Toll-Like Receptors, Hypertension, and an Antimalarial Drug

Madhu V. Singh[1]

The role of the immune system in the pathogenesis of hypertension is a growing area of research. Although the immune system had long been suspected to contribute to hypertension, only in the last decade have studies begun to define the roles of the participating immune cells and molecules involved in hypertension.[1–4] Toll-like receptors (TLRs)[5,6] are part of the innate immune system that recognize molecular patterns on various pathogens (pathogen-associated molecular patterns) and so called endogenous damage signals (damage-associated molecular patterns). These patterns may be proteins, lipids, carbohydrates, or nucleic acids. TLRs are expressed either on the cell surface or in intracellular endolysosomal compartment. Although the endogenous ligands of the TLRs that trigger hypertension have not been identified, disruption of TLR signaling has been shown to attenuate hypertension.

In a study published last year in "Cardiovascular Research," McCarthy *et al.* reported that intraperitoneal injection of a TLR9 inhibitory oligonucleotide ODN2088 in adult spontaneously hypertensive rats (SHR) decreased the systolic blood pressure, whereas injections of TLR9 agonist ODN2395 in normotensive rats increased the systolic blood pressure.[7] In their study published in the current issue of *American Journal of Hypertension*,[8] these investigators extend their findings by testing a mechanism of intracellular TLR activation, particularly TLR9, in SHR hypertension. Chloroquine, a drug known as an effective antimalarial therapeutic agent, blocks endosomal maturation[9] by inhibiting vesicular acidification that is also required for TLR9 signaling.[10] McCarthy *et al.* injected chloroquine in SHR to disrupt TLR9 signaling and measured expression of key molecular components of TLR signaling in mesenteric resistance vessels that significantly contribute to hypertension. The major finding of the study is that in young prehypertensive SHR (5 weeks old), disruption of intracellular TLR signaling by chloroquine decreased blood pressure. These findings are consistent with the authors' previous report as well as with the findings by Harwani *et al.*[11] demonstrating that the isolated splenocytes from prehypertensive SHR have an enhanced proinflammatory response to TLR 7/8 and 9 agonists in the presence of nicotine. In normotensive Wistar-Kyoto rats, nicotine was anti-inflammatory. Moreover, *in vivo* infusion of nicotine in young prehypertensive SHR induces premature hypertension.[12] An important concept which evolves from this work is that the innate immune system is important in SHR hypertension. This concept is also shown to work in angiotensin II (Ang II) induced hypertension where priming of dendritic cells, a member of the innate immune system, by Ang II contributes to hypertension.[13]

A second concept in this paper is the possible role of TLR adaptor protein MyD88. All TLRs, except intracellular TLR3, require MyD88 for signaling. Loss of MyD88 protein severely impairs TLR signaling and inflammatory responses.[14] In the study by McCarthy *et al.*, chloroquine decreased the expression of MyD88 in mesenteric vessels in prehypertensive SHR and reduced subsequent hypertension. This would mean that MyD88 acts as a proinflammatory signaling molecule that may contribute to the development of hypertension in SHR. This finding is in contrast to our study with Ang II infusion in mice showing that MyD88 is anti-inflammatory and TRIF is an inflammatory adaptor protein in Ang II-induced hypertension.[15] McCarthy *et al.* did not directly examine the role of MyD88 in established SHR hypertension. It is possible that MyD88 plays a different role in a genetically abnormal innate immune system of SHR than in the acquired Ang II hypertension.

A third concept emerges from the paradoxical finding that in contrast to the prehypertensive SHR, chloroquine treatment increased MyD88 expression in adult SHR. If so, then in adult SHR chloroquine treatment should increase blood pressure. The effect of chloroquine-induced increase in the expression of MyD88 on blood pressure of adult SHR needs to be examined. Did the blood pressure increase further (since MyD88 increased) or did it decrease? In the latter case, it would support the anti-inflammatory and antihypertensive role of MyD88 reported by Singh *et al.* paper.[15] This dilemma is unresolved at this time given the absence of data on blood pressure in adult SHR in the current study. This is a critical omission. There are other important questions that remain to be answered. For example, how specific is the effect of chloroquine on SHR hypertension? Does it also affect other mediators of inflammation?[16] Are there significant differences in the way the innate immune system responds in SHR and Ang II hypertension? Does MyD88 act as proinflammatory or anti-inflammatory in physiological vs. pathological context?

Corresponding author: Madhu V. Singh (madhu-singh@uiowa.edu).

Initially submitted September 19, 2016; accepted for publication September 19, 2016; online publication October 4, 2016.

[1]Department of Internal Medicine and Abboud Cardiovascular Research Center, Carver College of Medicine, University of Iowa, Iowa City, IA, USA

doi:10.1093/ajh/hpw128

Taken together, the results of this study identify changes in expression of TLR-signaling molecules in hypertension that may have a potential therapeutic lead into intracellular TLR signaling. At the same time, these results also underline the complexity of TLR system and its role in hypertension.

ACKNOWLEDGMENTS

The author thanks Drs Francois Abboud and Mark Chapleau for discussions and suggestions. Funded by NIH PPG (HL 14388) and AHA IRG (16IRG27260323).

DISCLOSURE

The author declared no conflict of interest.

REFERENCES

1. Goulopoulou S, McCarthy CG, Webb RC. Toll-like receptors in the vascular system: sensing the dangers within. *Pharmacol Rev* 2016; 68:142–167.
2. Schiffrin EL. Immune mechanisms in hypertension and vascular injury. *Clin Sci (Lond)* 2014; 126:267–274.
3. Singh MV, Abboud FM. Toll-like receptors and hypertension. *Am J Physiol Regul Integr Comp Physiol* 2014; 307:R501–R504.
4. Harrison DG. The immune system in hypertension. *Trans Am Clin Climatol Assoc* 2014; 125:130–138.
5. Blasius AL, Beutler B. Intracellular Toll-like receptors. *Immunity* 2010; 32:305–315.
6. Moresco EM, LaVine D, Beutler B. Toll-like receptors. *Curr Biol* 2011; 21:R488–R493.
7. McCarthy CG, Wenceslau CF, Goulopoulou S, Ogbi S, Baban B, Sullivan JC, Matsumoto T, Webb RC. Circulating mitochondrial DNA and Toll-like receptor 9 are associated with vascular dysfunction in spontaneously hypertensive rats. *Cardiovasc Res* 2015; 107:119–130.
8. McCarthy CG, Wenceslau CF, Goulopoulou S, Baban B, Matsumoto T, Webb RC. Chloroquine suppresses the development of hypertension in spontaneously hypertensive rats. *Am J Hypertens* 2016; e-pub ahead of print 13 September 2016.
9. Ohkuma S, Poole B. Cytoplasmic vacuolation of mouse peritoneal macrophages and the uptake into lysosomes of weakly basic substances. *J Cell Biol* 1981; 90:656–664.
10. Häcker H, Mischak H, Miethke T, Liptay S, Schmid R, Sparwasser T, Heeg K, Lipford GB, Wagner H. CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J* 1998; 17:6230–6240.
11. Harwani SC, Chapleau MW, Legge KL, Ballas ZK, Abboud FM. Neurohormonal modulation of the innate immune system is proinflammatory in the prehypertensive spontaneously hypertensive rat, a genetic model of essential hypertension. *Circ Res* 2012; 111:1190–1197.
12. Harwani SC, Chapleau MW, Abboud FM. Cholinergic stimulation of immune cells with nicotine induces renal inflammation and premature development of hypertension in young SHR. *The FASEB Journal* 2016; 30(Suppl 1):1237.3.
13. Kirabo A, Fontana V, de Faria AP, Loperena R, Galindo CL, Wu J, Bikineyeva AT, Dikalov S, Xiao L, Chen W, Saleh MA, Trott DW, Itani HA, Vinh A, Amarnath V, Amarnath K, Guzik TJ, Bernstein KE, Shen XZ, Shyr Y, Chen SC, Mernaugh RL, Laffer CL, Elijovich F, Davies SS, Moreno H, Madhur MS, Roberts J 2nd, Harrison DG. DC isoketal-modified proteins activate T cells and promote hypertension. *J Clin Invest* 2014; 124:4642–4656.
14. Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 1999; 11:115–122.
15. Singh MV, Cicha MZ, Meyerholz DK, Chapleau MW, Abboud FM. Dual activation of TRIF and MyD88 adaptor proteins by angiotensin II evokes opposing effects on pressure, cardiac hypertrophy, and inflammatory gene expression. *Hypertension* 2015; 66:647–656.
16. Browning DJ. Pharmacology of chloroquine and hydroxychloroquine. In, *Hydrochloroquine and Chloroquine Retinopathy*. Springer-Verlag: New York, 2014, pp 35–63.