Introduction

Insects are armed with an evolutionarily conserved cell mediated immune-defense mechanism, which may serves as a prototype of innate immunity in all phyla of the animal kingdom. The fruit fly, *Drosophila melanogaster*, with its philogenetically conserved innate immune system and well known genetic system, is one of the most frequently used model organisms to study regulation of cell mediated immune defense and has been widely used model organism to study host response to microbial and parasitic infections. Pathogens entering the hemocoel trigger a systemic immune, which results in the synthesis and secretion of a large set of humoral effector molecules, including antimicrobial peptides, the stress response proteins and the activation of the cell mediated immune response. The cell-mediated arm of the immune response is carried out by the hemocytes. The plasmatocytes engulf microorganisms, the crystal cells are involved in melanization and the lamellocytes participate in the encapsulation of large foreign particles, like the eggs of parasitoid wasps.

We developed a new septic injury method in *Drosophila melanogaster* to identify novel factors involved in the response. Using our method in a directed screen and we identified the *raspberry* as a new candidate involved in defense mechanisms. We examined the role of *raspberry* in the encapsulation reaction.

Aims

The focus of the presented work was to meet the following goals:

1. To develop a new septic injury method in *Drosophila melanogaster*, which imitates injury often occurring in the natural habitat, and is suitable to identify novel factors involved in host-pathogen interaction.
2. To construct a highly reproducible method, which is easy to perform and suitable for large scale genetic screens and allows screening for activity of potential antimicrobial drugs *in vivo*.
3. Validation of the method by using different *Drosophila* mutants known to be involved in the immune response.
4. To perform a directed screen for genes involved in defense mechanism.
5. Examination of a newly identified – using our septic injury method – gene in the cell-mediated immune response.

Methods

Wounding of adult flies was performed by cutting off the tarsal segments of the first pair of legs. The wounded flies were placed into vials containing bacterial lawn of Gram-negative (*Escherichia coli* and *Serratia marcescens*), and Gram-pozitív (*Bacillus cereus var. mycoides*). The survival rate of flies were monitored for 77 hours.

Using GFP expressing *Escherichia coli* bacteria, we monitored entry of the microbes through the wound into the body cavity by fluorescence microscopy.

We examined the effect of sterile injury on the survival of flies.

We validated our method by using mutations of different components of the immune response.

We tested the applicability of our septic injury assay for screening antimicrobial agents.

We performed a directed screen for searching new factors involved in host-pathogen interactions following a septic injury.

Using flow cytometry, we examined the role of *raspberry* (*ras*) in phagocytosis. Using the hipomorph ras^2 allel and RNA interference, we analyzed the role of *raspberry* in encapsulation reaction. We examined of pseudopod-like cytoplasmic extensions in hemocytes using fluorescence microscopy.

Results

1. We established a new method which imitates injury often occurring in the natural habitat. The method is a highly reproducible, easy to perform

2. The method in suitable to identify novel factors involved in host-pathogen interaction and allows screening for activity of potential antimicrobial drugs *in vivo*.

3. Using GFP expressing *E. coli*, we visually displayed how the infection takes place: bacteria enter into the body cavity through the wounded leg and spread within the animal.

4. The technique was validated by using different *Drosophila* mutants known to be involved in the regulation of the humoral immune response.

5. Our result showed that beside the important role of *Hemolectin* in larval hemolymph clotting, it is also involved in the wound healing of the adult *Drosophila*.

6. Using this novel septic injury method, we found that the *raspberry* gene is involved in the survival of the flies after septic injury since the survival rate of the mutants decreased significantly after *B. cereus* infection. This gene encodes the *Drosophila* inosine monophosphate dehydrogenase, which is a key enzyme of the *de novo* synthesis of guanine nucleotides.

7. We investigated the role of *raspberry* in the immune response of the *Drosophila* larvae, especially in the encapsulation reaction after infection with the parasitic wasp *Leptopilina boulardi* G486. The eclosion rate of wasps was higher in the *raspberry* mutants (ras^2) than in the wild type control group. After wasp infestation the proportion of living wasp larvae was significantly higher in the *rasberry* mutants compared to the control. The capsules in

rasberry mutants showed a loosened structure, the lamellocytes attached improperly to the wasp eggs causing a defective encapsulation reaction. We observed the same phenomena using two different *rasberry* RNAi constructs. The results show that *raspberry* has a role in the defense against the parasitic wasp *Leptopilina boulardi* and is required for a proper encapsulation response. Possible explanation for the role of *raspberry* in the encapsulation reaction is that it encodes the rate-limiting enzyme for GTP synthesis and thus influences the function of enzymes requiring GTP. It is known that G proteins and small GTPases are involved in the regulation of processes related to the immune response. However, further studies must be conducted to elucidate the exact pathways affected in the case of decreased IMPDH level in the encapsulation reaction.

Acknowledgements

I would like to thank Dr. István Andó for allowing me to join ad work in his lab. Thanks are due to my supervisor Dr. Éva Kurucz for her support, patience, professional help and her tenacious work. I would also like to take the opportunity to thank all current and former mebers of the lab for their constant support, be it professional or personal to Dr. Gábor Csordás, Dr. Viktor Honti, Dr. János Zsámboki, Dr. Róbert Márkus, Dr. Gyöngyi Cinege, Zita Lerner, Erika Gábor, Gergely István Varga. I am grateful to Anikó Képíró, Olga Kovalcsik, Anita Balázs and Szilvia Tápai, and for their technical assistance personal support. I am thankful to the *Drosophila* community of the BRC for their expertise, which proved indispensable to the finishing of this work, especially for Dr. Miklós Erdélyi, és Dr. Péter Vilmos, which played a significant part in the completion of this thesis. I thank Dr. Zsuzsanna Darula (Laboratory of Proteomics, BRC) and Edit Kotogány (Flow Citometry nad Cell Sorter Laboratory) for their help. I am deeply grateful to Dr. Margit Pál and Dr. Rita Sinka nak for critically reading and evaluating my thesis. I am grateful to my former biology teacher Anna Mária Leopold. Finally, I would like to thank my family, and my friends for standing by me over the years, and helping me in every way possible.

Publications supporting the dissertation:

1. Kari B, Zsámboki J, Honti V, Csordás G, Márkus R, Andó I, Kurucz É. 2013. A novel method for the identification of factors involved in host-pathogen interactions in *Drosophila melanogaster*. J Immunol Methods. 398-399:76-82. IF(2013): 2.225 MTMT: 2463198

2. Kari B, Csordás G, Honti V, Cinege G, Williams MJ, Andó I, Kurucz É. 2016. The *raspberry* gene is involved in the regulation of the cellular immune response in *Drosophila melanogaster*. PLoS ONE 11(3): e0150910. doi:10.1371/journal. pone.0150910 IF(2015): 3.54 MTMT: 3025315

Additional publications:

1. Vilmos P, Kristó I, Szikora S, Jankovics F, Lukácsovich T, Kari B, Erdélyi M. 2016. The actin-binding ERM protein Moesin directly regulates spindle assembly and function during mitosis. Cell Biol Int 40(6):696-707. doi: 10.1002/cbin.10607. IF(2015): 1.66 MTMT: 3071742

2. Kari B, Zsámboki J, Honti V, Csordás G, Márkus R, Andó I, Kurucz É. 2013. A szeptikus sérülést követő immunválaszt szabályozó genetikai faktorok azonisításának módszerei Drosopholában. Tudomány a vidék mindennapjaiban: Magyar Tudomány Ünnepe. ISBN: 978-963-306-245-6 (Book chapter/Electronic publication in hungarian) MTMT: 2519814