Posudek oponenta habilitační práce

Uchazeč	**Mgr. Adam Bajgar, Ph.D.**
Habilitační práce	Drosophila macrophages – opening gates for immunometabolic research in insects
Oponent (jméno a příjmení vč. titulů)	RNDr. Dominik Filipp, CSc.
Pracoviště oponenta, instituce	Ústav molekulární genetiky AV ČR

Over the past decade, Dr. Adam Bajgar, with his collaborators, including Gabriela Krejčová, has developed a highly original and influential body of research on macrophage physiology, immunometabolism, and evolutionary significance. Using *Drosophila* as a tractable model, his work has experimentally demonstrated that macrophage activation involves profound metabolic rewiring: bacterial challenge induces a shift toward aerobic glycolysis, increased reactive oxygen species, and secretion of systemic modulators such as the insulin antagonist ImpL2 and extracellular adenosine. These studies provide direct causal evidence that macrophage polarization toward a pro-inflammatory, bactericidal state requires metabolic adaptation, while also showing that these cells act as endocrine regulators, mobilizing nutrients from peripheral tissues to support immune defense. The work is a fascinating read, from which emerges a novel hypothesis linking nutritional phagocytosis to bactericidal function, suggesting that these dual roles of macrophages are evolutionarily conserved.

Beyond these experimental findings, Dr. Bajgar and collaborators have articulated a broader theoretical framework regarding macrophage functional plasticity. They propose that the dual roles of macrophages in inflammation and tissue repair are ancient traits, rooted in the primordial functions of amoeboid phagocytes. While this evolutionary hypothesis is currently speculative, Dr. Bajgar plans to actively address it through rigorous experiments using *Acanthamoeba* and *Dictyostelium*, and is considering a dedicated sabbatical to systematically evaluate and correlate these features with higher metazoans. Such work promises to provide strong experimental support for the idea that metabolic strategies and functional plasticity are fundamental, conserved features of phagocytes.

The translational relevance of this research is also notable. M1 polarization in *Drosophila* macrophages during bacterial phagocytosis mirrors human phenomena, where M1-like macrophages are activated by excess adipose tissue and circulating fatty acids, contributing to chronic low-grade inflammation. By highlighting these parallels, Dr. Bajgar's work bridges basic biology and human physiology, offering insights into immunometabolic disorders and the systemic consequences of macrophage activation.

The strengths of this body of work lie in the integration of modern experimental approaches, conceptual thinking, and evolutionary perspective. In vivo genetic manipulation in *Drosophila*, combined with systemic metabolic and molecular analyses, allows robust causal interpretation regarding macrophage polarization and metabolic rewiring. Framing these findings in an evolutionary context redefines macrophage biology, emphasizing that functional plasticity and metabolic strategies are conserved traits rather than mammal-specific phenomena. Limitations remain, particularly in the need for cross-species experimental validation and the constraints of *Drosophila* as a model for mammalian adaptive immunity and tissue complexity. Nevertheless, Dr. Bajgar's research has substantial scientific and public value: it advances understanding of immune coordination at cellular and organismal levels and provides valuable insight into the process of metabolic inflammation as well as compelling framework for

exploring the evolutionary origins of macrophage function. Ongoing and future work with unicellular organisms promises to rigorously test these evolutionary concepts, further strengthening the bridge between fundamental biology, evolutionary theory, and translational relevance.

It should be emphasized that, according to the attached analysis, no evidence was found indicating the presence of plagiarism in the work.

In conclusion, Dr. Adam Bajgar represents the full profile of an accomplished and promising academic. His scientific contributions in macrophage biology and immunometabolism are original, novel, and internationally recognized, as evidenced by his publication record, citation metrics, and invited talks. Beyond his research excellence, he is also a dedicated teacher, the guarantor of a Master's program, and an experienced mentor who has successfully guided bachelor's, master's, and PhD students. His role as organizer of scientific events, particularly those aimed at young researchers, further demonstrates his commitment to community building and the development of the next generation of scientists.

Dr. Bajgar's international collaborations, successful grant activity as principal investigator, and recognition through multiple awards underscore his standing as an established young researcher of high potential. His broad academic profile, combining innovative research, excellent teaching, effective mentorship, international networking, and leadership, makes him a complete and well-rounded candidate for habilitation. I am convinced that conferring the title of "*docent*" upon Dr. Bajgar will not only recognize his achievements but also benefit the faculty and the university, strengthening their visibility and reputation, and ensuring a productive, innovative, and inspiring environment for students and colleagues alike.

Dotazy oponenta k obhajobě habilitační práce

1. Evolutionary testing: Your hypothesis links nutritional phagocytosis to bactericidal function as a conserved trait of macrophages. What specific experiments with *Acanthamoeba* or *Dictyostelium* do you see as most decisive for testing this idea?
2. Translational link: You draw parallels between M1 polarization in *Drosophila* macrophages and obesity-associated inflammation in humans. How do you envision your findings contributing to therapeutic strategies for metabolic or inflammatory diseases?
3. Future directions: Looking forward, which part of your research, immunometabolism, evolutionary origins, or systemic regulation, do you believe has the greatest potential to change how we understand macrophage biology?
4. Self-reflection: Of all your findings in the past decade, which result surprised you the most, and how did it reshape your scientific perspective?

Závěr

Habilitační práce Mgr. et Mgr. Adama Bajgara, Ph.D. „Drosophila macrophages – opening gates for immunometabolic research in insects" **splňuje/~~nesplňuje~~** požadavky standardně kladené na habilitační práce v oboru **Molekulární a buněčná biologie a genetika.**

V Praze, dne 24.09.2025

[redacted]

podpis oponenta

The reviewer's evaluation of the habilitation thesis

Candidate	**Mgr. et Mgr. Adam Bajgar, Ph.D.**
Habilitation thesis	Drosophila macrophages – opening gates for immunometabolic research in insects
Reviewer	**Tina Mukherjee**
The reviewer's department, institution	**iBRiC-inStem, Bangalore, India**

(text of the evaluation, including a statement on the result of the anti-plagiarism check)

I would first like to start by congratulating Adam for the beautiful pieces of work presented. On a personal note, I have always enjoyed reading his manuscripts and the scientific insights they bring into an area that is still very new and relatively underexplored.

The thesis provides extensive and detailed information on macrophage biology, metabolic polarization, and systemic metabolic regulation by macrophages using *Drosophila melanogaster* as a key model system. It also offers deep insights into the evolutionary origins of these processes. Included are several key research publications by Adam and his collaborators, focusing on macrophage metabolic polarization and the role of adenosine signaling in immune-metabolic interactions during infection. This is undoubtedly an impressive body of work and, without question, deserves to be evaluated as excellent.

One of the highlights for me was the evolutionary perspective on macrophages. This section, right up front, set the tone and placed the work in the right scientific frame. The proposition that pro-inflammatory (M1-like) polarization derives from ancestral nutrient-gathering amoeboid cells — conserving traits such as aerobic glycolysis and bactericidal functions — while anti-inflammatory (M2-like) polarization and tissue remodeling evolved alongside multicellularity to support homeostasis and repair, was fascinating. These ancient origins explain both the plasticity and the pathological or homeostatic roles that macrophages can play.

The emphasis on homeostatic inflammation was also particularly insightful. The role of macrophage-driven inflammation for maintaining physiological homeostasis is only beginning to be fully understood. The fact that this underpins macrophage metabolic homeostasis is an intriguing concept, and one I believe will be central to future discoveries.

This conceptual framework connects strongly with the key findings on macrophage metabolic polarization in *Drosophila* upon bacterial infection. Much like in mammals, *Drosophila* macrophages (plasmatocytes) undergo a metabolic shift toward aerobic glycolysis (Warburg effect), mediated by the transcription factor HIF-1α/Sima in flies.

This polarization leads to changes in the secretome of these cells (may be?), helping coordinate whole-animal metabolism — another critical and novel insight. The identification of systemic metabolic changes driven by macrophage-secreted signals such as extracellular adenosine (e-Ado) and ImpL2 emphasizes the central regulatory role these cells play. Notably:

e-Ado, a signal of ATP consumption, is released by activated macrophages and acts through AdoR in distant tissues.

ImpL2, an insulin antagonist, is transcriptionally upregulated in response to HIF-1α-mediated glycolytic shift, further coordinating organismal metabolic responses.

The extension of this work to illustrate how macrophages contribute to nutrient recycling during metamorphosis, and the development of novel tools to specifically manipulate macrophages, are further significant contributions.

Looking ahead, the proposal to investigate macrophage–adipocyte cross-talk seems like a natural and exciting next step.

Overall, I believe this is an excellent and impactful body of work. I congratulate Adam once again for bringing these insights to the forefront not only for the *Drosophila* field but also for the broader immunology and metabolic biology communities.

The associated publications are outstanding.

Questions of the reviewer on the defence of the habilitation thesis

Here are a few questions I would be very interested to hear Adam's thoughts on:

1. **Why do macrophages continue to conduct these ancestral functions?** While their nutrient-recycling role may be primitive in origin, it is remarkable that macrophages retain and actively perform these roles even as other specialized tissues have evolved. Why do they remain nutrient recyclers?
2. **Does macrophage heterogeneity or ontogeny play a role in this context?** Are specific subsets of macrophages responsible for these distinct roles? How might origin (embryonic vs adult-derived) influence function in metabolic regulation?
3. **The metabolic shifts during infection are immune-driven but in metamorphosis, these seem developmentally regulated.**
   Could you elaborate on how macrophages mediate developmental homeostasis? Is there a developmental "checklist" or program they are executing?
4. **What's the next big question in this field that you would like to answer?** With the groundwork you've laid, what do you envision as the most important next discovery in the coming years?

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Conclusion

The habilitation thesis of **Mgr. et Mgr. Adam Bajgar, Ph.D.** „Antipredator behaviour of birds" **meets** the standard requirements for habilitation theses in the field of **Molecular and Cell Biology and Genetics.**

In BANGALORE, INDIA on .....

25th September 2025

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Posudek oponenta habilitační práce

Uchazeč	**Mgr. et Mgr. Adam Bajgar, Ph.D.**
Habilitační práce	Drosophila macrophages – opening gates for immunometabolic research in insects
Oponent (jméno a příjmení vč. titulů)	Prof. Miroslava Uhlířová, Ph.D.
Pracoviště oponenta, instituce	Institute for Genetics, Faculty of Mathematics and Natural Sciences, Cologne Excellence Cluster for Aging and Aging-Associated Diseases (CECAD), Cologne, Germany

First, I would like to state that I have no academic, family, or other relationship with the candidate. I know Dr. Adam Bajgar personally. However, I have not collaborated with him in the past, and there are no ongoing or planned collaborations at this time.

The submitted Habilitation Thesis comprises **44 pages of theoretical introduction** and an appendix documenting **9 original research articles** (3 first-author, 3 co-corresponding, 2 corresponding, and 1 co-authorship) and **2 reviews** (1 corresponding, 1 co-corresponding).

The introduction opens with a historical note on Élie Metchnikoff, who first described macrophages as "big eaters", phagocytes capable of protecting organisms against pathogens. Dr. Bajgar provides a concise overview of macrophage phenotypic plasticity with the classical M1 (pro-inflammatory) and M2 (anti-inflammatory) ends of the polarization spectrum, detailing their metabolic hallmarks: Hif-1α-driven aerobic glycolysis in M1 pro-inflammatory state, and AMPK-regulated oxidative phosphorylation in M2 state. He reviews evidence that manipulating these pathways and metabolism is both necessary and sufficient to induce or maintain macrophage polarization with major implications for health and disease. Importantly, he emphasizes that the M1 and M2 polarization states, although reproducibly and robustly induced using established *in vitro* protocols, do not fully reflect the polarization spectrum observed *in vivo*. In living organisms, macrophages residing in diverse tissues respond in a far more complex manner, involving a continuum of activation states shaped by their microenvironments, enabling dynamic responses to physiological and pathological stimuli. He illustrates this complexity with examples of specific function of tissue-resident macrophage populations. Disease contexts, such as obesity, non-alcoholic fatty liver disease and atherosclerosis, are presented to illustrate how chronic pro-inflammatory activation fuels metabolic syndrome, insulin resistance, hepatic fibrosis, and vascular pathology.

The next section discusses the evolutionary origins of macrophage polarization. Dr. Bajgar links an M1-like phenotype to nutrient-driven phagocytosis in unicellular amoebas and proposes that the emergence of multicellularity supported M2-like functions. This is exemplified by the facultative multicellular amoeba *Dictyostelium discoideum*, in which cell specialization support collective development. He concludes by introducing *Drosophila melanogaster*, his primary research model, and highlights his contributions to understanding the non-canonical roles of plasmatocytes, *Drosophila* macrophage-like cells, particularly how they integrate immune activation with systemic metabolic regulation in physiology and disease.

A major conceptual advance of Dr. Bajgar's work is the demonstration that infection-activated macrophages adopt aerobic glycolysis as an essential metabolic program for bactericidal activity, highlighting an evolutionarily conserved immunometabolic mechanism between insects and mammals. His work has shown that the energetic cost of this metabolic shift necessitates carbohydrates and lipid mobilization from the fat body, the central metabolic organ, mediated by macrophage-derived factors eAdo and ImpL2, the latter inducing FOXO-dependent production of lipoproteins and transient insulin resistance. Traditionally regarded as a hallmark of pathological states

such as chronic inflammation or cachexia, this phenomenon is reinterpreted as a context-dependent adaptive response that ensures adequate nutrient availability during acute infection. The discovery of ImpL2 homolog IGFPBP7, produced by infected mammalian macrophages and acting on neighboring hepatocytes, refines current models of immune-metabolic crosstalk and shed new light on metabolic disorders linked to chronic inflammation, including obesity and type-2 diabetes.

Moreover, Dr. Bajgar's group uncovered a previously unrecognized role of macrophages in nutrient recycling during insect metamorphosis. By phagocytosing histolyzing larval tissues, macrophages transform cellular debris into lipoproteins and storage peptides, thereby ensuring efficient resource reutilization. This discovery highlights the metabolic versatility of macrophages and suggests that similar processes may occur during vertebrate metamorphosis, embryonic development, and the continuous cellular turnover in mammals. The findings are exiting in light of the study of Franz *et al.*, (2018, *Dev Cell*) which demonstrated cooperation between fat body cells and hemocytes during wound repair in pupae.

Alongside his mechanistic studies, Dr. Bajgar has invested effort into developing tools for targeted delivery of molecular cargo into macrophages, enabling genetic and pharmacological cell-type specific manipulations. While these tools represent valuable proof-of-principle methods and have already expanded experimental possibilities, they remain open to further refinement and optimization, with the goal of broadening immune cell research beyond classical model organisms and offering potential applications to ecologically and medically relevant insects.

In the future, Dr. Bajgar aims to further exploit the *Drosophila* model for the study of adipose-tissue-macrophage interactions, a crosstalk of major importance for systemic metabolic health and biomedical relevance. By combining mechanistic insight with novel experimental strategies to manipulate immune cells in non-model species, his laboratory is well positioned to make further significant contributions to understanding the evolution of the immune cell functions and macrophage-tissue interactions with translational potential.

Remarks on the thesis

The publications have undergone rigorous peer review by experts in the field and have been accepted in highly visible journals, including *EMBO Journal*, *eLife*, *PLOS Biology*, and *Nature Metabolism*. Thus, there is no reason to question the scientific quality or novelty of the work. The combination of original research and review articles underscores Dr. Bajgar's strong expertise, deep interest, and engagement in the field.

That said, the section of the Habilitation Thesis describing the *Drosophila* model contains some simplifications and inaccuracies regarding balancer chromosomes, transgenes, and the citation of marginally related references instead of original articles (pp. 29-30). A few statements would also benefit referencing (first paragraph, p. 42). Given the central focus on macrophages and their functional complexity shaped by tissue residency, it would be worthwhile to include a concise overview of the origin and types of *Drosophila* hemocytes, especially considering recent scRNA-seq studies revealing cellular plasticity and changes in hemocyte populations across developmental stages. Finally, while the significance of research in model and non-model organisms is clear, it would be appropriate to acknowledge their limitations.

Statement on Plagiarism

As the text of the habilitation thesis is derived from the candidate's own published articles, it is to be expected that certain sentences may be identified as positive matches by plagiarism-detection software. Nevertheless, the thesis is an original work, and any allegation of plagiarism is therefore without merit.

Questions for the Habilitation Defense/ Dotazy oponenta k obhajobě habilitační práce

Bajgar et al., 2019, Biomater Sci. 7:4708-4719. doi: 10.1039/c9bm00539k.

1) The authors claim, "negligible effects on immune system activation", yet in two example micrographs of third instar larvae injected with the glucan-fluorescent-labelled particles (Figs. 1C and 2C), the sessile compartment appears visibly disturbed, suggesting hemocyte activation caused by injection and/or presence of the particles and/or manipulation during imaging.

Could Dr. Bajgar comment on the results and whether they assess morphology of hemocytes bled from glucan particle-injected animals as a proxy for activation?

2) "The dissection of immune tissue in the larval stage of *Drosophila* revealed a somewhat surprising presence of several positive cells also in the lymph gland (Fig. 2F). This observation suggests that some of the lymph gland cells are capable of active phagocytosis."

The observation of glucan particle-positive cells within the lymph gland and their distribution is unexpected given that the lymph gland is normally surrounded by a basal membrane. In the naive state *hml>GFP* labels differentiated hemocytes enriched in the cortical zone. However, the presented micrograph (Fig. 2F) shows expansion of *hml>GFP* into the cortical zone and some of these cells are glucan-positive, suggesting immune activation and accelerated differentiation.

Can Dr. Bajgar comment on the frequency of this phenotype and the possibility that immune activation compromises lymph gland integrity?

Krejčová et al., 2024, *Insect Mol Biol.* 33:323-337. doi: 10.1111/imb.12900.

3) Cholesterol metabolism is essential for macrophages influencing cell membrane properties, cellular signaling, cell-cell interactions, macrophage differentiation, activation, and motility. Furthermore, cholesterol-derived metabolites, such as oxysterols, are crucial immuno-metabolic modulators controlling cholesterol/lipid homeostasis, immune cell recruitment, polarization and pathogen responses linked to neurodegeneration, atherosclerosis and obesity.

Could Dr. Bajgar elaborate on the similarities and differences in cholesterol metabolism between insects and mammals, and speculate on the molecular mechanisms by which statin-mediated HMGCR inhibition acts in insects? Is there evidence that insects produce oxysterols with potential immunometabolic functions? Finally, is it plausible that macrophage-specific inhibition of the mevalonate pathway intersects with systemic endocrine regulation or influences developmental timing?

Conclusion/Závěr

Habilitační práce Mgr. et Mgr. Adama Bajgara, Ph.D. „Drosophila macrophages – opening gates for immunometabolic research in insects" **splňuje** požadavky standardně kladené na habilitační práce v oboru **Molekulární a buněčná biologie a genetika.**

Cologne, 22.09.2025

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podpis oponenta