Letter to the Editor

Anti-toll like receptor 4 (TLR4) therapy diminishes cardiac remodeling regardless of changes in blood pressure in spontaneously hypertensive rats (SHR)

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With the increasing global incidence of high blood pressure, arterial hypertension is becoming the main risk factor for cardiac remodeling and consequent end-organ damage [1]. Recent evidences have associated hypertension with a chronic low-grade inflammation and augmented levels of circulating pro-inflammatory molecules [2]. Among the pro-inflammatory pathways, toll-like receptors (TLRs) have been emerging as key regulators of chronic inflammation and associated disease, including cardiovascular disease. TLRs are important sources of pro-inflammatory molecules and they can recognize several damage-associated molecular patterns that are up-regulated during hypertension [3]. We recently described, for the first time, that hypertension is associated with increased TLR4 expression in mesenteric arterioles, kidneys and heart of adult spontaneously hypertensive rats (SHR) compared to pre-hypertensive SHR [4], as well as to normotensive Wistar [5]. Nevertheless, there is no direct evidence showing the role of TLR4 and TLR4-dependent signaling pathways in hypertension-associated cardiac remodeling, which is the aim of this study.

Male adult SHR (21-week-old) and age-matched Wistar rats were studied after a period of 15-day treatment with anti-TLR4 antibody or non-specific IgG antibody, as previously described [5]. This study was conducted in accordance with the ethical principles in animal research adopted by Brazilian College of Animal and experimentation. Left ventricles sections were stained with hematoxylin–eosin or picro-sirius to determine cardiomyocyte area, diameter and collagen deposition. mRNA and protein expression were determined by conventional qPCR and western blot techniques. Arterial blood pressure and heart rate were measured by carotid artery cannulation. Left ventricular developed pressure was measured by Langendorff under basal conditions and after isoproterenol stimulation. Analyses of mean ± SEM values were performed using two-way ANOVA or multiple t tests (post-hoc Sidak–Bonferroni), where appropriate. Data analysis was carried out using IBM SPSS 20 software. Values of p < 0.05 were considered statistically significant.

In this study, we provide the first direct evidence that TLR4 plays a key role in hypertension-associated cardiac remodeling. Our main findings demonstrate that anti-TLR4 treatment reduces TLR4 expression and TLR-dependent signaling pathways in left ventricles of hypertensive rats, leading to decrease in cardiomyocyte hypertrophy and fibrosis. The classic paradigm of hypertension-associated cardiac hypertrophy is that thickening of left ventricle occurs as a compensatory mechanism to minimize wall stress in response to elevated blood pressure [1]. In fact, with established hypertension, left ventricles of adult SHR (21-week-old) display augmented cardiomyocytes area and diameter as well as increased collagen deposition compared to age-matched normotensive Wistar rats (Fig. 1A) These morphological changes were paralleled by increased TLR4 mRNA expression in adult SHR in reference to age-matched Wistar (39 ± 8.5%, p < 0.05) or young SHR (28 ± 13%,
No differences in TLR4 expression were observed when comparing young (6-week-old) and adult (21-week-old) Wistar rats, reinforcing our hypothesis that there is a positive correlation between hypertension and increased TLR4.

Accordingly, our next step aimed to determine whether the anti-TLR4 therapy could counteract the hypertension associated cardiac remodeling. We found that anti-TLR4 therapy markedly reverses cardiomyocyte hypertrophy as well as decreases collagen deposition in left ventricles of 21-week-old SHR to levels observed in age-matched normotensive rats (Fig. 1A). No differences were found in saline-treated rats versus non-specific IgG antibody treatment (data not shown), confirming the efficacy and specificity of anti-TLR4 therapy to promote an anti-remodeling effect in the heart. The finding that anti-TLR4 therapy inhibits hypertensive cardiac remodeling without affecting blood pressure in SHR (Table 1) was intriguing. These results suggest that changes in ventricle morphology during hypertension can be triggered or maintained by mechanisms that are parallel and independent of pressure overload. Corroborating our findings, Ha et al. [6] have described a decrease of cardiomyocyte area following pressure overload in TLR4 knockout normotensive mice.

Cardiac hypertrophy is also associated with induction of genes expressed during embryonic development, such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and skeletal α-actin [7]. The precise mechanism that leads to this up-regulation remains unclear, but it is believed to be an adaptive process to protect the stressed heart [7]. Reduction in expression of those genes was observed after anti-TLR4 treatment in 21-week-old SHR (Fig. 1B). This response was paralleled by decreased expression of collagen types I and III, which may reinforce the protective role of anti-TLR4 therapy in reversing hypertension-induced cardiac remodeling. It is known that excessive collagen deposition increases ventricular stiffness and negatively affects cardiac contractility properties [1]. Nonetheless, no changes in the left

Fig. 1. (A) Histological analysis of cardiomyocyte area, cardiomyocyte diameter, and collagen deposition. Shown are representative images of hematoxylin and eosin (top) or pico-sirius (bottom) staining of left ventricles of SHR and Wistar rats treated with anti-TLR4 antibody or non-specific IgG antibody. Bar graphs show the results of calculated area (μm²) and diameter (μm) of cardiomyocytes, or the relative area stained with picro-sirius for collagen deposition. (B) mRNA levels of ANP, BNP, skeletal α-actin, collagen type I and collagen type III in left ventricles of SHR treated with anti-TLR4 antibody or non-specific IgG antibody. mRNA levels are expressed as 2^{- ΔΔCt} using β-actin as internal control and in reference to SHR IgG groups. Each data represents the mean ± SEM derived from 4 to 10 independent experiments. *p < 0.05.

Table 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>Heart rate (bpm)</th>
<th>LVDP basal (mm Hg)</th>
<th>LVDP isoproterenol (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar IgG</td>
<td>133.3 ± 3.8</td>
<td>99.7 ± 3.4</td>
<td>324.1 ± 15.2</td>
<td>131.3 ± 5.8</td>
<td>301.1 ± 3.9</td>
</tr>
<tr>
<td>Wistar anti-TLR4</td>
<td>132.7 ± 5.8</td>
<td>95.2 ± 5.8</td>
<td>335.4 ± 11.7</td>
<td>117.4 ± 7.0</td>
<td>273.3 ± 17.9</td>
</tr>
<tr>
<td>SHR IgG</td>
<td>208.8 ± 3.4</td>
<td>152.7 ± 2.8</td>
<td>367.6 ± 25.8</td>
<td>119.7 ± 7.3</td>
<td>307.1 ± 5.8</td>
</tr>
<tr>
<td>SHR anti-TLR4</td>
<td>211.5 ± 4.1</td>
<td>132.7 ± 2.8</td>
<td>360.6 ± 6.6</td>
<td>121.1 ± 6.4</td>
<td>309.9 ± 13.3</td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure; DBP: diastolic blood pressure; LVDP: left ventricular developed pressure. *p < 0.05 vs Wistar. Data represented as mean ± SEM (n = 4–13).
ventricular developed pressure were observed in SHR at the time point of this study (Table 1). These results are in accordance with Bing et al. [8], who showed that cardiac function of SHR can be preserved until 1 year of age despite hypertrophy.

We further demonstrated that TLR4 antagonism reduces TLR4 expression and TLR4-dependent pro-inflammatory signaling pathways in left ventricles of hypertensive rats (Fig. 2A). Initially we observed that anti-TLR4 treatment decreases MyD88 expression (Fig. 2A), a key adaptor molecule that interacts with TLR4 in the cell plasma membrane and mediates TLR4-signaling towards inflammation [9]. We also found that anti-TLR4 treatment decreases cardiac phosphorylation and activation of NF-κB (p65/total NF-κB: SHR 101 ± 1.6 vs SHR Anti-TLR4 92 ± 2.6, p < 0.05), as well as the expression of pro-inflammatory cytokines IL-1β and TNF-α (Fig. 2B). It has been established that inflammation contributes to cardiac remodeling [1]. The absence of IL-1β reduces left ventricular dilatation following myocardial infarction [10], and TNF-α over-expression leads to changes in collagen composition [11].

In summary, our results show for the first time that TLR4 plays a key role in hypertensive cardiac remodeling, via an inflammatory mechanism that increases cytokines (IL-1β and TNF-α), and which, moreover, is independent on changes in blood pressure levels. These findings shed new lights into the molecular mechanism that controls cardiac remodeling and suggests that targeting TLR4 may have major therapeutic implications to prevent hypertension-associated end-organ damage.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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