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Szkoła Główna Gospodarstwa Wiejskiego
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Określenie roli nowo poznanych pasożytniczych czynników hamujących migrację makrofagów w układzie *Dirofilaria repens* – żywiciel

Determination of the role of newly discovered parasitic macrophage migration inhibitory factors in the *Dirofilaria repens* - host system interactions

Rozprawa doktorska

Doctoral thesis

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Summary

Dirofilaria repens is the etiological agent of subcutaneous dirofilariasis, a disease that affects dogs, other carnivores, and humans. *D. repens* is widely distributed in Europe, and its range continues to expand. Despite the increasing threat posed by this parasite to human and veterinary health, the mechanisms of its interactions with the host's immune system remain poorly understood. One of the strategies employed by nematodes to survive for extended periods in host tissues involves the production of immunomodulatory proteins. The aim of this study was the molecular characterization of two homologues of the *Dirofilaria repens* macrophage migration inhibitory factor (*Dre*-MIF), analysis of gene expression of the homologues in microfilariae and adult *D. repens*, production of recombinant *Dre*-MIF-1 and *Dre*-MIF-2 proteins in a bacterial expression system, assessment of their immunogenicity in mice, evaluation of their reactivity with antibodies from dogs naturally infected with *D. repens*, and determination of the role of these proteins in macrophage activity in an *in vitro* model.

A bioinformatic analysis of the sequences of the two *D. repens* MIF homologues was conducted. The expression levels of *Dre-mif-1* and *Dre-mif-2* genes were examined at different parasite developmental stages. Recombinant r*Dre*-MIF-1 and r*Dre*-MIF-2 proteins were produced in a bacterial expression system, and their purification was optimized using affinity chromatography. The immunogenicity of r*Dre*-MIF-1 and r*Dre*-MIF-2 in mice, as well as their reactivity with sera from naturally infected dogs, were assessed using ELISA. The second part of the study investigated the effect of r*Dre*-MIF-1 on THP-1 macrophages through analysis of kinase phosphorylation, cytokine secretion profiles, and changes in the expression of selected pro- and anti-inflammatory markers following stimulation.

The bioinformatic analysis revealed that *Dre*-MIF-1 shares a higher level of amino acid sequence similarity with human and canine MIF than *Dre*-MIF-2. *Dre-mif-1* and *Dre-mif-2* showed higher expression levels in adult *D. repens* compared to microfilariae. r*Dre*-MIF-1 is more immunogenic than r*Dre*-MIF-2 in mice. In infected dogs, elevated levels of IgG1 subclass antibodies against both proteins were detected. r*Dre*-MIF-1 impacts macrophage activity; it inhibits pro-inflammatory cytokine release by LPS-stimulated macrophages, which confirms its anti-inflammatory and immunomodulatory effects.

Keywords: subcutaneous dirofilariasis, macrophage migration inhibitory factor, macrophage, immunomodulatory properties