Description for the general public

In 1990, Woese et al. introduced the concept of domains and divided the world of living organisms into *Bacteria*, *Archaea* and *Eucarya*. *Archaea* are single-celled prokaryotic microorganisms that live in extreme environments. These cells differ from bacteria by the unique structure of their cell wall, which contains characteristic pseudomurein instead of peptidoglycan, by the presence of an S layer made of proteins and/or glycoproteins, by the occurrence of gas vesicles in the cytoplasm and by unique extra-cellular structures, such as pili, archaella and hook-like filaments. One of the most interesting *Archaea* groups, which is being intensively studied recently, are halophiles - organisms that require salt concentrations higher than 0.2M NaCl for growth. Halophiles are found in environments all over the world - from natural brine in sea basins to salt mines, due to their well-developed cellular and enzymatic adaptations to the environment. The salt environment has been used for many years in halotherapy, which is a form of spa treatment, where salt is used in various forms. The positive effect of salt and the sea microclimate on human health has become the reason for the artificial creation of this climate in salt caves. Halotherapy is recommended for the treatment of airway diseases, such as asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD), as well as skin and cardiological diseases. The mechanism by which the halophiles interact with human cells is still unknown. The documented non-pathogenic effects of *Archaea*, as well as recently published studies on the presence of halophiles as a component of the human microbiota prompted us to wonder how halophiles interact with human immune cells. In the presented project two strains of halophilic *Archaea* will be studied: *Halorhabdus rudnickae* - isolated from a salt mine in Poland in 2016 by our team in collaboration with Prof. Milton S da Costa from the University of Coimbra in Portugal – a world-known expert in the field of extreme microorganisms - and *Natrinema salaciae*, isolated in 2012 by Prof. M.S. da Costa's group from Medee Lake in the Mediterranean basin. The role of dendritic cells (DC), as professional antigen-presenting cells that link both innate and adaptive responses, in the response to halophilic *Archaea* is still unknown. Therefore, the objective of the proposed study is to investigate the interaction of halophiles with DC in two independent *in vitro* models imitating the interactions of DC with airway pathogens: 1) initiated by superantigen SEB from *S. aureus* and 2) induced by LPS from *P. aeruginosa*. Both models have been selected due to the significant contribution of these pathogens in the development of respiratory diseases including sepsis, acute respiratory distress syndrome (caused by staphylococcal infections) and cystic fibrosis (where the patients are extremely susceptible to colonization by *P. aeruginosa*). In this project we will develop an innovative model, which will enable to use the halophiles as modulators capable of "silencing" the inflammatory response through their ability to induce "tolerogenic" properties of DC, leading to T-cell anergy. In this study 20 healthy blood donors (men and women) aged 25 to 40 will be enrolled. Monocytes will be isolated from human blood by magnetic separation techniques, and then incubated in the presence of IL-4 and GM-CSF to generate monocyte-derived DC. In the next step, DC will be stimulated with SEB from *S. aureus* or LPS from *P. aeruginosa* in the presence or absence of *H. rudnickae* or *N. salaciae*. First, the expression of CD86, CD80, HLA-DR, CD40 costimulatory molecules and pattern recognition receptors TLR2, TLR4, TLR9 will be studied on the surface of DC using flow cytometry. Subsequently, the production of cytokines (IL-10, IL-12 and IL-23) will be evaluated in DC culture supernatants. In mixed co-cultures of *S. aureus* SEB- and *P. aeruginosa* LPS–stimulated DC in the presence or absence of halophiles, and autologous CD4+ T-cells, the level of IFN-gamma, IL-10, IL-13, IL-17 and TNF-α will be determined by ELISA. Finally, human airway epithelial cells (hAEC) in an air-liquid interface (ALI) culture will be used to investigate how the halophilic *Archaea* presented by DC affect the integrity of tight junctions by analyzing the transepithelial electric resistance (TEER), paracellular flux (FITC-dextran) measurements and immunofluorescence staining of occludin and ZO-1 The results of the proposed study will help to understand the still poorly known interaction between DC and halophiles and will determine the immunomodulatory properties of halophiles in inflammation induced by airway pathogens. The gained knowledge on mechanisms by which the halophiles can act as modulators to silence inflammatory processes may help to understand the beneficial effects of halotherapy, including the role of halophilic organisms in this treatment.