Streszczenie w języku angielskim

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii*. This opportunistic, intracellular parasite is widespread throughout the world, and it is estimated that approximately 30% of the human population is infected with the protozoan. This parasitic infection poses a significant threat to immunocompromised individuals, with the potential to result in irreversible health complications or even fatal consequences. The parasite has the capacity to reproduce in all nucleated cells of the host, although it exhibits a high degree of tropism for the central nervous system. The protozoan has a complex life cycle, and its final hosts are felines, in which sexual reproduction of *T. gondii* occurs. Humans are most commonly infected with the parasite through the ingestion of oocysts excreted by cats, which are present in soil or water, and tissue cysts found in the meat of infected animals. Furthermore, the potential for transmission through the placenta should be considered, which may result in miscarriage or serious foetal malformations. Toxoplasmosis can also cause significant financial losses in animal husbandry.

There is currently no available vaccine for human use, and the only veterinary vaccine has significant limitations in its application. Consequently, there is ongoing intensive research to develop an effective vaccine that can protect against both acute and chronic toxoplasmosis.

The diagnosis of toxoplasmosis is usually based on serological tests, which primarily detect specific IgG and IgM antibodies that recognize native parasite proteins. However, the method based on native parasite antigens has certain limitations, which is why recombinant parasite proteins are of great interest as an alternative to the currently used antigen preparation.

The above problems motivated the research presented in this doctoral thesis, which aimed to evaluate the immunogenic and immunoprotective properties of recombinant *T. gondii* antigens and to determine their usefulness in the serodiagnosis of toxoplasmosis.

A total of 18 newly developed recombinant chimeric proteins and one single *T. gondii* antigen were tested to detect specific IgG antibodies in sera from infected humans and animals. Most of the developed proteins showed high diagnostic

potential, achieving 100% sensitivity and specificity in an indirect ELISA test. The effectiveness of the tested antigens in detecting IgG antibodies was similar or the same as that of native parasite antigens currently used in diagnostics.

The immunogenic and immunoprotective properties of recombinant chimeric *T. gondii* proteins composed of 3 or 4 different parasite antigens: SAG2-GRA1-ROP1 (SGR) and SAG1-MIC1-MAG1-GRA2 (SMMG) were also tested. A mouse model of experimental toxoplasmosis was used for this purpose. It was demonstrated that these proteins, administered with a safe adjuvant equivalent to an adjuvant approved for human use, are highly immunogenic and induce a long-lasting mixed Th1/Th2 post-vaccination response, leading to significant inhibition of parasite development after infection of immunised animals.

The promising results obtained for the recombinant SGR and SMMG antigens led to the development of DNA vaccines based on plasmids encoding these proteins. The animals were immunised with recombinant plasmids, alone or in combination with the developed liposomal carriers, to evaluate the efficacy of vaccination. The tested DNA vaccines did not show as high efficacy as recombinant proteins, and ultimately a significantly lower level of protection against *T. gondii* invasion was obtained.

Of the 18 newly developed recombinant chimeric antigens used for diagnostic testing, five were selected to determine their immunogenic and immunoprotective properties in a mouse *in vivo* model. The vaccines were highly effective, eliciting a mixed Th1/Th2 response and significantly inhibiting parasitic invasion in vaccinated mice. Trivalent chimeric antigens were also found to be superior to divalent one.

The studies on the efficacy of vaccination with recombinant antigens were further extended with a panel of *in vitro* tests, including the activation of innate response mechanisms, in order to comprehensively determine the immunogenicity of a given antigen, which may in the future enable the effective preselection of vaccine antigens.

Based on the results obtained, antigens with high diagnostic utility and high immunogenic potential were selected, which may form the basis for further research aimed at increasing their effectiveness, which in the future may contribute to the

improvement of methods used to detect *T. gondii* infection and the development of effective immunoprophylaxis against toxoplasmosis.

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