PIOF-GA-2011-298186 (Immune energy)

27/09/2012-26/03/2015

Final summary report

Title: Extracellular adenosine role in energetic metabolism during immune response

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Laboratory web pages: http://kmb.prf.jcu.cz/en/laboratories/en-dolezal-lab

The goals of the project were (1) to learn techniques of infection in genetic model organism *Drosophila melanogaster* at the host laboratory, (2) to set up these techniques at home institution and (3) to use the obtained knowledge for studying a role of extracellular adenosine in energy regulation during immune response. All these goals were fully achieved.

(1) During outgoing phase, researcher received a training of bacterial infection of adult Drosophila flies in laboratory of Dr. David Schneider at Stanford University School of Medicine. He learnt a microinjection technique, culture of various bacterial strains (*Listeria monocytogenes, Streptococcus pneumonia, Staphylococcus aureus, Salmonella typhimurium*), determination of bacterial growth within the host and the host survival. He optimized infection for different nutrient conditions and different genetic backgrounds of the host. Using these new techniques, he started to investigate the role of adenosine during infection. Researcher presented the project as invited lectures at the University of California, Berkeley and at the Buck Institute for Research on Aging, at two retreats of Microbiology & Immunology Department, Stanford School of Medicine and at two international conferences with the latter being selected for a platform presentation at the 55th Annual Drosophila Research Conference in San Diego 2014 (Dolezal, T., Bajgar, A., Kucerova, K., Jonatova, L., Schneider, D.S. - Extracellular adenosine regulates complex host-pathogen interactions through the energy release for the immune response).

(2) During return phase, researcher first established techniques within his home laboratory including installation of necessary equipment, setting up the biosafety level-2 regime, cultures of bacteria and microinjection. This also involved a training of research technician and research assistant that are now also able to use these techniques. The infections were optimized for new conditions (especially setting up different capillary puller and microinjector) and the results are now reproducing those obtained in the host laboratory. The techniques were therefore successfully set up in the home institution.

(3) The techniques established by this project are now being used for studying a role of extracellular adenosine in energy regulation during immune response. While training at Stanford, researcher selected two different kinds of bacterial infection of adult flies – one acute caused by extracellular pathogen *Streptococcus pneumoniae* and one chronic caused by intracellular pathogen *Listeria monocytogenes*. The third type of infection is represented by parasitoid wasp infection of Drosophila larvae. These three models of infection allow investigating different outcomes of manipulating energy on immune response. They all clearly demonstrated that adenosine is required for an effective immune response and without this signal, the resistance against different pathogens is markedly reduced. This project uncovered that adenosine is produced by immune cells upon their activation and mediates a systemic metabolic switch

which ensures a relocation of energy from the rest of the organism to immune system. This represents a first experimental support for a theoretical concept of **selfish immune system** in which immune system is seen as hierarchically above the rest of the organism in allocating energy during stress. Results of this project support this new concept and uncover a molecular mechanism, in which adenosine is used as a selfish signal produced by immune cells.

Blocking adenosine signaling decreases resistance against different types of infection while enhancing adenosine effects may increase resistance against acute streptococcal infection. However, enhancing adenosine effects is counterproductive during chronic infection caused by Listeria. Regulation of adenosine thus seems to be finely tuned to work sufficiently well for various types of infections. Such tight regulation also seems to be a tradeoff with longer lifespan under non-stressful conditions when decreasing adenosine regulation increases lifespan (an interesting byproduct of the bacterial infection studies, which is planned to be also published). These results thus uncovered complex host-pathogen interactions and adenosine-mediated role of energy regulation in these interactions; they were selected for platform presentation at 55th Annual Drosophila Research Conference 2014 (http://www.genetics-gsa.org/drosophila/2014/abstracts/text/f14531327.htm) and published in PLoS Biology (doi:10.1371/journal.pbio.1002135).

Proposed concept of selfish immune system may be found behind various pathologies at the border of energy and immunity associated with modern age, including diabetes, obesity, chronic inflammatory diseases and metabolic syndrome. Understanding molecular mechanisms behind the selfish behavior of immune system thus may have a profound impact on general public health. One of the most important results of this project is thus establishing a new in vivo model using various infections in Drosophila which can now be used to uncover these mechanisms. From this point of view, this project may represent a milestone in the researcher's scientific carrier.

Based on the results of this project, researcher is now applying for European Research Council Consolidator Grant 2015 (proposal number 681189, acronym SelfishImmunity). Proposal with title "Drosophila model for investigating role of selfish immune system in systemic physiology" aims to study molecular mechanisms leading to selfish behavior of the immune system, including newly identified extracellular adenosine as a selfish signal defined during this Marie Curie project. Significant part of the proposed project will be dependent on techniques of bacterial infection established by this project.

Results of this project allowed the researcher to start a habilitation process in Molecular and Cellular Biology and Genetics field at Faculty of Science, University of South Bohemia.

Results of this project were, upon publication in PLoS Biology, covered by several national media including national Czech Radio (http://prehravac.rozhlas.cz/audio/3376070) and daily newspapers (http://ceskobudejovicky.denik.cz/zpravy_region/musky-pomohly-pochopit-imunitni-reakce-20150506.html). Laboratory also organized The Day of Open Doors showing work with Drosophila (parasitoid wasp as Drosophila "alien") attracting roughly 150 people (including high-school students interested in studying natural sciences).

The most important new collaborations and contacts:
1. David S. Schneider – the host laboratory
2. Jason Tennessen (Indiana University, Bloomington, USA) – collaboration on studying Warburg effect
3. Rainer Straub (Univeristy Hospital Regensburg, Germany) – author of "Selfish Immune System" concept; close communication and mutual visits – important cross border to clinical research
4. Jindrich Chmelar (Faculty of Science, University of South Bohemia) – collaboration, testing the new ideas from the project in mouse model