

Analysis of blood cell lineages in *Drosophila melanogaster*

Ph.D. thesis summary

Gábor Csordás

Supervisors: Dr. István Andó and Dr. Viktor Honti

Ph.D. School in Biology

Biological Research Centre of the Hungarian Academy of
Sciences
Institute of Genetics

University of Szeged
Faculty of Science and Informatics

2014.

Szeged

Introduction

Insects are armed with a powerful innate immune response, which provides an effective barrier against invaders and tumors. The phylogenetically conserved immune functions, such as the phagocytosis of microbes and the encapsulation of large foreign particles are carried out by specialized immune cells, the hemocytes.

The development of the hemocyte lineages of *Drosophila melanogaster* is the result of a strictly regulated succession of intracellular and intercellular events. Studies on the *Drosophila* immune system have provided most of our knowledge on hematopoiesis and blood cell lineages in insects, and have shed light on some of the key features of blood cell differentiation in the animal kingdom.

The differentiation of hemocytes begins in the early embryonic stages. Two distinct mesodermal segments give rise to two independent embryonic hemocyte lineages: the procephalic mesoderm differentiates into embryonic macrophages and crystal cells, while the cardiogenic mesoderm forms the embryonic lymph gland. In the larval stages, hemocytes occupy three hematopoietic compartments: the lymph gland, the sessile tissue and the circulation. The lymph gland is a compact hematopoietic tissue consisting of paired lobes along the anterior end of the dorsal vessel. The sessile hematopoietic tissue localizes to the inner wall of the body cavity, and forms a banded pattern along the length of the larva. Both of these hematopoietic tissues contain differentiated effector cells, as well as precursor hemocytes. The third compartment, the circulation, comprises two effector hemocyte types: the plasmatocytes and the crystal cells. The plasmatocytes are phagocytic cells which engulf microbes and produce antimicrobial peptides, while crystal cells contain

enzymes necessary for the melanization cascade. Infestation by the parasitoid wasp *Leptopilina boulardi* results in the appearance of a third effector cell class, the lamellocytes, which form multilayered capsules around large foreign particles. Although the process of blood cell differentiation has been studied extensively, the origin of the hematopoietic compartments and effector hemocytes are still not well recognized.

Aims

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

- 1.) To identify the hemocyte lineages in the *Drosophila* embryo, which contribute to the formation of larval and adult hemocytes.
- 2.) To find the connection between these cell lineages and the larval and adult hemocyte compartments.
- 3.) To shed light on the compartmental origin of effector hemocytes that differentiate in the course of the immune response.
- 4.) To reveal the differentiative capacity of the different effector hemocyte lineages, and to identify factors, which are responsible for the regulation of this plasticity.
- 5.) To construct an experimental system, by which the fine structure and cellular composition of the hematopoietic compartments can be studied *in vivo*, with special emphasis on that of the sessile hematopoietic tissue.

Methods

1. Immune induction of *Drosophila* larvae
2. Preparation of hemocyte samples
3. Preparation of dissected larvae
4. Immunofluorescent staining
5. Microscopic analysis of hemocyte samples
6. Videomicroscopy of *Drosophila* embryos
7. Phagocytosis assay
8. Immobilization of larvae
9. Preparation of antibody mixtures for *in situ* immunofluorescence
10. Injection of antibody mixtures into *Drosophila* larvae
11. *In situ* confocal microscopy and videomicroscopy
12. Fluorescent staining of hemocyte samples with antibody-mixtures

Summary of the results

1. The origin of larval hemocyte compartments

In our experiments, we created a transgenic system, which enabled us to follow the embryonic hemocyte lineages in later developmental stages. We established that the larval circulation and sessile tissue can be traced back to the embryonic macrophage-lineage, while the lymph gland arises from the cardiogenic mesoderm. Our experiments revealed that in naive larvae, no cells exit or enter the lymph gland; however, upon immune induction by parasitic wasp, all three hematopoietic compartments take part in the differentiation of effector hemocytes, namely plasmatocytes and lamellocytes.

2. The plasticity of the plasmatocytes

We performed the detailed analysis of the hemocyte differentiation that follows the immune challenge. We observed hemocytes expressing lamellocytes specific markers just few hours after the induction. These cells represent an intermediate stage of differentiation between phagocytic plasmatocytes and encapsulating lamellocytes from both morphological, immunological and functional standpoint. We investigated this presumed transformation by tracking the fate of the plasmatocyte lineage *in vivo*, and found lamellocytes of clearly plasmatocyte origin in the parasite-infested larvae. From these results, we determined that in the course of the immune response, at least part of the lamellocyte differentiation can be attributed to phenomenon of macrophage plasticity.

3. The plasticity of the crystal cell lineage

Crystal cells are present in the circulation of naive larvae, and play a key role in the melanization processes following the immune challenge. By tracing the crystal cell lineage *in vivo*, we found that, unlike plasmatocytes, these cells do not transform into lamellocytes after parasitoid immune induction. However, we observed that by expressing factors, which directly induce the differentiation of effector blood cells, lamellocytes can be differentiated from the crystal cell lineage. The differentiation can be either cell autonomous, or non-cell autonomous, depending on the expressed factors.

4. The fate of hemocyte lineages in the pupa and the adult

The onset of pupariation triggers the spontaneous desintegration of the immobile hemocyte compartments. By tracking these changes with fluorescent reporters, we found that - similarly to the lymph gland - the structure of the sessile hematopoietic tissue also dissolves. In late pupae, we observed the rearrangement of this compartment in a pattern that resembles the adult stage.

With tracing of the embryonic hemocyte lineages, we also showed that pupal and adult hemocytes derive from both the procephalic mesoderm (embryonic macrophage-lineage) and the cardiogenic mesoderm (embryonic lymph gland-lineage), which also means that all three larval hemocyte compartments contribute to the adult blood cell pool.

From the presented data, we constructed a model to summarize the differentiation events that take place in response to parasitoid wasp infection. We augmented this model with further data to establish a genealogical map of hemocytes, which helps tracking the fate of the individual blood cell types and compartments during the development of *Drosophila*.

5. A novel technique to study larval hemocyte compartments *in vivo*

In recent years, confocal imaging techniques took a huge leap forwards, therefore it became possible to investigate live animals in great detail. Since the structure of the sessile hematopoietic tissue is severely damaged during the preparation of the larva, we created an *in vivo* method, by which the fine structure and composition of the hematopoietic tissues can be studied with fluorescent reporters. The basis of this method is the reversible immobilization of larvae, which allows in excess of an hour of videomicroscopic investigation. We combined the strength of the *in vivo* reporters with the specificity of molecular markers by injecting fluorescently labeled antibody-mixtures into live animals to create an *in situ* immunostaining assay. We confirmed the specificity of this technique by confocal microscopic experiments, and we identified different hemocyte types within the sessile compartment. Through the use of this method, we plan to characterize the dynamics of the hemocyte compartments in live larvae, and investigate the effect of different factors on the hemocyte compartments that regulate hematopoiesis in *Drosophila melanogaster*.

Acknowledgements

I would like to thank **Dr. István Andó** for allowing me to join his lab in 2006. He not only gave me the freedom to adapt a scientific way of thinking, but also supported me with his everlasting energy. I thank him for his patience and his help over the years.

I am grateful to **Dr. Viktor Honti**, by whom I caught a glimpse into the intricacy of *Drosophila* genetics. He taught me so much about professional approach, the real value of things and the way a researcher should think.

I thank **Dr. Róbert Márkus**, who supervised my undergraduate work, and set me on the track of *Drosophila* hematopoiesis.

Thanks are due to **Dr. Éva Kurucz** for her patience, professional help and her tenacious work, which ensured that performing experiments in the lab was always going smoothly.

I am thankful to my fellow Ph.D. student, **Gergely István B. Varga**, with whom we performed numerous experiments side by side, and had long discussions about science for the benefit of both of us.

I am deeply grateful to **Anita Balázs, Szilvia Tápai, Olga Kovalcsik** and **Anikó Képiró** for their technical assistance, which played a significant part in the completion of this thesis.

I would also like to take the opportunity to thank **all current and former members of the lab** for their constant support, be it professional or personal.

I thank **Dr. Izabella Bajusz** and **Dr. Henrik Gyurkovics** for the very constructive discussions, ideas and suggestions, which benefited both my work and me also.

I am grateful to **Dr. Ferenc Jankovics** for being my Ph.D. tutor, for his significant contribution to this work, and for his frequent professional and personal advices.

I am thankful to the ***Drosophila* community of the BRC** for their expertise, which proved indispensable to the finishing of this work.

I thank **Ferhan Ayaydin** and **Zsuzsa Kószó** for their help with confocal microscopy.

I would like to acknowledge our foreign collaborators, namely **Prof. Dan Hultmark, Prof. Utpal Banerjee, Dr. Michele Crozatier** and **Dr. Bruno Lemaitre**. They provided reagents and *Drosophila* lines which were instrumental in the work presented in the thesis.

I would like to thank **Dr. Miklós Erdélyi** for his career advices during my Ph.D. work, and for his help in scientific life.

I would also like to thank my **former teachers**, who helped me understand and appreciate biology, genetics, and the rationale of science. In particular, I am grateful

to **Dr. Béla Gál**, my biology teacher from high school, and to **Dr. Péter Maróy** and **Dr. Tibor Török**, who held fascinating Genetics seminars at the University.

I express my gratitude to **Dr. József Mihály** and **Dr. Attila Gácsér** for critically reading and evaluating my thesis on a very short notice.

I am deeply grateful to **Dr. Tibor Németh** for being a friend for over fifteen years, for his clarity, and for our lengthy discussion about life and science.

Finally, I would like to thank **my family, my relatives** and **my friends** for standing by me over the years, and helping me in every way possible. I thank you all.

List of publications

Publications supporting the dissertation:

V. Honti, G. Csordás, R. Márkus, É. Kurucz, F. Jankovics, I. Andó. Cell lineage tracing reveals the plasticity of the hemocyte lineages and of the hematopoietic compartments in *Drosophila melanogaster*. Mol Immunol., 47(11-12) (2010),pp. 1997-2004. **IF: 2.916** MTMT: 1921048

V. Honti, G. Csordás, É. Kurucz, R. Márkus, I. Andó. The cell-mediated immunity of *Drosophila melanogaster*: hemocyte lineages, immune compartments, microanatomy and regulation. Dev Comp Immunol. 42(1) (2014),pp. 47-56. (Review) **IF: 3.238** MTMT: 2372553

G. Csordás, G.I.B. Varga, V. Honti, F. Jankovics, É. Kurucz, I. Andó. *In vivo* immunostaining of hemocyte compartments in *Drosophila* for live imaging. PLOS One. 9(6) (2014): e98191. **IF: 3.534** MTMT: 2708773

Additional publications:

V. Honti, É. Kurucz, G. Csordás, B. Laurinyecz, R. Márkus, I. Andó. *In vivo* detection of lamellocytes in *Drosophila melanogaster*. Immunol Lett., 126(1-2) (2009),pp. 83-84. **IF: 2.906** MTMT: 1920812

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

V. Honti, Gy. Cinege, G. Csordás, É. Kurucz, J. Zsámboki, C.J. Evans, U. Banerjee, I. Andó. Variation of NimC1 expression in *Drosophila* stocks and transgenic strains. Fly (Austin). 7(4) (2013),pp. 263-266. **IF: 1.105** MTMT: 2372552

J. Zsámboki, G. Csordás, V. Honti, L. Pintér, I. Bajusz, L. Galgóczy, I. Andó, É. Kurucz. *Drosophila* Nimrod proteins bind bacteria. Centr Eur J Biol 8(7), pp. 633-645. **IF: 0.818** MTMT: 2326115

G. Csordás, V. Honti, R. Márkus R, F. Jankovics, G.I.B. Varga, É. Kurucz É, I. Andó. Az ecetmuslica (*Drosophila melanogaster*) véresejtképződése. Tudomány a vidék mindennapjaiban: Magyar Tudomány Ünnepe ISBN:978-963-306-245-6 (2013),pp. 23-28. (Book chapter/Electronic publication in Hungarian) MTMT: 2519808