Strain and dose specific effect of various *Propionibacterium acnes* strains on the cellular functions of HPV-KER cells

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INTRODUCTION

Acne vulgaris is the most common multifactorial inflammatory skin disease of the pilosebaceous follicles. Increased colonization by *Propionibacterium acnes (P. acnes)* and abnormal keratinocyte and sebocyte functions have been implicated in its pathogenesis.
Earlier it has been investigated that assorted *P. acnes* strains (*889, 6609* and *ATCC11828*) belonging to various phylogenetic subgroups within the species differentially affected the proliferation and viability of cultured normal human epidermal keratinocytes. (*Nagy et al. / Microbes and Infection 8 (2006) 2195e2205)*

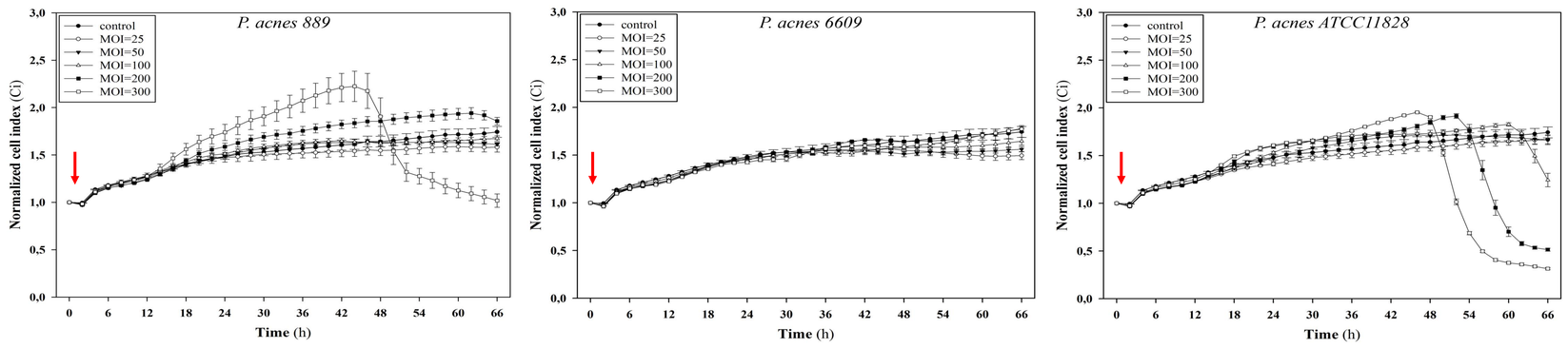
AIMS

To investigate the interaction between *P. acnes* bacterium and the keratinocytes. For that, we monitored the differential effects of the above *P. acnes* strains (*889, 6609, ATCC 11828*) on the proliferation and viability of an immortalized human keratinocyte cell line, the HPV-KER and investigated the underlying signaling events.

MATERIAL AND METHODS:

- **Cell type: HPV-KER immortalized human keratinocyte cell line**
- **Treatment: *P. acnes 889, 6609, ATCC 11828* strains (MOI: multiplicity of infection; 25, 50, 100, 200, 300)**
- **Real-time monitoring of the cell index (CI) changes of *P. acnes*-treated HPV-KER cells using the xCELLIgence system (RTCA analysis)**
- **Multiplicity of Infection (MOI)**
- **Analysis of:**
  - **cell number changes using Bürker-chamber**
  - **morphological changes and cell cytotoxicity using fluorescent microscopy**
  - **mRNA expression changes using real time RT PCR**
  - **NF-κB activation by western blotting**

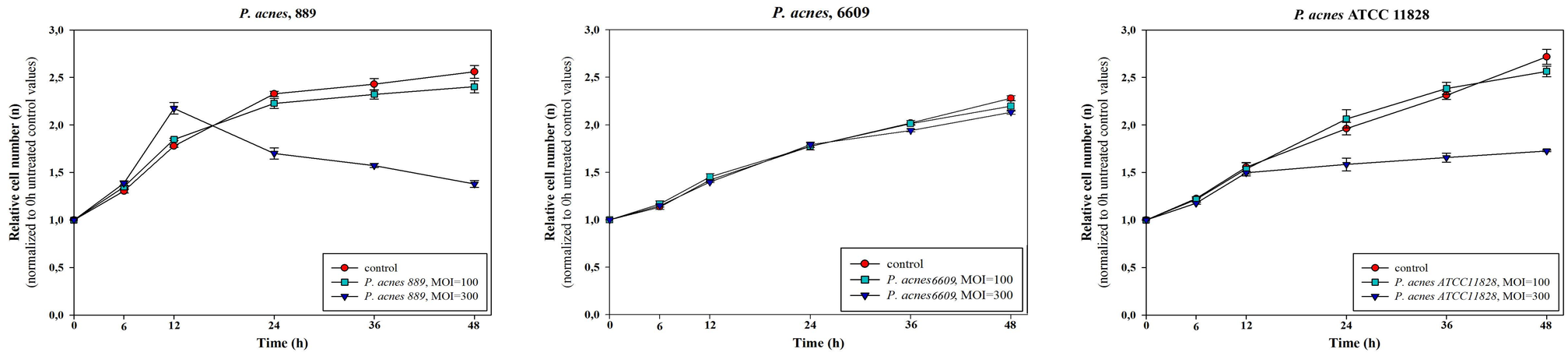
Real time label free cellular analysis (RTCA) of P. acnes-treated HPV-KER cells (xCELLigence analysis)



Plating of 10.000 cells/well, after 24h the cultures were treated with *P. acnes* bacterium (0 time point on the graph, red arrows). Based on the measured impedance values a cell index (CI) was calculated for each well, and normalized for the time point of the treatment. The applied *P. acnes* isolates differentially and dose dependently affected the CI values. Each treatments were performed in three technical triplicates. (Representative images of 2 parallel experiments)

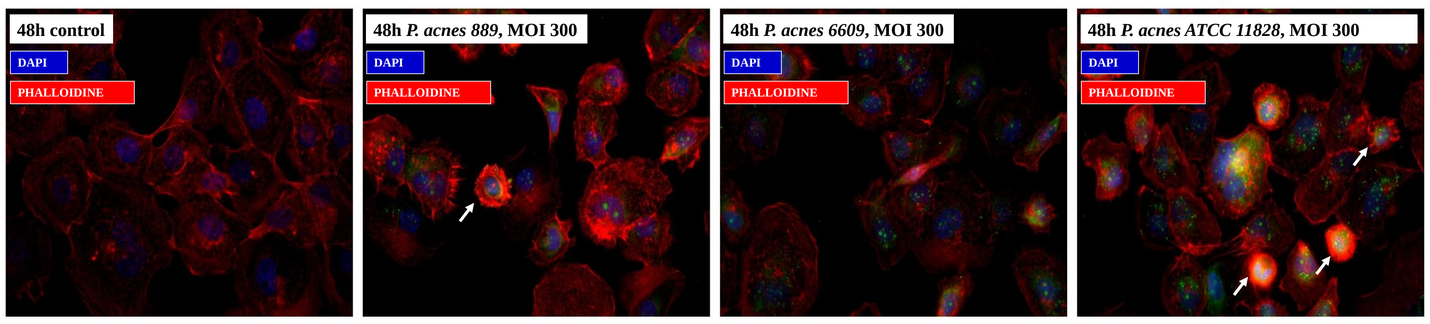
IDENTIFICATION OF THE CELLULAR AND MOLECULAR EVENTS LEADING TO THE MEASURED CI CHANGES

High MOI of the P. acnes 889 and ATCC 11828 cause cell number changes representing increased proliferation (889) and cytotoxicity (889, ATCC 11828)



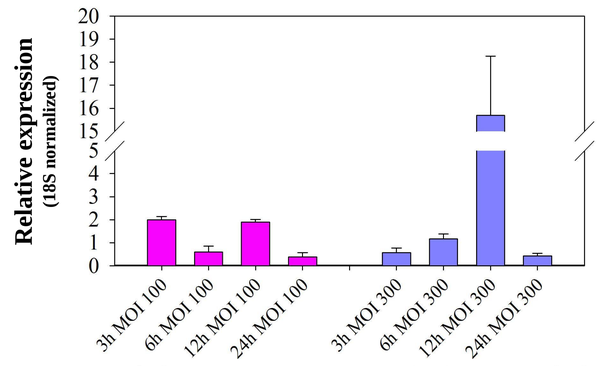
Various *P. acnes* strains differentially affected the proliferation and viability of HPV-KER cells, analyzed by following the cell number changes using a Bürker-chamber. Increased cell number changes upon *P. acnes 889* treatment was detected, the increasse was due to the enhanced proliferation, whereas a drop in the cell number was a result of cytotoxicity. Each treatments were performed in three technical triplicates. (Representative images of 2 parallel experiments)

High MOIs of the P. acnes 889 and ATCC11828 strains induce cell cytotoxicity



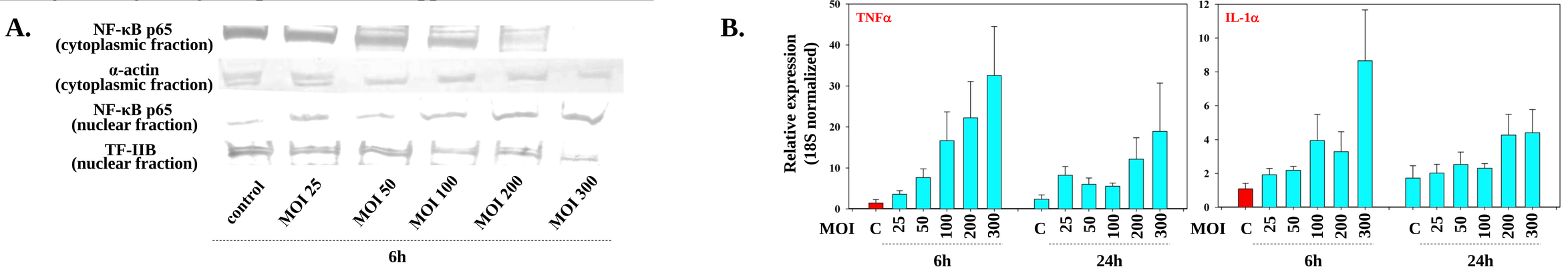
Only high MOI (300) of the *P. acnes 889* and *ATCC 11828* strains induced morphological changes in the actin cytoskeleton, and induced the cytotoxicity of the HPV-KER cells (white arrows), 48h after the bacterial treatment analyzed by fluorescent microscopy.

High MOI of P. acnes 889 strain induces increased cell proliferation



Only high MOI (300) of the *P. acnes 889* strain increased the expression of the proliferation marker Ki67 mRNA at 12 hour post-treatment.

The extent of TLR signaling is dependent on the applied P. acnes 889 dose



Parallel to the increase of the *P. acnes 889* dose, the activation of the NF-κB transcription factor increases (A; western blot). This will results in a dose dependent increases in the mRNA expressions of key pro-inflammatory cytokines: TNFα and IL-1α (B; Real-time PCR).

DISCUSSION

Assorted *P. acnes* strains have different strain- and dose-specific effects on the proliferation and viability of cultured HPV-KER cells. The number and type of bacterial cells that are present in the hair follicles may have an important role in determining the severity of acne lesions. Deeper understanding of the *P. acnes*-keratinocyte interaction can help to develop novel, more effective treatment modalities for acne.

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