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T regulatory cells and vascular function: the importance of their immunosuppressive action in hypertensive disease.

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Title 2: T regulatory cells and vascular function: the importance of their immunosuppressive action in hypertensive disease.

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The study by Mian *et al.* demonstrates that absence of T regulatory (Treg) cells within the T cell population exaggerated Angiotensin II (Ang II)-induced endothelial dysfunction, vascular remodeling, oxidative stress and inflammation. The extensive work displayed in this manuscript clarifies the protective role of Tregs in vessels and highlights how important is to distinguish the different populations of T cells in models of disease where chronic inflammation has a key role. The authors corroborate their initial hypothesis with exhaustive *ex vivo* experiments taking into account all the possible combinations of T cells transference into T and B cell-deficient recombination-activating gene 1 knockout (Rag1-/-) mice infused or not with Ang II. The transfer of T cells from the Scurfy mouse (Sf), a model which lacks Treg cells because of deficiency in the Foxp3 gene, to hypertensive Rag1-/- mice provides an interesting outcome regarding the mechanisms underlying the pathophysiology of hypertension and confirms the role of natural Tregs in protecting arterial function.

Emerging evidence from experimental and clinical studies indicates that alterations in immune cell populations play an important role in the pathogenesis of cardiovascular diseases (1-2). In patients suffering from coronary artery disease (CAD) the ratio between effective T cells (Th17) and suppressor T cells (Tregs) is increased due to a rise in the number of lymphocytes Th17 and the cytokines related with them, IL17 and IL23, and a decrease in the number of Tregs and in the levels of cytokines regulated by these, IL10 and TGFβ1 (2). Suppression of the adaptive immune system can attenuate hypertension in experimental animals and in humans (3). Moreover, in hypertensive patients the Th17 levels are augmented and the neutralization or deletion of IL-17, reduces hypertension induced by Ang II (4). In addition, it is reported that immune function perturbation by either pharmacological intervention or thymectomy prevented hypertension in experimental models (5). Seaberg *et al*. (6) demonstrated that suppression of CD4+ T cells caused by HIV infection is associated with a low incidence of hypertension. In rats with established hypertension (SHR), Rodriguez-Iturbe *et al.* (7) showed that intermittent administration of mycophenolate mofetil, a selective lymphocyte suppressor agent, reduced the renal inflammatory infiltration and hypertension.

The different populations of Tregs share a common characteristic of immunosuppressive capability, but differ in their cell surface markers, types, and site of formation. Among these populations, natural Tregs, characterized by the expression of CD4+CD25+ and the transcription factor (Foxp3) (8), 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 (9). The two cell surface molecules CD4+ and CD25+ were used to define this population of Tregs before the identification of the transcription factor (Foxp3) expression. Currently, Foxp3 is the most specific molecular marker for thymic or peripheral Tregs in rodents and humans. In humans, mutations in the gene encoding Foxp3 leads to a severe and fatal autoimmune disorder termed immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome (10). Scurfy is an analogous disease that occurs in mice due to a Foxp3 mutation (10). Recently, a novel marker for Tregs was identified, the C-type receptor CD69. The FoxP3(+)CD69(+) Treg subset is key for immunosuppressive function of Tregs and for the maintainance of immune tolerance (11).

Several groups demonstrated that Tregs are critical in the regulation of arterial blood pressure and microvascular function (12-15). In the current work presented by Mian *et al.*, the co-transfer of WT-natural Treg cells with Smurfy-T cells (Sf-T cells) delayed the onset of Ang II-induced systolic and diastolic blood pressure rise but did not reduce the systolic blood pressure after the first week of Ang II infusion. These results are controversial since others reported that adoptive transfer of Tregs, freshly isolated from normotensive mice, reduced the Ang II-induced BP rise in sustained fashion (12-15). This differed from a previous study performed by Kvakan *et al.* (16) in which, one single injection of adoptive Treg cells ameliorated cardiac damage independently of blood pressure-lowering effects. However, the dose of Angiotensin II that was used in this study was much higher than the doses described in the current and in the above mentioned studies. Authors explained this discrepancy by the difference in the Tregs injection protocol and by the inflammatory status of T cells. Sf-T cells present a high pro-inflammatory status as revealed by analysis of plasma pro-inflammatory cytokines and it is known that 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 (17). This fact is reflected in the current study, where the co-injection of Tregs and Sf T cells into the Rag1-/- mice could modulate the anti-inflammatory function of the first ones with time. However, further studies will be required to determine whether Tregs modulate the initiation or maintenance of hypertension.

Tregs release soluble factors such as IL-10, IL-35, and TGF-β with anti-inflammatory properties that may provide vascular protection by a paracrine effect (14, 18, 19). For instance, IL-35 has the dual ability to both expand functional Foxp3+IL-10-producing regulatory T cells and to suppress Th17 cell differentiation (18). Moreover, the suppressive action of Tregs is also exerted on other cell types, such as activated monocytes and macrophages (19). In previous studies, it was shown that the transfer of Tregs into hypertensive mice decreases inflammation, as evidenced by the reduction in inflammatory cytokines and macrophage infiltration into arteries. In this sense, Barhoumi *et al*. (12) reported a decrease in macrophages infiltration in adventitia and periadventitial fat after adoptive transfer of Tregs while Kvakan *et al.* showed that adoptive Treg cells transfer resulted in a marked reduction in cardiac CD4+, CD8+, and CD69+ cell and macrophage infiltration (16). In the present study, the authors observed that loss of Tregs shifted monocyte/macrophage polarization toward M1 while gain of Tregs caused the opposite effect in mesenteric arteries and renal cortex suggesting that Tregs could modulate the monocyte/macrophage polarization.

In conclusion, the study by Mian *et al.* demonstrates that specific deficiency of FOXP3-Tregs exaggerates Ang II-induced resistance artery endothelial dysfunction and remodeling, oxidative stress and inflammation, and influences hypertension development, by loss of function or gain of function approaches modulating innate and adaptive immune responses. The current study has the advantage of investigating both Tregs deficiency and replacement as a strategy and it may also be clinically relevant because it suggests the positive stimulation of Tregs as one of the critical therapeutic targets for preventing microvascular complications associated with hypertension. Further elucidation of the interactions between Tregs and the vascular bed by using large animal models and human samples is desired and will provide us with a better understanding of the roles and the potential interactions of the different T cells populations as part of the mechanisms responsible for the progression of cardiovascular diseases.

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Dear Editor,

Please enclosed find the Editorial Commentary requested to accompany the original paper “Deficiency of T regulatory cells exaggerates angiotensin II-induced microvascular injury by enhancing immune responses” by M.O.R. Mian et al. (JH-D-15-00549). We hope the Commentary fulfills the requirements of the Journal.

Sincerely yours,

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