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Dr hab. Magdalena Twarużek
Associate Professor
Kazimierz Wielki University
Faculty of Biological Sciences
Department of Physiology and Toxicology
Chodkiewicza Street 30
85064 Bydgoszcz

Opinion on the doctoral dissertation

"The dark side of endotoxin tolerance: increased susceptibility to cancer"

by MSc Konkonika Roy

PhD supervisor: dr hab. Sylwia Wrotek, prof. NCU
Assistant supervisor: Tomasz Jędrzejewski, PhD

Nicolaus Copernicus University in Torun
Faculty of Biological and Veterinary Sciences

Endotoxin tolerance (ET) is a well-documented immunosuppressive state that occurs after repeated exposure to bacterial lipopolysaccharide (LPS), leading to diminished pro-inflammatory responses. While this phenomenon helps prevent excessive inflammation and tissue damage during infections, emerging evidence suggests that ET may have a "dark side," including increased susceptibility to cancer. Endotoxin tolerance may promote cancer progression. While endotoxin tolerance protects against septic shock, its immunosuppressive effects may inadvertently foster cancer progression by weakening immune surveillance and promoting a tumor-friendly microenvironment. ET has garnered considerable attention recently due to its potential role in modulating immune responses,

particularly in chronic infectious conditions. However it is clear that there is a significant gap in understanding of ET within the context of cancer. The PhD student carried out a series of interesting studies seeks to fill this gap.

This dissertation is written in English, consists of 2 published articles and submitted one:

Roy, K.; Kozłowski, H.M.; Jędrzejewski, T.; Sobocińska, J.; Maciejewski, B.; Dzialuk, A.; Wrotek, S. Endotoxin Tolerance Creates Favourable Conditions for Cancer Development. Cancers 15 (20), 5113, 2023.

Roy, K.; Jędrzejewski, T.; Sobocińska, J.; Spisz, P.; Maciejewski, B.; Hövelmeyer, N.; Passeri,

B.; Wrotek, S. Divergent impact of endotoxinpriming and endotoxin tolerance on responses to cancer cells. Cellular Immunology 411-412, 104934, 2025.

Roy, K.; Maciejewski, B.; Jędrzejewski, T.; Spisz, P.; Sobocińska, J.; Di Pentima, M; Passeri, B.; Wrotek, S. Endotoxin tolerance enhances breast cancer aggressiveness and alters inflammatory marker expression in tumor and spleen of mice. International Immunology. (Manuscript ID: INTIMM-25-0140)

The doctoral dissertation submitted for evaluation is a series of publications, consisting of two original creative works, published in peer-reviewed scientific journals Cancers and Cellular Immunology indexed by the Journal Citation Report with a total impact factor (IF) of 8.2 and 300 MNiSW points, and one submitted to International Immunology (IF 3,2 and 140 Ministry points) article, which the PhD student gave the collective title: The dark side of endotoxin tolerance: increased susceptibility to cancer. My question is: Why does the PhD student provide for Article#3 submitted in 2025 values, IF from 2023 and Ministry points from 2024.

When assessing the series of publications, it should be stated that they constitute a coherent, homogeneous cycle of innovative and valuable research works. In all publications, the PhD student is the first author. The works are multi-authored, but I believe that this is a significant contribution and shows that it was the PhD student who was the main person in charge of the work contained in the attached publications. The

PhD student presents her contribution to the development of the publication. Unfortunately, there is no confirmation from the co-authors of these works. In two published manuscripts, it is possible to check it, unfortunately in Article#3 it is not possible.

These papers have been published in recognized international journals and have already received positive opinions from independent experts related to the given issues, which proves their reliability and high scientific value. All 2 already published articles were cited (with self-citations) only 4 times. The small number of citations may be due to the relatively recent publication date.

The doctoral dissertation begins with a list of the most important symbols and abbreviations. After the publication list, the MSc Konkonika Roy presents an abstract (in English and Polish) followed by a theoretical introduction, presenting the issues: molecular mechanism involved in the immune response to endotoxin; recognition of LPS in TLR4 signaling cascade, interaction LPS with TLR4, MyD88 dependent signaling, TRIF - dependent signaling, canonical and non-canonical inflammasome signaling, significance and mechanism of fever, molecular mechanism involved in endotoxin tolerance and the role of macrophages in infections and tumorigenesis. Next, the PhD student posted the aims of the study, a description of materials and methods. The discussion takes 7 pages of printout. The discussion is followed by a one and half page of conclusion.

The dissertation ends with a references, contained in 16 pages and covering 185 items of literature related to the topic and scope of the work, of which 43% comes from the last 10 years. On page 13 there are two citation methods: Harvard and Vancuver. References are arranged according to a numerical system, making it difficult to find several publications cited in the author-year method.

The work is illustrated with figures that are legible and significantly facilitate the reader to get acquainted with the large number of results obtained. The materials have been prepared in a transparent manner and in a way that allows for proper and complete assessment and familiarization with the essence of the scientific achievement. The issues discussed in the doctoral dissertation are very typical. The

research topic taken is original, innovative and important, both for basic research, and has practical significance in relations to understanding of ET within the context of cancer.

Brief description and assessment of the PhD thesis

a) The title is short, fine and informative
b) Structure of thesis is not the one of a classical PhD document, since the main part of the original research is reported in the journal articles, but it is fine in general
c) Bibliography is adequately covers the state of the knowledge.
d) Aims of the work is clearly specified in the thesis.

   The overarching goal of research was to explor the impact of ET on cancer, with specific objectives aimed at examining:

   - The impact of endotoxin tolerance on macrophages and cancer cells (Article #1)
   - The effect of the tumor microenvironment on endotoxin-tolerant macrophages (Article #2)
   - The effect of endotoxin tolerance on tumor development in mice (Article #3)

   I wonder how the PhD student established the goals of her work in this way, correlated with individual publications. In my opinion, this requires clarification.

e) Methodology is correct.

   When analyzing the methodologies in detail, it should be noted that the research was carried out using modern and well-selected methods that guarantee the reliability of the obtained results.

f) Assessment of results is provided in the first part of point 4 of this review. The scope and quality of results are appreciable for a PhD work.
g) The chapter Summary and Conclusion is a logical effect of the results obtained in the research. They were aptly captured and they prove the author's great ability to synthetically analyze the obtained results against the background of previous research.

The most important finding was that MoET, unlike non-treated with LPS cells (Mo NT), significantly supported tumor cell survival, increased their migratory capabilities, clonogenic potential, and spheroid-forming ability, all of which suggest pro-tumorigenic potential of ET. Furthermore, PhD demonstrated that in the tumor microenvironment, MoET adopt a phenotype similar to M2-type macrophages, which are considered immunosuppressive and pro-tumorigenic. These cells also displayed an altered metabolic profile, shifting away fromclassical glycolysis and oxidative phosphorylation pathways towards alternative metabolic routes.

The results of in vivo studies confirmed that ET significantly influences the cancer progression, leading to a reduction in survival and accelerated tumor growth in endotoxin-tolerant mice with breast cancer (ETBC group) compared to non-endotoxin-tolerized cancer-bearing mice (BCgroup).

h) Applicability of findings.

The obtained results provide evidence that ET locally reprograms macrophages towards a pro-tumoral phenotype and simultaneously modifies their response to tumor cells. Consequently, at the organismal level, ET shapes a systemic environment conducive to tumor development.

i) Imperfections, suggestions for improvements, questions.

Generally, the PhD document is carefully written and the Candidate has to be congratulated on the effort and its outcome. Concerning the questions related to the research work itself, some are formulated below, mainly as an invitation for the Candidate to look beyond and as a support for discussion at the PhD defense.

Controversies and Challenges

If ET may be protective in early cancer (preventing inflammation-driven mutations) but harmful in established tumors?

Does dependency: Low vs. high LPS exposure has opposite effects (hormesis)?

Animal vs. Human Data: Most evidence is murine; human ET signatures in cancer are poorly defined.

Key Research Questions

Does ET precede cancer (e.g., in chronic inflammatory conditions) or result from tumor-induced immunosuppression?

Can ET biomarkers predict immunotherapy response?

How do cancer treatments (e.g., chemo/radiotherapy) alter ET states?

Final conclusion

The doctoral dissertation presented by MSc Konkonika Roy provides a proof of her good knowledge of the about "the dark side" of endotoxin tolerance-specifically its connection to increased cancer susceptibility. The Candidate has demonstrated her capabilities to critically scrutinize the bibliography of the subject as well as various variants of computational approaches to turbulent flows. The thesis contains original analyses and novel findings beyond the state of the art. Judging by the PhD document, the Candidate has proven her good knowledge of the subject area, the professional skills, as well as the ability to think and work creatively. Given all the above, my final conclusion about MSc Konkonika Roy being a doctoral candidate is positive and I recommend that she orally defends the PhD dissertation with no reserve at all.

Taking into account the above arguments, I conclude that the doctoral dissertation submitted for evaluation by Ms. Konkonika Roy meets all the requirements specified in Article 187, paragraphs 1–2 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2024, item 1571). Therefore, I wholeheartedly recommend accepting the doctoral dissertation of Ms. Konkonika Roy and allowing the Doctoral Candidate to proceed with the next stages of the doctoral proceedings.

Twarużek

dr hab. Magdalena Twarużek, prof. Uczelni