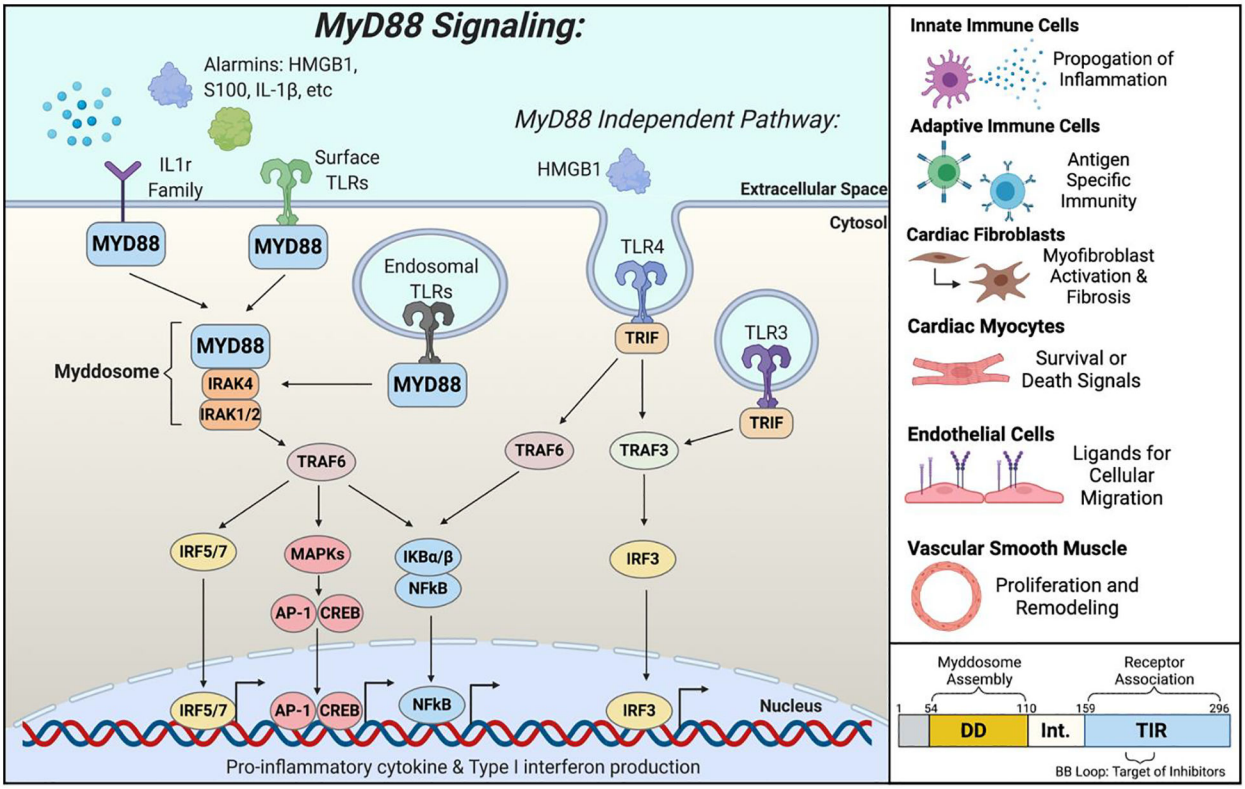

Figure 1: MyD88 Signaling Leads to Cell Specific Functions in Cardiovascular Disease.

Alarmins are recognized by TLRs and cytokine receptors, leading to activation of MyD88 and a subsequent immune response, along with cell specific functions (right panel). The MyD88 independent pathway contributes in parallel. The gene structure and domains of MyD88 are shown in the lower right panel. (DD = Death domain, Int = Intermediate domain, TIR = Toll-IL1R domain)

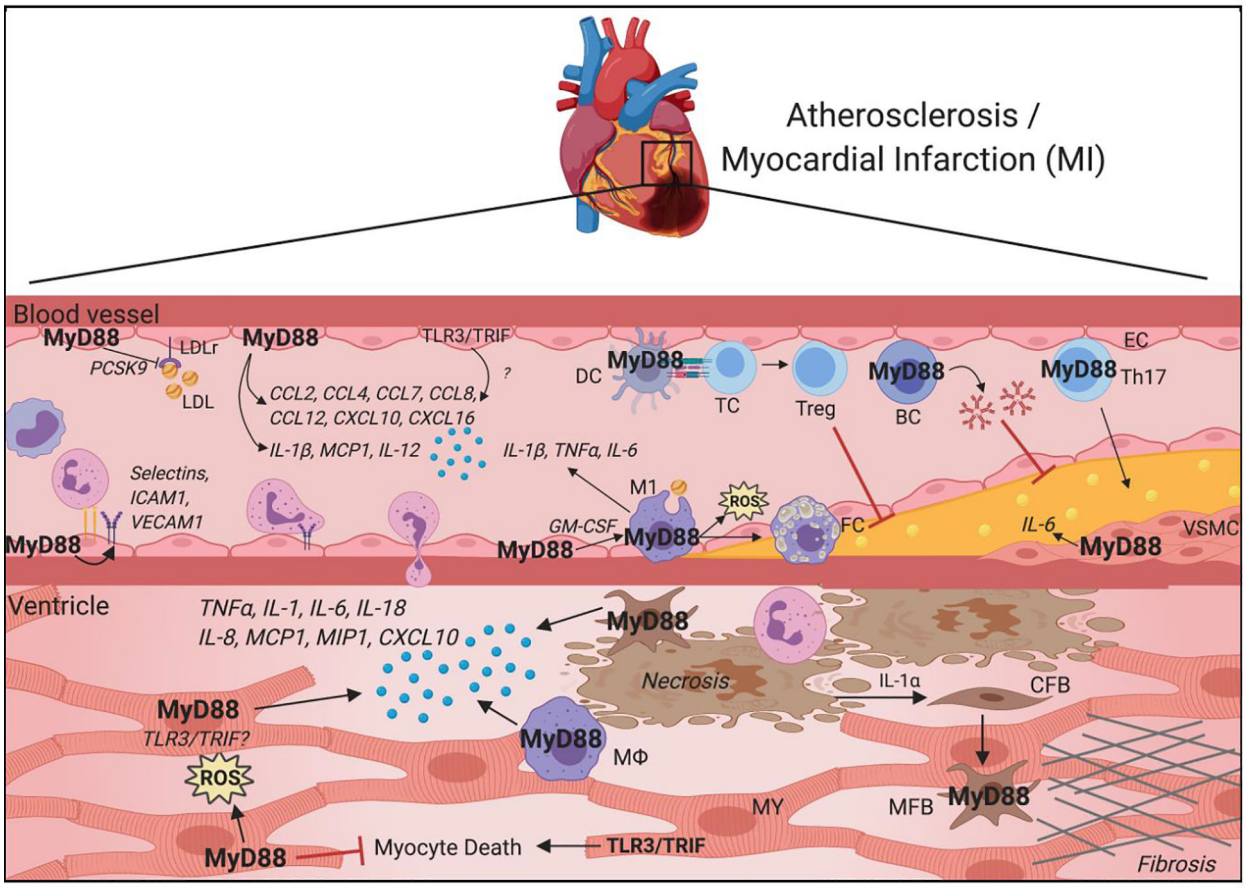

Figure 2: MyD88 Signaling Contributes to Atherosclerotic & Myocardial Infarction Associated Inflammation.

Different cell types affect the development of atherosclerotic plaques and inflammation, which can lead to myocardial infarction. Green arrows indicate protective mechanisms. DC = dendritic cell, TC = T-Cell, Treg = Regulatory T-Cell, BC = B-Cell, Th17 = Type 17 helper T-cell, EC = endothelial cell, M1 = type 1 macrophage, FC = foam cell, VSMC = vascular smooth muscle cell, MY = myocyte, MΦ = macrophage, CFB = cardiac fibroblast, MFB = myofibroblast

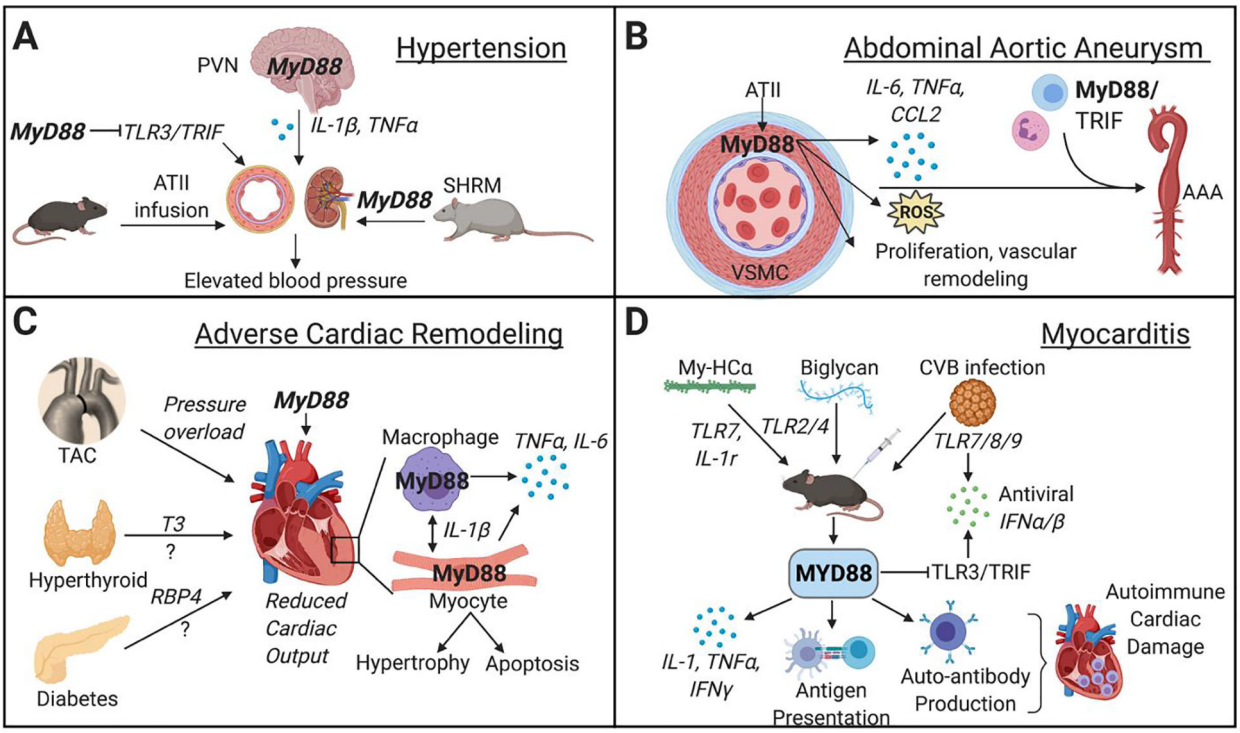

Figure 3: MyD88 Signaling in Other Forms of Cardiovascular Disease.

The role of MyD88 signaling in the development of hypertension (A), abdominal aortic aneurysm (B), adverse cardiac remodeling (C), and myocarditis (D)

Table 1

Pharmacologic Inhibition of MyD88 in Cardiovascular Disease.

Name	Mechanism	CVD Uses	Original Use	Sources
Direct Inhibitors of MyD88				
AS-1	IL-1r/MyD88 inhibitor	Prevention of cardiac hypertrophy in response to TAC, Ischemic injury prevention	–	58, 88, 116
ST2825	MyD88 dimerization inhibitor	Prevention of cardiac remodeling post MI	–	59, 117
T6167923	MyD88 dimerization inhibitor	–	–	118
TJ-M2010–5	MyD88 dimerization inhibitor	Cardiac allograft rejection prevention	–	119–121
LM9	MyD88 dimerization inhibitor	Atherosclerosis prevention, obesity related heart failure prevention	–	41, 87, 122
Existing Drugs Thai Indirectly Target the MyD88 Pathway				
Statins	Suppression of TLR4/MyD88	Cardiac Remodeling, Ischemic injury prevention Hyperlipidemia, Atherosclerosis	Hyperlipidemia, Atherosclerosis	129–133
ARBs	Suppression of TLR2/TLR4/MyD88	Ischemic injury prevention, hypertension	Hypertension	134–135
Chondroitan Sulfate	MyD88 inhibition	–	Osteoarthritis	136
Chloroquine, Hydroxychloroquine	MyD88 (TLR9?) inhibition	Hypertension	Autoimmune disease, malaria	137–138
Amitriptyline	Enhancement of TLR2/TLR4/MyD88	–	Antidepressant	139
Oxcarbazepine	Inhibition of TLR4/MyD88	–	Seizures	139
Opioids	Inhibition or Enhancement of TLR4/MyD88	–	Analgesia	140–141