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Mineralocorticoid receptors in immune cells: Emerging role in cardiovascular disease

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ABSTRACT

Mineralocorticoid receptors (MRs) 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.

1. 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 disproportionate 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

Abbreviations: 11β-HSD1, 11-betahydroxysteroid dehydrogenase type 1; 11β-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 deleted 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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stroke) has been known for some time and, more recently, inflammation has also been implicated in hypertension and heart failure. Although MR activation contributes to cardiovascular inflammation, 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 (DCs), 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.

1.1. 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 angiotensin converting enzyme (ACE). AngII acts through two angiotensin receptors (AT1R and AT2R) and specifically via the AT1R on adrenal cells to promote aldosterone release. Aldosterone activates renal MRs to enhance renal re-absorption of sodium and water, thereby increasing blood pressure [16]. 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 11β-hydroxysteroid dehydrogenase type 2 (11β-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 11β-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 11β-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 11β-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 the roles of MR in each immune cell in cardiovascular disease.

2. RAAS and MR in leukocytes

2.1. 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 expression [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 11β-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.

2.2. 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 TNFα, 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 TNFα and IL-6 in an MR-, but not in a GR-dependent manner [25]. The transcription factor NFκB regulates the expression of these cytokines in a variety of immune cells [26], and NFκB 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 NFκB in the same microglial cells [25].

Table 1
Expression of Renin–Angiotensin–Aldosterone System components in immune cells.

Cell type	Renin	ACE	AT1R	AT2R	ATN	MR	GR	11βHSD2
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 [31–34]	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; 11βHSD, 11β-hydroxysteriod-dehydrogenase.