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Mineralocorticoid Receptors in Immune Cells; Emerging Role in Cardiovascular Disease

Nicholas C. Bene^a, Pilar Alcaide^{a,b,c}, Henry H. Wortis^{a,b}, and Iris Z. Jaffe^{a,b,c}

^aTufts University School of Medicine, Boston, MA, USA

^bSackler School of Graduate Biomedical Sciences, Boston, MA, USA

^cTufts Medical Center, Molecular Cardiology Research Institute, Boston, MA, USA

Abstract

Mineralocorticoid receptors (MR) contribute to the pathophysiology of hypertension and cardiovascular disease in humans. As such, MR antagonists improve cardiovascular outcomes but the molecular mechanisms remain unclear. The actions of the MR in the kidney to increase blood pressure are well known, but the recent identification of MRs in immune cells has led to novel discoveries in the pathogenesis of cardiovascular disease that are reviewed here. MR regulates macrophage activation to the pro-inflammatory M1 phenotype and this process contributes to the pathogenesis of cardiovascular fibrosis in response to hypertension and to outcomes in mouse models of stroke. T lymphocytes have recently been implicated in the development of hypertension and cardiovascular fibrosis in mouse models. MR activation *in vivo* promotes T lymphocyte differentiation to the pro-inflammatory Th1 and Th17 subsets while decreasing the number of anti-inflammatory T regulatory lymphocytes. The mechanism likely involves activation of MR in antigen presenting dendritic cells that subsequently regulate Th1/Th17 polarization by production of cytokines. Alteration of the balance between T helper and T regulatory lymphocytes contributes to the pathogenesis of hypertension and atherosclerosis and the associated complications. B lymphocytes also express the MR and specific B lymphocyte-derived antibodies modulate the progression of atherosclerosis. However, the role of MR in B lymphocyte function remains to be explored. Overall, recent studies of MR in immune cells have identified new mechanisms by which MR activation may contribute to the pathogenesis of organ damage in patients with cardiovascular risk factors. Conversely, inhibition of leukocyte MR may contribute to the protective effects of MR antagonist drugs in cardiovascular patients. Further understanding of the role of MR in leukocyte function could yield novel drug targets for cardiovascular disease.

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Correspondence to: Iris Z. Jaffe, M.D./Ph.D. Tufts Medical Center, Molecular Cardiology Research Institute 800 Washington St, Box 80, Boston, MA 02111. ijaffe@tuftsmedicalcenter.org Phone: 617-636-0620 Fax: 617-636-1444.

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Keywords

mineralocorticoid receptor; aldosterone; macrophage; T lymphocyte; cardiovascular disease; hypertension

Introduction

The mineralocorticoid receptor (MR) is a member of the steroid receptor family of hormone activated transcription factors. The classical MR ligand is the adrenal hormone aldosterone that regulates blood pressure by activating MR in the kidney to promote renal sodium retention. Ample clinical data support that aldosterone and MR contribute to the risk and poor clinical outcomes for patients with hypertension, heart failure, myocardial infarction (MI), and stroke[1–3]. Indeed, MR antagonist drugs improve outcomes in cardiovascular patients out of proportion to modest changes in blood pressure and renal sodium handling, supporting extra-renal mechanisms[4–7]. Over the past two decades, it has become clear that the MR is expressed and functional in tissues outside the kidney[8–11] and that these extra-renal MRs contribute to cardiovascular disease. The role of vascular MRs in cardiovascular disease has been previously reviewed[12–14]. A role for inflammation in the initiation and progression of cardiovascular diseases has emerged. A critical contribution of chronic inflammation to atherosclerosis progression and complications (MI and stroke) has been known for some time and more recently inflammation has also been implicated in hypertension and heart failure. MR activation contributes to cardiovascular inflammation, however, the direct contribution of MR in immune cells to cardiovascular disease has only recently been considered and the available data in support of this new concept is summarized in this review. We specifically focus here on the role of MR in macrophages, dendritic cells (DC), T lymphocytes and B lymphocytes. As there is limited data on the role of MR in other immune cells in cardiovascular disease, including neutrophils and monocytes, these cells are not considered here.

The Renin-Angiotensin-Aldosterone System (RAAS)

The Renin-Angiotensin-Aldosterone system (RAAS) is a hormonal cascade that regulates electrolyte balance and blood pressure[15]. In response to decreased blood pressure, the kidney releases the protease renin to cleave circulating angiotensinogen, culminating in the production of angiotensin II (AngII) by the angiotensin converting enzyme (ACE). AngII acts through two angiotensin receptors (AT1R and AT2R) and via AT1R on adrenal cells, promoting aldosterone release. Aldosterone activates renal MRs to enhance renal re-absorption of sodium and water, thereby increasing blood pressure[17]. The classical MR ligand is aldosterone (or deoxycorticosterone (DOCA) in rodents), however, the receptor also binds glucocorticoids, including cortisol, with equal affinity. Since cortisol circulates at substantially higher concentrations than aldosterone, the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) is thought to be necessary for aldosterone responsiveness in epithelial cells by locally inactivating cortisol.[17]. With the discovery of MR in non-epithelial cells, the determination of the endogenous ligand in these cells has been controversial. In steroid free media *in vitro* or with high dose infusion *in vivo*, aldosterone will activate MR in all cells in which it is expressed. Some non-epithelial cells, including

vascular smooth muscle and endothelial cells, appear to express 11 β -HSD2 and hence are thought to be aldosterone-responsive[9;10], while cardiomyocytes do not and hence cortisol may be the cardiac MR ligand[18]. Thus, we also review what is known about expression of 11 β -HSD2 in leukocytes. Whether there are other mechanisms protecting MR from being occupied by cortisol, thereby conferring aldosterone-responsiveness to MR in leukocytes that do not express 11 β -HSD2, remains to be explored.

RAAS inhibitors, including ACE inhibitors, angiotensin receptor blockers, and MR antagonists, are used extensively in the treatment of hypertension and heart failure and to reduce the incidence of MI, stroke, and death[4–6;19;20]. Components of the RAAS have recently been identified in immune cells, raising the possibility that inhibition of RAAS in immune cells could be contributing to the beneficial effects of these drugs in cardiovascular patients. Here we review recent advances in our understanding of the role of MR in immune cells including macrophages, about which there is substantial new information, followed by dendritic cells, T lymphocytes and B lymphocytes about which less is known. In the first section, we summarize available data on expression of RAAS components in each type of leukocyte and the impact of MR activation on the immunologic functions of each immune cell. In the second section, we review what is known about roles of MR in each immune cell in cardiovascular disease.

I-RAAS and MR in Leukocytes

RAAS Expression in Macrophages

Macrophages are innate immune cells that arise from circulating monocytes upon infiltration into tissues. Bone marrow derived macrophages express both the MR and the related glucocorticoid receptor (GR). Levels of the two steroid receptors in macrophages are modulated in a stimulus specific way, with lipopolysaccharide (LPS) up-regulating GR expression and down-regulating MR expression, and interferon gamma (IFN- γ) increasing both MR and GR[21]. Table 1 summarizes what is known to date about macrophage expression of RAAS components. The AT1R is expressed constitutively in macrophages, while other components of the RAAS remain to be investigated [21](Table 1). Current evidence supports that macrophages do not express 11 β -HSD2[22;23] and therefore glucocorticoids, including cortisol, are predicted to be the activating ligand for MR in macrophages *in vivo*. However, the possibility that other protective mechanisms exist, resulting in a role for aldosterone as an MR ligand in macrophages under specific conditions, cannot be ruled out.

MR in Macrophage Function

Activated macrophages have diverse phenotypes that determine their effector functions. Classically activated macrophages, also called M1 macrophages, are activated by the cytokine IFN- γ , resulting in their potent microbicidal functions that also contribute to tissue inflammation, oxidative stress, and damage. Macrophages can be alternatively activated to the M2 phenotype that is involved in fibrosis and tissue remodeling[24]. Recent evidence indicates a role for MR in macrophage polarization. *In vitro* cultured thioglycolate-elicited mouse peritoneal macrophages were treated with aldosterone in steroid-depleted media, and

MR activation under these conditions resulted in increased expression of the M1 classical activation markers TNF α , RANTES, MCP1 and IL-12. The MR antagonist spironolactone prevented induction of these markers by LPS, supporting a role for macrophage MR[23]. Similarly, in an immortalized mouse microglial cell line, which are macrophage-like cells of the central nervous system, MR activation with aldosterone or low dose corticosteroids potentiated LPS-induction of the pro-inflammatory cytokines TNF α and IL-6 in an MR-, but not in a GR-dependent manner[25]. The transcription factor NF κ B regulates the expression of these cytokines in a variety of immune cells[26], and NF κ B is activated by aldosterone in macrophages in an MR-dependent manner, suggesting a potential mechanism for MR regulation of macrophage polarization. Conversely, GR-activation resulted in inhibition of NF κ B in the same microglial cells[25].

The role of macrophage MR was further studied in peritoneal macrophages taken from mice with MR specifically deleted from macrophages (Mac-MR-KO). MR-deficient macrophages showed reduced expression of M1 markers, decreased responsiveness to LPS-induced activation, and a shift toward the alternative-activated M2 phenotype[23](Figure 1). Classically activated macrophages produce reactive oxygen species (ROS) to enhance microbe destruction upon phagocytosis. However, this function contributes to the role of macrophages in cardiovascular disease, in which oxidative stress is an exacerbating factor. The role of macrophage MR in ROS production in the setting of cardiovascular diseases was studied in the context of atherosclerosis using Apolipoprotein E knockout mice (ApoE-KO). ApoE-KO mice treated with the MR antagonist eplerenone *in vivo* exhibited reduced atherosclerosis. Peritoneal macrophages isolated from eplerenone-treated ApoE-KO mice produced significantly less ROS and oxidized less low density lipoprotein (LDL) *in vitro*[27]. Overall, the available data support the conclusion that the MR in macrophages contributes to classical macrophage activation to the M1 pro-inflammatory phenotype, and that MR blockade or deletion in macrophages prevents classical macrophage activation and macrophage-induced oxidative stress(Figure 1).

RAAS Expression in T and B Lymphocytes

T and B lymphocytes are adaptive immune cells capable of recognizing and distinguishing antigens with functions that are characterized by specificity and memory. T and B lymphocytes arise from the bone marrow and populate the peripheral lymphoid organs (spleen and lymph nodes), where they complete their maturation and become activated in response to specific antigens presented by antigen presenting cells, including dendritic cells (DC). Upon antigen presentation, naïve lymphocytes can become effector cells or memory cells. Effector B lymphocytes produce antibodies that contribute to the elimination of extracellular microbes. Effector T lymphocytes can be separated into CD8+ cytotoxic T lymphocytes, which kill virus infected cells, and subsets of CD4+ T lymphocytes, including T helper (Th) cells that help other immune cells carry out their functions, and T regulatory (Treg) cells that suppress the functions of effector T lymphocytes. There are several well-defined subtypes of Th cells characterized by distinct regulatory transcription factors, production of different cytokines, and distinct effector functions. These include Th type 1 (Th1) cells that produce IFN- γ , which activates macrophages and other cells; Th type 17 (Th17) cells that produce IL-17, IL-21 and IL-22; and T helper 2 (Th2) cells that produce

IL-4, IL-5 and IL-13, help clear parasitic infections, and promote antibody production in B lymphocytes. In addition, these subtypes play different roles in disease, with Th1 and Th17 being highly pro-inflammatory and strongly associated with chronic inflammation, autoimmune disease, and cardiovascular disease, and Th2 with atopic diseases and allergic reactions[28].

In 1988, Armanini et al. observed that mouse splenic T lymphocytes contain proteins capable of binding to aldosterone and cortisol, supporting the presence of MR and GR proteins in T lymphocytes[29]. However, more recently, the level of MR expression in T lymphocytes was found to be very low when directly compared to kidney or dendritic cells, particularly in CD8⁺ T lymphocytes[30]. T lymphocytes also produce AngII and express AT1R, renin, the renin receptor, angiotensinogen, ACE, and the AT2R[31–34] (Table 1). 11 β -HSD2 expression has not been explored in T lymphocytes, so further studies are needed to determine whether the endogenous ligand for T lymphocyte MR is cortisol or aldosterone. Much less is known about the RAAS in B lymphocytes. Mouse and human B lymphocytes express MR, GR, and AT1R[29;34]. The affinity of MR and GR for ligand are similar in T and B lymphocyte populations from the human spleen, as determined by their dissociation constants and number of binding sites for each hormone[29]. However, further studies are needed to explore the expression of 11 β -HSD2 and other RAAS pathway components in B lymphocytes (Table 1).

Role of RAAS and MR in T and B Lymphocyte Function

T cells respond directly to AngII via the AT1R *in vitro*, resulting in enhanced proliferation of wild-type but not AT1aR-deficient mouse splenic naive T lymphocytes[31;32]. AngII treatment induced the expression of the early activation marker CD69 in mouse T lymphocytes *in vitro* and *in vivo*, indicating that naive T lymphocytes can be activated by AngII via the AT1aR[33]. Much less is known about the direct role of T and B lymphocyte MR in adaptive immune function, as there is very limited data exploring MR activation in lymphocytes *in vitro* and mice specifically lacking T or B lymphocyte MR have not been studied.

In vivo, DOCA treatment of mice increased polarization of T cells toward the Th1/Th17 phenotype with exacerbation of experimental autoimmune encephalomyelitis, an autoimmune disease that mimics human multiple sclerosis and is dominated by Th1 and Th17 effector immune responses. The effects of DOCA on Th17 polarization were inhibited by treatment with the MR antagonist eplerenone[30] or spironolactone[35], supporting a role for MR. However, data from Herreda et al. indicate that this is likely mediated by dendritic cells that also express MR[30]. Indeed, direct activation of T cells with anti-CD3 and aldosterone did not affect expression of T lymphocyte activation markers, including CD69 or IL-2. However, pretreatment of DC with aldosterone followed by co-culture with purified T cells resulted in CD8⁺ T lymphocyte activation, producing IL-2 and polarizing CD4⁺ T lymphocytes to produce IL-17. Aldosterone activation of DC MR increased DC expression of IL-6 and TGF β , and these factors likely mediate the effects of aldosterone-treated DCs on T lymphocyte Th1/Th17 polarization (Figure 1). DOCA treatment *in vivo* also decreased

Treg cell abundance in an MR-dependent manner[35]. However, the cell type in which MR activation mediates these effects remains unclear.

Despite the early reports showing MR expression in B lymphocytes, the role of MR in B lymphocyte function remains completely unexplored. While studies support the presence of functional AT1R and MR, the direct effects of aldosterone, DOCA or MR antagonists on B lymphocyte function remains to be studied.

II-Role of Leukocyte MR in Cardiovascular Diseases

Leukocyte MR in Hypertension

The new appreciation for the existence of functional MR in leukocytes supports the potential for leukocyte MR to contribute directly to cardiovascular disease, perhaps by promoting the pro-inflammatory M1 macrophage phenotype or by enhancing Th1/Th17 T lymphocyte polarization(Figure 2). MR contributes substantially to the development of hypertension by promoting volume retention in the kidney and likely also by increasing tone in the vasculature[36;37]. Hyperaldosteronism is thought to directly contribute to the cause of hypertension in 6 percent of patients with essential hypertension, 12 percent of those with severe hypertension, and over 20 percent of patients with resistant hypertension[38]. Moreover, the blood pressure lowering effects of MR antagonists do not correlate completely with evidence of renal electrolyte regulation, supporting the concept that extra-renal mechanisms contribute to MR control of blood pressure in humans[39]. A role for immune cells in RAAS-associated hypertension has recently been demonstrated, supporting the potential for leukocyte MR to directly contribute to blood pressure control[40–42].

T and B lymphocyte MR in Hypertension

In the absence of a pathophysiological stimulus, T and B lymphocytes do not appear to contribute to systemic blood pressure, as basal blood pressure is not altered in mice lacking all T and B lymphocytes (RAG1^{-/-} mice)[33]. However, both the “DOCA/salt” (uninephrectomy-DOCA-high salt) and the AngII infusion hypertension models are associated with an increase in circulating and vascular T lymphocytes[33;43]. DOCA/salt or AngII infusion are also associated with increased IL-17 production from T lymphocytes and IL-17 protein in the heart and vessel wall[35;44]. RAG1^{-/-} mice exhibit a blunted hypertension response to both of these RAAS-induced hypertension models that is rescued by adoptive transfer of T lymphocytes, but not B lymphocytes[33]. T lymphocytes require intact AT1R to reconstitute RAAS-associated hypertension[33]. IL-17 deficient mice also have a blunted hypertensive response to AngII[44] and IL-17 blocking antibody attenuates the hypertensive response to DOCA/salt[35]. Other T lymphocyte subtypes may protect from hypertension, as adoptive transfer of Treg prevented AngII-induced hypertension, vascular oxidative stress, and vascular inflammation in some[32;45] but not in all studies[46]. Human data also support a role for T lymphocytes in hypertension, as alloactivated T lymphocyte infusion in cancer patients increased blood pressure[47], and CD4⁺ T lymphocyte deficiency due to human immunodeficiency virus infection is associated with a lower than expected incidence of hypertension[48]. In addition, two recent reports indicate that high concentrations of sodium promote human and mouse Th17 cell

differentiation[49;50]. Thus, T lymphocytes, particularly the Th17 subset, contribute to RAAS-induced hypertension, whereas Treg cells are protective[40;41]. Although AT1R are necessary for T lymphocytes to contribute to hypertension and AT1R-MR crosstalk has been demonstrated in vascular cells[10;51], a direct role for T lymphocyte MR in RAAS-associated hypertension has not yet been explored. Experiments involving the specific deletion of T lymphocyte MR in the context of normal MR function elsewhere would provide greater clarity.

Macrophage MR in Hypertension

The availability of mice specifically deficient in macrophage MR has shed some light on the potential role of macrophage MR in blood pressure regulation, although some areas still remain controversial. Both Rickard et al. and Usher et al. demonstrated that basal blood pressure is not altered in mac-MR-KO mice compared to MR intact littermates[23;52]. Specifically, systolic and diastolic blood pressure, pulse pressure, and diurnal blood pressure variation are intact in mice lacking macrophage MR[23]. However, the role of macrophage MR in the blood pressure response in different hypertension models is more controversial. Using tail cuff plethysmography, Rickard et al. found that the significant rise in blood pressure in the DOCA/salt model was not observed at 4 weeks and likely attenuated at 8 weeks in mice lacking macrophage MR[52]. The authors concluded that macrophage MR contributes to the blood pressure phenotype in this hypertension model[52]. On the contrary, Usher and colleagues induced hypertension by treating mice with high salt and the nitric oxide synthesis inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) for 10 days and then adding AngII infusion for 5 days ("Ang II/L-NAME" model)[23]. In this study, the rise in blood pressure with salt/L-NAME/AngII as measured using the more accurate method of radiotelemetry was preserved in mac-MR-KO mice. In fact, the rise in systolic blood pressure and pulse pressure was exacerbated in mice mac-MR-KO mice in the sleep cycle, resulting in a loss of the diurnal variation in blood pressure[23]. These authors conclude that in the Ang II/L-NAME hypertension model, macrophage MR is not necessary for blood pressure elevation but might contribute to diurnal blood pressure variation. In a third model, the hypertensive response to salt/L-NAME was also preserved despite macrophage MR-deletion[53]. The reason for the differences between these studies may include the different hypertension models, the duration of hypertension, and the method of blood pressure measurement. Overall, substantial data supports that macrophage MR does not contribute to baseline blood pressure control. A role for macrophage MR in hypertension induced by enhanced RAAS activation is not completely clear, although in most models, macrophage MR does not appear to contribute to hypertension. In light of the substantial data supporting a role for T lymphocytes in RAAS-induced hypertension, the potential for macrophage MR to contribute to hypertension by regulating T lymphocyte activation also remains to be determined. As the role for MR-activation in clinical hypertension continues to grow, both from an increase in resistant hypertension[38] and the growing obese population in which the body-mass-index has been linked to hyperaldosteronism[1], further studies are needed to clarify the role of leukocyte MR in RAAS-induced hypertension and if these mechanisms could be targeted as novel therapies for high blood pressure.

Leukocyte MR in Cardiac and Vascular Fibrosis

Cardiac and vascular fibrosis refer to the inappropriate and enhanced deposition of collagen and other extracellular matrix proteins in the heart and in the vessel wall, respectively, usually in response to cardiovascular injury or risk factors. Cardiac fibrosis contributes to heart failure with preserved ejection fraction by decreasing cardiac compliance, thereby promoting diastolic dysfunction and contributes to heart failure with reduced ejection fraction by replacement fibrosis that leads to contractile dysfunction [54–56]. Vascular fibrosis enhances vascular stiffness that contributes to hypertension, particularly in the elderly [57] (Figure 2).

Macrophage MR in Cardiovascular Fibrosis

The role of macrophage MR in cardiovascular fibrosis has been explored using the mac-MR-KO mouse. Under basal conditions, macrophage MR deletion had no obvious effect on cardiac size, cardiac fetal gene expression (ANP, BNP, MHC, markers of heart failure), or cardiac fibrosis[23;53]. However, in multiple hypertension models (DOCA/salt[52], AngII/L-NAME[23], and salt/L-NAME[53]), macrophage MR-deletion attenuated hypertension-induced cardiac fibrosis. Further exploration of the mechanism revealed differences in the role of macrophage MR in cardiac macrophage recruitment. In the DOCA/salt model, cardiac inflammatory cell infiltration was preserved in mac-MR-KO mice[52], while a different group demonstrated in the AngII/L-NAME model that mac-MR deletion prevented cardiac macrophage infiltration and inflammatory gene expression (TNF α , RANTES)[23]. Pro-oxidant gene expression (Nox2 and p22phox) was increased in the heart in the salt/L-NAME model irrespective of the presence of macrophage MR[53]. However, in all three models, the degree of cardiac interstitial fibrosis and collagen gene expression were decreased in mac-MR-KO mice[23;52;53]. Mac-MR-KO hearts had decreased basal TGF β expression only in one study[52], whereas, in all cases, macrophage MR was necessary for hypertension-induced increases in cardiac expression of pro-fibrotic regulators including TGF β [23], plasminogen activator inhibitor-1 (PAI-1)[23], and connective tissue growth factor (CTGF)[53]. Overall, although cardiac macrophage infiltration was preserved in some models of hypertension, cardiac fibrosis was prevented by deletion of macrophage MR in all models, supporting the concept that direct effects of MR on macrophage function, potentially via modulation of macrophage M1/M2 polarization, contribute to hypertension-induced cardiac fibrosis.

In the vasculature, coronary perivascular inflammation, fibrosis, aortic thickening, inflammation, and fibrosis in response to AngII/L-NAME hypertension were also attenuated by Mac-MR-KO[23], as was the degree of coronary vascular CTGF staining in the salt/L-NAME model[53]. Overall, loss of macrophage MR exerted a protective effect on cardiac and vascular fibrosis in response to hypertension that mimics many of the protective effects of MR antagonism. These studies support the possibility that macrophage MR-inhibition may be contributing to the beneficial effects of MR antagonists in patients with heart failure or hypertension.

T and B Lymphocyte MR in Cardiac and Vascular Fibrosis

T lymphocytes may contribute to cardiac and vascular fibrosis in response to hypertension in part by contributing to the elevated blood pressure as described above. However, exploration of blood pressure-independent effects of T lymphocytes in cardiovascular fibrosis is also an active area of investigation[reviewed in 58]. Recent studies have implicated IL-17, the cytokine produced by Th17 cells, in cardiac fibrosis. IL-17 blockade prevented cardiac fibrosis and ventricular dysfunction without affecting blood pressure in the spontaneously hypertensive rat model[59] and also prevented cardiac fibrosis in the isoproterenol-induced heart failure model[60]. This might be due to paracrine effects of IL-17 made by T lymphocytes on cardiac fibroblasts[61]. Thus activation of MR resulting in increased Th17 cell polarization could contribute to cardiac fibrosis by this blood pressure-independent mechanism. Indeed, very recently, Amador et al. demonstrated that treatment with IL-17 blocking antibodies prevented DOCA/salt induced cardiac and renal fibrosis[35].

In the vasculature, Th17 lymphocytes promote vascular fibrosis[44], while Treg lymphocytes prevent many of the vascular remodeling effects of mineralocorticoid- and AngII-induced hypertension models[45;46]. Specifically, adoptive transfer of Treg lymphocytes attenuated AngII-induced cardiac hypertrophy and fibrosis, vascular medial thickening, and vascular oxidative stress. Overall, by changing the balance of T lymphocyte subsets with increased Th17 and decreased Treg lymphocytes, RAAS activation in the immune system could contribute to cardiac and vascular fibrosis. Again, the direct role of lymphocyte MR in this process is not clear, as dendritic cell MR appears to be necessary for T lymphocyte polarization by MR. Thus, future studies are needed to examine the role of lymphocyte MR in the mechanism of end organ fibrosis, thereby providing new insights into how lymphocytes might be manipulated to prevent cardiovascular fibrosis and heart failure in hypertensive patients.

Leukocyte MR in Atherosclerosis, MI and Stroke

Atherosclerosis is a systemic vascular disease initiated by cardiovascular risk factors that cause endothelial cell damage and recruitment of leukocytes to the vascular wall. Atherosclerotic plaques consist of a core of lipids and inflammatory cells with a fibrous cap of smooth muscle cells and extracellular matrix. Plaques with increased lipids and inflammatory cells are prone to rupture with thrombosis, the cause of MI in the coronary circulation or stroke in the cerebral circulation. High aldosterone levels are associated with increased incidence of MI and stroke[2;3], and RAAS inhibition prevents these outcomes in humans by unclear mechanisms[20;62;63].

Macrophage MR in Atherosclerosis, MI and Stroke

Animal studies and human data support a role for aldosterone and MR in atherosclerotic plaque formation[reviewed in 14]. MR activation by aldosterone administration or 11 β -HSD2 deletion in ApoE-KO mice results in increased atherosclerotic lesion size with enhanced plaque lipid content, vascular macrophage infiltration, and macrophage oxidative stress[64–66]. MR inhibition with eplerenone reduces oxidative stress in peritoneal macrophages, thus significantly decreasing the atherogenic properties of macrophages, including their ability to oxidize and take up LDL[27]. Thus, MR contributes to plaque

macrophage infiltration, which is a cause of rupture. However, whether these effects are mediated by MR in the macrophage and/or by MR in the kidney, vasculature or elsewhere requires further investigation. Macrophages also express the GR and transplantation of bone marrow from mac-GR-KO mice into a mouse model of atherosclerosis did not alter atherosclerotic lesion size or lesion inflammation, suggesting that any potential effects of glucocorticoids on macrophage contribution to atherosclerosis are likely mediated by the MR[67]. Although the specific role of macrophage MR has not been studied in MI models, it has been examined in a rodent model of stroke. In the mouse middle cerebral artery ischemia-reperfusion model, mac-MR-KO mice had decreased classically-activated M1 macrophages with increased M2 macrophages in the infarcted area of the brain, and was associated with a significantly decreased infarct volume[68]. Thus, by modulating the macrophage phenotype, macrophage MR activation might be contributing to adverse outcomes after stroke and MR antagonists could be beneficial.

T and B Lymphocyte MR in Atherosclerosis, MI and Stroke

Since the demonstration in the 1980s that T lymphocytes are present in human atherosclerotic plaques[69], extensive data from mouse models implicate T lymphocytes in vascular inflammation during atherogenesis[70]. Mouse models that genetically lack either Th1 cells or IFN- γ reveal that both contribute to atherogenesis in mice[71;72], and IFN- γ is indeed expressed in T lymphocytes isolated from human plaques[73]. In contrast to the pro-atherogenic role of effector T lymphocytes, Treg lymphocytes play a protective role, infiltrating the developing atherosclerotic plaque and decreasing in number as the lesion progresses[74]. Thus, MR regulation of T lymphocyte subset balance[30] could contribute to the progression of atherosclerotic disease and its complications. In the ApoE-KO mouse, aldosterone infusion induced splenomegaly and early T lymphocyte infiltration into atherosclerosis-prone vascular regions, such as the aortic arch[65]. These effects of aldosterone on the adaptive immune system leading to vascular inflammation were independent of changes in traditional cardiovascular risk factors, including blood pressure, glucose, and lipid levels, supporting the potential for a direct contribution of T lymphocyte MR. Furthermore, after MI, T lymphocytes also infiltrate the infarcted human myocardium[75], and these activated CD4⁺ T lymphocytes in the heart, while initially protective[76], may be detrimental as chronic inflammation persists[75]. Thus, there are many important roles for T lymphocytes in atherogenesis and the resulting complications. However, as discussed above, the specific role of T lymphocyte MR is not fully understood. Thus, further studies using T lymphocyte specific MR-KO mice, including adoptive transfer of MR-deficient T lymphocytes into recipient atherosclerosis-prone mice, are needed to explore the specific role of T lymphocyte MR in the development of atherosclerosis and in outcomes of MI and stroke.

B lymphocytes are also present in atherosclerotic plaques and antibodies play a role in atherosclerosis, but whether they exacerbate or ameliorate pathology appears to depend on their specificity[reviewed in 77]. In mice, IgM antibodies made by B-1 cells have been identified that react with phosphorylcholine or oxidation-specific epitopes and are protective from atherosclerosis, whereas other antibodies made by the larger B-2 cell population promote atherosclerosis. Depletion of B-2 but not B-1 cells in ApoE-KO mice attenuates

atherosclerosis by decreasing vascular inflammation[78]. Opinion is divided as to whether human B cells are divided into equivalent B-1 and B-2 populations. The role of MR in B lymphocyte function and in atherosclerosis has never been explored.

Summary and Future Directions

All together, the literature supports an active role for the immune system in the development, progression, and outcome of cardiovascular disease. Exploration of the specific roles of macrophages and of T and B lymphocyte subsets in cardiovascular function is an extremely active area of investigation. The fact that the MR is expressed in the innate and adaptive immune systems and modulates immune function opens new doors to understanding the pathogenesis of aldosterone-enhanced cardiovascular disease and the beneficial effects of MR antagonists. MR promotes macrophage activation to the pro-inflammatory M1 phenotype and this process is critical in the pathogenesis of cardiovascular fibrosis in response to hypertension and to the outcome of stroke. MR activation in dendritic cells promotes differentiation of T lymphocytes to the pro-inflammatory Th1 and Th17 phenotypes and MR activation decreases the proportion of Treg lymphocytes. This imbalance between Th and Treg lymphocytes contributes to the pathogenesis of hypertension and atherosclerosis and the associated complications. While significant progress has recently been made in understanding the role of immune cell MR in cardiovascular disease, much remains to be discovered. Expression of many components of the RAAS system in specific immune cells remains to be confirmed. Glucocorticoids may be the predominant MR ligand in macrophages due to lack of 11 β -HSD2, but the interplay between MR and GR effects on macrophage function and the ligand for the MR in T and B lymphocytes remains to be clarified. Despite multiple studies using macrophage-specific MR knockout mice, a role for macrophage MR in hypertension is unclear and the mechanism by which macrophage MR promotes cardiovascular fibrosis is still an area of active investigation. Although aldosterone induces macrophage and T lymphocyte infiltration into the atherosclerotic plaque, the specific role of the leukocyte MR remains to be explored by using macrophage- and T lymphocyte-specific MR deletion in the setting of atherosclerosis. Although B lymphocytes have been known to express MR for 25 years, very little is known about the function of MR in B cells. Finally, further studies are needed in human leukocytes to establish whether MR indeed contributes to immune cell functioning and cardiovascular disease progression in humans by the same mechanisms as it does in animal models.

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Abbreviations

(11 β -HSD1)	11-betahydroxysteroid dehydrogenase type 1
(11 β -HSD2)	11-betahydroxysteroid dehydrogenase type 2

(AngII)	angiotensin II
(ACE)	angiotensin converting enzyme
(ApoE-KO)	apolipoprotein E knockout mice
(DC)	dendritic cells
(DOCA)	deoxycorticosterone
(GR)	glucocorticoid receptor
(IFN- γ)	interferon gamma
(IL)	interleukin
(LPS)	lipopolysaccharide
(Mac-MR-KO)	mice with MR specifically delete from macrophages
(MR)	mineralocorticoid receptor
(MI)	myocardial infarction
(ROS)	reactive oxygen species
(RAAS)	Renin-Angiotensin-Aldosterone system
(Th)	T helper cells
(Treg)	T regulatory cells

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Highlights

MR is expressed in macrophages, dendritic cells, and T and B lymphocytes.

MR promotes the pro-inflammatory M1 macrophage phenotype.

Macrophage MR contributes to cardiovascular fibrosis in hypertension.

MR in dendritic cells increases pro-inflammatory Th1 and Th17 T cells and decreases anti-inflammatory Treg cells.

This Th and Treg cell imbalance contributes to hypertension and atherosclerosis.

Figure 1. Role of Leukocyte Mineralocortic Receptor (MR) in Immune Cell Function

MR has been shown to be expressed in macrophages, dendritic cells, T lymphocytes, and naive B lymphocytes. MR activation by hormone ligands (L) in macrophages promotes the M1 or classically activated macrophage phenotype, which expresses tumor necrosis factor α (TNF α), monocyte chemoattractant protein-1 (MCP1), RANTES, and interleukin (IL)-12. MR inhibition or deletion in macrophages promotes the alternatively activated M2 phenotype with increased expression of M2 markers. MR activation in dendritic cells enhanced dendritic cell production of transforming growth factor beta (TGF β) and IL-6, which promote differentiation of naive T lymphocytes to the Th1 and Th17 pro-inflammatory phenotypes. The function of MR in the B lymphocyte is not known. GR=glucocorticoid receptor.

Figure 2. Role of Leukocyte Mineralocorticoid Receptor (MR) Activation in Cardiovascular Disease

Activation of the MR in macrophages (solid arrows) promotes the M1 or classically activated macrophage phenotype and contributes to cardiac and vascular fibrosis. MR activation in dendritic cells leads to differentiation of T lymphocytes to the Th1 and Th17 pro-inflammatory phenotypes, which are involved in the pathogenesis of hypertension, cardiac fibrosis, and atherosclerosis (double solid arrows). The role of activation of B lymphocyte MR in cardiovascular disease is not known. Cardiac and vascular fibrosis and atherosclerosis have previously been found to contribute to heart failure, hypertension, stroke, and myocardial infarction (dashed lines). This schema provides novel mechanisms for MR-induced cardiovascular disease by leukocyte MR modulation of macrophage and T lymphocyte polarization.

Table 1

Expression of Renin-Angiotensin-Aldosterone System components in immune cells.

Cell type	Renin	ACE	AT1R	AT2R	ATN	MR	GR	11 β HSD2
Macrophage	Not tested	Not tested	yes ^{31,32,34,47}	Not tested	Not tested	Yes ^{23,52}	Yes ^{23,52}	No ^{23,52}
T lymphocyte	Yes ^{31,32}	Yes ^{31,32}	Yes ^{31,32,33,34}	Yes ³²	Yes ^{31,47}	Yes ²⁹	Yes ²⁹	Not tested
B lymphocyte	Not tested	Not tested	Yes ^{32,34,47}	Not tested	Not tested	Yes ²⁹	Yes ²⁹	Not tested

ACE=angiotensin converting enzyme, AT1R=angiotensin receptor, type 1, ATN=angiotensinogen, MR=mineralocorticoid receptor, GR=glucocorticoid receptor, 11 β HSD=11 β -hydroxysteroid-dehydrogenase