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Cellular Model for Porcine Host Responses Against *Actinobacillus pleuropneumoniae* Infection

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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 pleuropneumonia*.

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.

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 combining 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 monocyte/macrophage origin, previously established from a lung lavage [1] were tested for response against the bacterium (Figure 1).

CD14 which is present on the surface of 3D4/31 and 3D4/21 (Figure 2), is 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).

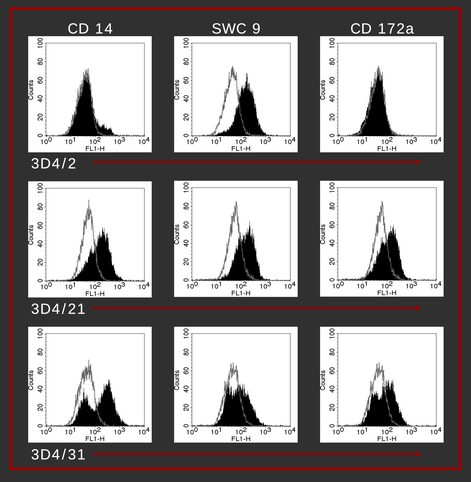

Figure 1. Flow cytometry analysis of various monocyte/macrophage markers to elucidate the developmental stage of the cells in each batch. CD14 gets down regulated on mature macrophages, CD172a is a monocyte marker and SWC9 is a mature macrophage marker

Grey open histograms show labeling with the isotype control for the respective marker, black filled histograms shows labeling with markers. Rows contains the respective cell line and columns contains the respective marker

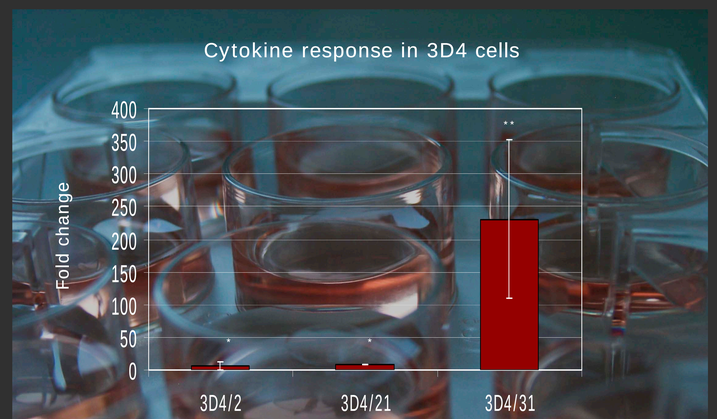

Figure 1. TNFα response in the infected cell cultures illustrated as fold expression change for each infected cell line compared to their respective un-infected control. Initial bacterial concentration of approximately 10 CFU per cell and incubation time of 3 hours. Error bars are +/- SEM, *n=3 and **n=5.

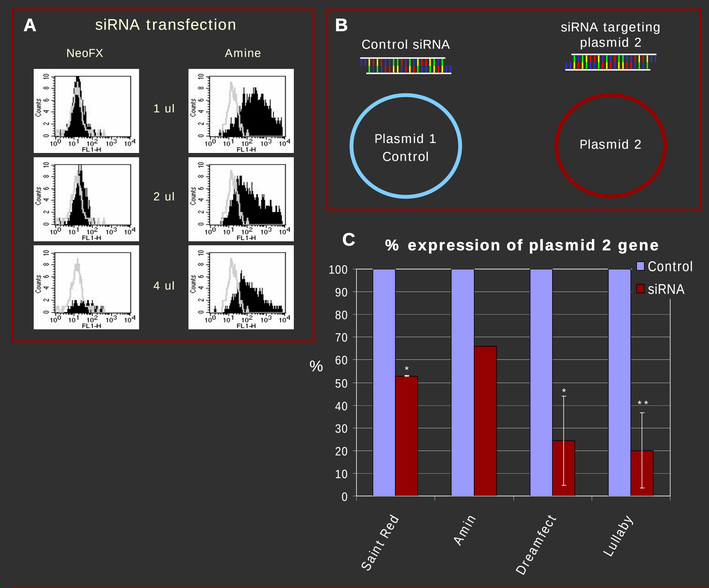

Figure 3. Evaluation of 5 different transfections reagents, NeoFX (Ambion), Amine (Ambion), Saint Red (Synvolux), Dreamfect (OZ Bioscience) and Lullaby (OZ Bioscience).

A. NeoFX and Amine were evaluated using FAM labelled siRNA and transfection efficiency was analyzed using flow cytometry. All reagents were analysed in this system as well (data not shown).

B. A two plasmid system, for evaluation of transfection agents, encoding one of two envelope glycoprotein genes from a viral fish pathogen; either – IHNV-G (plasmid 1) or VHSV-G (plasmid 2) [2]. Both plasmids are used in equal amounts in all transfection reactions. Plasmid 1 is used to normalize samples for variability in transfection efficiency in all experiments. Plasmid 2 is used to measure the degree of silencing. In control samples a negative control siRNA targeting GFP is used.

C. Comparison of the degree of reduction in VHSV-G gene expression using various transfection reagents. SD is indicated in errorbars, *n=2 and **n=4

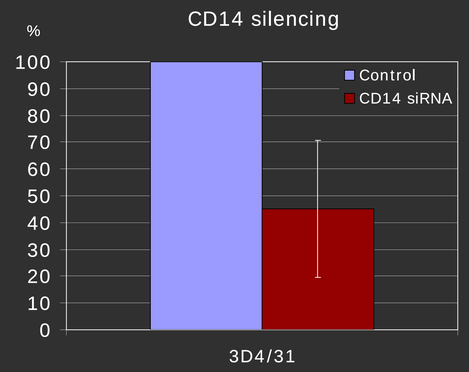

Figure 4. Silencing of CD14 in 3D4/31 cells using Lullaby transfection reagent. Error bars are SD, n=15.

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.

References cited
[1] Weingartl et al., J Virol Methods. 2002 Jul;104(2).
[2] Schyth et al., Molecular Therapy. 2007 Jul;15(7).