EFFECTS OF PLANT ANTIMICROBIAL PEPTIDES ON INNATE IMMUNE RESPONSE DURING HOST-PATHOGEN INTERACTIONS

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Introduction. Host antimicrobial peptides have an important immunomodulatory function besides their antibacterial activity. However, the actions of plant antimicrobial peptides (PAP) against innate immune response (IIR) of mammals are poorly known. In this work, we evaluated the effects of PAP γ-thionin (BEC-g) and thionin Thi2.1 (BEC-T) produced by transgenic bovine endothelial cells on elements of innate immune response on two host-pathogen models: 1) bovine mammary epithelial cells (BMEC) infected with *Staphylococcus aureus*; and 2) Caco-2 cells (human intestinal epithelium) infected with *Escherichia coli*. We have previously demonstrated that BEC-T inhibits the viability of *S. aureus* endocyted by BMEC after 24 h of treatment, while BEC-g does not have this property (1). We analyzed the expression of several genes representative of IIR of mammals, which include: chemokines, pro- and anti-inflammatory cytokines and host antimicrobial peptides (AP).
The objective of this work was to evaluate the effect of PAP on IIR of mammals previous or during infection.

Methods. We evaluated by real-time PCR the effect of 3.125 μg/ml of protein of conditioned media (CM) from BEC-T and BEC-g. In BMEC-*S. aureus* we analyzed the expression of the bovine genes: interleukin (IL)-8, IL-10, tumour necrosis factor- α (TNF-α), tracheal antimicrobial peptide (TAP) and β-defensin 5 (BNBD5). We achieved two different experimental approaches: 1) pre-treated BMEC during 2 and 6 h with CM and after infected for 2 h with *S. aureus* (time necessary for bacterial internalization); 2) BMEC infected for 2 h and then treated with CM from BEC producers of PAP during 2 and 6 h. As control, we used the CM of BEC non-transfected (NT). A similar approach was performed in Caco-2 infected with *E. coli,* but only during 2 h of infection. Experiments were performed at least three times in triplicates.

Results. We detected a better IIR of BEC-g at 2 h treatments, which are represented in Figure 1. Briefly, 2 h pre-treatment of BMEC with CM favors a pro-inflammatory response, since TNF-α expression is induced (Fig. 1A). If then, these cells are infected by *S. aureus*, an anti-inflammatory response is induced (higher levels of IL-10, Fig. 1B), besides other anti-inflammatory pathways are activated (IL-8). The concomitant treatment of PAP and infection reduces IIR of BMEC (Fig. 1C). On other hand, 6 h-treatments reduce anti- and pro-inflammatory IIR and favors AP production, mainly BNBD5 by BEC-T. In the human model only BEC-T induced IL-10 at 6 h treatment (data not shown).

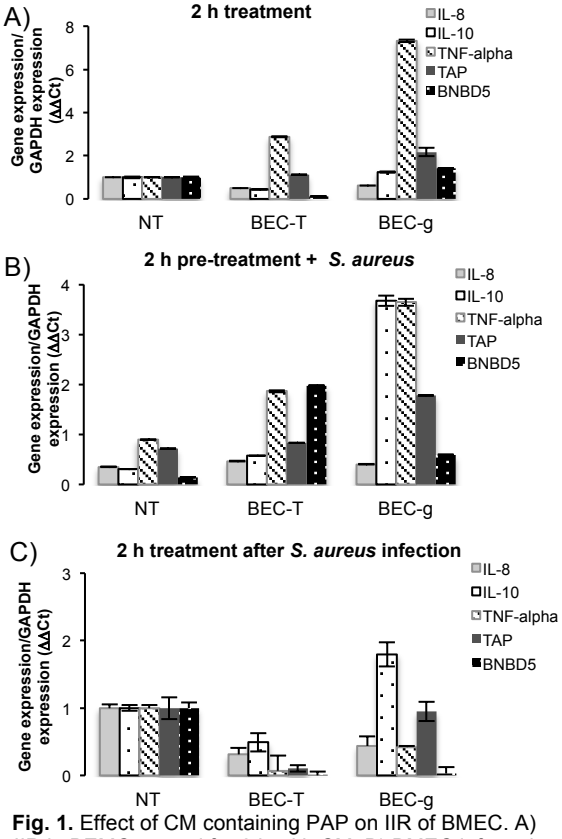

Fig. 1. Effect of CM containing PAP on IIR of BMEC. A) IIR in BEMC treated for 2 h with CM; B) BMEC infected with *S. aureus* and 2 h-treated with CM; and C) treated with CM after infection with *S. aureus.*

Conclusions. PAP can modulate IIR of mammals, and the pre-treatment induces a better response than when infection has been established.

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