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CD₄+CD₂₅⁺Foxp3 regulatory T cells and vascular dysfunction in hypertension

Modar Kassan^a, Andrea Wecker^a, Philip Kadowitz^b, Mohamed Trebak^c, and Khalid Matrougui^a

^aDepartment of Physiological Sciences, Eastern Virginia Medical School, Norfolk, Virginia

^bDepartment of Pharmacology, Tulane University, New Orleans, Louisiana

^cCollege of Nanoscale Science and Engineering, University at Albany, Albany, New York, USA

Abstract

Endothelial dysfunction plays a key role in the development and progression of cardiovascular disease. In patients with hypertension, endothelial dysfunction is characterized by a decrease of vasodilator factors release. Recent evidence highlights the involvement of regulatory T cell in the cardiovascular physiology and pathology. An increasing body of data suggest that an imbalance in the immune system triggers inflammation and compromises the cardiovascular homeostasis. In this mini-review, we will highlight the role of immune regulatory T cells in hypertension-induced vascular dysfunction.

Keywords

hypertension; regulatory T cell; vascular dysfunction

INTRODUCTION

Elevated blood pressure is a serious health problem in the United States and is one of the most important risk factors for the development of cardiovascular disease, which leads to increased risk of death from stroke, myocardial infarction, and vascular complications [1]. According to a study published in 2011, approximately 30% of adults in the United States suffer from hypertension, and 60% of the population is prehypertensive [2]. In most hypertensive patients, systemic vascular resistance is increased associated with vascular dysfunction. It is well known that calcium channel antagonists, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin II type 1 (AT-1) receptor antagonists are not efficient in a great number of hypertensive patients, suggesting the presence of patients who are resistant to these medications [3]. Therefore, it is crucial to discover new avenues for hypertension-induced vasculopathy.

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Correspondence to Khalid Matrougui, PhD, Department of Physiological Sciences, Eastern Virginia Medical School, 700W Olney Road, Norfolk, VA 23501, USA. Tel: +1 757 446 5278; matrouk@evms.edu.

Conflicts of interest

There are no conflicts of interest.

ENDOTHELIAL DYSFUNCTION IN HYPERTENSION

Endothelial cells play an important role in the regulation of vascular tone by releasing relaxing factors such as nitric oxide [4], prostacyclin, and endothelium-derived hyperpolarizing factor [5]. In hypertension, endothelium-dependent relaxation is impaired in mice and in humans [6], especially at the level of the microcirculation vascular bed [7], in which it is characterized mainly by downregulation of endothelial nitric oxide synthesis (eNOS) and/or inactivation of nitric oxide [8]. In arterial hypertension, the interaction of nitric oxide with superoxide anions is largely responsible for the decrease in nitric oxide bioavailability [9]; the superoxide anion reacts with the endogenous nitric oxide, thereby promoting vasoconstriction and leading to an increase in the systemic vascular resistance and elevated blood pressure. NADPH oxidase (NOX) is known to be the major source of superoxide ($O_2^{\bullet-}$) in the vasculature [10], and reactive oxygen species (ROS) produced by NOX enzymes have been implicated in several models of experimental hypertension [11]. NOX can be activated by angiotensin II via the action of AT1 receptor, and as a consequence releases $O_2^{\bullet-}$ [12]. Another important agonist for vascular oxygen radical generation is tumor necrosis factor alpha (TNF- α) [13], which activates NOX in endothelial cells [14]. In addition, apart from that $O_2^{\bullet-}$ reduces nitric oxide bioavailability and impairs vascular function, $O_2^{\bullet-}$ also causes enhanced renal sodium reabsorption, which increases plasma volume and contributes to hypertension [15].

IMMUNE SYSTEM IMPLICATION IN HYPERTENSION-INDUCED VASCULOPATHY

The role of the immune system is to protect our body from pathogens and toxins. This defense mechanism is provided by two pathways, called the innate and the adaptive system. The difference between these two systems is at the level of how quickly and for how long they respond to pathogens as well as for their specificity for different types of microbes.

The main components of the innate immune system, also called the nonspecific immune system, are dendritic cells, physical phagocytic leukocytes, epithelial barriers, natural killer (NK) cells, and circulating plasma proteins secreted from the liver. These cells and proteins are the first line of defense; they are always present in the body and ready to protect and fight against microbes in the site of infection. Thus, on the one hand, the innate system provides a generic but immediate defense against toxins and pathogens. On the other hand, the main components of the adaptive immune system are B and T lymphocytes. These cells are the second line of defense and they are activated when the pathogens get past the first line of defense. Once these compounds are activated, they create and provide a more effective defense that consists in neutralizing and destroying the pathogens. Adaptive immunity also includes a 'memory' that makes future responses against a specific antigen more efficient.

The interaction between the innate and adaptive immune systems is carried out by cytokines that bind to cells, and by cell-cell interactions between dendritic cells and lymphocytes in lymph nodes. This interaction is crucial so that the adaptive response cannot occur without an innate immune system. This network is very important to communicate the innate to the adaptive immune system as well as between components within each of these immune systems. Importantly, there is evidence that the interaction between both immune systems can be bidirectional. Thereby, the innate and adaptive immune systems are not separable, but are intimately intertwined to regulate immune responses.

NOX and AT1 receptors have been localized on the T lymphocytes [16]. It has been reported that suppression of the adaptive immune system can attenuate hypertension in

experimental animals and in humans [17]. Innate and adaptive immunity are involved in Ang II-induced hypertension and end-organ damage and it has been demonstrated that Ang II infusion in rats caused recruitment of T cells in the kidney, an effect prevented by Ang II type 1 receptor blockers [18]. Furthermore, Seaberg *et al.* [19] demonstrated that suppression of CD4⁺ T cells caused by HIV infection is associated with a low incidence of hypertension. It has been reported that immune function perturbation by either pharmacological intervention or thymectomy was able to prevent hypertension in several experimental models [20], and emerging evidence from experimental and clinical studies indicates that alterations in immune cell function play an important role in the pathogenesis of cardiovascular diseases [21]. Finally, an increasing body of knowledge suggests that an imbalance in the immune system induces inflammation that leads to cardiovascular diseases [22].

Natural regulatory T cells (Tregs) are an important component of the immune system, and are characterized by the expression of CD4⁺CD25⁺ and the transcription factor (Foxp3) [23]. Tregs play a crucial role in maintaining immunologic self-tolerance and protection from autoimmune diseases as well as in regulating immune responses to different pathogens [24]. The process of T-cell differentiation is first initiated by signaling from dendritic cell to T cell in the lymph node to produce proliferation and differentiation. The adhesion molecules from the naïve T cells allow them to migrate to all the secondary lymphoid organs where the virgin T cells get in contact with the invaders that entered the body and as a consequence, it will trigger its activation. Once activated, T cells must leave the secondary lymphocytes and migrate to the sites of infection and inflammation to exert the appropriate defense mechanisms [25].

A number of different types of Treg subsets, such as natural Treg [26], Th3[27], Tr1 [28], CD8 Treg [29], and natural killer Treg (NK Treg) [30], have been identified in the human immune system. These T-cell populations share a common characteristic of immunosuppressive capability, but differ in their cell surface markers, types, and site of formation. Among these populations of Tregs, natural Tregs, known as CD4⁺CD25⁺ T cells, have been well studied and accumulating evidence suggests that this population plays a crucial role in the maintenance of immunological self-tolerance and negative control of pathological as well as physiological immune responses [31]. Apart from natural Tregs, Tr1 and Th3 cells, known as inducible Tregs, can be induced from the naïve T cells in the periphery. These inducible Tregs do not have the classical CD4⁺CD25⁺ T-cell phenotype and they are defined based on their cytokine profile. Whereas Th3 cells are known to secrete transforming growth factor β (TGF- β), Tr1 cells are characterized by their secretion of interleukin-10 (IL-10). A study by Chen *et al.* [32] showed that peripheral CD4⁺CD25⁻ naïve T cells can be converted to regulatory CD4⁺CD25⁺ by TGF- β released by Th3 and they speculate that this conversion is mediated through the induction of Foxp3 expression.

However, not only IL-10 and TGF- β play a role in Tregs activity and protective effects, it has been shown that Tregs activity depends on the influence of cytokines responsible for their differentiation, maintenance, and function. Thus, Chen *et al.* [32] reported that naïve human CD4⁺ T cells are induced by TGF- β to develop a robust suppressor activity and Bettelli *et al.* [33] showed that a combination of IL-6 and TGF- β supports differentiation toward Th17 cells. Additionally, IL-21 is an autocrine cytokine necessary for Th17 development and differentiation. IL-21 together with TGF- β can differentiate naïve CD4⁺ T cells into Th17 cells even in the absence of IL-6 [34]. Moreover, it has been shown that IL-2 plays an important role in maintaining a balanced natural Treg activity *in vivo* [35]. IL-27 is another cytokine that has multiple functions including the suppression of IL-2 expression, and antagonism of IL-6, which is required for TH-17 cell differentiation [36]. IL-1 is another cytokine that has been reported to play an important role in Tregs expansion and

differentiation of CD₄⁺CD₂₅⁺Foxp3 T cells from CD₄⁺CD₂₅⁺Foxp3⁻ T cells [37]. Valencia *et al.* [38] showed that TNF has the novel action of modulating autoimmunity by inhibiting CD₄⁺CD₂₅⁺ Tregs activity. Niedbala *et al.* [39] showed that Tregs highly express and constitutively secrete IL-35. The mechanisms by which Tregs produced this novel anti-inflammatory cytokine which promotes a suppressive activity appear to depend on the dual capacity of IL-35 to both expand functional Foxp3⁺IL-10-producing regulatory T cells and suppress Th17 cell differentiation, both *in vitro* and *in vivo*. In summary, Tregs number and activity can be modulated positively or negatively by several cytokines, with certain cytokines promoting the differentiation and function of Tregs, and other cytokines antagonizing those activities. Natural Tregs are CD₄⁺ T cells that originate during T-cell development in the thymus and constitutively express the alpha chain of the IL-2 receptor CD₂₅ [23]. Tregs are generated in the thymus as a functionally distinct and mature subpopulation of T cells and persist in the periphery with stable function, and their generation is, at least in part, developmentally controlled.

Congenital deficiency of Tregs results in serious impairment of self-tolerance and immunoregulation leading to severe autoimmunity and immunopathology in humans. The natural presence of Tregs in the immune system as a phenotypically distinct population makes them a good target to treat or prevent immunological diseases and to control pathological as well as physiological immune responses.

Tregs express a variety of cell surface molecules such as CD₂₅CD₄₅Rb^{low}, CD₆₂L⁻, CD₁₀₃, cytotoxic T-lymphocyte antigen-4 (CTLA-4, or CD₁₅₂), and glucocorticoid-induced TNF receptor family-related gene (GITR), making it possible to isolate and characterize them. Before the identification of the transcription factor (Foxp3) expression, the two cell surface molecules CD₄⁺ and CD₂₅⁺ were used to define this population of Tregs; therefore, these cells were often referred to as CD₄⁺CD₂₅⁺ Tregs.

It has been demonstrated recently that Foxp3 is very important for the development and function of Tregs [40]. Currently, Foxp3 is the most specific molecular marker for thymic or peripheral Tregs in rodents and humans. Mutation of the gene encoding Foxp3 leads to severe autoimmune diseases. When the Foxp3 mutation occurs in human, it leads to a severe and fatal autoimmune disorder termed immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome resulting in watery diarrhea, eczematous dermatitis, and insulin-dependent diabetes mellitus and leads to death. Scurfy is an analogous disease that occurs in mice due to a Foxp3 mutation. There is evidence indicating that Foxp3 is necessary for Tregs development in mice, but in human Foxp3 alone is not sufficient to indicate regulatory activity of CD₄⁺CD₂₅⁺ cells. This hypothesis is supported by a study made by Seidel *et al.* [41], which demonstrates that in humans, mRNA for Foxp3 was detected in recently activated CD₄⁺CD₂₅⁺ cells lacking regulatory function. Another study defined an additional potential marker for Tregs, termed IL-17 receptor alpha chain (CD₁₂₇). In this study, it was demonstrated that CD₁₂₇ expression inversely correlates with Foxp3 and suppressive function of human CD₄⁺ Tregs [42]. Furthermore, cells separated based only on CD₄⁺ and CD₁₂₇ expression showed comparable suppressive capabilities to CD₄⁺CD₂₅⁺ Tregs [43]. Tregs disturbance is implicated in many immune diseases: in this review, however, we will focus only on the relationship between Tregs and hypertension-induced vascular endothelial dysfunction.

NATURAL REGULATORY T CELLS AND ENDOTHELIAL DYSFUNCTION IN HYPERTENSION

To address the question of whether a defect in Tregs number and function is linked to vascular endothelial dysfunction in hypertension, it is important to appreciate the potential

means by which such defect may occur and how these defects affect endothelial function. The results of studies regarding the role of Tregs in cardiovascular disease are still controversial. Moreover, the significance and role of Tregs in microvascular endothelial dysfunction in hypertension is an important question that remains unanswered. Lack of such knowledge is a fundamental problem, because, without it, vascular endothelium-dependent relaxation impairment that is involved in coronary artery disease and stroke will continue to be a high risk factor for myocardial infarction and cerebral vascular disease in hypertensive patients.

It has been reported that lymphocyte stimulation by angiotensin II contributes to the pathogenesis of hypertensive kidney disease [44], suggesting the involvement of the immune system in the regulation of arterial blood pressure. Recent studies have demonstrated the role of T cells in the genesis of hypertension, vascular dysfunction, and cardiac fibrosis [3,45,46]. Mice lacking T and B cells (RAG-1^{-/-} mice) do not develop hypertension or abnormalities in vascular function during angiotensin II infusion [3]. Blood pressure elevation and vascular inflammation were associated with dysfunction of Treg suppressor effects in Dahl salt-sensitive rats [47]. Consomic SSBN2 rats, which have the salt-sensitive rat genome and chromosome 2 from normotensive Brown Norway rats, had improved Treg function, decreased hypertension, and rescue vascular and systemic inflammatory mediators compared to salt-sensitive rats [48]. Another study has reported that one single injection of Tregs to angiotensin II-induced hypertensive mice reduced cardiac fibrosis independent of an effect on arterial blood pressure [48]. These results provide new insights into the pathogenesis of hypertensive cardiac damage and could, therefore, lead to new therapeutic approaches that involve manipulation of the immune system.

We recently demonstrated that Tregs play an important role in the regulation of endothelium-dependent relaxation in coronary arterioles and in the regulation of arterial blood pressure [46,49]. Thus, the transfer of Tregs, freshly isolated from normotensive mice, into hypertensive mice three times a week for 2 weeks significantly reduced arterial blood pressure and improved endothelium-dependent relaxation in coronary arterioles [46,49]. This differed from a previous study by Kvakani *et al.* [48] in the number of Tregs injected. Recently, other groups found similar data, strengthening the concept that Tregs are critical for the regulation of arterial blood pressure and microvascular function. Additionally, in-vitro results with cultured Tregs isolated from normotensive mice showed that stimulation with angiotensin II for 24 h significantly reduced the number of Tregs by an apoptosis-dependent mechanism [50].

It is unclear, however, how Tregs regulate arterial blood pressure and microvascular function. It has been shown that Tregs release soluble factors such IL-10, IL-35, and TGF- β [51]. Moreover, the suppressive action of Tregs is also exerted on other cell types, such as activated monocytes and macrophages [52]. In our previous study, we showed that the transfer of Tregs into hypertensive mice decreases inflammation, as evidenced by the reduction in inflammatory cytokines and macrophage infiltration into coronary arterioles. Our data have been recently supported by Barhoumi *et al.* [50], who reported a decrease in macrophage infiltration in adventitia and periadventitial fat after adoptive transfer of Tregs.

Our recent data demonstrated that microvessels from hypertensive mice incubated with conditioned media of Tregs have significantly improved endothelium-dependent relaxation responses mediated through an IL-10-dependent mechanism [49]. These data indicate that Tregs regulate vascular function by a paracrine-dependent mechanism. In addition, the beneficial effect of Tregs conditioned media on endothelium-dependent relaxation was blocked after the use of IL-10 receptor antagonist, IL-10-neutralizing antibodies, and vessels from IL-10-knockout mice. One of the IL-10 mechanisms thought to be involved in the

control of vascular endothelium-dependent relaxation involves the reduction of oxidative stress through the inactivation of NOX [49]. Future studies are needed to delineate the factors, released by Tregs, and their mechanism in the regulation of vascular function and therefore blood pressure.

CONCLUSION

The main function of Tregs is the maintenance of immunological tolerance, which has been found to be involved in the pathogenesis of many cardiovascular diseases [53,54]. Impaired Tregs function or number is hypothesized to be involved in several autoimmune and inflammatory diseases such as type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, atherosclerosis, and vascular dysfunction in systemic and pulmonary hypertension [21,55]. Atherosclerosis is a chronic inflammatory disorder regulated by Tregs [56]. Tregs imbalance regulates vascular inflammation and may be an important factor in development of atherosclerotic plaque destabilization and the onset of acute coronary syndrome and myocardial infarction. As Tregs imbalance may be a major factor in vascular endothelial dysfunction, plaque rupture, and myocardial infarction, Tregs replacement by infusion may represent a new therapeutic strategy for the treatment of cardiovascular diseases, and Tregs could represent an important and highly promising new target for modifying cardiovascular physiopathology function. More information about the role of Tregs in cardiovascular disease pathophysiology is needed.

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Abbreviations

Ang II	angiotensin II
Tregs	natural regulatory T cells

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