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Publication date:
2008

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Cellular Model for Porcine Host Responses Against *Actinobacillus pleuropneumoniae* Infection

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In this study we established an in vitro cell line model that was able to respond to the presence of the gram negative bacterium *Actinobacillus pleuropneumoniae*. The cellular response was evaluated by quantitative PCR, and a 230 fold up-regulation of TNF-alpha gene expression was found.

Two out of five siRNA transfection reagents, Lullaby and Dreamfect, were selected based on the two plasmid siRNA system. With Lullaby it was possible to obtain a 55% reduction in gene expression of CD14.

Results

Evaluation of the capability of the cells to respond to the presence of *A. pleuropneumoniae* was based on TNF-alpha gene expression (Figure 1). 3D4/31 showed a remarkably higher response than the two other cell lines with a 230 fold up-regulation as opposed to the two others having a 6 and 8 fold change up-regulation respectively.

Evaluation of the five different transfection reagents with the Fam labelled siRNA revealed that all reagents had similar transfection efficiencies, except for neoFX which had close to no transfection efficiency and high toxicity (Figure 3A).

Only the four best reagents were analysed in the two plasmid system (Figure 3B&C) which revealed Lullaby and Dreamfect to be the best transfection reagent candidates for this cellular system, with 80% and 77% inhibition of gene expression, respectively.

It was possible to obtain a 55% reduction of CD14 expression in 3D4/31 cells (Figure 4) and the obtained silencing ranged from 18% to 97% in individual experiments (n=15). Thus, this low degree of silencing may very well be related to variable transfection efficiencies among the batches with the silencing itself...