

## Reduced endothelial dependent vascular relaxation in vessels from TLR4<sup>-/-</sup> mice is associated with increased superoxide generation

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Toll like receptor (TLR) 4 is a pattern recognition receptor expressed on cells such as endothelial cells, and is responsible for sensing endotoxin. We have previously shown that absence of TLR4 is associated with reduced endothelial dependent vasodilatation, even though TLR4<sup>-/-</sup> mouse mesenteric arteries have an intact endothelial layer and comparable expression of NOSIII protein as wild type mice (Harrington *et al* 2007). The mechanism behind reduced endothelial cell function in TLR4<sup>-/-</sup> mice is not known, but could be related to increased NADPH oxidase (NOX) activity (Zhang *et al* 2006).

Female wild type (C57 Black6) or TLR4<sup>-/-</sup> mice (Hoshino *et al* 1999) were killed by cervical dislocation (8 to 14 weeks old) and mesenteric arteries mounted in isometric wire myographs.

Arteries were contracted with an EC<sub>80</sub> of U46619 (3x10<sup>-8</sup>mol/L) and acetylcholine (Ach) responses measured (Fig 1A). To image and quantify superoxide levels, some arteries were exposed to the superoxide dismutase mimetic MnCl<sub>2</sub> (3x 10<sup>-4</sup>mol/L) for 20 minutes prior to contracting with U46619. After

10 minutes, dihydroethidium (DHE; 10<sup>-5</sup>mol/L) which binds to superoxide to form ethidium, was added for 20 minutes followed by increasing concentrations of Ach. Tissues were fixed with 2% paraformaldehyde, and nuclei stained using DAPI (25µg/ml). Arteries were mounted, and images captured using a confocal

microscope. Mesenteric arteries from TLR4<sup>-/-</sup> mice had a reduced endothelial dependent relaxant response (Fig 1A) and increased superoxide levels (Fig 1B) when stimulated with Ach. Increased levels of superoxide seen in vessels from TLR4<sup>-/-</sup> mice were reduced to control levels in the presence of MnCl<sub>2</sub> (Fig 1B).

**Fig 1 A.** Data shown as mean ± SEM, n=10-11; \* p<0.05 by two way ANOVA. **B.** Data are mean pixel count ± SEM, n=6; \* p<0.05 by one way ANOVA with Bonferroni's Multiple Comparison Test.

These observations suggest increased superoxide generation in TLR4<sup>-/-</sup> vessels reduces the activity of endothelial derived NO and thereby explains the cardiovascular phenotype in TLR4<sup>-/-</sup> mice.

Harrington *et al*, J Card Pharm Ther 2007, 12:322-326;  
Hoshino *et al*, JI 1999, 162:3749-3752;  
Zhang *et al*, JCI 2006, 116:3050-3059.

