Abstract of the dissertation for a doctoral degree, entitled: *Hyperthermia and Immune Response: exploring the immunoregulatory potential of fever-range temperatures*, by mgr inż. Henryk Mikołaj Kozłowski

For centuries, medical practitioners have recognized the beneficial effects of fever, leading to the development of techniques aimed at intentionally inducing elevated body temperatures for therapeutic purposes. While this type of treatment called fever-range hyperthermia (FRH) is currently in use, its full effects are not yet well understood.

In my studies I employed *in vivo* and *in vitro* methods to investigate the potential of FRH to affect immune system. I developed a new model of FRH in rats to investigate the impact of FRH on hematology profile and the expression of regulatory molecules, including cytokines and miRNA. I observed that FRH significantly influences lymphocytes and granulocytes what was associated with changed expression of regulatory molecules, including macrophage inhibitory factor (MIF-1α) and granulocyte colony-stimulating factor (G-CSF). I also found that hyperthermia affected the miRNA machinery, resulting in changes in miRNA-155 expression in the serum.

Since it is known that macrophages are heat sensitive cells, I decided to identify the phenotype of these cells. My initial in vitro studies showed, that FRH induced expression of CD163 and arginase-1 (Arg-1), markers specific to M2 macrophages. Furthermore, FRH inhibited the expression of inducible nitric oxide synthase (iNOS), a marker for M1 macrophages. Interestingly, FRH also increased the expression of pro-inflammatory markers such as IL-1β, IL-6, reactive oxygen species (ROS), cyclooxygenase 2 (COX-2), and Toll-like receptor 4 (TLR-4), indicating that FRH induces an M2b phenotype. Thus, FRH treated cells exhibit both pro-inflammatory and anti-inflammatory properties and therefore are considered regulatory cells.

As TLR-4 pathway is essential for inflammation, I investigated the effect of FRH on immunomodulators such as Mistletoe Extract (ME) and LPS that are TLR-4 dependent. I found that FRH could prevent ME-induced cell death, improve macrophage viability, and activate the NF-κB pathway, leading to the development of a pro-inflammatory response. Although LPS is widely recognized for its ability to induce the polarization of macrophages into the M1 phenotype, I found that FRH can effectively reverse this effect and instead promote the

induction of a regulatory M2b phenotype. However, I also observed that this mechanism was not solely TLR-4 dependent.

In conclusion, my research on FRH has revealed that the biological effects of hyperthermia are complex and multifaceted, extending beyond the previously known immunomodulatory effects observed via the TLR-4 pathway. Hyperthermia causes changes at the gene, protein, cell, and organism levels, affecting leukocyte populations, cytokines, and miRNA expression. My findings suggest that macrophages play a critical role in the response to FRH, exhibiting an M2b phenotype that has both pro-inflammatory and anti-inflammatory properties. Based on my results, far-reaching conclusions can be drawn that fever-associated increase in body temperature may be a switch for macrophages, that change M1 macrophages towards M2 phenotype to gradually finish inflammation.

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