

Targeting Toll-like Receptor 2: polarization of porcine, equine, ovine monocyte-derived macrophages by three diverse synthetic diacylated lipopeptide

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Introduction

Toll-like receptor 2 (TLR2) ligands are attracting attention as prophylactic and immunopotentiator agents against pathogens. We previously reported that a synthetic diacylated lipopeptide (Mag-Pam2Cys-P48) polarized porcine macrophages towards a proinflammatory antimicrobial phenotype. Here, we investigated the role of Mag-Pam2Cys-P48, alongside two other lipopeptides (Mag-Pam2Cys-P80 and Mag-Pam2Cys-Mag1000) on monocyte-derived macrophage (moMΦ) from three diverse species: pig, sheep, horses.

Materials and methods

In this work, healthy pigs, sheep, horses were used as blood donor and moMΦ were generated in vitro using human M-CSF or autologous plasma. Cells were left untreated or stimulated with Mag-Pam2Cys-P48, Mag-Pam2Cys-P80 or Mag-Pam2Cys-Mag1000 (all at 100 ng/mL) for 24 h, then immunomodulatory properties of these molecules on moMΦ were investigated using flow cytometry, microscopy, multiplex ELISA, qPCR (Figure 1).

Results

First, analysis assessing the homology between the three different TLR2 were carried out. The results of TLR2's aminoacidic (AA) sequences comparison (average AA identity: 77%) coupled with quantitative assessment of protein structure similarity (average TM-score: 0.88), suggested a high conservation among the three different species. Then, in vitro data revealed that all tested TLR2 agonists polarized porcine macrophages towards a pro-inflammatory phenotype, with enhanced expression of activation markers (MHC II DR, MHC I, CD14), increased phagocytic activity, and induction/release of pro-inflammatory cytokines (Figure 2, Figure 3). In parallel, we observed that all TLR2 ligands decreased expression of several TLRs in porcine moMΦ (Figure 3). Induction of pro-inflammatory cytokines (IL-1, IL-6, CXCL8, TNF) were also observed in ovine and equine moMΦ 24h post-stimulation with these diacylated lipopeptides (data not shown).

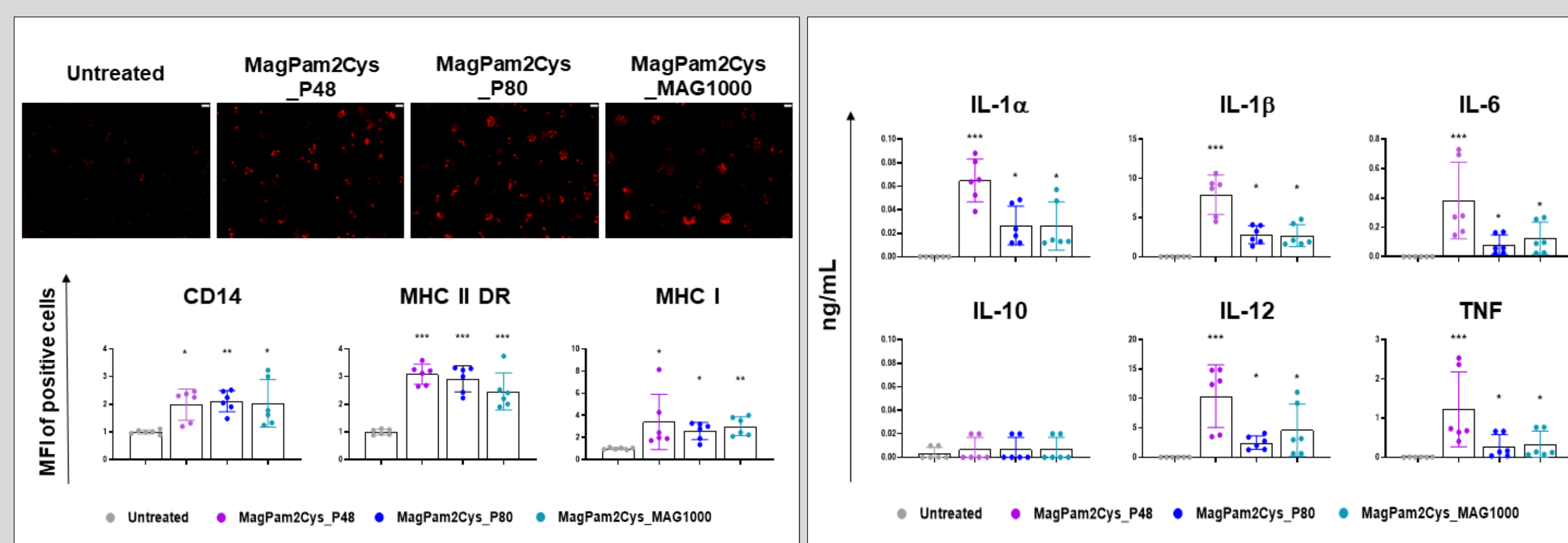


Figure 2. Effect of TLR2 ligands on porcine moMΦ phenotype and functionality. Porcine moMΦ were left untreated or activated with TLRs agonists. 24h later, expression of activation markers, phagocytic activity, and release of key cytokines was assessed with flow cytometry, microscopy, and multiplex ELISA, respectively.

Conclusions

All tested TLR2 agonists polarized porcine, sheep and equine macrophages towards a pro-inflammatory phenotype. Preliminary data in pigs suggest that the inflammatory activity evoked by Mag-Pam2Cys could be regulated to avoid potentially harmful consequences, although further experiments are required. We hope that our in vitro results will lay the foundation for the further evaluation of these diacylated lipopeptide as an immunopotentiator in vivo.

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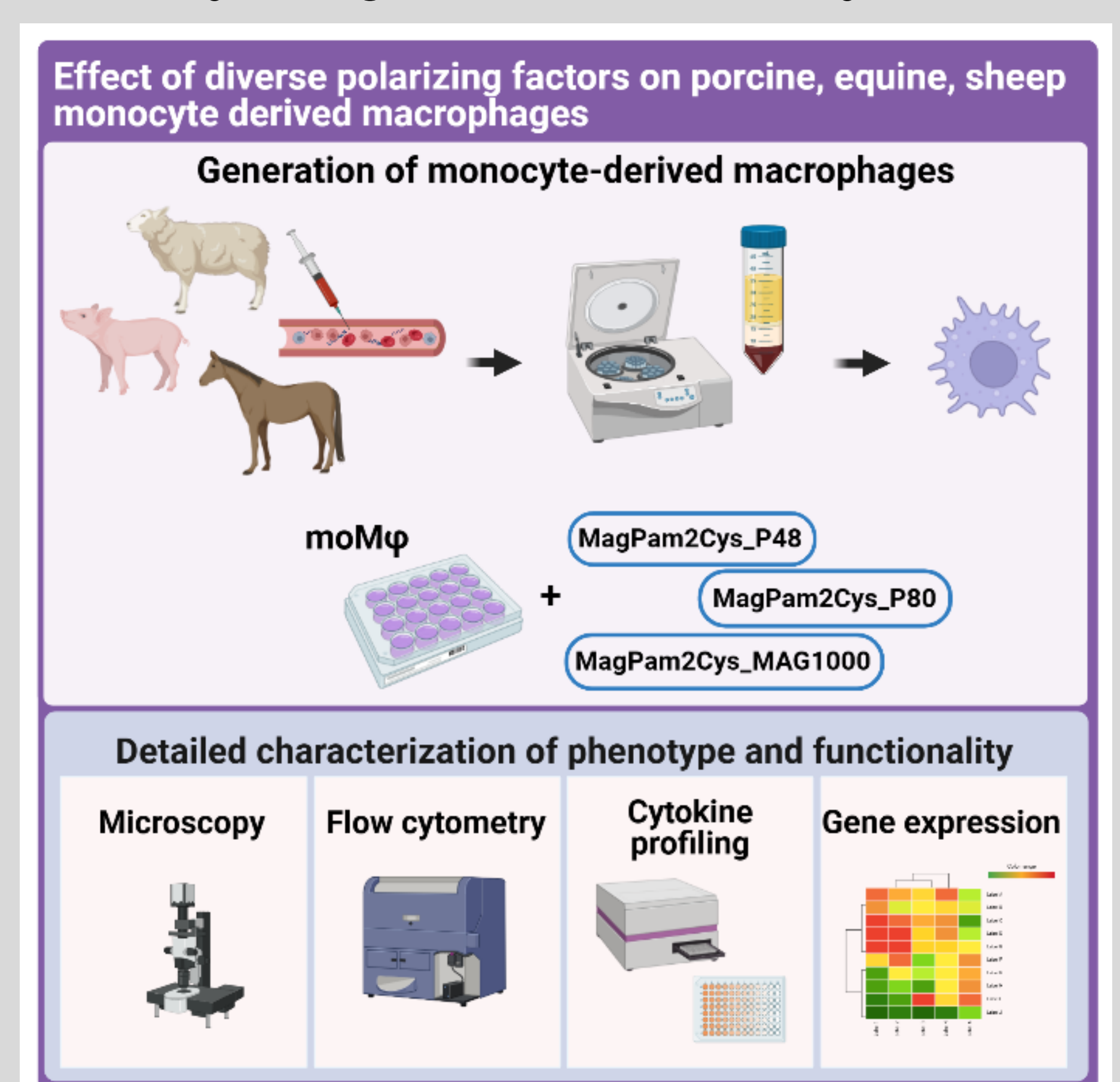


Figure 1. Figure scheme. Created with BioRender.com.

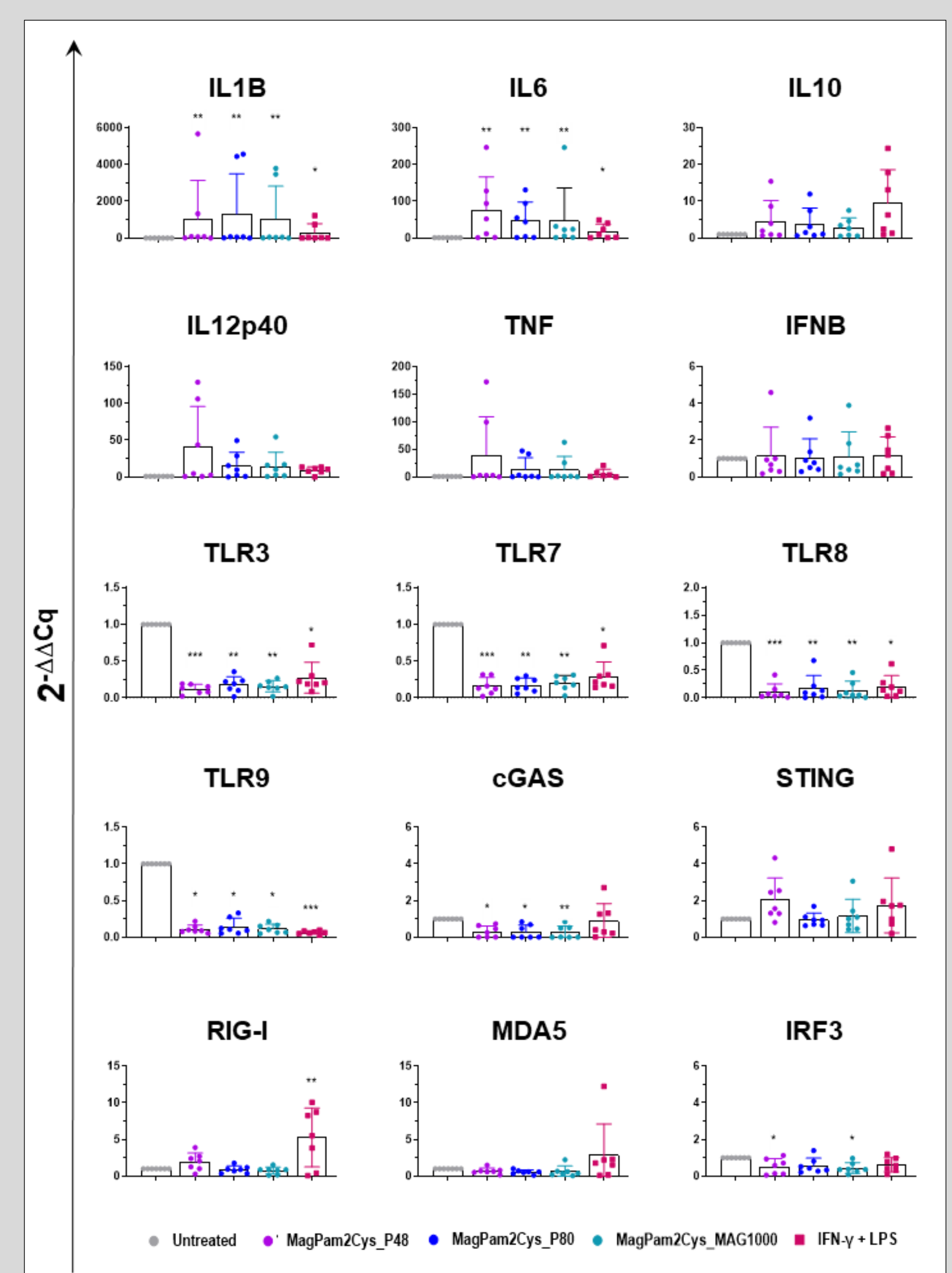


Figure 3. Effect of TLRs agonists on expression of key immune gene. Porcine moMΦ were left untreated or activated with TLRs agonists. 24h later, expression of key immune genes was assessed with qPCR.