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Opponent's opinion on the habilitation thesis of Dr. Michal Pastorek,
entitled *Neutrophil extracellular traps in health and disease*

The habilitation thesis of Dr. Michal Pastorek on the topic of neutrophil extracellular traps is elaborated in English on approximately 100 pages in the form of a review study. It includes five published scientific articles of his, four original and one review article, dealing with the same topic.

Some formal shortages need to be pointed out in the beginning.

First, there are no title and acknowledgement pages included so when, wherein and with whom the experimental work had been performed is not revealed to a reader; second, both the list of publications, with exceptions of the papers to be involved, and the list of up-to-date author's positions and experience are as well missing, which would also be instrumental for the reader to put together an overall picture of the author's scientific career; third, the reader would definitely appreciate more detail figure legends, e.g. scales are standardly integral parts of microscopic images; and lastly, in some cases it would be more approachable for the reader if the original works and not just review papers were cited, e.g. when a circadian rhythm of replenishment is referred.

Nevertheless, the work as such is written with clarity and brevity, it is scientifically sound and without flaws, which is anyway documented by a number of included papers not mine to review but which had been found by peers to meet the highest publication level.

Now, to the scientific topic; neutrophil extracellular traps (NETs) are very important tools of the innate immunity in combating pathogen, but when temporally and spatially unresolved, they might be behind several severe disorders, such as autoimmune diseases or thrombosis. The goal of the habilitation thesis of Dr. Pastorek was to characterise these differences at the molecular level.

In particular, he and his colleagues focused both on the analysis of samples from patients with various diseases, and on the using of mouse models of these diseases. As the most important discovery within their research I consider the *in vitro* elucidation of mitochondrial signals and counterpart receptors implicated in the sterile NET formation which might be one of the triggers of serious inflammatory pathologies.

Not only provide the findings described in this thesis insights into the molecular composition and mechanisms of NET formation but also would contribute to identification of possible pharmacological modulators of this process.

In conclusion, the habilitation dissertation of Dr. Michal Pastorek fulfils all the requirements and I recommend it for the defense.

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Upon reading, several scientific questions arose in my mind, many of them just to be answered on the next page. Here, I take my chance to ask some of those questions that remained unanswered to me:

1) According to author's notion, traps serve to confine pathogens on site. However, isn't it a double edge sword? Doesn't it capture also useful migratory immune cells?
2) As mentioned, netosis is a type of programmed cell death. However, the latter should not induce inflammation since it does not disclose damage signals. Isn't in this respect netosis closer to necrosis?
3) As author's put it, neutrophils are not easy to handle. Is it possible to apply or generate the human neutrophil-like cell line(s) instead of primary cells from donors?
4) As described, neutrophils are capable of three major tasks - phagocytosis, degranulation, and netosis. What is decisive in choosing the one of them? Is it a donor, cellular subtype or microenvironment?
5) According to author's notion, murine neutrophils produce lower amount and much smaller NETs than their human counterparts. Does it stem from the genetic differences between species or simply from sterile living conditions in animal facilities? Have the similar differences been observed also between primates and humans?
6) Author applied the terms fever and hypothermia in the context of *in vitro* experiments. Is that correct when considering the systemic complexity whereby the body temperature is physiologically controlled?
7) The role of metabolic pathways is pinpointed within the thesis. In this respect, how does Warburg effect affect the NET formation?

I thank for the opportunity to evaluate this very important work.

Dr. Vladimir Leksa, 19.2.2024, Bratislava