Summary of Ph.D. Dissertation

The Characterization of the Multinucleated Giant Hemocyte, a Novel Player in Innate Immunity

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Összefoglaló

A rovarok immunitása sok szempontból hasonlít a gerincesek veleszületett immunitásához. Közös jellemzőjük, hogy a patogének felismerésében és kiiktatásában humorális és sejtközvetítette immunfolyamatok egyaránt részt vesznek.

A természetben a *Drosophila* fajokra a legnagyobb veszélyt a parazitoid darazsak jelentik. A gazda-parazitoid koevolúciót a túlélésért folytatott harc formálja, amely a parazitoidok elleni változatos immunválaszok tárházát eredményezi. A *Drosophila* fajok és parazitoidjaik együttes evolúciója az effektor vérsejtek sokféleségét idézte elő, változó hatékonyságú immunreakciókkal.

Kimutattuk, hogy a sokmagvú óriás vérsejtekkel rendelkező *Drosophila* fajok parazitoid darázsfertőzésekkel szemben kivételesen ellenállóak.

A sokmagvú óriás vérsejtek nagyfokú ultraszerkezeti hasonlóságot mutatnak az emlős óriássejtekkel. Citoplazmájukban nagyszámú savas vezikulát tartalmaznak, amelyek összefüggő réteget alakítanak ki a parazitoidok felszínén. A *Z. indianus*

sokmagvú óriás vérsejtek nagyszámú sejtmag nélküli fragmentumot bocsátanak a hemolimfába, amelyek felhalmozódnak a sebzés helyén, így hasonlóan az emlősök csontvelő-óriássejt eredetű vérlemezkéihez részt vesznek a sebgyógyulási folyamatokban.

A nagyfokú hasonlóság a transzkriptom összetételében is tükröződik. Az emlős óriássejtek működéséhez szükséges gének ortológjai magas szinten fejeződnek ki a *Drosophila* sokmagvú óriás vérsejtekben.

Kimutattuk továbbá, hogy egyes sokmagvú óriás vérsejteket differenciáló *Drosophila* fajok genomja a bakteriális eredetű horizontális géntranszferrel beépült citoletális duzzasztó toxin B (*cdtB*) alegységét és Hemolysin E pórusképző toxinokat kódoló géneket (*hl*) hordoz, amelyek kifejeződése parazitoid darázsfertőzést követően szignifikánsan megemelkedett és a kódolt fehérjék jelenlétét kimutattuk a parazitoidok felszínén. Eredményeink arra utalnak, hogy a bakteriális eredetű CdtB és HL toxinok a rovarokban részt vesznek a parazitoidok elleni immunválaszban.

Eredményeink a veleszületett immunitás eddig ismeretlen sejtes és humorális elemeinek szerkezeti,

molekuláris és funkcionális tulajdonságaira derítettek fényt.

I. Introduction

Drosophila is a well-established model organism to study a broad range of biological phenomena, including innate immunity. The innate immune system of drosophilids is comprised of humoral and cellular components, which act in concert to recognize and eliminate invaders. The main humoral organ, the fat body, secretes into the hemolymph various molecules involved in pathogen recognition, immune regulation, and the elimination of microbes. The blood cells in *Drosophila melanogaster* are the phagocytic plasmatocytes, the crystal cells that participate in melanization, and the lamellocytes responsible for the isolation and elimination of large particles, including parasitoids. Infection by parasitoid wasps is a frequent danger encountered in nature by drosophilids. The parasitoids lay their eggs in *Drosophila* larvae and pupae and use them as a source of nutrients during their development. The fight between the

parasitoid and the host is life or death therefore an effective immune defense against the pathogen is essential for survival. Co-evolution of drosophilids with their parasitoids has generated a broad diversity of effector cells, providing immune reactions with variable effectiveness. In several species of the *ananassae* subgroup of *Drosophilidae* and in the invasive *Zaprionus indianus*, our research group had previously described a so far unrecognized cell type, the multinucleated giant hemocyte (MGH), a motile encapsulating blood cell.

II. Aims

The aims of my studies were explore the molecular mechanisms behind the highly effective immune response of MGHs, and to gain insights into their role in two representative species: in *D. ananassae* and in *Z. indianus*. More specifically:

- compare the parasitoid resistance of MGH differentiating *D. ananassae* and *Z. indianus*, and lamellocyte differentiating *D. melanogaster*,

- determine the factors that induce MGH differentiation,
- characterize the structural components of MGHs,
- gain insights into the genetic background of the effective immune response mounted by MGHs,
- search for putative humoral factors involved in the elimination of the parasitoid and determine their evolutional origin.

III. Methods

- Parasitization and survival assay
- Microbead injection, sterile and septic injury
- Indirect immunofluorescence assay
- Transmission electron microscopy
- Acidic compartment assay using LysoTracker dye
- Transcriptome analysis
- Quantitative RT-PCR
- Western blot analysis
- LDH cytotoxicity assay

IV. Results

- We have shown that the drosophilids possessing multinucleated giant hemocytes (MGHs) exhibit higher resistance to parasitoid wasp infections than *D. melanogaster*.
- In *D. ananassae*, mature MGHs differentiate only after parasitoid infection, and their formation cannot be induced by cuticle wounding.
- Transmission electron microscopic assays revealed that *D. ananassae* MGHs possess a vesicle-rich cytoplasm with a large number of electron-dense vesicles forming multiform dense bodies. The electron-dense vesicles are acidic, and they form a continuous dense layer on the contact surface of the MGH and the parasitoid.
- The MGHs release exosomes and microvesicles, which could act as carriers of effector molecules involved in isolation and elimination of parasitoids.
- Transcriptome analysis of *D. ananassae* hemocytes revealed the high energy demand of the MGHs and showed that the elements of the JNK signaling pathway are expressed in these cells.

- We have shown that the drosophilids differentiating MGHs, express horizontally acquired genes of microbial origin encoding the Cytolethal distending toxin B (CdtB), and a family of pore-forming toxins, the hemolysin E-like (HL) proteins.
- The expression of *hl* genes and the encoded proteins is elevated after parasitoid wasp infection, with primary expression in the main immune tissues, the blood cells and the fat body. We also detected the presence of HL proteins on the surface of the parasitoid larvae. These findings suggest the involvement of these proteins in the anti-parasitoid immune response. The evolutionary strategy to acquire genes rapidly from prokaryotes might provide a powerful advantage to the species of the *ananassae* subgroup in the coevolutionary race with parasites. In particular, the acquisition of toxins, such as CdtB and HL, confers a benefit in immune defense reactions and therefore increases the fitness of the host.
- MGHs share similar features with mammalian giant cells (GCs), which usually form with the fusion of macrophages. The ultrastructural analysis of MGHs uncovered their characteristic cytoplasm with several

vesicles, electron dense bodies, autophagosomes, and multivesicular bodies, all typical for mammalian GCs and megakaryocytes.

- We discovered an emperipolesis-like phenomena, when plasmatocytes are encased in the cytoplasm of MGHs, a process first described between neutrophils and megakaryocytes.
- Orthologs, crucial for mammalian GC function are expressed at high level in MGHs, such as genes encoding for β integrin subunits, vacuolar type H+-ATPase subunits and Cysteine proteinase-1.
- We showed that several features of *Z. indianus* MGHs resemble those of mammalian megakaryocytes as both cell types possess a characteristic cytoplasm with an elaborate system of canals that communicates with the extracellular space, and both cell types release a large number of anucleated cytoplasmic fragments.
- We provided evidence for a novel function of the MGHs, as the anucleated fragments released by these cells accumulated at wound sites. Similarly to the platelets originated from the megakaryocytes, the

anucleated fragments released by the giant hemocytes of *Z. indianus* could be involved in wound healing.

- Transcriptome analysis of *Z. indianus* hemocytes revealed constitutive expression of several genes, which, based on their homology to *D. melanogaster* or mammalian genes, could be involved in wound healing and blood coagulation.

List of publications

MTMT ID: 10069445

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Peer-reviewed international publications required for the fulfilment of the doctoral process

Cinege, G.*, **Magyar, L.B.***, Kovács, A.L., Lerner, Z., Juhász, G., Lukacsovich, D., Winterer, J., Lukacsovich, T., Hegedűs, Z., Kurucz, É., Hultmark, D., Földy, C., Andó, I., 2021. Broad Ultrastructural and Transcriptomic Changes Underlie the Multinucleated Giant Hemocyte Mediated Innate Immune Response against Parasitoids. Journal of Innate Immunity 14, 335–354. **IF 7.111** https://doi.org/10.1159/000520110

* - authors contributed equally to this work

Verster, K.I., Cinege, G., Lipinszki, Z., **Magyar, L.B.**, Kurucz, É., Tarnopol, R.L., Ábrahám, E., Darula, Z., Karageorgi, M., Tamsil, J.A., Akalu, S.M., Andó, I.,

Whiteman, N.K., 2023. Evolution of insect innate immunity through domestication of bacterial toxins. Proceedings of the National Academy of Sciences 120, e2218334120. **IF 11.1** https://doi.org/10.1073/pnas.2218334120

Other peer-reviewed international publications

Cinege, G., Lerner, Z., **Magyar, L.B.**, Soós, B., Tóth, R., Kristó, I., Vilmos, P., Juhász, G., Kovács, A.L., Hegedűs, Z., Sensen, C.W., Kurucz, É., Andó, I., 2019. Cellular Immune Response Involving Multinucleated Giant Hemocytes with Two-Step Genome Amplification in the *Drosophilid Zaprionus indianus*. Journal of Innate Immunity 12, 257–272. **IF 4.932** https://doi.org/10.1159/000502646

Cinege, G., **Magyar, L.B.**, Kovács, H., Varga, V., Bodai, L., Zsindely, N., Nagy, G., Hegedűs, Z., Hultmark, D., Andó, I., 2023. Distinctive features of *Zaprionus indianus* hemocyte differentiation and function revealed by transcriptomic analysis. Front Immunol 14, 1322381. **IF 7.3** https://doi.org/10.3389/fimmu.2023.1322381

Cinege, G., Fodor, K., **Magyar, L.B.**, Lipinszki, Z., Hultmark, D., Andó, I., 2024. Cellular immunity of *Drosophila willistoni* reveals novel complexity of the insect anti parasitoid defense. Cells 13(7), 593. **IF 6.0** https://doi.org/10.3390/cells13070593

Tarnopol, R., Tamsil, J., Cinege, G., Ha, J., Verster, K.I., Ábrahám, E., **Magyar, L.B.**, Kim, B.Y., Bernstein, S.L., Lipinszki, Z., Andó, I., Whiteman, N.K. Retracing the

horizontal transfer of a novel innate immune factor in Drosophila. Manuscript submitted, Nature.

Magyar, L.B., Ábrahám, E., Lipinszki, Z., Tarnopol, L.R. Whiteman, N.K., Varga, V., Hultmark, D., Andó, I., Cinege, G., Pore forming prokaryotic toxin-like proteins in the anti-parasitoid immune response in Drosophila. Manuscript in preparation.

Társszerzői nyilatkozat

Kijelentem, hogy ismerem Magyar Lilla Brigitta Ph.D. fokozatra pályázó doktorjelölt „The characterization of the multinucleated giant hemocyte, a novel player in innate immunity" című disszertációját. Továbbá, igazolom, hogy Magyar Lilla Brigitta jelentős mértékben hozzájárult a publikációk eredményeihez, és a disszertációjában közölt eredményeket más Ph.D. tudományos fokozat megszerzéséhez nem használjuk fel.

1. Cinege, G.*, **Magyar, L.B.***, Kovács, A.L., Lerner, Z., Juhász, G., Lukacsovich, D., Winterer, J., Lukacsovich, T., Hegedűs, Z., Kurucz, É., Hultmark, D., Földy, C., Andó, I., 2021. Broad Ultrastructural and Transcriptomic Changes Underlie the Multinucleated Giant Hemocyte Mediated Innate Immune Response against Parasitoids. Journal of Innate Immunity 14, 335–354. **IF 7.111** https://doi.org/10.1159/000520110

* - megosztott első szerző

2. Verster, K.I., Cinege, G., Lipinszki, Z., **Magyar, L.B.**, Kurucz, É., Tarnopol, R.L., Ábrahám, E., Darula, Z., Karageorgi, M., Tamsil, J.A., Akalu, S.M., Andó, I., Whiteman, N.K., 2023. Evolution of insect innate immunity through domestication of bacterial toxins. Proceedings of the National Academy of Sciences 120, e2218334120.

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