Abnormal CD161$^+$ immune cells and retinoic acid receptor–related orphan receptor γt–mediate enhanced IL-17F expression in the setting of genetic hypertension

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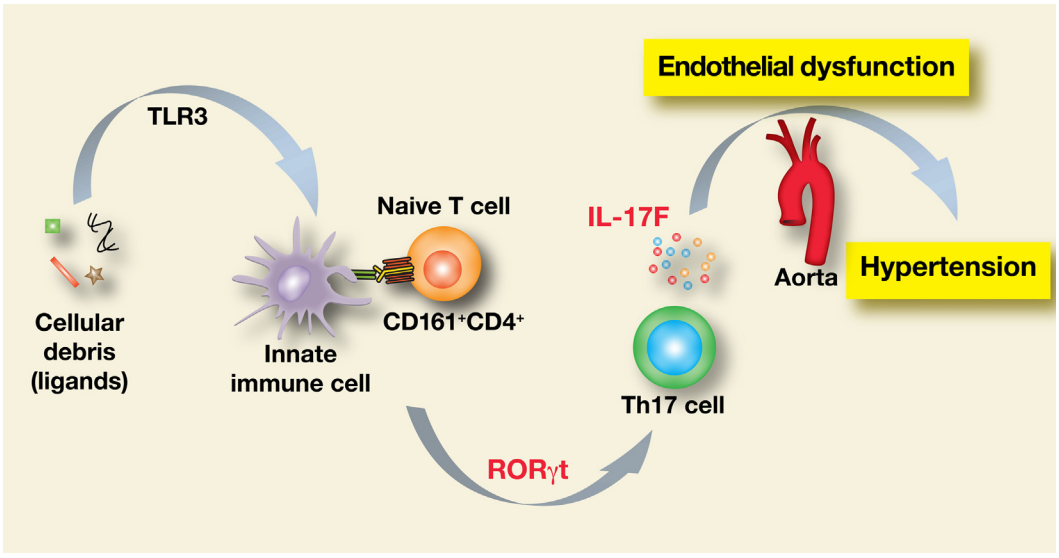
GRAPHICAL ABSTRACT



Background: Hypertension is considered an immunologic disorder. However, the role of the IL-17 family in genetic hypertension in the spontaneously hypertensive rat (SHR) has not been investigated.

Objective: We tested the hypothesis that enhanced T_H17 programming and IL-17 expression in abundant CD161$^+$ immune cells in SHRs represent an abnormal proinflammatory adaptive immune response. Furthermore, we propose that this response is driven by the master regulator retinoic acid receptor–related orphan receptor γt (RORγt) and a nicotinic proinflammatory innate immune response.

Methods: We measured expression of the CD161 surface marker on splenocytes in SHRs and normotensive control Wistar-Kyoto (WKY) rats from birth to adulthood. We compared expression of IL-17A and IL-17F in splenic cells under different conditions. We then determined the functional effect of these cytokines on vascular reactivity. Finally, we tested whether pharmacologic inhibition of RORγt can attenuate hypertension in SHRs.

Results: SHRs exhibited an abnormally large population of CD161$^+$ cells at birth that increased with age, reaching more than 30% of the splenocyte population at 38 weeks. The SHR splenocytes constitutively expressed more RORγt than those of WKY rats and produced more IL-17F on induction. Exposure of WKY rat aortas to IL-17F impaired endothelium-dependent vascular relaxation, whereas IL-17A did not. Moreover, *in vivo*

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inhibition of RORγt by digoxin decreased systolic blood pressure in SHRs.
Conclusions: SHRs have a markedly enhanced potential for RORγt-driven expression of proinflammatory and prohypertensive IL-17F in response to innate immune activation. Increased RORγt and IL-17F levels contribute to SHR hypertension and might be therapeutic targets. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Hypertension, innate immune system, Toll-like receptor, retinoic acid receptor–related orphan receptor γt, digoxin, T cells, T_H17, CD161, IL-17, spontaneously hypertensive rat, gene expression*

Abbreviations used
nAChR: Nicotinic acetylcholinergic receptor
PE: Phycoerythrin
Poly-IC: Polyinosinic-polycytidylic acid
RORγt: Retinoic acid receptor–related orphan receptor γt
SBP: Systolic blood pressure
SHR: Spontaneously hypertensive rat
SNP: Sodium nitroprusside
TLR: Toll-like receptor
Treg: Regulatory T
WKY: Wistar-Kyoto

Multiple organ systems involving the kidneys, vasculature, central nervous system, and immune system contribute to the genesis and maintenance of hypertension. The spontaneously hypertensive rat (SHR) is a widely studied rodent model of genetic hypertension that faithfully displays pathologic changes seen in human disease. SHRs show progressive increases in blood pressure, increased sympathetic tone, renal dysfunction, and a dysregulated immune system.[1,2] The role of inflammation and the immune system in hypertension has long been known, but the underlying mechanisms are just beginning to be understood.

SHR immune cells display an inherently enhanced inflammatory response. Cultured splenocytes from prehypertensive SHRs produce greater amounts of proinflammatory cytokines than those from normotensive Wistar-Kyoto (WKY) rats when stimulated by Toll-like receptor (TLR) 7/8 and TLR9 agonists in the presence of angiotensin II or nicotine.[1] In contrast, nicotine suppresses inflammatory cytokine release in WKY rats. In addition, nicotine treatment of cultured splenocytes from SHRs increases the relative abundance of a $CD161^+$ cell population. However, the distribution of CD161 cell-surface markers in the immune system of the SHR and its potential role in hypertension-related inflammation have not been examined.

Originally identified as a rodent homolog of a cell-surface marker for human natural killer cells,[3] the CD161 marker correlates with activation of orphan nuclear receptor retinoic acid receptor–related orphan receptor γt (RORγt), which functions as a master regulator of polarization of IL-17–producing T_H17 cells.[4-6] IL-17A and IL-17F are potent proinflammatory cytokines that drive inflammatory processes in patients with autoimmune and cardiovascular diseases, including hypertension,[7,8] and end-organ damage.[9,10] Angiotensin II–induced hypertension is not sustained in *Il17*$^{-/-}$ mice.[11] Dermal overexpression of IL-17A in psoriasis-like skin disease induces endothelial dysfunction and arterial hypertension.[12] In addition, infusion of IL-17 causes Rho kinase–mediated endothelial dysfunction and hypertension.[13] Antihypertensive treatment with telmisartan and statin in patients with carotid atherosclerosis reduces blood pressure and numbers of IL-17–producing T_H17 cells.[14] Deoxycorticosterone acetate–salt diet–induced organ damage is attenuated by blocking IL-17.[15]

The IL-17 family of cytokines consists of 6 members (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F). IL-17A (commonly known as IL-17) plays a key role in several autoimmune diseases and has been studied extensively. Relatively little is known about IL-17F, which is the closest homolog of IL-17A and forms a heterodimer with it. IL-17A and IL-17F share a common receptor but are recognized to have nonoverlapping functions.[16] Despite a presumed role of the immune system, inflammation, and T_H17 cells in the setting of hypertension, the effect of IL-17F in a hypertension-related physiologic process has not been demonstrated.

The autonomic nervous system has immunomodulatory effects on expression of proinflammatory cytokines and proliferation of many immune cells.[17] This immunomodulation exerts a tonic inhibition of proinflammatory immune cells by acetylcholine binding to nicotinic acetylcholinergic receptors (nAChR).[18,19] However, the effects of nicotine, an agonist of nAChR, on different cell types can vary depending on the expressed subunit and pharmacology of the receptors.[20-22] T_H17 cells are differentiated from a $CD4^+$ immune cell lineage that expresses nAChR and are subject to the regulatory effects of nicotine.[23-25] The inhibitory effect of nicotine on proinflammatory responses to TLR activation seen in splenocytes of normotensive WKY rats is abrogated in SHR splenocytes and replaced by a more pronounced proinflammatory response.[1]

Here we tested the hypothesis that enhanced T_H17 programming by RORγt transcription factor and IL-17F expression in $CD161^+$ immune cells represent an abnormal proinflammatory adaptive immune response in SHRs. Furthermore, we proposed that this response is driven by the master regulator RORγt and a nicotinic proinflammatory innate immune response.

Our results demonstrate that the CD161 cell-surface marker is widely and profusely expressed on SHR immune cells, including $CD4^+$ and $CD8^+$ T lymphocytes. There are more infiltrating $CD161^+$ cells in SHR kidneys and aortas than in WKY rats. $CD4^+CD161^+$ immune cells constitutively overexpress RORγt and induce IL-17F in response to inflammatory stimulation. Inhibition of RORγt expression with digoxin reduces systolic blood pressure (SBP) and renal infiltration of CD3 cells in kidneys of SHRs. We also report that nicotine, an anti-inflammatory agonist in WKY rats, actually increases $CD4^+CD161^+$ cell numbers in SHRs and potentiates their TLR3-mediated activation and *Il17f* expression. Finally, we identified a significant impairment of cholinergic endothelium-mediated vascular relaxation in response to IL-17F that was not seen with IL-17A.

We conclude that both the innate and adaptive immune systems are inherently dysregulated in SHRs. The dysregulation involves $CD161^+CD4^+$ immune cell RORγt transcription factor, a master regulator of IL-17A and IL-17F expression, that contributes to hypertension. The dysregulation also involves a nicotine-mediated increase in numbers of $CD4^+CD161^+$ splenocytes and expression of IL-17F that disrupts cholinergic endothelial vasorelaxation.