Cardiovascular Research (2016) **112**, 426–428
doi:10.1093/cvr/cvw199

EDITORIAL

Conflicting mechanisms of AT2 cardioprotection revealed

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This editorial refers to 'The angiotensin II type 2 receptor activates flow-mediated outward remodelling through T cells-dependent interleukin-17 production', by A. Caillon *et al.*, pp. 515–525.

Coronary artery disease is a major cause of mortality in the developed world due to the death of cardiac muscle because of inadequate delivery of oxygen-rich blood. The knowledge gained from basic research on preventing cardiac muscle loss by enhancing the formation of new arteries in the heart (angiogenesis) has not translated to date into clinical benefit. An alternative, perhaps more promising strategy is to foster arteriogenesis, which refers to the remodelling of pre-existing collateral resistance vessels to form arteries that effectively bypass major arterial obstructions.[1–4] Indeed, a positive correlation is observed between the number of collateral arteries and extent of their coverage and survival in patients with coronary artery disease,[4] although similar remodelling if it occurs in an atherosclerotic artery may increase the propensity for plaque destabilization and rupture.[5] Notably, several pro-arteriogenic mechanisms or treatments promote atherosclerotic plaque development and instability, while conversely anti-atherogenic factors may inhibit arteriogenesis.[6]

The structural changes in the vessel wall that occur with arteriogenesis reflect outward remodelling, which is an increase in lumen diameter and relative reduction in wall thickness. The primary stimulus that drives arteriogenesis is sheer stress due to increases in blood flow and entails activation of endothelial cells (EC), basal membrane degradation, infiltration of immune cells, proliferation of vascular smooth muscle cells (VSMC), and changes in the extracellular matrix.[1] Specific factors involved in the outward remodelling include nitric oxide (NO), vascular endothelial growth factor, chemokine (C-C) motif ligand 2 (CCL2; also known as monocyte chemoattractant protein 1 or MCP-1), and matrix metalloproteinases (MMPs; especially MMP-9),[1,5] although our understanding of how arteriogenesis progresses *in vivo* is rudimentary.

Collateral vessel growth is driven by local inflammation and involvement of both the innate and adaptive immune systems.[1,2] Infiltration of both macrophages and lymphocytes into the vessel wall occurs with M2 macrophages playing an essential role in collateral remodeling.[1,7,8] NK cells and $CD4^+$ effector T helper cells are predominant players as well,[9] although regulatory T cells ($CD4^+CD25^+FOXP3^+$ T cells) were found to have only a moderate effect on arteriogenesis.[10] However, the function of different $CD4^+$ helper lymphocyte subsets in arteriogenesis is not well studied. In this issue, Caillon *et al.*[11] report the novel observation that memory T helper 17 (Th17) cells that produce interleukin (IL)-17 and express the angiotensin II type 2 (AT2) receptor are critical for outward arterial remodelling to occur *in vivo* in the mouse mesenteric arterial bed after ligation of feed arteries supplying collateral pathways. Normal outward remodelling that occurs in wild-type mice in response to high flow was absent in AT2 receptor knockout mice, as well as in nude mice (which lack T cells). Outward remodelling in response to high flow could be reproduced in AT2 receptor knockout mice if they were also infused with exogenous IL-17. These findings strongly implicate an important role for the AT2 receptor, T cells, and IL-17 in the outward remodelling of blood vessels in response to high flow. *In vitro* studies further implicated a role for AT2-induced IL-17 production from Th17 cells. IL-17 release could be induced from memory T cells, but not naïve T cells, following incubation with angiotensin II (Ang II) and this response could be inhibited by the presence of PD123319, a selective AT2 receptor antagonist. These data suggest that AT2 receptor signalling is required for IL-17 production in Th17 cells in response to Ang II. Of particular interest, treatment of older mice with the AT2 receptor agonist C21 was able to restore outward remodelling that is otherwise lost with aging.

There is a growing list of studies showing a protective effect of the AT2 receptor on the heart and vasculature that involves in part anti-inflammatory actions.[12,13] In the heart, expression levels of AT2 on cardiac myocytes, fibroblasts, and EC are thought to increase under stress conditions. However, little is known about the presence of AT2 receptors on subsets of infiltrating immune cells. Recently, a novel regulatory T cell subset expressing the AT2 receptor was reported to have a beneficial impact on heart function and infarct size after myocardial infarction in the mouse.[14] The impact of AT2 on atherosclerosis is somewhat controversial, although the bulk of evidence supports a protective role of the AT2 receptor via anti-inflammatory actions in this setting as well, such as reduced ROS formation and attenuated expression of adhesion molecules by EC.[15,16] In contrast, the work of Caillon *et al.*[11] reveals the potential importance of the AT2 receptor in protecting the heart involving an inflammation-driven process (*Figure 1*).

The critical role of IL-17 in outward remodelling reported by Caillon *et al.*[11] is consistent with the earlier observation of Madhur *et al.*[17] on

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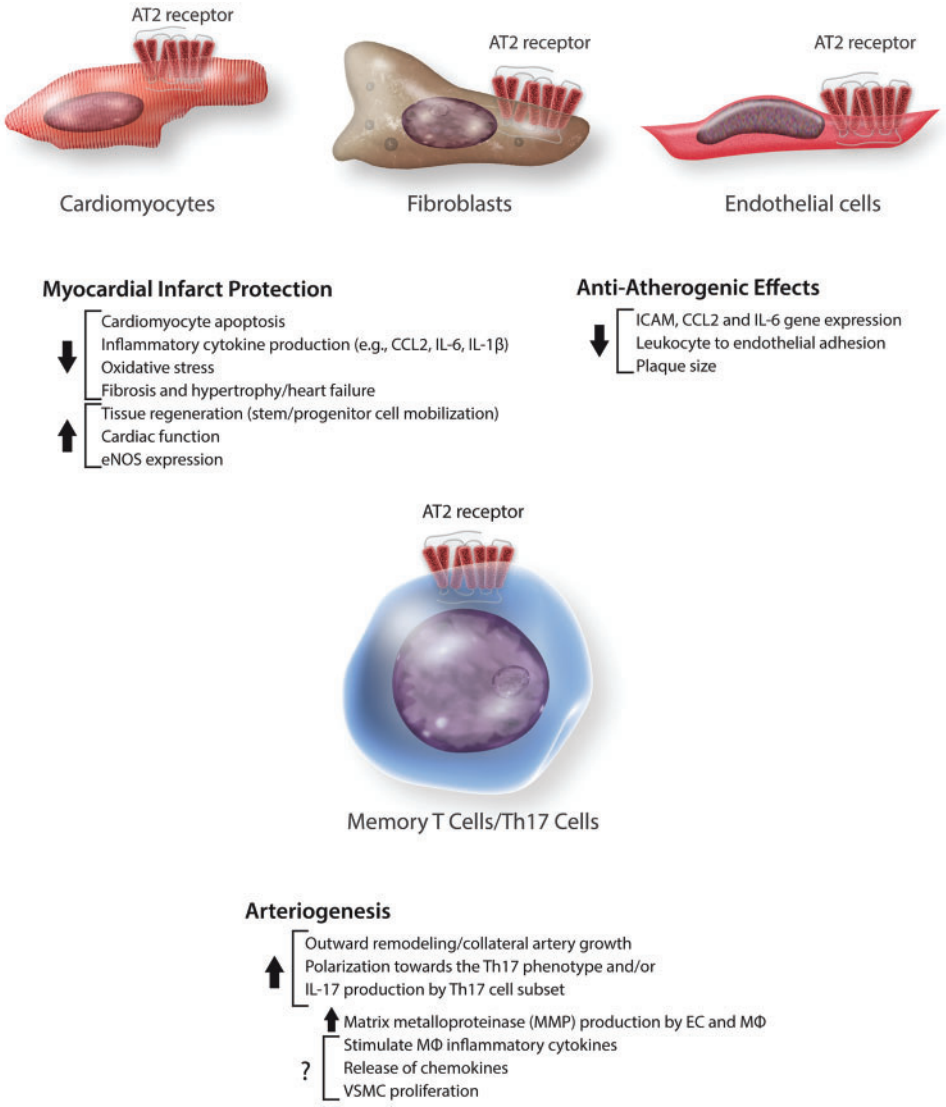

Figure 1 *Yin and yang* of the angiotensin II type 2 (AT2) receptor in cardiovascular protection. Activation of the AT2 receptor has been implicated in protecting the heart from damage and adverse remodeling before or after ischemic assault. Many of the beneficial actions of AT2 in this context are attributed to anti-inflammatory or anti-oxidative mechanisms, involving inhibition or activation of various genes. Protection involves cardiac cells and infiltrating leukocytes or stem/progenitor cells that express AT2 receptors. Recent evidence also links the AT2 receptor to anti-atherogenic effects due to inhibition of endothelial inflammation and leukocyte adhesion. In contrast to these protective, 'anti' actions of AT2, Caillon *et al.*[11] provide evidence in this issue that memory T cells/Th17 cells promote arteriogenesis by producing IL-17 *via* an AT2-driven process. This finding indicates that the AT2 receptor may also be linked to pro-inflammatory events in protecting the heart. EC, endothelial cells; HF, heart failure; MΦ, macrophages.

attenuated outer remodelling in IL-17 knockout mice in the carotid ligation model of atherosclerosis. Caillon *et al.*[11] present evidence to support the conclusion that one role of IL-17 in outward remodelling is to enhance endothelial nitric oxide synthase (eNOS) expression and activity, although there is evidence that other sources of NO, such as inducible NOS (iNOS), may substitute for eNOS activation in driving arteriogenesis.[1] Caillon *et al.*[11] also report that IL-17 activates MMP-2 and MMP-9 activity in macrophages and EC. IL-17 may also activate VSMC oxidative stress, migration, or proliferation.[18,19] In general, the actions of IL-17 on EC and VSMC are pro-inflammatory, and while there is evidence that IL-17 may be atheroprotective, IL-17 is generally considered atherogenic.[20] Overall, the actions of IL-17 in atherosclerosis are pleotropic and even contradictory, likely reflecting concentration, spatiotemporal, and immune cell type-dependent considerations. Obviously, the impact of AT2 expressing Th17 cells on atherosclerosis needs to be established, before the utility of targeting these cells to facilitate arteriogenesis is considered. Nonetheless, the nuances associated with the role of IL-17 in atherosclerosis, may preclude targeting this route to stimulate arteriogenesis.

Additional questions raised by the study of Caillon *et al.*[11] include: what is the mechanism that couples the AT2 receptor to IL-17 production, whether sex differences occur, and what is/are the antigen(s) responsible for activation of the AT2-positive Th17 cells? Identifying the chemoattractant factors involved, which are likely organ-specific, might

facilitate their therapeutic targeting as well. It also remains to be established whether a similar scenario also occurs in the heart as well as other non-mesenteric vascular beds.

In summary, the study by Caillon *et al.*[11] provides convincing pharmacological and genetic evidence of a novel role for AT2 receptor activation on certain memory Th17 cells in supporting a sub-inflammatory state and IL-17 production that drives outward remodelling and arteriogenesis in response to high blood flow. More broadly, translating this knowledge to the clinic may be challenging in the context of coronary artery disease and atherosclerosis based on the pleotropic and to a large extent detrimental actions of IL-17.

Acknowledgements

The authors are very appreciative of the thoughtful suggestions and comments of A.M.G. The authors acknowledge the generous support of the Department of Pharmacology and Toxicology of UMMC.

Conflict of interest: None declared.

Funding

This work was supported by a grant from the National Heart, Lung, and Blood Institute at the National Institutes of Health [grant number 5R01HL107632-04] to SPD.

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