The role of the mineralocorticoid receptor in immune cells in cardiovascular disease

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Funding information
MEMORY consortium grant of the ERA-CVD Joint Transnational Call 2018 by the Dutch Heart Foundation, Grant/Award Numbers: JTC2018, 2018T093, 2018T093, JTC2018; German Research Foundation, Grant/Award Number: WE 1688/19-1; IMPRESS DCVA, Grant/Award Number: 2020B,004; Dutch Heart Foundation IN-CONTROL II CVON, Grant/Award Number: CVON2018-27

Chronic low-grade inflammation and immune cell activation are important mechanisms in the pathophysiology of cardiovascular disease (CVD). Therefore, targeted immunosuppression is a promising novel therapy to reduce cardiovascular risk. In this review, we identify the mineralocorticoid receptor (MR) on immune cells as a potential target to modulate inflammation. The MR is present in almost all cells of the cardiovascular system, including immune cells. Activation of the MRs in innate and adaptive immune cells induces inflammation which can contribute to CVD, by inducing endothelial dysfunction and hypertension. Moreover, it accelerates atherosclerotic plaque formation and destabilization and impairs tissue regeneration after ischaemic events. Identifying the molecular targets for these non-renal actions of the MR provides promising novel cardiovascular drug targets for mineralocorticoid receptor antagonists (MRAs), which are currently mainly applied in hypertension and heart failure.

LINKED ARTICLES: This article is part of a themed issue on Emerging Fields for Therapeutic Targeting of the Aldosterone-Mineralocorticoid Receptor Signaling Pathway. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.179.13/issuetoc

KEYWORDS
aldosterone, atherosclerosis, cardiovascular disease, immune system/inflammation, mineralocorticoid receptor

Abbreviations: 11β-HSD2, hydroxysteroid 11-β dehydrogenase 2; 18F-FDG, [18F]fluorodeoxyglucose; Arg, arginase; BrdU, bromodeoxyuridine; CV, cardiovascular; CVD, cardiovascular disease; DAMP, danger-associated molecular pattern; GPER, G protein-coupled oestrogen receptor; GR, glucocorticoid receptor; HDL, high-density lipoprotein; HRE, hormone response element; hsCRP, high sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; IMT, intima-media thickness; LDLR, low-density lipoprotein receptor; LPS, lipopolysaccharide; LysM, lysozyme M; MR, mineralocorticoid receptor; MRA, mineralocorticoid receptor antagonist; MR-KO, myeloid MR knock-out; NFAT, nuclear factor of activated T-cells; NGAL, neutrophil gelatinase-associated lipocalin; NLRP3, neutrophil-to-lymphocyte ratio pyrin domain containing 3; oxLDL, oxidized low-density lipoprotein; PA, primary aldosteronism; PCSK9, proprotein convertase subtilisin / kexin type 9; PET-CT, positron emission tomography–computed tomography; RAAS, renin-angiotensin-aldosterone system; SR-A, scavenger receptor A; SR-BI, scavenger receptor B1; TH17, T-helper cell 17; Treg, regulatory T-cell; VEGF-C, vascular endothelial growth factor-C; VSMC, vascular smooth muscle cell.

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1 | INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of loss of disability-adjusted life years and deaths worldwide (Joseph et al., 2017). It accounts for approximately one third of all deaths globally, with CVD-related morbidity having increased by over 10% in the last decade. Importantly, the majority of cardiovascular events is not prevented by current therapeutic regimens, despite a growing number of pharmacological agents targeting classical cardiovascular risk factors, such as hyperlipidaemia and hypertension (Joseph et al., 2017). Not surprisingly, the impetus to further delineate the pathophysiological mechanisms driving CVD to identify novel targets for personalized therapies is high.

In the last two decades, research has changed our vision on the pathophysiology of CVD and highlighted its complex pathophysiology. Perhaps the most novel change was that chronic low-grade inflammation was acknowledged as an important additional risk factor for CVD (Swirski & Nahrendorf, 2013). Inflammatory cells seem to affect, overall, the atherosclerotic burden and tissue regeneration. Anti-inflammatory drugs hold promising value for treatment of CVD. Canakinumab (a monoclonal antibody against IL-1β) and colchicine significantly reduced morbidity and mortality in high CV risk patients. Other anti-inflammatory drugs failed to prevent or reduce the CVD burden (Ridker et al., 1997). General loss of immune function may underlie increased susceptibility to infection and needs further investigation, as well as identification of targets for CVD-specific modulation of inflammation.

One promising target is the mineralocorticoid receptor (MR) that primarily binds the mineralocorticoid aldosterone, but has an additional high affinity for the glucocorticoid cortisol and androgens. General MR activation is undisputedly associated with CVD. First, the autonomous adrenal overproduction of aldosterone (primary aldosteronism, PA) results in a two to three-fold increased risk of cardiovascular events (Monticone et al., 2018). Second, treatment of patients in heart failure with a MR antagonist (MRA) reduced CVD morbidity and mortality (Pitt et al., 1999, 2003). Moreover, in patients with established CVD but without PA, higher plasma aldosterone levels were associated with an increased risk of myocardial infarction, stroke, and cardiovascular mortality (Ivanov et al., 2012). Although the MR is mainly known by its classical effect—to promote renal sodium reabsorption in epithelial cells in the distal nephron after binding of aldosterone and to regulate blood pressure and salt homeostasis (Funder & Zennaro, 2017)—these effects appear in part to be independent of blood-pressure levels. Ongoing research showed the MR to be present on virtually all cells of the cardiovascular system, including endothelial cells, vascular smooth muscle cells (VSMCs), and cells of the adaptive as well as innate immune system (van den Berg et al., 2014). Activation of these extra-renal MRs seems to have additional detrimental effects on cardiovascular health (Bene et al., 2014; Faulkner & Belin de Chantemele, 2019; van den Berg et al., 2014; van der Heijden, Deinum, et al., 2018).

This review aims to summarize the effects of the MR on innate and adaptive immune cells on the process of atherosclerosis and CVD, in order to highlight promising novel drug targets to prevent CVD.

2 | THE MR AND ITS EXPRESSION IN THE CARDIOVASCULAR SYSTEM

The MR is a cytosolic receptor, which after ligand binding shuttles into the nucleus to bind to a DNA sequence known as the hormone response element (HRE) (Figure 1). Upon binding, the MR forms homodimers, or combines with the glucocorticoid receptor (GR) to form heterodimers—thus initiating different transcriptional pathways (Ong & Young, 2017; Savory et al., 2001). Next to a ligand-binding and DNA-binding domain, it contains an amino terminal domain, which interacts with cofactors to alter specificity of gene activation. MR activation can also result in rapid effects which suggest non-genomic routes. Moreover, mineralocorticoids may also function as a ligand of cell membrane-associated receptors, such as the G protein-coupled oestrogen receptor (GPER). These complex MR signalling cascades and mineralocorticoid–receptor interaction have been extensively reviewed elsewhere (Ong & Young, 2017; Ruhs et al., 2017).

In the cardiovascular system, the MR is expressed by various cell types. In immune cells, the MR is expressed in monocytes and macrophages (innate immune cells), and most studies on MR-induced phenotypic modification of immune cells focus on this myeloid MR. However, the MR is also expressed and functional in dendritic cells (Bene et al., 2014), which link the innate and adaptive immune system, as well as in T- and B-cells (Armanini et al., 1988) (adaptive immune cells). MR expression in lysozyme M (LysM) positive cells of innate immune cells has been shown by PCR and Western blotting (Rickard et al., 2009). Moreover, MR expression was found by PCR in myeloid as well as T- and B-cells. In addition, MR protein expression was shown in myeloid cells (Montes-Cobos et al., 2017). Also, MR expression in myeloid cells was found by PCR and Western blotting (Usher et al., 2010). The same was seen for the MR in dendritic cells (Herrada et al., 2010).

Expression of the MR in lymphocytes has been shown by radio-receptor assay (Armanini et al., 1988) others showed expression of the MR in T-cells by FACS analysis, PCR, and Western blotting (Sun et al., 2017). Therefore, there are convincing data that the MR is expressed in cells of the innate and adaptive immune system, although, to our knowledge, the presence of MRs in neutrophils has not been studied.

Apart from the kidney and immune cells, the MR is also expressed in several other cells of cardiovascular tissues. Experimental studies using cell type-specific gene targeting of the MR gene in mice have revealed the importance of this extra renal aldosterone signalling in cardiomyocytes, endothelial cells, and vascular smooth cells. As this is not the focus of the present manuscript, the reader is referred to recent reviews (Blower et al., 2019; Lother et al., 2015; van den Berg et al., 2014). Knockout of the MR gene in fibroblasts was without effect in cardiac hypertrophy (Lother et al., 2011).

Recent data suggest that some of the sex differences found in CVD are related to the endothelial MR. An interaction of the MR with oestrogen receptors could be the underlying mechanisms, as reviewed previously (2019). However, translation of these findings to clinical practice is still lacking. In the recent trial on the effect of finerenone on chronic kidney disease outcomes in Type 2 diabetes, 1/3 of...
participants were female. However, no significant gender difference was found for reduction of primary composite endpoint by finerenone (Bakris et al., 2020). More work is necessary to establish the role of MRs in gender differences in patients with CVD.

3 | EVOLUTIONARY CLUES THAT THE MR IS IMPORTANT FOR MORE THAN BLOOD PRESSURE ALONE

Generally, aldosterone is seen as the main physiological MR ligand. Nonetheless, evolutionary clues suggest that the MR originated to serve different ligands: Cartilaginous and bony fish exhibit the MR but no mineralocorticoids. In these fish, cortisol is likely to be the main ligand of MRs, important in stress responses. Aldosterone was first discovered in lungfish, who developed millions of years later (Funder, 2017).

In mammals, the MR still has a similarly high affinity for the glucocorticoids (cortisol in humans and corticosterone in rodents) (Figure 1) (Arriza et al., 1987). In the physiological state, the plasma concentration of cortisol is much higher than that of aldosterone. Therefore, mechanisms exist to exert specificity at the MR. The most important one is co-expression of the enzyme hydroxysteroid 11β dehydrogenase 2 (11β-HSD2) that converts cortisol into cortisone which has negligible affinity for the MR. In the vasculature, 11β-HSD2 is expressed by vascular smooth muscle cells and endothelial cells (van den Berg et al., 2014). However, monocytes and macrophages do not express 11β-HSD2, while in lymphocytes, its presence has not
been studied. Interestingly, even in cells that do not express 11β-HSD2, the MR is more sensitive to aldosterone than to cortisol. Multiple post-binding mechanisms orchestrate this ligand-specific functional outcome of MR-ligand binding, which results in significantly stronger MR transcriptional activation at lower concentrations when bound to aldosterone than to cortisol (Arriza et al., 1987). These mechanisms, which are complex and incompletely understood, have been extensively reviewed elsewhere (Fuller et al., 2017). For cells of the immune system, it is yet to be investigated which of these mechanisms are of importance. However, regardless of the ligand, MR signalling is available for drug-targeting.

Moreover, it is broadly acknowledged that immune sensors are also detecting and rectifying deviations from cell homeostasis to maintain cell physiology. Blood pressure control and host defence are essential for evolutionary survival of mammals, and one might speculate that it is therefore not surprising that evolution incorporated immune cells as active participants in the regulation of blood pressure and cardiovascular homeostasis. Infection can cause hypotension via fluid loss during fever, tachypnoea, and diarrhoea and vascular hyperpermeability. Thus, the risk of hypotension related to inflammation might have favoured selection of mechanisms that link immune and MR activation to blood pressure increases for short-term survival benefits. Such an evolutionary force may explain why important antimicrobial effectors like monocytes/macrophages and lymphocytes could have direct hypertensive effects by promoting vasoconstriction or sodium retention (Wenzel et al., 2016).

4 IMMUNE CELLS IN CARDIOVASCULAR DISEASE

Conceptual and technological innovations have importantly increased our understanding of CVD as a chronic inflammatory disorder (Swirski & Nahrendorf, 2013). Atherosclerosis is the pathological process that underlies most CVD, and is driven by leukocytes, in particular monocytes and macrophages which are the most abundant cell types in the plaque, together with vascular smooth muscle cells (VSMC). Single-cell RNA sequencing in a murine low-density lipoprotein receptor deficient (LDL−/−) mouse model of atherosclerosis recently identified 30% of plaque leukocytes in early, and 50% in more mature plaques to be macrophages, while CD8+ T-cells accounted for another 20% (Cochain et al., 2018). Preventing monocyte influx into the arterial wall prevents the formation of atherosclerotic plaques (Moore et al., 2013) and progression of established lesions in preclinical atherosclerosis models (Inoue et al., 2002). Communication of these innate immune cells with cells of the adaptive immune system and local non-immune cells (i.e., endothelial cells and vascular smooth muscle cells) is necessary to initiate and maintain a local pro-inflammatory environment that promotes expansion and destabilization of atherosclerotic lesions, while in the steady state, the interaction between these cell types is pivotal to maintain a healthy local vascular environment (Moore et al., 2018). This illustrates the remarkable plasticity of this system and its cells, where the balance can shift from homeostasis to disease in the wrong circumstances.

When pro-atherosclerotic factors, including disturbed shear stress and hypertension, activate endothelial cells, circulating monocytes are the first to adhere to leukocyte adhesion molecules that are expressed on these activated endothelial cells, to migrate into the intima. Their differentiation into macrophages that engulf oxidized lipid particles, such as oxidized LDL (oxLDL) via scavenger receptors, mainly CD36 and the scavenger receptor-A (SR-A, CD204) to form foam cells, further accelerates atherosclerotic plaque formation (Moore et al., 2013). Stimulation of plaque macrophages by local danger associated molecular patterns (DAMPs) induces a (long-term) pro-inflammatory behaviour of macrophages and induces production of various pro-inflammatory chemokines and cytokines that attract more inflammatory cells to the site and induce phenotypic changes in neighbouring non-immune cells. Moreover, cholesterol crystals are able to activate the macrophage NLRP3 inflammasome — cytoplasmic protein sensors to potential dangerous exogenous or (in this case) endogenous triggers. NLRP3-dependent production of IL-1β is central in the inflammatory cascade. Moreover, several components of the extracellular matrix are able to induce myeloid cell activation through the toll-like receptors (TLR). Together, this results in a vicious circle of low-grade, sterile inflammation. Production of matrix metalloproteinases (MMPs) by immune and non-immune cells destabilize the fibrous cap overlying the atherosclerotic plaque (Nahrendorf & Swirski, 2016).

Importantly, although present in lower numbers and studied to a lesser extent in atherosclerosis models than macrophages, for virtually every immune cell, a role in atherosclerosis has been suggested, either athero-promoting or athero-protective (Swirski & Nahrendorf, 2013). In brief, dendritic cells reside in the aorta and affect atherogenesis, probably through interaction with T-cells. T-cells can exert diverse effects on the atherosclerotic process, being either athero-promoting (T helper 1 and T helper 17 subsets) or athero-protective (T helper 2 and regulatory T-cell subsets) (Swirski & Nahrendorf, 2013). Neutrophils, amongst others, produce a range of chemotactic agents that attract monocytes, produce myeloperoxidase that oxidizes lipoproteins, and produce neutrophil extracellular traps (Silvestre-Roig et al., 2020).

Immune cells are important not only in the atherosclerotic process itself but also in determining one of the most important risk factors for its development, hypertension. Here, it is mainly the adaptive immune system that plays an important role. The first conclusive evidence for a role of the adaptive immune system in the pathogenesis of arterial hypertension was provided by Guzik et al., by showing that the increase in blood pressure caused by angiotensin II infusion was significantly blunted in mice lacking T- and B-cells (Guzik et al., 2007). This finding was confirmed in several other laboratories and genetic models (Ji et al., 2014; Madhur et al., 2020; Mattson et al., 2013). Surprisingly, we did not observe resistance to angiotensin II in B6. Rag1−/− mice (that do not have mature T- and B-cells) (Seniuk et al., 2020). The Sandberg laboratory also reported that Jackson B6. Rag1−/− mice lost their resistance to angiotensin II-induced hypertension (Ji et al., 2017). There is no doubt that lymphocytes play a role in...
hypertension. The negative data illustrate that there are important, but still unidentified confounders (Madhur et al., 2020; Rios et al., 2020).

Last, tissue regeneration after a CVD event is importantly regulated by immune cells (Swirski & Nahrendorf, 2013). Most studied is the role of leukocytes after myocardial infarction. After the onset of ischaemia, endothelial cells up-regulate adhesion molecules facilitating neutrophil influx in the injured heart, which accelerates local inflammation. This influx of inflammatory neutrophils quickly overwhelms the population of macrophages (Pinto et al., 2012) and dendritic cells (Choi et al., 2011) that reside in the healthy heart. Inflammatory monocytes are the other subclass of immune cells that infiltrate the injured heart in the early stages after myocardial infarction, further releasing pro-inflammatory mediators and inducing proteolysis that causes tissue destabilization. After several days, they are replaced by anti-inflammatory monocytes that supportangiogenesis and extracellular matrix synthesis (Swirski & Nahrendorf, 2013). Monocytes also infiltrate the non-injured myocardium where they can exert either protective effects through induction of angiogenesis or destructive effects through mediators that induce cardiac dilatation and fibrosis (Swirski & Nahrendorf, 2013).

Although not a part of the cardiovascular system, the recent discovery of the increasing levels of MR expression during (white) adipose cell differentiation (Fu et al., 2005) is also particularly relevant to CVD. MR activation has been suggested to potentiate white adipose tissue inflammation, oxidative stress, fibrosis, and insulin resistance, although part of these effects are attributed to effects of MR-induced activation of myeloid cells embedded in adipose tissue, with pro-inflammatory macrophages promoting a local inflammatory environment and decreasing insulin sensitivity (Wada et al., 2017). Knockout of the MR in adipocytes in mice fed a high-fat diet decreased the expression of genes involved in adipogenesis (Ferguson et al., 2020).

5 | ANTI-INFLAMMATORY THERAPY REDUCES CARDIOVASCULAR DISEASE IN THOSE AT RISK

Although preclinical data on the importance of inflammation in CVD are plentiful, only recently, the first landmark trial showed that systemic anti-inflammatory treatment reduced cardiovascular morbidity and mortality in those at high risk. Canakinumab, an antibody directed against the macrophage-derived driver of inflammation, IL-1β, reduced cardiovascular events, by an additional 15%, when added to standard therapy in patients with a recent myocardial infarction, at the expense of increased fatal infections (Ridker et al., 2017). A “broad-spectrum” anti-inflammatory approach using low dose colchicine in those with coronary disease in different stages also gave promising results (Tardif et al., 2019). However, low-dose methotrexate was unable to reduce atherosclerotic cardiovascular events in a similar population (Ridker et al., 2019), illustrating that the mechanisms linking chronic inflammation to atherogenesis and adverse outcomes are only partly understood. Unravelling these is essential in the development of new drug therapies.

6 | IMMUNOMODULATION THROUGH THE MR OF INNATE IMMUNE CELLS AFFECTS CARDIOVASCULAR DISEASE

The studies discussed below describe the current insights in the inflammatory effects of the myeloid MR, as well as the MR of dendritic cells. The immunomodulatory effect of the monocyte/macrophage MR is also summarized in Figure 1. Moreover, clinical and experimental data are summarized in Table 1.

Upon activation by various stimuli, monocytes differentiate into macrophages with a diverse spectrum of phenotypes. For practical reasons, these macrophage phenotypes are often divided into “classically activated” inflammatory M1-macrophages and “alternatively activated” or “anti-inflammatory” M2-macrophages. Importantly, these phenotypes are oversimplifying biology and should be considered as two extremes of an extensive spectrum (Nahrendorf & Swirski, 2016) but, in general, M1 macrophages are also considered pro-atherogenic, while pro-fibrotic characteristics might be present in both (Nahrendorf & Swirski, 2016).

Various murine models combine in vivo, ex vivo, and in vitro techniques to characterize the potential of MR signalling to modify the monocyte and macrophage phenotype. These models use either aldosterone infusion, DOCA (deoxycorticosterone acetate, a mineralocorticoid)/salt infusion, or sodium restriction (which results in an endogenous activation of the renin-angiotensin-aldosterone system [RAAS]) to induce MR (over)stimulation, or myeloid-specific MR knockout or MRAs to reduce MR signalling in vivo. Many of these models suffer from the lack of a hypertensive control group, and therefore, in theory, some of the observed changes might be secondary to hypertension and release of mediators after vascular stress rather than to MR stimulation per se. In knockout models, the cre/lox-recombination system allows the selective deletion of the MR from inflammatory cells, and the LysM promoter is widely used to drive cre expression in monocytes/macrophages. Of note, the use of the LysM promoter does not allow strict macrophage-specific gene inactivation, but in parallel targets dendritic cells, neutrophils, and monocytes. Human data investigating the immunomodulatory effects of the MR are scarce and consist of in vitro studies (co)incubating monocytes and monocyte-derived macrophages with aldosterone or MRAs. Additionally, some studies report on ex vivo of monocytes obtained from patients with primary aldosteronism. Some studies of the effects of hyperaldosteronism on circulating markers of inflammation, such as hsCRP and circulating IL-6, largely show a heightened systemic inflammatory state as reflected in these biomarkers (Remde et al., 2016; Staermose et al., 2009; Tzamou et al., 2013) (although some disagree; Somloova et al., 2016; van der Heijden, Groh, et al., 2020). However, these studies are unable to answer the question which cell type and mechanisms are responsible for the induction
| Species   | Model                                      | Immunological effects                                                                 | Reference                                                                 |
|-----------|--------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| **Systemic inflammation and innate immune cells** |                                            |                                                                                       |                                                                           |
| Human     | In vivo                                    | PA patients                                                                          | Circulating hsCRP ↑, IL-6 ↓, Arterial wall FDG-uptake ↑, hsCRP = IL-6 = CCL2 = | (Bruder-Nascimento et al., 2016; Remde et al., 2016)                        |
|           | ET patients, aldosterone ↑                 | PA patients                                                                          | Circulating hsCRP ↑, leukocyte count ↑,                                        | (Tzamou et al., 2013)                                                      |
|           | PA patients                                 |                                                                                       |                                                                           | (van der Heijden, Smeets et al., 2020)                                   |
| Ex vivo   | PA patients, Mφ, LPS stimulated             | IL-6 ↑, TNF-α ↑, IL-1β ↑                                                              | (Krysiak & Okopien, 2012)                                                  |
|           | PA patients, Mφ, oxLDL stimulated           | IL-6 and TNFA ↑                                                                      | (van der Heijden, Smeets et al., 2020)                                   |
|           | PA patients, PBMCs, stimulated              | IL-6 =, TNF-α =, IL-1β                                                                | (van der Heijden, Smeets et al., 2020)                                   |
|           | PA patients, Mφ                             | Inflammasome activation                                                               | (Bruder-Nascimento et al., 2016)                                          |
| In vitro  | Mo derived Mφ, aldosterone stimulated       | M1-marker iNOS ↑, ROS production ↑                                                    | (Labuzek et al., 2013)                                                    |
|           | Mo derived Mφ, eplerenone stimulated        | M2-markers ↑ (ARG1, mannose receptor), M1-marker iNOS (--) ROS production (--)       | (Bendtzen et al., 2003; Labuzek et al., 2013; Sonder et al., 2006)         |
|           | PMNs, eplerenone + LPS stimulated           | M1-markers and pro-inflammatory cytokines/Chemokines ↓                                 |                                                                           |
|           | Mo derived Mφ, aldosterone-trained          | IL-6 and TNF-α ↑ after TLR2/4 re-stimulation                                          | (van der Heijden, Keating et al., 2020)                                   |
| Mouse     | Combined                                   | Sodium restriction, ApoE KO                                                           | Circulating IL-6 ↑, Mφ foam cell formation ↑                               | (Raz-Pasteur et al., 2014; Tikellis et al., 2012)                          |
|           | Aldosterone infusion, ApoE KO               | CCl2 ↑                                                                                |                                                                           | (Keidar et al., 2004; McGraw et al., 2013)                                |
|           | Aldosterone stimulated Mφ                   | Mφ: NADPH activity ↑, superoxide anion production ↑, LDL oxidation ↑                  |                                                                           |                                                                           |
|           | Pre-incubation with MR antagonist           | mRNA: Tnfa ↑, Ccl5 ↑, Il12 ↑, Ccl2 ↑ (M1-markers)                                    | (Usher et al., 2010)                                                      |
|           | Myeloid specific MR-KO, Mφ                  | LPS-induced Tnfa ↑                                                                   |                                                                           |                                                                           |
|           | Myeloid MR-KO, cardiac fibrosis, heart      | M2-markers ↑ (i.e., Fizz1), M1-markers (i.e., TNFα and Il1β), Augmentation of M2-profile when costimulated with IL-4 | (Bienvenu et al., 2012; Usher et al., 2010)                               |
|           | homogenates                                 |                                                                                        |                                                                           |                                                                           |
|           | Eplerenone treatment, ApoE KO               | M2-markers ↑ (i.e., Arg1 and Il10), M1-markers (i.e., Il6 and Tnfa ↓)                 | (Raz-Pasteur et al., 2012)                                                |
|           | Myeloid MR-KO, Mφ                           | Migratory capacity ↑, proliferation ↑, efferocytosis ↑, Foam cell formation ↑, Cholesterol efflux receptors ↑, cholesterol uptake receptors (--) (i.e., CD36 and SR-A) | (Shen, Chen, et al., 2016b; Sun et al., 2016; Usher et al., 2010)          |
| Species                  | Model                                                                 | Immunological effects                                                                 | Reference                          |
|-------------------------|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------|------------------------------------|
|                         | DOCA/salt infusion, kidney homogenates                                 | *Nlrp3/pro-caspase-1/pro-IL1β*, hypertension                                         | (Krishnan et al., 2016)            |
|                         | NLRP3 receptor inhibitor/Nlrp3 deficiency                              | Attenuation of effect                                                                | (Krishnan et al., 2016, 2019)      |
|                         | Aldosterone stimulation, BMDM                                          | *Nlrp3*, IL1β                                                                     | (Bruder-Nascimento et al., 2016)   |
|                         | Aldosterone infusion, WT                                              | Circulating IL-1β, Vascular inflammation and remodelling, Mφ (peritoneal cavity): Inflammasome activation | (Bruder-Nascimento et al., 2016)   |
|                         | Bone marrow transplantation from NLRP3−/− mice                        | Attenuation of effect                                                                |                                    |
|                         | Aldosterone infusion, dendritic cell depleted mice                    | NGAL, profibrotic and pro-inflammatory effects                                       | (Araos et al., 2019)              |
|                         | Aldosterone stimulated DC                                             | DC-mediated CD8(+) T-cell priming, DC-mediated induction of Th17 phenotype CD4(+) T-cells (IL-17 secretion) |                                    |
| Rat                    | Combined Aldosterone infusion, kidney homogenates                     | M1-markers (e.g., CD68, TNFa, Arg2, IFN-γ, iNOS), MMP-2 (M2-markers)                | (Doi et al., 2014; Martin-Fernandez et al., 2016) |
|                         | Aldosterone infusion, kidney homogenates                              | Inflammasome                                                                        | (Doi et al., 2014)                |
|                         | Aldosterone stimulated DC                                             | DC-mediated CD8(+) T-cell priming (IL-2 secretion), DC-mediated induction of Th17 phenotype CD4(+) T-cells (IL-17 secretion) | (Herrada et al., 2010)           |

**Adaptive immune cells**

| Species | Combined | Abdominal aortic constriction, T-cell MR-KO | Cardiac inflammation, T-cell number (heart), T-cell activation markers | (Li et al., 2017). |
|---------|----------|---------------------------------------------|------------------------------------------------------------------------|-------------------|
| Combined| AngII infusion, T-cell MR-KO                | Blood pressure, renal and vascular damage, IFN-γ-producing T-cells       | (X. N. Sun et al., 2017)                                             |
| Rat     | Combined DOCA/salt, heart and kidney homogenates | Th17 cell activation, forkhead box P3 mRNA                               | (Amador et al., 2014)                                               |

**Note:** ↑, increase; ↓, decrease; (−), no effect.

**Abbreviations:** AngII, angiotensin II; DC, dendritic cell; ET, essential hypertension; KO, knockout; Mφ, macrophage; MMPs, matrix metalloproteinases; Mo, monocyte; MR mineralocorticoid receptor; PMNs, peripheral mononuclear cells; TLR, Toll-like receptor, ROS, reactive oxygen species.
of inflammation, apart from the fact that many do not include adequate control groups.

Aldosterone infusion in murine models enhances the expression of classical M1 markers (i.e., *Ifng, Inos, Tnfa*, and *Arg2*) in the kidney (Martin-Fernandez et al., 2016) and peritoneal macrophages (Usher et al., 2010), respectively (Figure 1). In these models, macrophage reactive oxygen species (ROS) production was also increased. Production of ROS by macrophages contributes to endothelial dysfunction and the oxidation of LDL. Oxidized lipoproteins are subsequently ingested by macrophages to form foam cells that characterize the atherosclerotic plaque (Victor et al., 2009). Aldosterone infusion in mice increased the production of ROS from peritoneal macrophages (Figure 1), which enhanced their ability to oxidize LDL (Keidar et al., 2004). Administration of MRA in the presence of aldosterone attenuated the pro-oxidative effects (Keidar et al., 2004), and pre-incubation of human monocytes with eplerenone reduced aldosterone-induced ROS production. MR antagonists or myeloid MR knock-out (MR-KO), without aldosterone excess, might not be able to reduce ROS formation completely (Labuzek et al., 2013; Shen, Morgan, et al., 2016) although current data are conflicting (Keidar et al., 2003).

Interestingly, circulating IL-1β is increased in mice after aldosterone infusion (Bruder-Nascimento et al., 2016). This is particularly relevant since the CANTOS trial showed improvement of cardiovascular morbidity and mortality in high risk patients treated with IL-1β targeted therapy, as mentioned previously (Ridker et al., 2017). IL-1β is a pro-inflammatory cytokine derived from macrophages, which is high in the inflammatory signalling cascade. Its production is dependent of the NLRP3 inflammasome, which activates caspase-1 to cleave pro-interleukin-1β (and pro-IL-18). Aldosterone/salt infusion in rats, and DOCA/salt infusion in mice, increased the expression of inflammasome components in the kidneys (Doi et al., 2014; Krishnan et al., 2016). Mice treated with a NLRP3 receptor inhibitor, or that are deficient in an adapter protein of NLRP3, are protected against the hypertension and renal inflammation seen after DOCA/salt infusion (Krishnan et al., 2016). Surprisingly, *Anakirna* (an IL-1 receptor antagonist) lowered blood pressure in mice treated with DOCA/salt, but it did not alter the renal immune cell infiltration. In mice, aldosterone increased caspase-1 activation, and NLRP3 and IL-1β gene expression in bone-marrow derived macrophages. This activation was prevented by NLRP3 knockout (Ling et al., 2017). In vivo, caspase-1 deficiency prevented vascular dysfunction, aldosterone induced vascular cell adhesion protein 1 (VCAM-1) and ICAM-1 expression and macrophage adherence to the arterial wall and vascular remodelling after aldosterone infusion. To further distinguish whether NLRP3 inflammasome activation of the vasculature or immune cells were responsible for these effects, they transplanted the bone marrow from NLRP3−/− mice into wild-type mice. Aldosterone-induced changes in vascular reactivity were partly blocked in these mice, and some protection from aldosterone-induced vascular inflammation was seen. Overall, these findings suggest that NLRP3 inflammasosome in myeloid cells contributes to vascular damage induced by aldosterone (Figure 1) (Bruder-Nascimento et al., 2016). In line with the findings described above, models in which MR signalling is reduced (either through myeloid-specific MR knockout or MRA) show a decrease of expression of macrophage M1 markers (Bienvenu et al., 2012; Rickard et al., 2009), while some report an additional increase in expression of macrophage M2 markers (i.e., *IL-10* and *Arg1*) (Raz-Pasteur et al., 2014; Usher et al., 2010). These findings are summarized in Figure 1. Moreover MRA treatment dampened ex vivo inflammatory cytokine production upon stimulation with the TLR4 ligand, lipo polysaccharide (LPS) (Raz-Pasteur et al., 2014; Usher et al., 2010), even without aldosterone present in the culture medium. This was confirmed by human ex vivo stimulation studies (Keidar et al., 2004; Sonder et al., 2006; Sun et al., 2016). This, together with findings in other models reviewed elsewhere (Funder, 2013), suggests that the MRA can act as inverse agonist. Inverse agonists have the ability to not only block a receptor for binding with a true agonist, but to exert a (pharmacological) response opposite to that of the agonist after binding. This has important consequences for their use. In the context of inflammation, this would imply that MRA treatment could exert anti-inflammatory effects, independent of aldosterone excess, and therefore be of interest to a varied patient population.

We recently showed that aldosterone is able to induce a long lasting pro-inflammatory effects in human monocyte-derived-macrophages, a phenomenon known as trained immunity, which was partly abolished by co-incubation with the MRA *spironolactone*. Trained immunity initially described the ability of human monocytes to build a long-term immunological memory upon stimulation with microbial products (Netea et al., 2016), driven by broad epigenetic and immunometabolic changes (van der Heijden, Noz, et al., 2018). The concept has been extended to include pro-atherogenic, sterile compounds such as oxLDL (Bekkering et al., 2014), lipoprotein (a) (van der Valk et al., 2016), and catecholamines (van der Heijden, Groh, et al., 2020). In vivo, trained immunity was confirmed in a murine model, using a Western-type diet (Christ et al., 2018), and it has been implicated in the low-grade, long-term inflammation that is characteristic of atherosclerosis (Christ et al., 2016; Leentjens et al., 2018). Monocytes isolated from patients with PA showed heightened responsiveness ex vivo in some studies. We could not confirm these findings (van der Heijden, Smeets, et al., 2020), but instead showed that monocytes obtained from PA patients that were differentiated into macrophages ex vivo for a week expressed more TNFA and IL6 after stimulation with oxLDL (van der Heijden, Smeets, et al., 2020), suggestive of some immunological memory.

Last, recent interesting data showed an important role for the MR of dendritic cells in vascular damage. The neutrophil gelatinase-associated lipocalin (NGAL) is an important novel marker of CVD. Immune cell depletion of NGAL protected against cardiovascular fibrosis and a proinflammatory phenotype induced by aldosterone/salt infusion in mice (Buonafine et al., 2018; Tarjus et al., 2015). Moreover, in an aldosterone/salt infusion mice model, dendritic cell depletion prevented NGAL increases and the associated profibrotic and pro-inflammatory effect, while in vitro aldosterone up-regulated NGAL expression in dendritic cells (Araos et al., 2019).
THE MYELOID MR ACCELERATES ATHEROSCLEROSIS

Although data on the relationship between MR activation in myeloid cells and atherosclerotic burden in humans are lacking, surrogate markers for atherosclerosis, such as the carotid artery intima-media thickness (IMT), are increased in PA patients (Holaj et al., 2007; Strauch et al., 2006). These changes reverse after adrenalectomy, although no effect was seen of spironolactone treatment (Strauch et al., 2008). We recently showed that patients with PA have an increased signal from the large arteries using PET-CT with [18F]deoxyglucose, compared to controls with essential hypertension, suggestive of macrophage infiltration of the arterial wall (van der Heijden, Smeets, et al., 2020). The total number of plaque macrophages importantly contributes to plaque size and vulnerability. It reflects the balance between infiltration by circulating monocytes and local proliferation of resident macrophages and macrophage egress, apoptosis, and efferocytosis (the clearance of apoptotic macrophages by phagocytic leukocytes) (Swirski & Nahrendorf, 2013). In murine models, aldosterone increased (McGrath et al., 2013), and myeloid-specific MR-KO decreased, the macrophage numbers in the atherosclerotic plaques (Shen, Morgan, et al., 2016). Moreover, in inflammatory and NO-deficiency models of kidney and heart fibrosis, MRA treatment (Martin-Fernandez et al., 2016) and MR-KO (Usher et al., 2010) reduced macrophage infiltration, although this finding was not confirmed by all groups (Bienvenu et al., 2012). Macrophage infiltration in femoral arteries after wire-injury was significantly less in myeloid-specific MR-KO than in wild-type mice (Sun et al., 2016). Although it is known that aldosterone induces the expression of adhesion molecules on vascular endothelial cells, transwell experiments showed that the migratory ability of peritoneal macrophages from MR-KO mice was markedly decreased, indicating that intrinsic changes through the macrophage MR further contribute to migratory capacity. Also, the MR was shown to influence the proliferative potential of macrophages (Sun et al., 2016). Myeloid-specific MR deficiency was also shown to significantly decrease the number of apoptotic cells in atherosclerotic lesions and increase efferocytosis (Shen, Morgan, et al., 2016).

Foam cell formation—the ingestion of (partly modified) lipoproteins by plaque macrophages—also importantly adds to the expansion of the atherosclerotic plaque and formation of an unstable necrotic core, prone to rupture.

After oxidation, the oxidized LDL (oxLDL) is mainly ingested in macrophages through the scavenger receptors CD36 and scavenger receptor class A (SR-A), while the ATP-binding cassette transporters A1 (ABCA1), G1 (ABCG1), and scavenger receptor BI (SR-BI) mediate cholesterol efflux. Up-regulating RAAS activity with a low sodium diet augmented cholesterol accumulation in circulating and peritoneal mice macrophages, an effect which was inhibited by eplerenone. Others also report a decreased oxLDL content of peritoneal mouse macrophages after eplerenone treatment (Keidar et al., 2003). In myeloid-specific MR-KO mice receiving a high-fat diet, foam cell formation in vitro and in vivo was decreased (Mattson et al., 2013). In this model, gene expression of cholesterol efflux receptors was up-regulated in myeloid specific MR-KO macrophages while expression of genes involved in oxLDL uptake was not significantly altered, indicating that the MR mainly influences cholesterol efflux pathways (Figure 1). This was also reported in a different myeloid-specific knockout model (Usher et al., 2010).

Several intrinsic factors determine the stability of the atherosclerotic plaque. Next to apoptosis of foam cells that leads to the formation of a necrotic core, plaque composition with a high number of inflammatory cells contributes to instability. Plaque instability is further caused by local production of matrix metalloproteinases (MMPs) that lead to degradation of the protective fibrous cap overlying a thrombogenic core (Kojima et al., 2014). MMP-1, MMP-2, MMP-9, and MMP-12, are all produced by macrophages (next to other cell types), and play a role in the pathogenesis of atherosclerosis and atherothrombosis (Ketelhuth & Back, 2011; Wagsater et al., 2011). The role of the MR in determining MMP production has been little studied. MMP-2 protein levels were higher in purified kidneys from rats treated with aldosterone (Wenzel et al., 2016), but many MMP producing cell types could have contributed to this finding, since in several non-immune cells, the MR was suggested to induce metalloproteinase expression (Rude et al., 2005; Sun et al., 2016). In a murine model of cardiac tissue remodelling, intact MR signalling in macrophages was required to increase MMP-12 production above baseline levels (Figure 1) (Shen, Chen, et al., 2016a). The aldosterone-to-renin ratio and circulating MMP-2 concentrations were correlated in patients with PA in vivo, but no control group with normal aldosterone values was included (Lim et al., 2016) and adrenalectomy did not reduce MMP-2 levels in PA patients (Hung et al., 2015). We did not find differences in MMP-2 plasma concentrations between PA patients and controls with essential hypertension (van der Heijden, Smeets, et al., 2020).

HYPERTENSION AND CARDIOVASCULAR FIBROSIS ARE MODULATED BY MYELOID MR SIGNALLING

The observation that immune cells are important in inducing hypertension is a relative novel finding. Although most studies that investigate the role of the immune system in hypertension focus on lymphocytes, cells of the myeloid system are suggested to contribute likewise. Selective ablation of lysozyme M-positive myelomonocytic cells markedly blunts angiotensin-induced infiltration of these cells into the vascular wall and attenuates hypertension and vascular dysfunction (Wenzel et al., 2011). Osteopetrotic mice, in which macrophages are functionally deficient, exhibit reduced hypertension and vascular remodelling in response to angiotensin or DOCA salt (De Ciuces et al., 2005; Ko et al., 2007).

The role of the myeloid MR has been studied in different models of hypertension. Knockout of the MR in monocytes and macrophages uniformly showed a decrease in vascular and cardiac fibrosis (Rickard...
et al., 2009; van der Heijden, Smeets, et al., 2020) (Figure 2), independent of the model of hypertension used. Cardiac hypertrophy and fibrosis induced by aortic constriction were also significantly attenuated in myeloid MR-deficient mice. Macrophage infiltration in the heart was inhibited, and the expression of inflammatory genes decreased in the deficient mice. In addition, aortic fibrosis and inflammation were also attenuated (Li et al., 2014). However, the effects on blood pressure are conflicting. MR knockout mice showed less increase in blood pressure and cardiac fibrosis in compared to wild-type mice after 8 weeks of DOCA salt (Rickard et al., 2009; Shen, Morgan, et al., 2016). In contrast, no effect on blood pressure was found in MR knockout mice in a model of hypertension induced by administration of the NOS inhibitor, N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) and salt. LysM cre MRflox mice paradoxically even displayed a higher systolic blood pressure than wild-type mice (Usher et al., 2010) in a model of hypertension mediated by administration of L-NAME, in combination with angiotensin II infusion and high salt diet, although vascular and cardiac fibrosis were decreased. In summary, these studies clearly demonstrate a role for the MR in macrophages in hypertensive cardiovascular remodelling. In the DOCA salt model of hypertension, the monocyte/macrophage MR also regulates blood pressure, while in models with NOS inhibition, the data are less clear.

Intriguingly, macrophages have been proposed as a factor in the control of salt sensitivity by modulating the lymphatic response in the skin (Machnik et al., 2009). The interstitium of the skin has emerged as important organ involved in maintaining body sodium balance. Skin monocytes and macrophages regulate non-osmotic storage of salt in the skin by up-regulating NFAT5 in T-cells and secreting vascular endothelial growth factor-C (VEGF-C). The latter increases lymph capillary density which acts as a fluid buffer and attenuates the blood pressure response to high salt. Failure of this macrophage-driven escape results in skin electrolyte overload and hypertension (Wenzel et al., 2021). No data are available on the role of the MR in skin myeloid cells and non-osmotic salt storage in the skin, but it is tempting to speculate that the blood pressure reducing effect of myeloid cell MR-KO, which occurs specifically in DOCA salt models, might occur through this pathway. In contrast, NO deficiency promotes hypertension independently of macrophage function and MR deficiency in these cells has therefore no effect on blood pressure. Importantly, the

![FIGURE 2](image_url)

**FIGURE 2** Effect of MR signalling in monocytes/macrophages on cardiovascular outcome. MR signalling in monocytes/macrophages and lymphocytes affects blood pressure, hypertension-induced end organ damage and atherosclerosis as well as cardiac tissue remodelling after ischaemia. In models of hypertension, inactivation of the MR reduced blood pressure and cardiac fibrosis. In atherosclerosis, macrophage infiltration into the vascular wall is increased, both through intrinsic changes in the macrophage itself as well as through MR mediated effects on endothelial cells. The combination of increased foam cell formation, reduced efferocytosis and increased production of MMPs results in an unstable plaque phenotype. In the (post)ischaemic heart, intact myeloid MR signalling importantly contributes to cardiac fibrosis.
development of cardiac fibrosis in the models of NO inhibition and the protection by monocyte/macrophage MR deficiency are independent of blood pressure changes.

9 | TISSUE REMODELLING AFTER MYOCARDIAL, RENAL, AND CEREBRAL ISCHAEMIA IS IMPROVED AFTER MYELOID-SPECIFIC MR-KO

Mice with myeloid cell-restricted MR deficiency displayed improved cardiac function and remodelling associated with enhanced infarct neovascularization and scar maturation. Mechanistic experiments showed that inactivation of the macrophage MR promotes myocardial infarct healing through enhanced efferocytosis of neutrophils, the suppression of free radical formation, and the modulation of fibroblast activation state (Fraccarollo et al., 2019). Restenosis after percutaneous coronary intervention is a serious medical problem. Mice with myeloid restricted MR deficiency showed reduced intima area and intima/media ratio, Ki67- and BrdU-positive vascular smooth muscle cells, expression of pro-inflammatory molecules, and macrophage accumulation after wire injury (Sun et al., 2016). Myeloid-specific MR deficiency improved renal dysfunction, inflammation, and damage in a model of progressive glomerulonephritis (Huang et al., 2014). Similarly, selective ablation of myeloid MRs protected against subsequent chronic dysfunction and fibrosis induced by an episode of bilateral kidney ischaemia/reperfusion in mice. This protection was associated with increased expression of M2-anti-inflammatory markers in the MR-deficient macrophages (Barrera-Chimal et al., 2018). In addition, the infarct volume after middle cerebral artery occlusion, an animal model of stroke, was also reduced in these mutant mice (Frieler et al., 2011).

10 | THE LYMPHOCYTE MR IS IMPLICATED IN HYPERTENSION AND CARDIAC AND RENAL FIBROSIS

Studies investigating the lymphocyte MR in CVD are less plentiful than those on the myeloid cell MR and restricted to the MR of T-cells. Nonetheless, they have provided interesting data on the role of signalling via the MR in these cells, mainly in the context of hypertension and fibrosis in the kidney and the heart (Amador et al., 2014; Li et al., 2017; Sun et al., 2017) (Figure 2). Li et al. and Sun et al. found an important role for the MR in T-cells in two studies. First, they showed that knockout of the T-cell MR in mice resulted in decreased cardiac hypertrophy, fibrosis and dysfunction, compared with littermate control mice after abdominal aortic constriction. T-cell MR-KO mice displayed less cardiac inflammation, which was illustrated by decreased accumulation of myeloid cells and reduced expression of inflammatory cytokines. Lower numbers and less activation of T-cells were observed in the heart of T-cell MR-KO mice after abdominal aortic constriction (Torre et al., 2017). Second, they demonstrated that MR deficiency in T-cells strikingly decreased both systolic and diastolic blood pressure and attenuated renal and vascular damage in angiotensin II-infused mice (Torre et al., 2017). Flow cytometric analysis showed that T-cell MR-KO mitigated angiotensin II-induced accumulation of IFN-γ-producing T-cells, particularly CD8+ population, in both kidneys and aortas. At the molecular level, MR interacted with the transcription factors NFAT1 and AP-1 in T-cells (Sun et al., 2017), as summarized in Figure 1.

MR signalling may constitute a new signal 3 in T-cells (Figure 2). T-cells are known to require three signals for activation, referred to as signals 1, 2, and 3. Signal 1 is the recognition by T-cell receptors of specific antigenic peptides, presented on antigen-presenting cells. Signal 2 is co-stimulation, involving interactions of CD80 and CD86 on antigen-presenting cells with CD28 on T-cells. Signal 3 involves stimulation of receptors outside the immunological synapse by hormones, cytokines, and danger signals present in the inflammatory milieu (Barbaro et al., 2017). Thus activation of the MR in lymphocytes could be a new signal 3 for these cells, in the context of arterial hypertension, as the MR in T-cells stimulates production of IFN-γ resulting in hypertension and cardiovascular fibrosis (Figure 2).

Next to CD8+ T-cells, also Th17 might be phenotypically dependent on MR signals. Th17 differentiation in a rat DOCA model was suggested to be MR-dependent (Amador et al., 2014). Moreover, in this model, MRA inhibited DOCA-induced suppression of the Foxp3 transcription factor that controls regulatory T-cell (Treg) differentiation. This leads to the hypothesis that the MR could control the Th17/Treg balance, in the presence of mineralocorticoid abundance. In addition, data from an earlier study by Herrada et al. (Herrada et al., 2010), suggest that both the enhancement of CD8+ T-cell activation and promotion of Th17-polarization by the MR are critically dependent on modulation of dendritic cell responses by aldosterone. In addition, adoptive transfer of regulatory T-cells ameliorated vascular and renal effects of aldosterone infusion in mice (Kasal et al., 2012). DOCA treatment in vivo also decreased the abundance of regulatory T-cells in a MR-dependent manner (Amador et al., 2014). However, the cell type in which MR activation mediates these effects remains unclear.

11 | FUTURE PERSPECTIVES

Two decades ago, two hallmark trials showed that treatment with MRA substantially reduced morbidity and mortality in patients with heart failure. The observed effect was larger than would have been expected from the modest decrease in blood pressure or the diuretic effect (Pitt et al., 1999; Pitt et al., 2003). This underscores the presence of direct adverse cardiovascular effects of the MR, independent of blood pressure regulation, as discussed here. Targeting of the MR in a cell-specific manner could further improve the benefit to risk (side effect) ratio of intervention with MR signalling.

In humans, replication of the evidence from experimental animals that the MR in inflammatory cells causes CVD is a major challenge. A better and deeper understanding of the role of the MR in these cells
is important and clinically relevant for the identification of new therapeutic interventions. Clinical studies which could verify current experimental findings are necessary, but also difficult to undertake. Inflammatory cells serve many different functions in host defence. Accordingly, their non-selective inhibition, as a strategy to treat CVD disease, may result in severe and unwanted immunosuppressive effects. A better understanding of the exact cellular, temporal and spatial contributions of the MR in the immune system to CVD is needed. Only after this step has been taken, can the novel therapeutic approaches targeting the MR in immune cells to combat CVD be addressed in drug development programs. This will require an approach that integrates epidemiological data, data sets about genetic variations in patients and the functional probing of cytokines and immune cells, as well as their crosstalk (Wenzel et al., 2021).

Non-steroid MR antagonists, such as finerenone, have stronger anti-inflammatory effects and less effects on potassium levels, than the steroid MR antagonists. It is primarily thought that this is mediated by a differential cofactor recruitment and tissue distribution (Agarwal et al., 2021). Whether the stronger anti-inflammatory effects of the non-steroid MR antagonists are mediated by preferential inhibition of the MR in inflammatory cells is not known but needs to be further explored.

In the future, we expect nanotechnology to provide important therapeutic advances by specifically targeting myeloid cells in CVD (Duivenvoorden et al., 2019). Next to targeted delivery of drugs to distinct cell populations, the ability to label nanoparticles in order to follow their migration to and accumulation in, tissues of interest is an important additional advantage in research. Although the use of nanotechnology is still seldom used in the field of cardiovascular diseases, over 20 nanotechnological approaches for the delivery of therapeutic modalities have been FDA-approved in various areas of medicine (Duivenvoorden et al., 2019), mainly in the field of oncology and infectious diseases. In the cardiovascular field, research into the application of nanotechnology includes trials investigating the targeting of PCSK9 with small interfering RNA (siRNA), for cholesterol lowering and angiotensinogen targeting for blood pressure reduction (Fitzgerald et al., 2014). Alternatively, nanoparticles could be used to deliver immunomodulating drugs (such as MRAs) to plaque macrophages, as has already been explored in a HDL nanoparticle model for the delivery of simvastatin (Duivenvoorden et al., 2014). Again, when designing myeloid-cell targeting nanoparticles, the most important challenge is to ensure development of technologies with enough precision as not to affect the host defence system, in general.

12 | CONCLUSION

Thirty-five years after the cloning of the MR, there is clear evidence that it plays an important role in inflammatory CVD. Virtually, all cells in the cardiovascular system express the MR. Especially for myeloid cells, and to a lesser extent lymphoid cells, strong phenotypic changes are induced by MR signalling. Under the influence of increased MR signalling, monocytes and macrophage polarize towards an M1-like phenotype, with increased pro-inflammatory cytokine and chemokine production. Lymphocytic MR signalling promotes IFN-γ production and hypertension. Although preclinical data are plentiful, the lack of human data makes it difficult to translate these findings to clinical practice. As MR antagonists are readily available and the number of patients in which they are administered is growing, answering many of the questions that remain on the role of the MR on immune cells in hypertension, atherosclerosis and tissue remodelling seems within reach. The growing field of nanotechnology will help to target inflammation in a cell- and tissue-specific manner, and nanotechnological targeting of the myeloid and lymphoid MR is an interesting novel and topical for future investigations.

12.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos, et al., 2021; Alexander, Cidlowski, et al., 2021; Alexander, Fabbro, et al., 2021a, 2021b; Alexander, Kelly, et al., 2021a, 2021b).

ACKNOWLEDGEMENTS

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Niels P. Riiksen is supported by the Dutch Heart Foundation IN-CONTROL II CVON grant (CVON2018-27) and the IMPRESS DCVA grant (2020B,004) and the MEMORY consortium grant of the ERA-CVD Joint Transnational Call 2018 by the Dutch Heart Foundation (UTC2018, project MEMORY; 2018T093). Marlies Bode and Ulrich Wenzel are supported by the German Research Foundation (WE 1688/19-1).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

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REFERENCES

Agarwal, R., Kolchkof, P., Bakris, G., Bauersachs, J., Haller, H., Wada, T., & Zannad, F. (2021). Steroidal and non-steroidal mineralocorticoid receptor antagonists in cardiorenal medicine. European Heart Journal, 42, 152–161. https://doi.org/10.1093/eurheartj/ehaa736
Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Abbracchio, M. P., Alexander, Fabbro, et al., 2021a, 2021b; Alexander, Kelly, et al., 2021a, 2021b).
reveals the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis. Circulation Research, 122, 1661–1674. https://doi.org/10.1161/CIRCRESAHA.117.312509

De Cuieis, C., Amiri, F., Brassard, P., Endemann, D. H., Touyz, R. M., & Schiffrin, E. L. (2005). Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II–infused macrophage colony-stimulating factor–deficient mice: Evidence for a role in inflammation in angiotensin-induced vascular injury. Arteriosclerosis, Thrombosis, and Vascular Biology, 25, 2106–2113. https://doi.org/10.1161/01.ATV.0000181743.28028.57

Dol, T., Dol, S., Nakashima, A., Ueno, T., Yokoyama, Y., Kohno, N., & Masaki, T. (2014). Mibozirib ameliorates renal injury and hypotension along with the attenuation of renal caspase-1 expression in aldosterone-salt-treated rats. PLoS ONE, 9, e93513. https://doi.org/10.1371/journal.pone.0093513

Duivenvoorden, R., Senders, M. L., van Leent, M. M. T., Perez-Medina, C., Doi, T., Doi, S., Nakashima, A., Ueno, T., Yokoyama, Y., Kohno, N., & van der HEIDEN ET AL. (2017). Macrophage colony-stimulating factor signalling diversity. The Journal of Endocrinology, 234, T23–T34. https://doi.org/10.1530/JEO-17-0060

Funder, J. W. (2013). Mineralocorticoid receptor antagonists: Emerging roles in cardiovascular medicine. Integr Blood Pressure Control, 6, 129–138. https://doi.org/10.2147/IBPC.S13783

Funder, J. W., & Zennaro, M. C. (2017). 30 years of the mineralocorticoid receptor: The scientific impact of cloning the mineralocorticoid receptor: 30 years on. The Journal of Endocrinology, 234, E3–E6. https://doi.org/10.1530/JEO-17-0264

Guzik, T. J., Hoch, N. E., Brown, K. A., McCann, L. A., Rahman, A., Dikalov, S., Goronyz, J., Weyand, C., & Harrison, D. G. (2007). Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. The Journal of Experimental Medicine, 204, 2449–2460. https://doi.org/10.1084/jem.20070657

Herrada, A. A., Contreras, F. J., Marini, N. P., Anadó, C. A., Gonzalez, P. A., Cortes, C. M., Cortés, C. M., Riedel, C. A., Carvajal, C. A., Figueroa, F., Michtea, L. F., & Fardella, C. E. (2010). Aldosterone promotes autoimmune damage by enhancing Th17-mediated immunity. Journal of Immunology, 184, 191–202. https://doi.org/10.4049/jimmunol.0802886

Holaj, R., Zelinka, T., Wichterle, D., Petrak, O., Strauch, B., & Widmisky, J. Jr. (2007). Increased intima-media thickness of the common carotid artery in primary aldosteronism in comparison with essential hypertension. Journal of Hypertension, 25, 1451–1457. https://doi.org/10.1097/HJH.0b013e3281268532

Huang, L. L., Nikolic-Paterson, D. J., Han, Y., Ozols, E., Ma, F. Y., Young, M. J., & Tesch, G. H. (2014). Myeloid mineralocorticoid receptor activation contributes to progressive kidney disease. Journal of the American Society of Nephrology, 25, 2231–2240. https://doi.org/10.1681/ASN.2012111094

Hunag, C. S., Chou, C. H., Wu, X. M., Chang, Y. Y., Wu, V. C., Chen, Y. H., Chang, Y. S., Tsai, Y. C., Su, M. J., Ho, Y. L., Chen, M. F., Wu, K. D., Lin, Y. H., & TAIPAI Study Group. (2015). Circulating tissue inhibitor of matrix metalloproteinase-1 is associated with aldosterone-induced diastolic dysfunction. Journal of Hypertension, 33, 1922–1930 discussion 1930. https://doi.org/10.1097/HJH.0000000000000619

Inoue, S., Egashira, K., Ni, W., Kitamoto, S., Usui, M., Otani, K., Ishibashi, M., Hiasa, K. I., Nishida, K. I., & Takeshita, A. (2002). Antimonocytokine chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E–knockout mice. Circulation, 106, 2700–2706. https://doi.org/10.1161/01.CIR.0000038140.80105.AD

Ivanës, F., Susen, S., Mouquet, P., Pigny, P., Cuilleret, F., Sautiere, K., Collet, J. P., Beygui, F., Hennache, B., Ennezat, P. V., & Juthier, F. (2012). Aldosterone, mortality, and acute ischaemic events in coronary artery disease patients outside the setting of acute myocardial infarction or heart failure. European Heart Journal, 33, 191–202. https://doi.org/10.1093/eurheartj/ehr176

Ji, H., Pai, A. V., West, C. A., Wu, X., Speth, R. C., & Sandberg, K. (2017). Loss of resistance to angiotensin II-induced hypertension in the Jackson laboratory recombination-activating gene null mouse on the C57BL/6J background. Hypertension, 69, 1121–1127. https://doi.org/10.1161/HYPERTENSIONAHA.117.09063

Ji, H., Zheng, W., Li, X., Liu, J., Wu, X., Zhang, M. A., Umans, J. G., Hay, M., Speth, R. C., Dunn, S. E., & Sandberg, K. (2014). Sex-specific T-cell regulation of angiotensin II-dependent hypertension. Hypertension, 64, 573–582. https://doi.org/10.1161/HYPERTENSIONAHA.114.03663

Joseph, P., Leong, D., McKee, M., Anand, S. S., Schwalm, J. D., Teo, K., Mente, A., & Yusuf, S. (2017). Reducing the global burden of cardiovascular disease, part 1: The epidemiology and risk factors. Circulation, 121, 677–694. https://doi.org/10.1161/CIRCRESAHA.117.308903

Kasal, D. A., Barhoumi, T., Li, M. W., Yamamoto, N., Zdanovich, E., Rehman, A., Neves, M. F., Laurant, P., Paradis, P., & Schiffrin, E. L. (2012). T regulatory lymphocytes prevent aldosterone-induced
vascular injury. Hypertension, 59, 324–330. https://doi.org/10.1161/HYPERTENSIONAHA.111.181123

Keidar, S., Hayek, T., Kaplan, M., Pavlotzky, E., Hamoud, S., Coleman, R., & Aviram, M. (2003). Effect of eplerenone, a selective aldosterone blocker, on blood pressure, serum and macrophage oxidative stress, and atherosclerosis in apolipoprotein E-deficient mice. Journal of Cardiovascular Pharmacology, 41, 955–963. https://doi.org/10.1097/00005344-200306000-00019

Keidar, S., Kaplan, M., Pavlotzky, E., Coleman, R., Hayek, T., Hamoud, S., & Aviram, M. (2004). Aldosterone administration to mice stimulates macrophage NADPH oxidase and increases atherosclerosis development: A possible role for angiotensin-converting enzyme and the receptors for angiotensin II and aldosterone. Circulation, 109, 2213–2220. https://doi.org/10.1161/CIRCULATIONAHA.104.509183

Ketelhut, D. F., & Back, M. (2011). The role of matrix metalloproteinases in atherothrombosis. Current Atherosclerosis Reports, 13, 162–169. https://doi.org/10.1007/s11883-010-0159-7

Ko, S. H., Pan, H., Grigoropoulos, C. P., Luscombe, C. K., Fréchet, J. M., & Poulikakos, D. (2007). All-inkjet-printed flexible electronics fabrication on a polymer substrate by low-temperature high-resolution selective laser sintering of metal nanoparticles. Nanotechnology, 18, 345202. https://doi.org/10.1088/0957-4484/18/34/345202

Kojima, Y., Downing, K., Kundu, R., Miller, C., Dewey, F., Lancero, H., Razz, U., Perić, L., Hedin, U., Schadt, E., Maegdefessel, L., Quertermous, T., & Leeper, N. J. (2014). Cyclin-dependent kinase inhibitor 2B regulates efferocytosis and atherosclerosis. The Journal of Clinical Investigation, 124, 1083–1097. https://doi.org/10.1172/JCI70391

Krishnan, S. M., Dowling, J. K., Ling, Y. H., Diep, H., Chan, C. T., Ferens, D., Kett, M. M., Pinar, A., Samuel, C. S., Vinh, A., Arumugam, T. V., Hewitson, T. D., Kemp-Harper, B. K., Robertson, A. A. B., Cooper, M. A., Latz, E., Mansell, A. S., Obey, C. G., & Drummond, G. R. (2016). Inflammasome activity is essential for one kidney/deoxycorticosterone acetate/salt-induced hypertension in mice. British Journal of Pharmacology, 173, 752–765. https://doi.org/10.1111/bph.13230

Krishnan, S. M., Ling, Y. H., Huuskes, B. M., Ferens, D. M., Saini, N., Chan, C. T., Diep, H., Kett, M. M., Samuel, C. S., Kemp-Harper, B. K., Robertson, A. A. B., Cooper, M. A., Peter, K., Latz, E., Mansell, A. S., Obey, C. G., Drummond, G. R., & Vinh, A. (2019). Pharmacological inhibition of the NLRP3 inflammasome reduces blood pressure, renal damage, and dysfunction in salt-sensitive hypertension. Cardiovascular Research, 115(4), 776–787. https://doi.org/10.1093/cvr/cvy252

Krysiak, R., & Okopien, B. (2012). The effect of treatment on monocyte and lymphocyte cytokine release in patients with aldosteronoma. Hypertension Research, 35(1), 123–125. https://doi.org/10.1038/hr.2011.142

Labuzek, K., Liber, S., Buldak, L., Machnik, G., Liber, J., & Okopien, B. (2013). Eplerenone promotes alternative activation in human monocyte-derived macrophages. Pharmacological Reports, 65, 226–234. https://doi.org/10.1007/s13194-013-0908-9

Leentjens, J., Bekkering, S., Joosten, L. A. B., Netea, M. G., Burgner, D. P., & Rispens, N. (2018). Trained innate immunity as a novel mechanism of organ damage in primary aldosteronism compared with essential hypertension. Endocrinology and Metabolism (Seoul), 31, 567–576. https://doi.org/10.3803/EmN.2016.31.4.567

Ling, Y. H., Krishnan, S. M., Chan, C. T., Diep, H., Ferens, D., Chint-Dusting, J., Kemp-Harper, B. K., Samuel, C. S., Hewitson, T. D., Latz, E., Mansell, A., Sobey, C. G., & Drummond, G. R. (2017). Anakinra reduces blood pressure and renal fibrosis in one kidney/DOCA/salt-induced hypertension. Pharmacological Research, 116, 77–86. https://doi.org/10.1016/j.phrs.2016.12.015

Lother, A., Berger, S., Gilsbach, R., Rösner, S., Ecke, A., Barreto, F., Bauersachs, J., Schütz, G., & Hein, L. (2011). Ablation of mineralocorticoid receptor in myocytes but not in fibroblasts preserves cardiac function. Hypertension, 57, 746–754. https://doi.org/10.1161/HYPERTENSIONAHA.110.163287

Lother, A., Moser, M., Bode, C., Feldman, R. D., & Hein, L. (2015). Mineralocorticoids in the heart and vasculature: New insights for old hormones. Annual Review of Pharmacology and Toxicology, 55, 289–312. https://doi.org/10.1146/annurev-pharmtox-010814-124302

Machnik, A., Neuhof, W., Jantsch, J., Dahlmann, A., Tammela, T., Machura, K., Park, J. K., Beck, F. X., Müller, D. N., Derer, W., Goss, J., Ziomber, A., Dietsch, P., Wagner, H., van Rooijen, N., Kurtz, A., Hilgers, K. F., Altitalo, K., Eckardt, K. U., ... Titze, J. (2009). Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. Nature Medicine, 15, 545–552. https://doi.org/10.1038/nm.1960

Madhur, M. S., Kirabo, A., Guzik, T. J., & Harrison, D. G. (2020). From rags to riches: Moving beyond RAG1 in studies of hypertension. Hypertension, 75, 930–934. https://doi.org/10.1161/HYPERTENSIONAHA.119.14612

Martin-Fernandez, B., Rubio-Navarro, A., Cortegano, I., Ballesteros, S., Alia, M., Cannata-Ortiz, P., Olivares-Alváro, E., Egido, J., de Andrés, B., Gaspar, M. L., & de Las Heras, N. (2016). Aldosterone induces renal fibrosis and inflammatory M1-macrophage subtype via mineralocorticoid receptor in rats. PLoS ONE, 11, e0145946. https://doi.org/10.1371/journal.pone.0145946

Mattson, D. L., Lund, H., Guo, C., Rudemiller, N., Geurts, A. M., & Jacob, H. (2013). Genetic mutation of recombination activating gene 1 in Dahl salt-sensitive rats attenuates hypertension and renal damage. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 304, R407–R414. https://doi.org/10.1152/ajpregu.00304.2012

McGraw, A. P., Bagley, J., Chen, W. S., Galayda, C., Nickerson, H., Armani, A., Caprio, M., Carmeliet, P., & Jaffe, I. Z. (2013). Aldosterone increases early atherosclerosis and promotes plaque inflammation through a placental growth factor-dependent mechanism. Journal of the American Heart Association, 2, e000018. https://doi.org/10.1161/JAHA.112.000018

Montes-Cobos, E., Schweingruber, N., Li, X., Fischer, H. J., Reichardt, H. M., & Lühder, F. (2017). Deletion of the mineralocorticoid receptor in myeloid cells attenuates central nervous system autoimmunity. Frontiers in Immunology, 8, 1319. https://doi.org/10.3389/fimmu.2017.01319

Monticone, S., D’Ascenzo, F., Moretti, C., Williams, T. A., Veglio, F., Gaita, F., & Mulatero, P. (2018). Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: A systematic review and meta-analysis. The Lancet Diabetes and Endocrinology, 6, 41–50. https://doi.org/10.1016/S2213-8587(17)30319-4

Moore, K. J., Koplev, S., Fisher, E. A., Tabas, I., Bjorkgren, J. L. M., Doran, A. C., & Kovacic, J. C. (2018). Macrophage trafficking, inflammatory resolution, and genomics in atherosclerosis: JACC macrophage...
in CVD series (part 2). *Journal of the American College of Cardiology*, 72, 2181–2197. https://doi.org/10.1016/j.jacc.2018.08.2147

Moore, K. J., Sheedy, F. J., & Fisher, E. A. (2013). Macrophages in atherosclerosis: A dynamic balance. *Nature Reviews. Immunology*, 13, 709–721. https://doi.org/10.1038/nri3520

Moss, M. E., Carvajal, B., & Jaffe, I. Z. (2019). The endothelial mineralocorticoid receptor: Contributions to sex differences in cardiovascular disease. *Pharmacology & Therapeutics*, 203, 107387. https://doi.org/10.1016/j.pharmthera.2019.06.009

Nahrendorf, M., & Swirski, F. K. (2016). Abandoning M1/M2 for a network model of macrophage function. *Circulation Research*, 119, 414–417. https://doi.org/10.1161/CIRCRESAHA.116.309194

Netea, M. G., Joosten, L. A., Latz, E., Mills, K. H., Natoli, G., Stunnenberg, H. G., O’Neill, L. A., & Xavier, R. J. (2016). Trained immunity: A program of innate immune memory in health and disease. *Science*, 352, aaf1098. https://doi.org/10.1126/science.aaf1098

Ong, G. S., & Young, M. J. (2017). Mineralocorticoid regulation of cell function: The role of rapid signalling and gene transcription pathways. *Journal of Molecular Endocrinology*, 58, R33–R57.

Pinto, A. R., Paolicelli, R., Salimova, E., Gospocić, J., Slonimsky, E., Bilbao-Cortes, D., Godwin, J. W., & Rosenthal, N. A. (2012). An abundant tissue macrophage population in the adult murine heart with a distinct alternatively-activated macrophage profile. PloS One, 7, e36814. https://doi.org/10.1371/journal.pone.0036814

Pitt, B., Remme, W., Zannad, F., Neaton, J., Martinez, F., Roniker, B., Bittman, R., Hurley, S., Kleiman, J., Gatin, M., & Eplerone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study Investigators. (2003). Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *The New England Journal of Medicine*, 348, 1309–1321. https://doi.org/10.1056/NEJMoa030207

Pitt, B., Zannad, F., Remme, W. J., Cody, R., Castaigne, A., Perez, A., Palensky, J., & Wittes, J. (1999). The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *The New England Journal of Medicine*, 341, 709–717. https://doi.org/10.1056/NEJM199909023411001

Raz-Pasteur, A., Gamliel-Lazrovich, A., Coleman, R., & Keidar, S. (2012). Eplerenone reduced lesion size in early but not advanced atherosclerosis in apolipoprotein E-deficient mice. *Journal of Cardiovascular Pharmacology*, 60(6), 508–512. https://doi.org/10.1097/JCP.0b013e3182652525

Raz-Pasteur, A., Gamliel-Lazrovich, A., Gantman, A., Coleman, R., & Keidar, S. (2014). Mineralocorticoid receptor blockade inhibits accelerated atherosclerosis induced by a low sodium diet in apolipoprotein E-deficient mice. *Journal of the Renin-Angiotensin-Aldosterone System*, 15, 228–235. https://doi.org/10.1177/1071441714525196

Remde, H., Dietz, A., Emeny, R., Aebi, P., Peters, A., de las Heras, A., & Rothe, H. (2014). The cardiovascular markers copeptin and high-sensitive C-reactive protein decrease following specific therapy for atherosclerosis. *Jour- nal of Endocrinology*, 229(2), 327–335. https://doi.org/10.1530/EJENDO-13-0651

Rommel, C., Heier, K., Wirth, J., Janssens, S., Jaffe, I. Z., Kreutz, A., & Gavrilovic, J. (2004). Loss of the mineralocorticoid receptor leads to a robust wild-type hypertensive phenotype in response to Ang II (angiotensin II). *Hypertension*, 75, 1110–1116. https://doi.org/10.1161/HYPTENSIONAHA.119.13773

Rude, M. K., Duhaney, T. A., Kuster, G. M., Judge, S., Heo, J., Colucci, W. S., Siwik, D. A., & Sam, F. (2005). Aldosterone stimulates matrix metalloproteinases and reactive oxygen species in adult rat ventricular cardiomyocytes. *Hypertension*, 45, 555–561. https://doi.org/10.1161/01.HYP.0000176236.55322.18

Ruhs, S., Holze, A., Hübschmann, R., & Grossmann, C. (2017). 30 years of the mineralocorticoid receptor: Nongenomic effects via the mineralocorticoid receptor. *Journal of Endocrinology*, 234(1), T107–T124.

Savory, J., P lespreux, G. G., Lamprecht, C., Liao, M., Walther, R. F., Lefebvre, Y. A., & Haché, R. J. (2001). Glucocorticoid receptor homodimers and glucocorticoid-mineralocorticoid receptor heterodimers form in the cytoplasm through alternative dimerization interfaces. *Molecular and Cellular Biology*, 21, 781–793. https://doi.org/10.1128/MCB.21.3.781-793.2001

Seniuk, A., Thiele, J. L., Stubbe, A., Oser, P., Rosendahl, A., Bode, M., Meyer-Schwesinger, C., Wenzel, U. O., & Ehmk, H. (2020). B6 RAG1 knockout mice generated at the Jackson Laboratory in 2009 show a robust wild-type hypertensive phenotype in response to Ang II (angiotensin II). *Hypertension*, 75, 1110–1116. https://doi.org/10.1161/HYPTENSIONAHA.119.13773

Shen, J. Z., Morgan, J., Tesch, G. H., Rickard, A. J., Chrissobolis, S., Drummond, G. R., Fuller, P. J., & Young, M. J. (2016). Cardiac tissue injury and remodeling is dependent upon MR regulation of activation pathways in cardiac tissue macrophages. *Endocrinology*, 157, 2313–2322. https://doi.org/10.1210/endo-2016-1040

Shen, Z. X., Chen, X. Q., Sun, X. N., Sun, J. Y., Zhang, W. C., Zheng, X. J., Zhang, Y. Y., Shi, H. J., Zhang, J. W., Li, C., & Wang, J. (2016a). Mineralocorticoid receptor deficiency in macrophages inhibits atherosclerosis by affecting foam cell formation and efferocytosis. *The Journal of Biological Chemistry*, 292, 925–935.

Shen, Z. X., Chen, X. Q., Sun, X. N., Sun, J. Y., Zhang, W. C., Zheng, X. J., Zhang, Y. Y., Shi, H. J., Zhang, J. W., Li, C., Wang, J., Liu, X., & Duan, S. Z. (2016b). Mineralocorticoid receptor deficiency in macrophages inhibits atherosclerosis by affecting foam cell formation and efferocytosis. *The Journal of Biological Chemistry*, 292–935. https://doi.org/10.1074/jbc.M116.739243

Silvestre-Roig, C., Braster, Q., Ortega-Gomez, A., & Soehnlein, O. (2020). Neutrophils as regulators of cardiovascular inflammation. *Nature Reviews. Cardiology*, 17, 327–340. https://doi.org/10.1038/s41569-019-0326-7

Somlova, Z., Petrak, O., Rosa, J., Strauch, B., Indra, T., Zelinka, T., Haluzík, M., Zíkan, V., Holaj, R., & Wdowski, J. Jr. (2016). Inflammatory markers in primary aldosteronism. *Physiological Research*, 65, 229–237.

Sonder, S. U., Mikkelsen, M., Rieneck, K., Hedegaard, C. J., & Bendtzen, K. (2006). Effects of spirinolactone on human blood mononuclear cells: Mineralocorticoid receptor independent effects on gene expression and late apoptosis induction. *British Journal of Pharmacology*, 148, 46–53. https://doi.org/10.1038/sj.bjp.0706700

Staermose, S., Marwick, T. H., Gordon, R. D., Cowley, D., Dowling, A., & Stowasser, M. (2009). Elevated serum interleukin 6 levels in normotensive individuals with familial hyperaldosteronism type 1. *Hypertension*, 53, e31–e32. https://doi.org/10.1161/HYPERTENSIONAHA.108.128512

Strauch, B., Petrak, O., Wichterle, D., Zelinka, T., Holaj, R., & Wdowski, J. Jr. (2006). Increased arterial wall stiffness in primary aldosteronism in
comparison with essential hypertension. American Journal of Hypertension, 19, 909–914. https://doi.org/10.1016/j.amjhyper.2006.02.002

Strach, B., Petrak, O., Zelinka, T., Wichterle, D., Holaj, R., Kasalicky, M., Safarik, L., Rosa, J., & Widimsky, J. (2008). Adrenalecytomy improves arterial stiffness in primary aldosteronism. American Journal of Hypertension, 21, 1086–1092. https://doi.org/10.1016/j.ajh.2008.243

Sun, J. Y., Li, C., Shen, Z. X., Zhang, W. C., Al T. J., Du, L. J., Zhang, Y. Y., Yao, G. F., Liu, Y., Sun, S., & Naray-Fejes-Toth, A. (2016). Mineralocorticoid receptor deficiency in macrophages inhibits neointimal hyperplasia and suppresses macrophage inflammation through SGK1-AP1/NF-kappaB pathways. Arteriosclerosis, Thrombosis, and Vascular Biology, 36, 874–885. https://doi.org/10.1161/ATVBAHA.115.307037

Sun, X. N., Li, C., Liu, Y., Du, L. J., Zeng, M. R., Zheng, X. J., Zhang, W. C., Liu, Y., Zhu, M., Kong, D., & Zhou, L. (2017). T-cell mineralocorticoid receptor controls blood pressure by regulating interferon-gamma. Circulation Research, 120, 1584–1597. https://doi.org/10.1161/CIRCRESAHA.116.310480

Swirsik, F. K., & Nahrendorf, M. (2013). Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science, 339, 161–166. https://doi.org/10.1126/science.1230719

Tardif, J. C., Kouz, S., Waters, D. D., Bertrand, O. F., Diaz, R., Maggioni, A. P., Pinto, F. J., Ibrahim, R., Gomara, H., Kiwan, G. S., Berry, C., Lopez-Sendon, J., Ostadal, P., Koenig, W., Angoulvant, D., Gregoire, J. C., Lavoie, M. A., Dubé, M. P., R manh, D., R. Roubille, F. (2019). Efficacy and safety of low-dose cloficarb after myocardial infarction. The New England Journal of Medicine, 381, 2497–2505. https://doi.org/10.1056/NEJMoa1912388

Tarjus, A., Martinez-Martinez, E., Amador, C., Latouche, C., El Moghrabi, S., Berger, T., Mak, T. W., Fay, R., Farman, N., Rossignol, P., & Zannad, F. (2015). Neutrophil gelatinase-associated lipocalin, a novel mineralocorticoid- induced biotarget, mediates vascular profibrotic effects of mineralocorticoids. Hypertension, 66, 158–166. https://doi.org/10.1161/HYPERTENSIONAHA.115.05431

Tikellis, C., Pickering, R. J., Tsorotes, D., Huet, O., Chin-Dusting, J., Cooper, M. E., & Thomas, M. C. (2012). Activation of the Renin-Angiotensin system mediates the effects of dietary salt intake on atherosclerosis in the apolipoprotein E knockout mouse. Hypertension, 60(1), 98–105. https://doi.org/10.1161/HYPERTENSIONAHA.112.191767

Torre, C., Abrave, P., Tsomitsa, L. L., Mottola, G., Lepolard, C., Trouplin, V., Gimenez, C., Desrousseaux, J., Gempp, S., Levasseur, A., Padovani, L., Lemiche, E., & Ghigo, E. (2017). Staphylococcus aureus promotes Smad-4/PGR-2/Smed-setd8-1 methyltransferase Signalling in planarian neoblasts to sensitize anti-bacterial gene responses during re-infection. eBioMedicine, 20, 150–160. https://doi.org/10.1016/j.ebiom.2017.04.031

Tzamou, V., Vyssouls, G., Karpanou, E., Kyvelou, S. M., Gialernios, T., & Stefanadis, C. (2013). Aldosterone levels and inflammatory stimulation in essential hypertensive patients. Journal of Human Hypertension, 27, 535–538. https://doi.org/10.1038/jhh.2013.13

Usher, M. G., Duan, S. Z., Iwaschenko, C. Y., Frieler, R. A., Berger, S., Schütz, G., Lumeng, C. N., & Mortensen, R. M. (2010). Myeloid mineralocorticoid receptor controls macrophage polarization and cardiovascular hypertrophy and remodeling in mice. The Journal of Clinical Investigation, 120, 3350–3364. https://doi.org/10.1172/JCI41080

van den Berg, T. N., Rongen, G. A., Frolich, G. M., Deinum, J., Hausenloy, D. J., & Riksen, N. P. (2014). The cardioprotective effects of mineralocorticoid receptor antagonists. Pharmacology & Therapeutics, 142, 72–87. https://doi.org/10.1016/j.pharmthera.2013.11.006

van der Heijden, C., Deinum, J., van Buul, J. D., Ravidani, A., Nederveen, A. J., Verberne, H. H., Scipione, C., & Nieuwdorp, M. (2016). Oxidized phospholipids on lipoprotein(a) elicit arterial wall inflammation and an inflammatory monocyte response in humans. Circulation, 134, 611–624. https://doi.org/10.1161/CIRCULATIONAHA.116.020838

Victor, V. M., Rocha, M., Sola, E., Banuls, C., Garcia-Malpartida, K., & Hernandez-Mijares, A. (2009). Oxidative stress, endothelial dysfunction and atherosclerosis. Current Pharmaceutical Design, 15, 2988–3002. https://doi.org/10.2174/138161209789058093

Wada, T., Ishikawa, A., Watanabe, E., Nakamura, Y., Aruga, Y., Hasegawa, H., Onogi, Y., Honda, H., Nagai, Y., Takats, K., Ishii, Y., Sasahara, M., Koya, D., Tsuneki, H., & Sasaoka, T. (2017). Eplerenone prevented obesity-induced inflammatory activation and glucose intolerance. The Journal of Endocrinology, 235, 179–191. https://doi.org/10.1530/JOE-17-0351

Wagstaff, D., Zhu, C., Bjorkgren, J., Skogsberg, J., & Eriksson, P. (2011). MMP-2 and MMP-9 are prominent matrix metalloproteinases during atherosclerosis development in the LDLr−/−/Apob(100/100) mouse. International Journal of Molecular Medicine, 28, 247–253. https://doi.org/10.3892/ijmm.2011.693

Wenzel, K., Rajakumar, A., Haase, H., Geusens, N., Hubner, N., Schulz, H., Brewer, J. Roberts, L., Hubel, C. A., Herse, H., Herling, L., Qadri, F., Lindschau, C., Wallukat, G., Pijnenborg, R., Heidecke, H., Riemekasten, G., Luft, F. C., Muller, D. N., … Deckend, R. (2011). Angiotensin II type 1 receptor antibodies and increased angiotensin II intolerance. Circulation Research, 62, 364–375. https://doi.org/10.1161/CIRCULATIONAHA.110.185711

Wenzel, U., Turner, J. E., Krebs, C., Kurtz, C., Harrison, D. G., & Ehmkhe, H. (2016). Immune mechanisms in arterial hypertension. Journal of the American Society of Nephrology, 27, 677–686. https://doi.org/10.1681/ASN.2015050562

Wenzel, U. O., Ehmkhe, H., & Bode, M. (2021). Immune mechanisms in arterial hypertension. Recent advances. Cell and Tissue Research, 385, 393–404. https://doi.org/10.1007/s00441-020-03409-0

How to cite this article: van der Heijden, C. D. C. C., Bode, M., Riksen, N. P., & Wenzel, U. O. (2022). The role of the mineralocorticoid receptor in immune cells in cardiovascular disease. British Journal of Pharmacology, 179(13), 3135–3151. https://doi.org/10.1111/bjp.15782