Title of the doctoral dissertation: The dark side of endotoxin tolerance: increased susceptibility to cancer

Doctoral dissertation abstract:

Endotoxin tolerance (ET) is a mechanism that develops in organism due to repeated or prolong endotoxin exposure, for example during infections caused by Gram-negative bacteria. A hallmark of ET, among others is, a significant suppression of pro-inflammatory cytokine synthesis and a weakened febrile response. Many years ago, it was observed that a persistently weakened febrile response during infection could predispose individuals to the development of cancer. However, the causes of this medically important phenomenon remained unknown. Considering that one of the characteristic features of ET is the absence of a febrile response, a hypothesis was proposed that ET may be responsible for the reduced fever-related immune response observed in cancer patients. Therefore, the aim of this study was to investigate whether ET promotes cancer progression.

I began my research by developing ET models in two systems: cellular and organismal. Under *in vitro* conditions, I analyzed macrophages exhibiting endotoxin tolerance (Mo_{ET}) by assessing the levels of various markers associated with the inflammatory response, such as CD14, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nitric oxide (NO), and reactive oxygen species (ROS). Compared cells exposed to LPS only one (Mo_{LPS}), Mo_{ET} showed significantly reduced expression of iNOS and COX-2. Moreover, despite decreased NO production in the tumor microenvironment, ROS levels in these cells remained significantly elevated. Then, I focused on analyzing the interactions between tumor cells and Mo_{ET}. My most important finding was that Mo_{ET}, 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, I demonstrated that in the tumor microenvironment, Mo_{ET} adopt a phenotype similar to M2-type macrophages, which are considered immunosuppressive and protumorigenic. These cells also displayed an altered metabolic profile, shifting away from classical glycolysis and oxidative phosphorylation pathways towards alternative metabolic routes.

In the next stages of the research, I validated the *in vitro* results using an animal model. 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 (BC group). Assessment of immune mediators in spleens collected from ETBC mice revealed decreased expression of interleukin (IL) 6 and interferon (IFN) γ, alongside increased expression of iNOS (NOS2), IL-1β, COX-2, STAT6, and colony-stimulating factor 1 (CSF-1) compared to BC mice. Similarly, analysis of tumors from ETBC mice showed elevated expression of IL-10, NOS2, IL-1β, vascular endothelial growth factor (VEGF), COX-2 compared to BC mice.

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.

Key words: endotoxin tolerance; pro-inflammatory factors; M1/M2 macrophage phenotype; cancer; immunosuppression; tumor microenvironment

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