

# Microglia-Mediated Neuroinflammation: A Potential Target for the Treatment of Cardiovascular Diseases

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**Abstract:** Microglia are tissue-resident macrophages of the central nervous system (CNS). In the CNS, microglia play an important role in the monitoring and intervention of synaptic and neuron-level activities. Interventions targeting microglia have been shown to improve the prognosis of various neurological diseases. Recently, studies have observed the activation of microglia in different cardiovascular diseases. In addition, different approaches that regulate the activity of microglia have been shown to modulate the incidence and progression of cardiovascular diseases. The change in autonomic nervous system activity after neuroinflammation may be a potential intermediate link between microglia and cardiovascular diseases. Here, in this review, we will discuss recent updates on the regulatory role of microglia in hypertension, myocardial infarction and ischemia/reperfusion injury. We propose that microglia serve as neuroimmune modulators and potential targets for cardiovascular diseases.

**Keywords:** neuroimmune, autonomic nervous system, central-peripheral crosstalk, sympathetic nervous system

## Introduction

Microglia, commonly known as brain-resident immune cells, are ubiquitously present in the central nervous system (CNS)<sup>1</sup> and participate in the monitoring of the microenvironment. Microglia are abundant within the brain and comprise up to approximately 20% of the total glial cells.<sup>2</sup> They are present in the white and gray matter of the brain, but their distribution in the brain is uneven, and the cell density between different brain regions may vary substantially. The highest concentration was found in areas such as the hypothalamus and neostriatum, and the lowest densities of microglial cells were observed in the cerebellum, medulla oblongata and spinal cord.<sup>3</sup> Recent findings have shown that microglia establish direct contact with different compartments of neurons. Microglia are involved in almost all brain diseases, including neurodegenerative diseases, traumatic brain injury, and mental illness. After activation, microglia can secrete pro-inflammatory and anti-inflammatory mediators and play a broad role during CNS injury.<sup>4</sup>

The autonomic nervous system, which comprises the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), contributes to the regulation of cardiac function.<sup>5</sup> Sympathetic outflow is controlled by key cerebral nuclei and neural circuits in the CNS, predominantly the rostral ventrolateral medulla (RVLM),<sup>6</sup> the nucleus tractus solitarius (NTS), and the hypothalamic paraventricular nucleus (PVN).<sup>7</sup> The imbalance between the SNS and PNS, especially the continuous activation of the SNS, is one of the main contributors to pathological cardiac remodeling.<sup>8-10</sup> However, the upstream regulators of SNS activity remain largely unknown. Recently, studies have shown that microglia may play an important role in regulating SNS activities and cardiovascular function by releasing various substances, including cytokines, chemokines, and growth factors.<sup>2</sup> Given the importance of the SNS in cardiovascular function, this article mainly reviews the changes in the expression and activity of microglia in different cardiovascular diseases and how microglia contribute to the regulation of cardiovascular disease.

## Origin and Functions of Microglia

Microglia are cells of mesodermal origin that populate the central nervous system during an early developmental stage. During early development, erythrocytes/myeloid progenitor cells in the yolk sac differentiate into tissue-resident macrophage progenitor cells. These cells with an amoeboid morphology migrate into the brain and reside in the brain tissue as microglial cells.<sup>11</sup> By retaining the ability to divide and synthesize DNA, microglia are capable of self-renewal during inflammation in the case of cell depletion. In addition, when microglia are depleted and unable to self-replenish, bone marrow-derived monocytes are also capable of replenishing tissue-resident macrophages in the CNS.<sup>12</sup>

Microglial cells have been described as branched, tissue-resident macrophages in the brain. Researchers have attempted to construct a system defining the complexity of microglial activation, and the M1-M2 classification, which was applied to peripheral macrophages, divides the microglial activation state into classical activation (M1) or alternative activation (M2).<sup>13</sup> According to the morphology and function of microglia, microglia are divided into three categories: M0, M1, and M2. The M0 phenotype represents microglia that are highly active in their presumed resting state, commonly known as the “resting” microglia phenotype, which monitors the presence of pathogens in the local environment and the changes in extracellular concentrations of constitutively expressed neurochemicals.<sup>14</sup> In addition, highly dynamic synapses allow them to sense the microenvironment by interacting with blood vessels, neurons, ependymal cells, and other glial cells, such as astrocytes.<sup>15</sup> The M1 phenotype is characterized by the production of pro-inflammatory cytokines (such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ), chemokines and reactive oxygen species (ROS), leading to an acute immune response. The M2 phenotype is characterized by the production of anti-inflammatory cytokines (such as IL-4 and IL-13), which promote tissue repair, debris removal, wound healing, and the restoration of brain homeostasis.<sup>16</sup> As members of the mononuclear macrophage family, microglia also have the function of macrophages, including the identification and monitoring of dead cells, pathogenic microorganisms, and endogenous or exogenous compounds.

During development, microglia clear dead cells through “eat me signals”, which are produced by apoptotic cells and transmitted to microglia.<sup>17</sup> The phagocytic activity of microglia also contributes to the homeostasis of synapses.<sup>18,19</sup> When infection, tissue damage, or stimulatory signals are present in the microenvironment, microglia are activated and undergo phenotypic and morphological changes and migrate to the injury or stimulation site to produce an inflammatory response. Activated microglia, namely, the M1/M2 phenotypes, release neurochemicals with neuroprotective or neurotoxic effects.<sup>20,21</sup>

As mentioned above, the simplicity of classifying microglial polarization into the M0/M1/M2 concept is based on the classification of peripheral macrophages and is mainly applied to inflammatory reactions in diseased tissue. A series of reports have illuminated that the expression profiles, functions, survival and ultrastructural characteristics of microglia and monocyte-derived macrophages differ dramatically, even when their morphology and surface markers display similarities, in different immune microenvironments.<sup>22,23</sup> These findings have drawn further attention to the classification of microglia and macrophages.<sup>24,25</sup>

Recently, the emergence of novel single-cell techniques, such as cytometry by time-of-flight mass spectrometry (CyTOF) and single-cell RNA sequencing, revealed the heterogeneity of microglia and facilitated the understanding of microglial diversity.<sup>26</sup> The resident macrophages in the CNS, according to the anatomical area, could further be divided into two major populations, microglia and CNS-associated macrophages (CAMs, also named border-associated macrophages [BAMs]).<sup>27</sup> At E9.5, a phenotypically similar primitive macrophage population could be observed in the yolk sac. However, at E10.5, two macrophage populations distinguishable by the expression of CD206 were detected in the yolk sac. Later, at E12.5 and E14.5, CD206<sup>+</sup> macrophages, the CAMs, mainly resided in the developing choroid plexus and the meninges, while CD206<sup>-</sup>/P2Y12<sup>+</sup> macrophages were detected in the developing parenchyma, corresponding to microglia. These findings indicate early segregation of brain macrophages giving rise to CAMs and microglia.<sup>28</sup>

## The Potential Role of Microglia in Cardiovascular Diseases

### The Potential Role of Microglia in Hypertension

Increased neuroinflammation and sympathetic tone contribute to the incidence and maintenance of hypertension. Targeting the neuroinflammatory response with an anti-inflammatory reagent or overexpression of interleukin-10 in the brain attenuates hypertension.<sup>29,30</sup> However, the cellular mechanisms by which neuroinflammation regulates blood

pressure (BP) remain unclear. In a chronic systemic inflammation-induced hypertension model, sustained hypertension was induced after LPS infusion for 14 days, and the activation of microglia, increased IL-1 $\beta$ , IL-6 and TNF- $\alpha$  expression, and O<sub>2</sub><sup>-</sup> production in the RVLM were observed. All of these changes were blunted by inhibiting microglial activation.<sup>31</sup> In addition, the activation of microglia was observed in the PVN and motor cortex of both angiotensin II- and L-N<sup>G</sup>-nitro-L-arginine methyl ester-induced hypertension models.<sup>32</sup> Targeted depletion of microglia significantly attenuated neuroinflammation in the PVN, the plasma vasopressin level, kidney norepinephrine concentration, and BP.<sup>32</sup>

Other studies using minocycline (50 mg/kg/day, oral administration), an inhibitor of microglial activation, to directly inhibit the activation of microglia reported the effective inhibition of sympathetic activity and the attenuation of hypertension both in spontaneously hypertensive rat (SHR) models (normal diet, duration of minocycline treatment 4–6 weeks) and in chronic angiotensin II (Ang II, 200 ng/kg/min)-infused rats (normal diet, duration of minocycline treatment 3–7 weeks).<sup>33</sup> These results provide direct evidence that microglia are central to neuroinflammation and neuronal regulation of hypertension. However, other studies have reported that either systemic (25 mg/kg/day) or central administration of minocycline (0.5  $\mu$ g/50 nL) into the PVN failed to decrease BP, although microglial activation was observed in the PVN in Ang II (high salt diet, 150 ng/kg/min, 2 weeks)-induced hypertensive rats and in stroke-prone spontaneously hypertensive rats (SHRSP) from 15 weeks old,<sup>34,35</sup> which is possibly related to the diet (normal diet vs high salt diet), the animal model (SHRs vs SHRSP), the concentration and duration of Ang II used for modeling (200 ng/kg/min for 3–7 weeks vs 150 ng/kg/min for 2 weeks) and the dose/route of minocycline administration (50 mg/kg/day, oral administration vs 25 mg/kg/day or 0.5  $\mu$ g/50 nL, systemic or central administration).

In addition to the pathological state discussed above, changes in microglia have been observed during a physiologically receptive state in acute hypertension and during the hypotension response.<sup>36</sup> Increases and decreases in BP trigger alertness in the physiology of microglia in the brainstem region, inducing changes in the microglial spatial distribution and the number of synapses in contact with the microglial end processes. After 6 hours of acute hypertension, the number of synapses in contact with microglia increased by 30% in both regions of the brainstem, the CVLM and RVLM. Induction of acute hypotension for 6 hours caused microglia to reduce the number of synaptic contacts by >20% in both the CVLM and RVLM. However, these changes were not accompanied by characteristic morphological changes of the microglia, and the numbers of M1 or M2 microglia were not changed.<sup>36</sup> This observation further indicates that the M1/M2 microglial classification cannot fully clarify the function of microglia.

Several key molecules that regulate hypertension by targeting microglia have been identified. The brain (pro)renin receptor (PRR) is a novel component of the renin-angiotensin system. The immunoreactivity of PRR is significantly correlated with systolic BP but not the use of antihypertensive drugs, suggesting that PRR might be a key initiator of the pathogenesis of hypertension.<sup>37,38</sup> The subfornical organ (SFO) is one of seven circumventricular organs in the human and rodent brain that lacks a traditional blood–brain barrier (BBB), indicating that the SFO senses circulating factors such as Ang II or prorenin and plays a key role in the regulation of BP.<sup>39,40</sup> In the SFO, most neurons and microglia, but not astrocytes, express PRR. At the same time, targeted knockdown of PRR attenuates the development of Ang II-induced hypertension in mice,<sup>41,42</sup> while other work reported that minocycline could fully abolish the (pro)renin-elicited increases in pro-inflammatory cytokine expression *in vitro*,<sup>43</sup> indicating that an intervention targeting PRR on microglia may be an effective method for the treatment of hypertension.

C-X3-C motif chemokine receptor 1 (CX3CR1), a microglial biomarker, is a chemokine receptor that binds to its ligand C-X3-C motif chemokine ligand 1 (CX3CL1). A previous study reported that CX3CL1 microinjection produces a cardiovascular response in the NTS of normal rats.<sup>44</sup> Intracerebroventricular (ICV) administration of AZD8797, a CX3CR1 inhibitor, attenuates fructose-induced hypertension and the expression of pro-inflammatory cytokines.<sup>45</sup>

Kinins are considered potent vasoactive hormones and inflammatory mediators, and the expression of its extracellular amino terminal Kinin B1 receptor (B1R) is well documented on neurons, microglia, and astrocytes within the brain and spinal cord. B1R is markedly upregulated in the presence of inflammation or tissue injury,<sup>46</sup> and its specific antagonist R715 (70  $\mu$ g/kg/day) could reduce BP, decrease sympatho-excitation and exert a significant inhibitory effect on neuroinflammation in a DOCA-salt-induced hypertension mouse model.<sup>47</sup> However, the effect of B1R antagonists on BP remains controversial. Acute injection of the B1R antagonist Leu<sup>8</sup>-des-Arg<sup>9</sup>-BK (12 nmol) into the fourth cerebral ventricle does not change the BP in Wistar Kyoto (WKY) rats or female SHRs.<sup>48</sup> In contrast, the same B1R antagonist, Leu<sup>8</sup>-des-Arg<sup>9</sup>-BK (0.1–10  $\mu$ g), infused

into the lateral cerebral ventricle through an intracerebral guide cannula, was shown to reduce the BP and heart rate (HR) in male SHR. <sup>49</sup> Explanations for the conflicting results may be attributed to the differences in sex (female vs males) of the animals used in the two studies, the dose of pharmacological agents (12 nmol vs 0.1–10 µg) used and the route of agent administration (injection into the fourth cerebral ventricle vs the lateral cerebral ventricle). Nevertheless, a subsequent study reported that the B1R antagonist SSR240612 caused a pronounced antihypertensive effect in both SHR and Ang II-treated rats, <sup>50</sup> suggesting a potential role of B1R in the pathogenesis of hypertension. <sup>51</sup>

Triggering receptor expressed on myeloid cells 2 (TREM2) is a receptor that recognizes phospholipids, apoptotic cells and lipoproteins. <sup>52</sup> Previous studies revealed that TREM2 deficiency exacerbates inflammatory cytokine release from activated M1 microglia and neuronal apoptosis, while TREM2 overexpression markedly attenuated inflammation and neuronal death in AD models. <sup>53,54</sup> Recently, TREM2 was reported to be significantly upregulated in microglia in a hypertension model induced by Ang II infusion, and the overexpression of microglial TREM2 mitigated the microglial inflammatory response, suggesting its possible beneficial effects on BP regulation. <sup>55</sup>

Interventions targeting phenotypic changes in microglia also contribute to the progression of hypertension. High mobility group box protein 1 (HMGB1) is synthesized and released after the activation of microglia, functions as an alarming protein or damage-associated molecular pattern (DAMP) in response to neuroinflammation and is considered a potential mediator priming stress-induced microglia. <sup>56</sup> Evidence has shown that the ablation of HMGB1 and the advanced glycation end-product receptor (RAGE) attenuates persistent chronic noise-induced M1-type microglial activation and hypertension, <sup>57</sup> which theoretically suggests that reducing neuroinflammation and SNS activity in prehypertensive individuals may be a new strategy for the treatment of hypertension. In mice with Ang II-induced hypertension, supplementation with TGF-β significantly inhibited neuroinflammation and renal norepinephrine levels and increased BP. TGF-β regulates microglia to maintain brain homeostasis in response to hypertensive disorders, which shifts microglia to the immunosuppressive phenotype, namely, resting M0 microglia, and thus resists the increase in BP during the onset of hypertension. <sup>58</sup> Based on these findings, TGF-β and its signal transduction pathway may be potential targets for controlling neurogenic hypertension, and resting microglia may play a key role in curbing neuroinflammation. Vitamin D (VitD), a generally recognized pleiotropic hormone, has been reported to possess anti-inflammatory, antioxidant and neuroprotective properties, in addition to its classic functions in calcium and phosphorus homeostasis. <sup>59</sup> Although no significant difference in the trend of BP reduction was observed, chronic calcitriol treatment shifted microglial polarization from the pro-inflammatory M1 phenotype to the immunoregulatory M2 phenotype in SHR, indicating the neuroprotective mechanisms of VitD in the hypertensive brain. <sup>60</sup>

TLR4, a pathogen recognition receptor, is expressed on leukocytes, cardiomyocytes, and endothelial cells and contributes to the activation of innate immunity. TLR4 is expressed primarily on microglia and sparsely on astrocytes and neurons. <sup>61,62</sup> The binding of TLR4 to appropriate ligands activates microglia, induces a local inflammatory response and promotes the expression of pro-inflammatory cytokines. <sup>61</sup> A previous study showed that exogenous Ang II stimulates TLR4 via Ang II type 1 receptor (AT1R), which could induce the activation of hypothalamic microglia *ex vivo*. <sup>63</sup> Recently, it was demonstrated that TAK-242 (TLR4 inhibitor, 2 weeks) administration could abolish microglial activation and preserve BBB integrity in the PVN, RVLM, and NTS in SHR. <sup>64</sup> Moreover, TLR4 blockade attenuated the progression of MAP increases in SHR and protected against autonomic dysfunction, suggesting that TLR4 is a viable alternative target in the treatment of hypertension.

Recently, the concept of an association between dysbiotic gut microbiota and hypertension has been established in both animal and human studies. <sup>65–67</sup> A published study showed that intracerebroventricular administration of chemically modified tetracycline-3 (CMT-3), a tetracycline derivative with effective anti-inflammatory activity, could inhibit microglial activation and neuroinflammation in the PVN, decrease sympathetic activity and attenuate the increased mean arterial pressure in Ang II rats. In addition, the antihypertensive function of CMT-3 may be attributed to its regulatory effects on selective gut microbial communities and gut wall histopathology. <sup>68</sup> Kefir, a probiotic obtained from the fermentation of milk by kefir grains, was shown to decrease BP and improve endothelial dysfunction in SHR. <sup>69,70</sup> One study indicated that the antihypertensive effects of kefir treatment, mediated at least in part through improved structural and functional integrity of the intestinal wall, abolished microglial activation and protection against neuroinflammation within the PVN and RVLM. <sup>71</sup> These observations suggested the involvement of microglial activation in the regulation of selective gut microbiota and implicated these cells in BP control and brain-gut communication dysfunction in hypertension.

Additionally, aerobic training restores PVN autonomic nerve dysfunction, HMGB1 content, microglial activation, and inflammation to normal in SHR. Aerobic training reduces microglial activation and the expression of pro-inflammatory cytokines, ultimately improving autonomic control and reducing BP and HR in SHR.<sup>72</sup> Some relevant clinical evidence also suggests the feasibility of aerobic training in attenuating hypertension.<sup>73</sup> A comprehensive summary of these findings is shown in Table 1.

## The Potential Role of Microglia in Myocardial Infarction

After myocardial infarction, microglial activation in the PVN of the hypothalamus has been observed,<sup>74</sup> and increased levels of pro-inflammatory cytokines in the PVN then activate the hypothalamus-pituitary-adrenal axis, increase the activity of the sympathetic nervous system and contribute to the acute pro-inflammatory response in the myocardium after myocardial infarction.<sup>75</sup> In addition, activated microglia were also detected in the RVLM, NTS and periaqueductal gray (PAG), regions known to have important cardiovascular regulatory functions.<sup>76</sup> In a rat myocardial infarction model, the average number of microglia was not changed, but the proportion of activated microglia in the PVN was increased. The activation of microglia starts at 4 weeks and is sustained until 16 weeks after myocardial infarction.<sup>77</sup> At 24 h and 1 week after myocardial infarction, a significant increase in the proportion of activated microglia was not observed. Furthermore, an ICV infusion of minocycline, beginning one week prior to infarction, significantly attenuated the increase in microglial activation by at least 50% in the PVN, RVLM, PAG and NTS, and neuronal activation was significantly reduced by 50% in the PVN and virtually abolished in the PAG, RVLM and NTS.<sup>76</sup> Thus, myocardial infarction potentially induces microglial activation, and activated microglia contribute to increased neuronal activity.

P2X receptors are recognized as ligand-gated ion channels that respond to extracellular ATP.<sup>78</sup> Among them, the P2X<sub>7</sub> purinergic receptor (P2X<sub>7</sub>R) has been identified as a key mediator of inflammation.<sup>79</sup> Based on accumulating evidence, P2X<sub>7</sub>R is involved in regulating cardiovascular activity both in peripheral and central regions.<sup>79,80</sup> Colocalization of P2X<sub>7</sub>R with the microglial marker Iba-1 suggests that P2X<sub>7</sub>R is expressed on microglia rather than on neurons, and an intraperitoneal injection of P2X<sub>7</sub>R antagonists or P2X<sub>7</sub> siRNA attenuates the increased levels of pro-inflammatory cytokines in the PVN and the augmented sympathetic nervous system activity after myocardial infarction, which may contribute to improved cardiac function.<sup>81</sup>

Macrophage-induced type C lectin (Mincle) is a key C-type lectin receptor that was originally discovered based on the potent induction of macrophages by inflammatory stimuli. It is rarely expressed under normal conditions but is strongly activated after stimulation with apoptotic fragments, necrotic cells, heat shock proteins, and nucleic acid fragments.<sup>82</sup> Recently, Mincle expression was reported to be localized in microglia within the PVN, and its expression was markedly increased at 24 hours post-MI, together with sympathetic hyperactivity. Targeted knockdown of Mincle expression in the PVN attenuated microglial activation and sympathetic nerve activity, which contributed to decreased ventricular arrhythmia susceptibility post-MI.<sup>83</sup> Furthermore, the NOD-like family NLRP3/IL-1 $\beta$  axis in the PVN mediates the cardioprotective effects of Mincle inhibition. Targeting the Mincle signaling pathway in the PVN represents a novel approach to reduce the sympathetic hyperactivity post-MI, likely limiting the complications associated with MI.

The effect of TLR4 on microglia was also reported for MI. In a rat model of MI, TLR4 was primarily localized in microglia, and its expression increased markedly within the PVN at 3 days post-MI. TLR4 knockdown via shRNA microinjection into the PVN resulted in a decreased degree of microglial activation, decreased activation of Fos protein (+) neurons in the PVN and ameliorated sympathoexcitation after MI.<sup>84</sup> In addition, TLR4 knockdown in the PVN decreased the incidence of malignant ventricular arrhythmias following MI. However, another study showed that TLR4 colocalizes with GRP78, a marker of endoplasmic reticulum stress, in PVN neurons, and acute LPS treatment increases the expression of the TLR4 and TNF- $\alpha$  proteins in the PVN, which contributes to an increased HR and plasma norepinephrine concentration and decreased heart rate variability (HRV) and high frequency (HF) components of HRV.<sup>61</sup> Further inhibition of TLR4 or endoplasmic reticulum stress attenuates LPS-induced microglial activation, indicating that TLR4 signaling promotes autonomic dysfunction, inflammation and microglial activation through neuronal ER stress in the PVN. Thus, the exact mechanisms by which central TLR4 regulates neuroinflammation and sympathetic activity require further research. A comprehensive summary of these findings is shown in Table 2.

**Table I** The Potential Role of Microglia in Hypertension

Model of Hypertension		Treatment Details	Major Findings	Conclusion	References
Animals	Protocol				
Male C57BL/6 and CD11b-DTR mice (8–10 weeks old)	Subcutaneous infusion of Ang II (1000 ng/kg/min) or oral administration of L-NAME (1.5 ng/mL in drinking water) for 4 weeks	DT ICV (1000 pg/g/d) to CD11b-DTR mice	The loss of microglia led to downregulated IL-1 $\beta$ and TNF- $\alpha$ expression in the CNS and decreased the levels of plasma vasopressin, kidney NE, and NMDA.	Microglia are the major cellular factors involved in neuroinflammation and BP regulation.	[32]
Male C57BL/6 mice (12–16 weeks old)	Implanted subcutaneously with a DOCA-silicone sheet (DOCA 1 mg/g body weight) and receiving 1% NaCl drinking water	1. Kinin B1 receptor knockout; 2. R715, a specific B1R antagonist (70 $\mu$ g/kg/day)	1. DOCA administration upregulated the expression of Kinin B1R in the PVN and RVLM. 2. Kinin B1R deletion or blockade decreased BP, attenuated neuroinflammation and oxidative stress, and restored autonomic function.	Kinin B1R blockade may represent a novel strategy to reduce neuroinflammation, oxidative stress, and sympatho-excitation in neurogenic hypertension.	[47]
Male C57BL/6j mice (10 months old, 30–35 g)	1. Infusion of Ang II (0.5 ng/kg/day in 9% NaCl) via an osmotic mini pump. 2. TREM2 overexpression in vitro	None.	1. Ang II induced hypertension exacerbated $\beta$ -amyloid deposition and neuronal apoptosis, increased the number of activated microglia in the cortex and hippocampus of mice, and upregulated microglia TREM2. 2. TREM2 overexpression reversed M1 microglia-induced neuronal toxicity and decreased neuroinflammation.	1. TREM2 plays an anti-neuroinflammatory role in microglia. 2. Controversy remains regarding whether TREM2 participates in the regulation of BP.	[55]
Male C57BL/6 mice (25 $\pm$ 5 g)	Electric foot shock with noises induced a stressed condition	RAGE knockout via Cre-CX3CR1/RAGE <sup>fl/fl</sup> mice	1. Stress exposure increased the cytoplasmic translocation of HMGB1 in microglia. 2. Microglia-specific knockout of RAGE decreased M1 phenotypic polarization, MAP and sympathetic activities.	Reducing neuroinflammation and SNS activity is associated with BP regulation	[57]
Male C57BL/6, CD11b-DTR and CX3CR1-GFP mice (8–10 weeks old)	ICV infusion of Ang II (500 ng/kg/min)	ICV infusion of a TGF- $\beta$ neutralizing antibody (50 $\mu$ g/day), recombinant TGF- $\beta$ 1 (50 ng/day), or DT (800 pg/g BW/day)	1. Blockade of TGF- $\beta$ signaling further increased BP in Ang II-treated mice. 2. The recombinant TGF $\beta$ 1 treatment reversed the increased levels of MHC-II and TNF- $\alpha$ , reduced BP reduction, and upregulated the expression of pSMAD 2/3 and COX-2.	Surveillant microglia are tightly regulated by TGF $\beta$ , which are critical for maintaining the homeostasis of the CNS and blood pressure.	[58]

Male Wistar–Kyoto rats and SHR rats (6 months old, 200–230 g)	Spontaneously hypertensive rats	Gavage of calcitriol (100 ng/kg)	Calcitriol treatment had no significant effect on BP regulation but it significantly decreased the number of Iba-1 <sup>+</sup> cells and levels of IL-1 $\beta$ and TNF $\alpha$ , and shifted microglia polarization from the M1 to M2 phenotype	VitD is neuroprotective in the hypertensive brain by modulating the brain ACE2/Ang(1–7)/MasR axis	[60]
Male Wistar–Kyoto rats and SHRs (7–8 weeks old, 175–225 g)	Spontaneously hypertensive rats	Intraperitoneal (i.p.) administration of the TLR4 antagonist TAK-242 (2 mg/kg/d) for 2 weeks	1. TLR4 inhibition abolished microglia activation and preserved the BBB integrity in the PVN, RVLM, and NTS of SHRs. 2. TLR4 blockade attenuated the progression of MAP increases in SHRs and protected against autonomic dysfunction.	TLR4 represents a viable alternative target in the treatment of hypertension.	[64]
Male Sprague–Dawley rats (250–280 g); Male SHRs, Wistar–Kyoto rats (6 weeks old)	Subcutaneous infusion of Ang II (200 ng/kg/min).	ICV infusion of CMT-3 (3.5 $\mu$ g/h)	1. The ICV infusion of CMT-3 decreased the number of microglia and percentage of activated microglia. 2. Microglia inhibition also contributed to reduced expression of IL-1 $\beta$ , TNF- $\alpha$ , and TIMP-1, and decreased MAP.	The link between microglia and certain microbial communities may have implications for the treatment of HTN.	[68]
SHRs and Wistar–Kyoto rats (12 weeks old)	Spontaneously hypertensive rats	Aerobic training for 5 days/wk, 1 h/day for 2 wks	Short-term aerobic training contributed to the decrease in HR, Iba-1 <sup>+</sup> cells, levels of pro-inflammatory cytokines, and the HMGB1 content and CXCR4 signaling in the PVN.	Aerobic training regulates microglia activation and the production of pro-inflammatory cytokines in the presence of hypertension	[72]

**Abbreviations:** DTR, diphtheria toxin receptor; Ang II, angiotensin II; L-NAME, N( $\omega$ )-nitro-L-arginine methyl ester; DT, diphtheria toxin; ICV, intracerebroventricular injection; IL-1 $\beta$ , interleukin-1 $\beta$ ; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; CNS, central nervous system; NE, norepinephrine; NMDA, N-methyl-D-aspartate; BP, blood pressure; RVLM, rostral ventrolateral medulla; SHRs, spontaneously hypertensive rats; CMT-3, chemically modified tetracycline-3; TIMP-1, tissue inhibitor of metalloproteinase-1; MAP, mean arterial pressure; HTN, hypertension; CVLM, caudal ventrolateral medulla; RA, renin-angiotensinogen transgenic; AGT, angiotensinogen; AAV, adeno-associated virus; eGFP, enhanced green fluorescent protein; AT1, Ang II type 1; AVP, arginine vasopressin; CX3CR1, C-X3-C motif chemokine receptor 1; sFKN, soluble fractalkine; NTS, nucleus tractus solitarius; IL-6, interleukin-6; DOCA, deoxycorticosterone acetate; TREM2, triggering receptor expressed on monocytes 2; RAGE, advanced glycation end product receptor; HMGB1, high-mobility group Box 1; SNS, sympathetic nervous system; TGF- $\beta$ , transforming growth factor- $\beta$ ; MHC-II, major histocompatibility complex-II; pSMAD2/3, phosphorylated mothers against decapentaplegic 2/3; COX-2, cyclooxygenase-2; Iba1, ionized calcium-binding adapter molecule 1; VitD, vitamin D; ACE2, angiotensin I-converting enzyme 2; Ang (1–7), angiotensin (1–7); CXCR4, C-X-C chemokine receptor type 4; HR, heart rate; PVN, hypothalamic paraventricular nucleus.

**Table 2** The Potential Role of Microglia in Myocardial Infarction and Cardiac I/R Injury

Model of MI or I/R		Treatment Details	Major Findings	Conclusion	References
Animals	Protocol				
Male Sprague–Dawley rats	Ligation of the left anterior descending coronary artery	Infusion of minocycline (172 ng/mL, 0.3 µL/h)	1. After 12 weeks, MI contributed to dramatically increased numbers of activated microglia. 2. Minocycline treatment decreased the number of Iba-1 <sup>+</sup> and FRA <sup>+</sup> cells in the PVN and the percentage of activated microglia in the PAG and RVLM. 3. No evidence of improved heart function with minocycline.	Inflammation occurs in brain nuclei, and inhibition of microglia activation may not be sufficient to ameliorate cardiac dysfunction.	[76]
Male Sprague–Dawley rats (200–250 g)	Coronary artery ligation	Intraperitoneal (i.p.) administration of the P2X7R antagonist Brilliant Blue-G (BBG, 25 or 50 mg/kg injection per day for 5 days) before surgery	1. Colocalization of P2X7R with Iba-1 in the PVN rather than neurons was observed after MI surgery. 2. BBG application reduced the expression of P2X7R, IL-1β and TNF-α in the PVN, and improved cardiac function by decreasing MAP, HR, IVP and LVEDP, and the LF/HF ratio.	Inhibition of P2X7R activation in the PVN may be an effective method for the current treatment of AMI.	[81]
Male Sprague–Dawley rats (50–60 days, approximately 280 g)	Coronary artery ligation	PVN microinjection of Mincle siRNA 24 hours prior to MI surgery (250 pmol/50 nL)	1. The upregulation of Mincle receptor, NLRP3/IL-1β pathway were observed post-MI, and IF suggested that the Mincle receptor colocalized with microglia within the PVN. 2. Mincle knockdown decreased the number of Iba-1 <sup>+</sup> cells, inhibited the NLRP3/IL-1β pathway in the PVN and reduced RSNA and NE concentrations.	Inhibition of Mincle ameliorates sympathetic hyperactivity mediated by the NLRP3/IL-1β pathway.	[83]
Male Sprague–Dawley rats (50–60 days, approximately 260 g)	Coronary artery ligation of LAD	Silence the TLR4 gene in microglia of the PVN via a shRNA	1. After MI, TLR4 was activated predominantly in microglia in the PVN, and NF-κB signaling and ROS production were upregulated. 2. TLR4 gene silencing contributed to the decreased levels of NE and RSNA, and induced the downregulation of NF-κB, IL-1β, and TNF-α expression.	Inhibition of TLR4 attenuated sympathoexcitation.	[84]
Sprague–Dawley rats (250–300 g)	Ligation of the LAD for 30 min and reperfusion for 3 h	LED light source (610 nm) illumination	LED illumination significantly inhibited LSG neural activity and decreased microglia activation and the levels of IL-1β, TNF-α and NGF	LED therapy reduced microglia activation and pro-inflammatory cytokine expression after cardiac I/R	[90]

**Abbreviations:** MI, myocardial infarction; FRA, fos-related antigens; IVP, intraventricular pressure; LVEDP, left ventricular end-diastolic pressure; NLRP3, NOD-like receptor protein 3; RSNA, renal sympathetic nerve activity; TLR4, Toll-like receptor 4; NF-κB, nuclear factor kappa-B; LAD, left anterior descending; I/R, ischemia/reperfusion; LED, light emitting diode; NGF, nerve growth factor.

## The Potential Role of Microglia in Cardiac Ischemia/Reperfusion Injuries

Studies have shown increased microglial activity in the caudate putamen and hippocampus after cardiac I/R injury.<sup>85,86</sup> The timing of microglial activation following cardiac I/R was investigated. It was demonstrated that after coronary artery ligation for 30 min followed by various reperfusion durations, the level of microglial activation peaked at 3 days after reperfusion,<sup>87</sup> suggesting a potential role of microglial activation in cardiac I/R injury.

Light-emitting diode (LED) therapy has been shown to attenuate neuroinflammatory responses by inhibiting the activation of microglia.<sup>88,89</sup> Thus, LED therapy may protect against myocardial I/R injury by attenuating microglia and sympathetic activation. Recently, our studies showed that LED therapy (2.0 J/cm<sup>2</sup>, 610 nm) located at the skull surface of the hypothalamic PVN through the scalp and skull from 30 min before ischemia to 3 h after reperfusion could significantly attenuate the ischemia and infarct size following cardiac I/R.<sup>90</sup> In addition, LED illumination significantly reduced the inducibility of ventricular arrhythmias after I/R injury. The attenuated activation of microglia and subsequently decreased peripheral sympathetic activity contribute to the protective effects of LED therapy against cardiac I/R injury.

## Conclusions

In summary, microglia play an important role in the crosstalk between the CNS and the peripheral nervous system, and interventions targeting microglia may represent promising potential therapies for cardiovascular diseases, including hypertension, myocardial infarction, heart failure, cardiac ischemia/reperfusion and ventricular arrhythmias.

## Data Sharing Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests.

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