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Cellular Model for Porcine Host Responses Against Actinobacillus pleuropneumoniae Infection

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Introduction
In order to investigate molecular actors of the innate immune response to gram-negative bacterial infections a simple cellular model was established with the intention of combing exposure to bacteria with inhibition of eukaryotic expression by siRNA.

In this model a bacterial species causing severe pleuropneumonia in pigs, Actinobacillus pleuropneumoniae was used as infectious agent and three cell-lines of porcine alveolar macrocyte/macrophage origin, previously established from a lung lavage [1] were tested for response against the bacterium (Figure 3).

CD14 which is present on the surface of 3D4/31 and 3D4/2 (Figure 2), is a part of the LPS receptor complex in cell membrane, that mediates a cellular response to LPS. As LPS is a primary component of the outer membrane in gram-negative bacteria it would be interesting to silence this component in order to elucidate its role in the immediate immune response.

Five different transfection reagents were investigated for siRNA transfection and silencing efficiency by using a FAM labelled siRNA as well as a two-plasmid system containing siRNA against one of the plasmids [2] (Figure 3).

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), however 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 rather than to problems with the silencing itself.

Conclusion
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 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.

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