NN 118207

Project closing report

Summary

The aim of the studies was to identify and construct novel molecular markers to reveal the functional heterogeneity of *Drosophila* blood cell populations. In order to gain this goal we built immunological and genetic systems and combined them with deep learning technology. We identified novel epitopes on prophenoloxidases, which are present in *Drosophila* and in the honey bee (*Apis mellifera*) blood cells enabling the identification of crystal cells in the honey bee. The function of members of the Nimrod gene cluster (Somogyi et al., 2010), the largest synthenic gene cluster identified by us in the framework of previous NKFI grants, were uncovered (Cinege et al., 2017, and Melcarne et al., 2019). We showed that the proteins encoded by the *Vajk* genes are essential components of the cuticle, the mechanical barrier facing the challenges by pathogen. We also showed that the NimC1 and Eater proteins interact with each other in the phagocytosis of microorganisms Melcarne et al., 2019). The function of the *Drosophila* gene *headcase*, with human homolog (HECA) was revealed. It was found, that its expression is developmentally regulated and its regulatory function is achieved through its interaction with the Hedgehog and the Decapentaplegic pathways (Varga et al., 2019). We identified a so far unrecognized differentiation pathway of blood cells with the involvement of two types of effector cells lamellocytes type I and lamellocyte type II (Anderl et al., 2016). The methodology and findings were combined with deep learning technology, and the basis for single cell isolation and transcriptome analysis at an analytical scale was established (Szkalisity et al., *in press*). Using the molecular markers we invented single cell mass spectrometry for immune profiling of Drosophila blood cells (Balog et al., 2021). This method permits the identification of hemocyte subsets and to reveal their connections and developmental relationships with an exceptional resolution. The method can be generalized and used in other insect species, where genetic lineage tracing cannot be applied.

Detailed report

Aim i) Identifying and constructing novel molecular and genetic markers for further analysis of the immune system in *Drosophila melanogaster* and characterizing the identified novel molecules with respect to their function.

In the search for new hemocyte markers we analyzed the expression profile of genes of the previously identified Nimrod chromosomal region (Somogyi et al. 2010) which is acknowledged to encode for immunity-related proteins. The analysis of the Vajk-proteins revealed (Cinege at al., 2017) that they are expressed in cuticular structures of the late embryo and the late pupa, indicating that they contribute to cuticular barrier functions. We showed that they are essential components of the cuticle, the mechanical barrier facing the challenges by pathogen. On the basis of these findings we suppose that the cuticular localization of the Vajk proteins might also serve as the first, mechanical barrier to protect the organism from the harmful environmental aspects, thus achieved a concerted action with the previously characterized proteins encoded by the immunity related Nimrod cluster genes. Analyzing the function of other Nim-related genes, *NimC1* and *eater*, we revealed that they synergistically contribute interact in the phagocytosis of microorganisms (Melcarne et al., 2019).

For hemocyte isolation we established a combined approach using genetic and immunological markers in flow cytometry and microscopy. Applying the combined approach we identified several transitory steps in hemocyte development and showed that the combination is suitable to investigate the dynamics of hematopoiesis after immune induction (Anderl et al., 2016) and demonstrated that it will permit the isolation of several hemocyte populations. We identified two effector cell populations involved in the encapsulation reaction, as lamellocytes type I and lamellocytes type II. The development of the enhancer trap system enabled the production of genetic markers of hemocytes, and

of specific antibodies for the different hemocyte classes. Construction of hemocyte specific GAL4 constructs and fluorescent enhancer-reporter fusions further expanded our genetic toolbox, allowing the observation of hemocytes or specific hemocyte subclasses in vivo. We invented genetic markers in combination with fluorescent hemocyte markers using flow cytometry and showed that this combination of the toolkits is an accurate and fast method for the differential counting of *Drosophila* blood cell populations from single larvae, and is potentially useful for the high throughput analysis and separation of hemocyte phenotypes in genetic screening, or drug testing in vivo.

We identified Headcase (human homolog is HECA) as a marker for plasmatocytes and revealed that its expression is developmentally regulated. Functional studies on the developmentally regulated expression led to the conclusions that headcase is a novel regulator of blood cell fate and its regulatory function is achieved through its interaction with the Hedgehog and the Decapentaplegic pathways (Varga et al., 2019).

We identified several immunological markers of the honey bee (*Apis mellifera*) and two novel markers for the crystal cells and lamellocytes in *D. melanogaster*, as phenoloxidases (Gabor et al., 2017 and Gabor et al, 2020). As the antibodies define hemocyte subsets in the honey bee too, they will be basic standard reagents in studies of honey bee cell mediated immunity. The homology searches for hemocyte antigens revealed high homology of hemocyte antigens within the arthropod phylum. The analysis of the hemolectin gene showed that Hemolectin is highly conserved is many insect species, including *Bombyx mory* and the *A. mellifera*. We showed (Gábor et al., 2017) that Hemolectin as a marker molecule reveals a clear functional heterogeneity of hemocytes, allowing for the analytical separation of hemocyte classes, and could promote the molecular identification of hemocyte lineages in *A. mellifera*.

Aim ii) Performing a gene expression profile analysis of hemocyte activation and differentiation in D*rosophila melanogaster* and selecting candidates for further analysis.

We invented a novel technology, the SingleCell Mass Cytometry for *D. melanogaster* blood cells in collaboration with the Laboratory of Functional Genomics, the BRC, Szeged (Balog et al., 2021). The method reveals cellular diversity at protein level. Cells are labeled by antibodies, conjugated with stable monoisotopic heavy metals loaded on a polymer. Detection is based on the atomic mass of heavy metals offering the possibility to analyze up to 37 proteins simultaneously in a single sample. We have conjugated six IgG antibodies to hemocyte surface antigens (L1, P1, Hemese, 4A12/A9, 1F12/H5, H18), two IgG antibodies against intracellular antigens (3A5/B6-1, 31A4/180) and one anti-IgM t for the detection of the L6 surface antigen specific IgM antibody. We have already titrated the aforementioned antibodies in order to characterize the different developmental stages of *D. melanogaster* hematopoietic cells by these 9 markers in one single sample. We also aim to investigate the compartment specific immune composition in *D. melanogaster*. Not only wild type but also strains bearing different genetic modifications are considered for analysis (Balog et al. 2021). Combination of the genetic system developed in collaboration with the Hultmark laboratory (Anderl et al. 2016) with deep learning technology, it was possible to monitor continuous events of blood cell differentiation with a high resolution, permitting the recording analysis of continuous biological events (Szkalisity et al., 2021).

(Comment: in the present version the funding by grant NN118207 is not indicated, however I copy here the letter of the Corresponding Author, Professor Péter Horváth . horvath.peter@brc.hu

On Mon, Feb 8, 2021 at 10:23 AM <ando@brc.hu> wrote:

Kedves Péter!
El kell készítenem a 118207-es OTKA pályázat zárójelentését. A bírálat alatt álló közleményt is szeretném betenni a jelentésbe, ezért kérlek, hogy biztosíts arról, hogy a pályázat száma bekerül/t a kéziratba.
Üdvözlettel,
István

Igen, lasd alabb:

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Peter Horvath, Ph.D.
Director
Institute of Biochemistry
Biological Research Centre, Szeged
w: www.brc.hu/sysbiol/horvath-peter-lab-index.html
t: +36 70 5120015

Institute for Molecular Medicine Finland (FIMM), Helsinki
t: +358 50 4480947

Aim iii) Finding vertebrate homologs of the newly identified immune-genes-molecules via computational analysis. Report on headcase, see under Aim i).

On the basis of a transcriptome analysis of the encapsulating cells we identified and selected 52 genes specifically expressed in the encapsulating cells in Drosophila species which mount cell mediated immune reaction with the contribution of multinucleated giant hemocytes, as encapsulating effector cells, *Drosophila ananassae* and *Zaprionus indianus* (Cinege et al. 2017). We identified the corresponding homologs in *D. melanogaster* and also found homologies in the mouse and in the human genome. The validation of the genes, construction of the genetic system for their analysis are in progress.

Aim iv) Maintaining and developing further the multidisciplinary research team and the undergraduate and postgraduate training unit with a broad interest in molecular immunology based on the research and teaching experience of our already existing multidisciplinary team at the Innate Immunity Group of the Department of Genetics of the Biological Research Centre Szeged and the Department of Functional Genomics and Cancer, Institute of Genetics and Molecular and Cell biology, Strasburg, France.

In the time frame of the support the following students received PhD degree: Beáta Kari, Erika Gábor, Gergely István Varga, Zita Lerner - Thesis submitted.

The developed experimental systems and basic findings permitted the foundation of an independent, new research group at the Biological Research Center, the *Drosophila* Blood Cell Differentiation Laboratory.

The joint work with the original partner did not work out as planned, however the grant provided a basis for broadening the international collaborations. We showed that NimC1 and Eater transmembrane proteins synergistically contribute to phagocytosis of bacteria and also showed that the Headcase protein is a crucial factor in determining the fate of effector cells in cell mediated immunity. Although the transcriptome analysis with the international partner did not work out as planned, we set a system using machine learning (Szkalisity et al., 2021) (based on our other collaborative work – (Anderl et al., 2016) - for single cell transcriptome analysis and were inventing multidimensional single cell mass cytometry for analysis of hemocyte subsets (Balog et al. 2021), for the first time in non-vertebrate species. Also, the results gained by the analysis of *D. ananassae* hemocytes laid the basis for collaborative projects with California University, Berkeley. USA, Csaba Földy, Tamás Lukacsovich, Laboratory of Neural Connectivity, Brain Research Institute, University of Zürich, Switzerland.