

FINAL REPORT ON THE SHORT-TERM RESEARCH FELLOWSHIP

The influence of *Mycoplasma pneumoniae* (MP) and CARDS toxin on the human airway epithelium: The effect of surfactant protein A on the MP pathogenicity in vitro

Research Fellow: Tatsiana Hlinkina, PhD Student of Department of Epidemiology and Microbiology of Belarusian Medical Academy of Post-Graduate Education

Host supervisor: Marek L. Kowalski, Professor, M.D., Ph.D. Chairman, Department of Immunology, Rheumatology and Allergy Medical University of Lodz

Duration: 3 months (02.04.2018 - 29.07.2018)

The working schedule: Monday – Friday, from 8.00 a.m. to 15.00 p.m.

Location: Department of Immunology, Rheumatology and Allergy of Medical University of Lodz, Poland

During the research fellowship we aimed at developing a model of MP and CARDS toxin interaction with human bronchial epithelial cells (HBECs) in order to determine the effect on airway epithelial cells response to MP infection and to assess the role of human surfactant protein A (SP-A) in the modulation of pathogenicity of MP in vitro.

Major research techniques which I learned and successfully implemented

1. Establishing of airway epithelial cell cultures

BEAS-2B cells purchased from ATCC (CRL-9609) were grown on 75 cm² tissue culture flasks in Minimal Essential Medium (Sigma) supplemented with 10% FBS and 1% antibiotics Penicilin/Streptomycin (Sigma, with 10000 units of penicillin and 10 mg streptomycin per 1 ml of 0,9% NaCl) at 37°C in the incubator with 5% CO₂. When the growth of cells was too dense, the cells were subcultured by trypsinization.

2. Preparation of *Mycoplasma pneumonia* for infection of BEAS-2B cells

Mycoplasma pneumonia strain (ATCC 15531) was grown in *Mycoplasma* broth base (Thermo Scientific. Oxoid) supplemented with *Mycoplasma* supplements (Thermo Scientific. Oxoid) for 21 days at 37°C in 5% CO₂. Color change of the medium was observed every day. If the color of the medium changed from red to yellow, *Mycoplasma* growth can be confirmed.

3. Infection of BEAS-2B cells with *Mycoplasma pneumonia*

After BEAS-2B cells reached 100% of cell confluence on the flasks in monolayers they were plated for experiments in 24-well plates at a cell density of 10⁵ cells per well and maintained for 24 hours until reaching 80%-90% of cell confluence at 37°C in 5% CO₂. The concentrations of *Mycoplasma pneumoniae* were adjusted to McFarland standards 0.5; 0.25 and 0.1. The cells were inoculated with *Mycoplasma pneumonia* for 2.5 hours and checked after 24, 48 and 72 of incubation.

Preliminary results

1. I was able successfully grow the epithelial cells and infect them with MP.
2. I documented that interaction of MP with epithelial cells resulted in dose-dependent changes in the cell morphology.

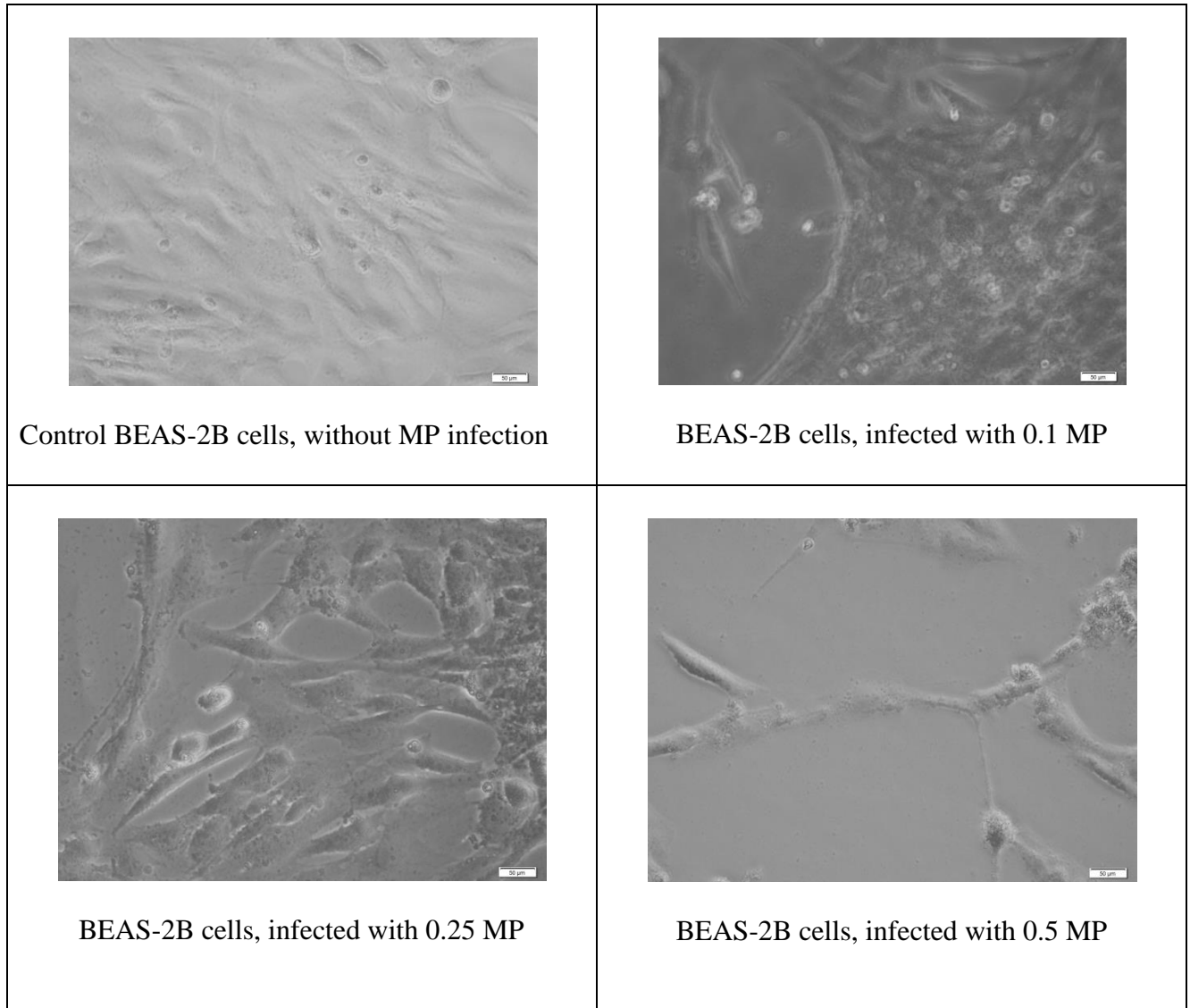


Figure – Morphology of control and MP infected BEAS-2B cells

3. I established optimal timing and concentrations of MP for BEAS-2B cells infection.
4. Thanks to courtesy of Dr Alastair Watson, Research Fellow in Pulmonary Immunology, Southampton University, United Kingdom I obtained naïve human SP-A which will be used in further experiments.

My future research plans based on the experience gained during the EAACI fellowship

During the research fellowship period, I managed to advance the project and made plans for further project development.

I will be able to visit the Laboratory of Professor Marek L. Kowalski for short term visit during the next 6 months, which will allow me to complete remaining tasks.

Summary

The scientific fellowship in the Department of Professor Marek L. Kowalski gave me the opportunity to learn and carry out numerous new techniques. I learned a lot about the organization of laboratory, in which cells are cultured and infected with respiratory significant pathogens. Due to careful and detailed explanation of the scientific members of Laboratory I have learned the techniques of cells defrosting, all niceties of BEAS-2B cells growing on flasks, cells passaging, counterstaining, determination of cells viability, preparation of biological material for ELISA and PCR.

Let me admit that possibility of work with *Mycoplasma pneumoniae* was the essential part of the project.

During the period of the stay I have reviewed the scientific literature and wrote the draft of a research paper ready to be submitted:

Hlinkina T., Pawełczyk M., Brauncajs M., Jarzębska M., Szulc A., Watson A., Kostiuk S.A., Kowalski M.L. Role of *Mycoplasma pneumoniae* in the pathogenesis of airway inflammation associated with asthma

Personally, I would like to admit that the work in the Department was a great scientific experience for me, I studied a lot, was able to use all necessary methods for cell culturing and analysis of a cell response. I was daily in communication with the members of research group of Laboratory, so it was easy to develop the skills of application of any method. I am fully excited to continue the scientific collaboration in the future in the frame of a fellowship or a grant program.

I acknowledge with pleasure my scientific supervisor Professor Marek Kowalski and his scientific group members for providing help and support during my stay at the Department of Immunology, Rheumatology and Allergy of Medical University of Lodz, Poland as well as EAACI for the support during fellowship program.

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