Corticosteroids, Heart Failure, and Hypertension: A Role for Immune Cells?

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Aldosterone and its receptor the mineralocorticoid receptor (MR) are best known for their regulation of fluid and electrolyte homeostasis in epithelial cells. However, it is now clear that MR are also expressed in a broad range of nonepithelial tissues including the cardiovascular system. In the heart and vascular tissues, pathological activation of MR promotes cardiovascular inflammation and remodeling for which there is increasing evidence that macrophages and other immune cells (e.g. T cells and dendritic cells) play a significant role. While the glucocorticoids and their receptors have well-described antiinflammatory actions in immune cells, a role for aldosterone and/or the MR in these cells is largely undefined. Emerging evidence, however, suggests that MR signaling may directly or indirectly promote proinflammatory responses in these immune cells. This review will discuss the current understanding of the role of corticosteroid receptors in macrophages and their effect on cardiovascular diseases involving inflammation. **(*Endocrinology* 153: 5692–5700, 2012)**

More people die from cardiovascular disease worldwide than any other cause with an estimated 17.3 million deaths per year (1). The prognosis of symptomatic heart failure remains dismal with average 1-yr and 5-yr mortality rates of 35% to 50%, respectively, despite major therapeutic advances (2–4). Moreover, the economic burden of heart failure is increasing globally in terms of healthcare requirements, disability, and premature deaths (5, 6).

Vascular and target organ inflammation from tissue injury has long been recognized to underlie the complex pathophysiology of cardiovascular disease regardless of the initiating event such as ischemia, autoimmunity, or activation of the renin-angiotensin-aldosterone system. The immune system and endogenous corticosteroids are important modulators of this inflammatory process with recent studies describing a novel role for the macrophage mineralocorticoid receptor (MR) in modulating cardiac remodeling and systolic blood pressure (7–9). The aim of this review is to highlight the growing role of the immune system in modulating the outcome of MR signaling in cardiovascular tissues and of MR signaling in modulating the immune response.

Corticosteroids and Their Receptors

The corticosteroid receptors, glucocorticoid and mineralocorticoid receptors (GR and MR) are members of the steroid hormone receptor subgroup of the nuclear receptor superfamily of ligand-activated transcription factors (10, 11). A range of nongenomic responses have also been described for steroid hormone receptor and involve rapid intracellular signaling responses (12, 13). MR bind both endogenous glucocorticoids (cortisol in human, corticosterone in rodent) and mineralocorticoids (aldosterone) with high affinity (14). However, the epithelial MR selectivity for the physiological mineralocorticoids (*e.g.* aldosterone) is conferred by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) conversion of cortisol to cortisone, which is inactive at the MR (15, 16). In contrast, the absence of 11βHSD2 in nonepithelial tissues

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Abbreviations: DOC, Deoxycorticosterone; GR, glucocorticoid recptor; 11βHSD2, 11β-hydroxysteroid dehydrogenase type 2; MR, mineralocorticoid receptor; MRKO, MR knockout; NAME, nitro-arginine methyl ester; NF-κB, nuclear transcription factor-κB; PA, primary aldosteronism; VSMC, vascular smooth muscle cell.

(*e.g.* cardiomyocytes, monocytes/macrophages) allows physiological glucocorticoids to modulate MR signaling in these tissues. The specific role of MR signaling in these nonepithelial tissues is now recognized as an important mediator of cardiac pathology and a role for the nonepithelial MR as a second receptor for cortisol in normal physiology is increasingly being appreciated.

The corticosteroid hormones, via their effects on MR and GR, play an important role in cardiovascular inflammation. GR signaling is predominantly antiinflammatory in many systemic and local disease models, mainly due to its potent immunosuppressive effects. Despite the postulated antiinflammatory benefits in cardiovascular tissues, adverse cardiovascular effects are seen in glucocorticoid excess states such as Cushing's syndrome and patients on therapeutic exogenous glucocorticoids. The effect of glucocorticoid excess on cardiovascular disease is complex and multifactorial, and the independent effects of GR signaling remain difficult to interrogate (reviewed in Ref. 17). Whereas genomic and nongenomic MR regulation of proinflammatory and profibrotic pathways have been described in vascular endothelial and smooth muscle cells (18–20) and cardiomyocytes (21, 22), the exact role of the MR in immune cells such as macrophages has not been completely elucidated. Recent studies, however, suggest that MR signaling in macrophages also mediates proinflammatory and profibrotic gene expression (7, 9). This review will focus on MR-mediated cardiovascular pathology and the modulating role of the immune system on the underlying inflammation.

MR Activation and Cardiovascular Disease

Sustained activation of the MR by elevated aldosterone levels leads to cardiovascular dysfunction and pathology. Primary aldosteronism (PA) is characterized by autonomous mineralocorticoid excess and is a common cause of secondary hypertension (23, 24). Patients with primary aldosteronism experience disproportionately more cardiovascular events than those with essential hypertension matched for blood pressure levels (25). Under normal circumstances, MR signaling in renal tubules leads to sodium and water retention and thus maintains extracellular volume and blood pressure. Aldosterone excess mediates hypertension not only via potentiating renal effects but also via MR in the central nervous system (26, 27) and in the vascular wall. Activation of vascular MR directly regulates vascular tone and thus total peripheral resistance (28); in the longer term, elevated aldosterone contributes to remodeling of the vessel wall which, in turn, contributes to the maintenance of a higher blood pressure (19, 29, 30). Vascular smooth muscle cell (VSMC) MR can also independently regulate blood pressure in aged animals in which changes in renal sodium handling and vascular structure are unaffected (30).

The detrimental effects of MR activation in the heart are also clearly illustrated by clinical studies using MR antagonists. Large randomized controlled trials (RALES, EPHESUS, and EMPHASIS) have demonstrated the unequivocal mortality and morbidity benefits of MR antagonist treatment in both mild and moderately severe symptomatic heart failure, implicating MR signaling as a key mediator of heart disease (32–34). However, the ligand responsible for MR activation had been the subject of debate given that circulating levels of aldosterone in these patients were barely elevated (35). This raises the possibility that the cardiovascular benefits conferred by aldosterone antagonists were at least partly independent of aldosterone itself and lend support to a role for cortisol in pathological states such as heart failure (36). Cardiac MR expression has also been found to be increased in failing hearts in humans and experimental animals (37, 38). The clinical benefits of MR antagonists are further supported by extensive animal studies which illustrate the effects of an MR activated state on cardiac inflammation and remodeling after aldosterone or deoxycorticosterone (DOC) plus salt (aldosterone/salt, DOC/salt) (39–41) or angiotensin II administration and/or L-nitro-arginine methyl ester (angiotensin II/L-NAME) (9, 42, 43). These studies again show that mineralocorticoid-dependent and -independent MR activation promote tissue inflammation, remodeling, and left ventricular dysfunction. The current review aims to highlight the interactions between the immune system and cardiovascular tissue in both the clinical setting and these experimental models.

MR Signaling in Cardiac Inflammation and Remodeling

Progressive heart failure is determined by pathological cardiac remodeling that occurs after tissue injury from myocardial infarct, acute or chronic inflammation, pressure and volume overload, and activation of the renin-angiotensin-aldosterone system (44). MR activation promotes tissue oxidative stress and inflammation, which is characterized by a perivascular mononuclear infiltrate and increased adhesion markers with chemokine and cytokine expression (39, 45, 46) (Fig. 1). This is a continuous process with subsequent remodeling and interstitial fibrosis.

Myocardial reduced nicotinamide adenine dinucleotide phosphate oxidase is a source of reactive oxygen species mediating oxidative stress that plays a significant

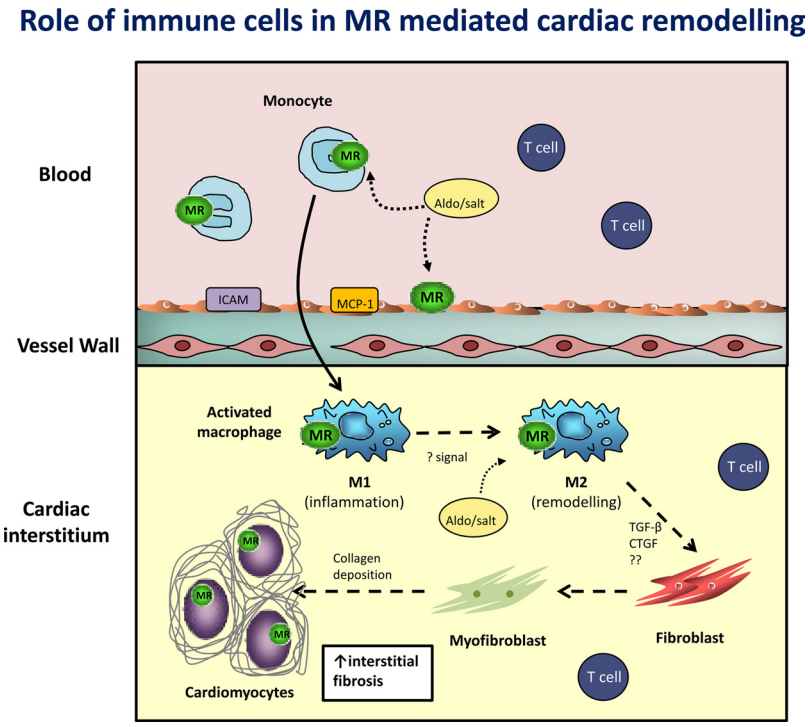

FIG. 1. Proposed schema of the role of immune cells in MR-mediated cardiac remodeling. Activation of MR by aldosterone and salt leads to expression of vascular adhesion markers (e.g. ICAM) and chemokines (e.g. MCP-1). Recruitment and activation of M1 pro-inflammatory macrophage follows which further generate cytokines to amplify and perpetuate tissue inflammation and damage in early phase. An as yet undefined regulatory signal promotes M2 pro-fibrotic (wound healing) macrophage recruitment and proliferation in the continuous presence of aldosterone and salt. Activation and differentiation of cardiac fibroblasts into myofibroblasts in response to growth factors [e.g. TGF-β, connective tissue growth factor (CTGF)] facilitates exuberant collagen deposition leading to interstitial fibrosis. Although there is an increase in T cell infiltration, their exact modulating role in the MR activation model remains uncharacterised.

role in cardiac pathology. Increased reduced nicotinamide adenine dinucleotide phosphate oxidase expression and activity are localized to cardiomyocytes and endothelium in heart failure (47, 48), and these are thought to activate redox-sensitive signaling pathways such as Src kinases and MAPK (ERK1/2) in pressure overload hypertrophy and failure (49). Inflammatory and fibrotic responses to aldosterone were blunted in Nox2-null mice, which were also shown to have reduced nuclear transcription factor-κB (NF-κB) activation (50). It has also been proposed that cardiomyocyte MR activation potentially leads to dysregulation of cellular handling of calcium, magnesium, and other ions, which induces high mitochondrial calcium and reactive oxygen species, resulting in necrosis and replacement fibrosis (51). Consistent with these studies, cardiomyocyte MR-null mice showed blunted oxidative stress and tissue injury responses to ischemia, suggesting that MR activation in these cells drives key oxidative responses (52, 53).

Activation and proliferation of fibroblasts in response to inflammatory cytokines and growth factors in high mineralocorticoid states promotes collagen deposition (fibrosis) leading to structural and functional remodeling in the myocardium (45, 46, 54). Repeated injury occurs with persistent inflammation and also leads to extensive interstitial scarring (55), which is reversible by MR antagonists (40, 56). These models have also established that the remodeling benefits of MR blockade are independent of their blood pressure-lowering effects as well as plasma aldosterone level (57, 58). MR signaling in cardiomyocytes has also been shown to up-regulate genes involved in extracellular matrix remodeling (59, 60). Recent studies with cardiomyocyte MR-null mice demonstrate protection from cardiac failure after pressure overload from transaortic constriction or coronary artery ligation (52, 61). Whereas the first study shows no protective effect on cardiac inflammation or fibrosis, the latter demonstrates improvement in cardiac contractility, reduction in oxidative stress (see below), and protection from adverse remodeling with loss of cardiomyocyte MR. Similarly, cardiomyocyte MR-null mice are also protected from DOC/salt cardiac remodeling (53). Together, these studies suggest that cardiomyocyte MR has a role in the regulation of structural and functional remodeling. In contrast, the lack of cardiac protection in fibroblast MR-null mice is consistent with the lack of functional MR in these cells (61).

Immune cells have now been shown to play a critical role in mediating proinflammatory and profibrotic effects in the setting of MR activation in cardiac tissues. MR are expressed in immune cells of the myeloid lineage including monocytes/macrophages (62), a subset of dendritic cells (63), and potentially neutrophils (64), making them non-classical targets of aldosterone and potentially cortisol due to the absence of 11βHSD2 (65). A significant inflammatory component with marked macrophage recruitment had been characterized in cardiovascular remodeling in MR-mediated and other cardiac pathophysiology (66–68). MR signaling induces oxidative stress in macrophages (69) via activation of NF-κB, which generates a proinflammatory macrophage phenotype (M1), responsible for amplifying tissue inflammation with consequent injury (39, 45). Although the dynamic nature of the mac-

rophage phenotype in tissue injury and healing is not fully understood in the DOC/salt model, tissue repair and remodeling processes in other experimental disease models suggest M1 is subsequently replaced by profibrotic macrophage phenotype (M2) likely in response to tissue signals or T cell cytokines (70–72). M2 releases profibrotic factors involved in cardiac remodeling such as TGF-β, which promotes fibroblast activation and collagen deposition (73–75) (Fig. 1). We and others have demonstrated an independent and central role for MR in macrophages (7–9). Selective deletion of MR in these cells protects against adverse cardiac remodeling in the DOC/salt and angiotensin II or angiotensin II/L-NAME models of hypertension and, interestingly, also reduces infarct volume in the cerebral ischemia model (76). These highlight a central role for macrophage MR in the pathogenesis of tissue remodeling.

An important modulator of these macrophage functions is the T cell-macrophage interaction (71, 73, 74). However, the exact role of T cells in DOC/salt model of cardiac remodeling is uncertain. Potential mechanisms include direct regulation of dendritic cell MR by aldosterone to influence the differentiation of the $CD4^+$ T helper cell (a subset of T cells) (63). CXCR4 antagonism was recently demonstrated to protect mice treated with DOC/salt from cardiac fibrosis and hypertension. Because CXCR4, a chemokine receptor, is widely expressed on T cells, this study suggests an important mechanistic role for T cells in MR-mediated disease (77). Studies from various disease models indicate that $CD4^+$ T helper cells perform a key role in modulating the immune cell response in tissue inflammation and remodeling, *e.g.* T helper 1 (T_H1) and T helper 2 cells (T_H2) driving M1 and M2, respectively (78–81). Adoptive transfer of regulatory T helper cell (T_{reg}) has also been shown to be cardiac protective in hypertensive mice treated with transaortic constriction by down-regulating immune response (82). The nature of the interaction between the various members of the immune system in MR-mediated pathology is an exciting and emerging area of research.

Therefore, MR signaling, in the setting of inappropriately high salt, mediates oxidative stress in various cell types in the myocardium, resulting in activation of the immune system and chronic inflammation, which initiates concurrent tissue repair and remodeling.

Immune Cells in MR-Mediated Cardiac Pathology: Insights from Transgenic Mice Models

Recently, Rickard *et al.* (7) and others (8, 9) demonstrated a role for macrophage MR and provided insights into macrophage phenotypes in cardiovascular remodeling by analyzing the effects of DOC/salt or angiotensin II/L-NAME treatment on transgenic mice with selective deletion in myeloid MR [myeloid MR knockout (MRKO)]. Given that cre recombinase is driven by the Lysozyme M promoter, this deletion affects cells of myeloid lineage, in particular monocytes, macrophages, and a subset of dendritic cells (83)

Peritoneal MR-null macrophages, elicited by thioglycolate stimulation, show reduced M1 but elevated M2 marker expression (9). Taken together with the demonstration of reduced inducible NOS-positive macrophages in hearts from L-NAME/salt-treated myeloid MRKO mice (8) as well as an earlier study by Keidar *et al.* (69), these data verify that MR signaling contributes to expression of proinflammatory M1 cytokines in macrophages. Although there is a good suggestion that macrophages from myeloid MRKO adopt a typical M2 phenotype (9), other *in vivo* studies demonstrate a reduction in M1 markers but unchanged (rather than increased) M2 markers (7, 8), highlighting some variation in *in vivo* and *ex vivo* responses of the different macrophage populations. These *in vivo* studies raise the possibility that macrophages from myeloid MRKO mice may be atypical M2 or M2-like (regulatory macrophages) phenotype because the mice were protected from cardiac fibrosis. In support, stimulation of myeloid MRKO macrophages with glucocorticoid, typically known to polarize macrophages toward M2-like phenotypes (73, 74), results in synergistic expression of M2 markers (9). This also illustrates the opposing effects of MR and GR signaling in macrophages. Therefore, more *in vivo* macrophage data are needed to verify the phenotype and function in the context of myeloid MRKO or general MR antagonism. It is likely that the chronic MR activation due to aldosterone or DOC in wild-type cardiac tissue yields a dynamic mixture of typical M1 and M2, and intermediate phenotypes at different phases of the remodeling process resulting in aberrant remodeling and fibrosis. Interrupting this process by generating atypical M2 or M2-like macrophages by MR KO intuitively accounts for the benefits seen in these studies (7–9).

The effect of myeloid MR deletion on dendritic cells in these models has not been explored. Studies using mice deficient in dendritic cells given DOC/salt model would enable assessment of the role of dendritic cells and may indirectly provide clues into the role of T cells in modulating immune responses to DOC/salt cardiovascular tissue remodeling.

MR-Mediated Inflammation and Remodeling in the Vessel Wall

Comprehensive reviews on MR signaling in the vessel wall have previously been published (29, 84). Vascular

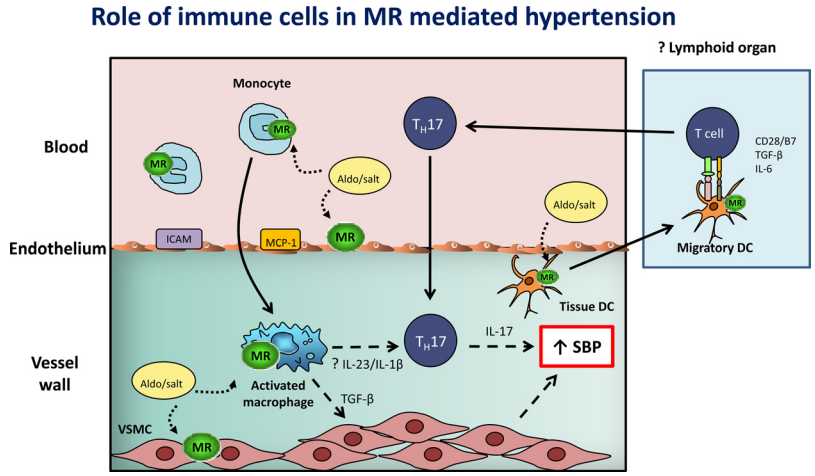

FIG. 2. Proposed schema of the role of immune cells in MR mediated hypertension. Initial vascular inflammation due to MR signaling from aldosterone and salt promotes vascular dendritic cell activation and migration to the proposed secondary lymphoid organ as well as recruitment and activation of macrophage into the vessel wall. Antigen presentation to naive CD4+ T cell in the context of costimulatory molecules and TGF-β and IL-6 promotes differentiation into TH17 which migrate out and into the vessel wall. Further essential priming of TH17 with IL-23 or IL-1β, produced by the proposed recruited macrophage, enables IL-17 expression and secretion. This amplifies vascular inflammation leading to systolic hypertension. Vascular remodeling mediated by direct or indirect MR activation on vascular smooth muscle cells contributes to the maintenance of systemic hypertension.

inflammation as a result of MR activation promotes recruitment of monocytes and lymphocytes via expression of vascular adhesion markers, chemokines, and cytokines (Fig. 2). Activation of the vascular NF-κB and Rho-kinase signaling pathways by oxidative stress is thought to play an additional role. There is increasing evidence for a role for activated T cells in mediating vascular inflammation and hypertension in the setting of aldosterone treatment (85, 86).

Remodeling of the vessel wall contributes to the underlying pathophysiology of MR-mediated hypertension. Direct role of VSMC MR in blood pressure regulation has been demonstrated using mice with SMC-specific MR deficiency (30). MR signaling in VSMC stimulates migration, proliferation, and secretion of extracellular matrix (reviewed in Ref. 29). Multiple factors such as angiotensin II, platelet derived growth factor, endothelin-1, and placental growth factor interact with MR and activate both genomic and nongenomic MR signaling pathways to result in remodeling (29, 87, 88). This leads to vascular fibrosis and stiffness that is characteristic of longstanding hypertension, particularly in patients with untreated and/or unrecognized PA.

Role of Macrophages in MR-Mediated Hypertension

Previous animal models have implicated macrophages as key players mediating vascular inflammation and hypertension. Mice studies employing techniques to exclude the presence of macrophages in tissues demonstrated reduced vascular inflammation, decreased oxidative stress and remodeling, and protection from hypertension when treated with angiotensin II (89, 90). A role for macrophage MR signaling in blood pressure regulation has also been illustrated in studies involving macrophage MR deletion. These mice are clearly protected from DOC/salt-induced hypertension (7) but not in angiotensin II and/or L-NAME-dependent hypertension (8, 9). These differences may reflect the direct vascular endothelial oxidative stress and inflammatory effects of L-NAME, independent of MR activation. Studies from this laboratory (7) suggest that macrophage MR play a pathogenic role in oxidative stress, inflammation, and vascular dysfunction, thus exacerbating hypertension. Macrophages are a rich source of TGF-β, which is potentially up-regulated during MR-mediated vascular inflammation and injury. Recent *in vivo* studies demonstrate the ability of TGF-β to stimulate VSMC proliferation via downstream Smad3 signaling (91, 92). Thus, in addition to mediating vascular inflammation, macrophages may play a direct role in vascular remodeling and thus contribute to the persistence of hypertension.

Role of T cells in MR-Mediated Hypertension

There is mounting evidence for a role for T cells in hypertension. Guzik *et al.* (85) demonstrated that RAG-1 $-/-$ mice (lacking T and B cells) are protected from both angiotensin II and DOC/salt-induced vascular inflammation and hypertension and that this can be reversed by adoptive transfer of T cells. However, a role for macrophages was not investigated. Madhur *et al.* (86) subsequently illustrated that infiltrating T cells in the vascular wall may be a T_H17 subset by using *IL-17* $^{-/-}$ mice and showing loss of a pressor effect and preserved vascular function in response to angiotensin II. T_H17 is a differentiated effector T helper cell, increasingly recognized for its role in many chronic inflammatory/autoimmune diseases (inflammatory bowel disease, rheumatoid arthritis, *etc.*) (93). The provision of IL-6 and TGF-β by dendritic cells is critical for this T_H17 differentiation (94). However, IL-17 production is enabled only after further priming by cyto-

kines such as IL-23 or IL-1β (95, 96). Because macrophages are known to be a rich source of IL-23 or IL-1β, whether they interact with T_H17 to promote the final functional phenotype in vascular tissues is currently uncertain. A recent *ex vivo* study demonstrated bone marrow-derived dendritic cells treated with aldosterone are able to prime naive $CD4^+$ T cells and promote T_H17 differentiation (63). This highlights the functional significance of MR signaling in myeloid dendritic cells (97, 98) and the ability of aldosterone to indirectly promote T_H17 differentiation. Vinh *et al.* (99) demonstrated the critical role of costimulatory molecules (CD28/B7) in angiotensin II and DOC/salt-mediated hypertension, which points to the existence of T cell priming by dendritic cells. Thus one may propose the pathway of T_H17 induction in Madhur's study (Fig. 2), which was not specifically designed to investigate the nature of the presented antigens or the specificity for T_H17 priming.

T_{reg} are suppressors of T_H1, T_H2, and T_H17 likely via effects of IL-10 (100). They are primed in an environment rich in TGF-β and retinoic acid in the absence of IL-6, and at the expense of effector T cells, particularly T_H17 (101). The importance of the balance or ratio of T_H17 to T_{reg} has been illustrated in many autoimmune/inflammatory diseases such as systemic lupus erythematosus, autoimmune arthritis, and IgA nephropathy (102–104). From a therapeutic perspective, it is worthy to note that mice given angiotensin II or aldosterone treatment demonstrate a reduction in $Foxp3^+$ T_{reg} and a reciprocal increase in $CD3^+$ T cell infiltration ($\uparrow$ $CD3^+$ T to T_{reg} ratio) in the aorta. Moreover, T_{reg} adoptive transfer ameliorates vascular adverse effects of both agents (31, 105). Modulating the shift from proinflammatory T cells to T_{reg} may therefore be a potential therapeutic approach to suppress MR-mediated vascular inflammation and injury that promote hypertension.

Conclusions

The emerging role of immune cells in MR-mediated disease is one of the many areas that have expanded the role of MR beyond physiological salt and water regulation. Moreover, MR and GR signaling in macrophages and other nonepithelial tissues appear to have opposing effects. Thus the net effect of MR and GR signaling in macrophages (*i.e.* the immune response) may be determined, in part, by the relative intracellular abundance of MR *vs.* GR ligands. Whereas the role of immune cells has been firmly established in other systemic disorders, and to a lesser extent in other cardiac disease models, our current understanding in MR-mediated cardiac models remains deficient. Nevertheless, the discovery of the role of immune cells in MR-mediated cardiac remodeling and hypertension in several experimental mice models represents a new frontier in our research direction and understanding of its pathophysiology. Whether therapeutic selective immunomodulation, already in clinical practice for other autoimmune/inflammatory diseases, will be a useful novel approach in heart failure and hypertension remains to be seen.

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