

**TITLE:** Macrophages response to *Ureaplasma diversum* and its membrane-associated lipoproteins is mediated by toll-like receptors (TLR) 2 and TLR4 signaling

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### **ABSTRACT:**

*Ureaplasma diversum* is an opportunistic pathogen that causes severe inflammation in the reproductive tract of cows, interfering with bovine reproduction. Thus, the objective of this study was to evaluate the immunological response generated against *U. diversum* in bovine macrophages culture. For this, the viable and heat-inactivated strains ATCC 49782 and IC-GOTA and their respective isolated lipoproteins were inoculated into macrophage cultures in the presence or absence of toll-like receptors (TLR) 2, TLR2/4 and nuclear factor kappa B (NF- $\kappa$ B) signaling blockers. The nitric oxide concentration (NO) was measured from the culture supernatant. The cells were processed for gene expression analysis of interleukin 1 beta (IL-1 $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), TLR2 and TLR4. The strains (incubation for 6, 12 and 24 hours) and different concentrations of lipoproteins (incubation for 2, 6 and 12 hours) induced higher gene expression in relation to uninfected cultures. There was also induction of NO secretion. The inoculation of *U. diversum* into macrophages in the presence of blockers inhibited the expression of IL-1 $\beta$  and TNF- $\alpha$  in all treatments. These data provide strong evidence that *U. diversum* and its isolated lipoproteins stimulate NO production and interact with TLR4 in a signaling type involving TLR2. This interaction active NF- $\kappa$ B that acts stimulating the expression of pro-inflammatory cytokines. To date, no studies evaluating the immunological response generated against *U. diversum* in bovine macrophages culture have been found. These results may contribute to a better understanding of the immunomodulatory activity and the pathogenicity of these infectious agents.

**Keywords:** *Ureaplasma diversum*; Macrophages; NK- $\kappa$ B; TLR.