

Purification and Identification of *Neospora caninum* Membrane Glycoconjugates

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Neospora caninum is an obligate intracellular parasite of great importance in veterinary medicine, causing neuromuscular disorders in dogs, abortion in cows and economic impact. In neosporosis, pathogenic mechanisms are closely related to interactions between host cell surface receptors and parasite antigenic molecules. In previous works developed by our laboratory, we found that primary cultures of glial cells, when infected by *N. caninum* tachyzoites, release high levels of anti-inflammatory cytokines. Nowadays, glycoconjugates are well described in many protozoans and pointed an immunomodulation role of these molecules in different parasitic infections. The goal of this study is to purify and identify glycoconjugates of *N. caninum*, in order to clarify their role in the immune response in central nervous system to this parasite. Tachyzoite of *N. caninum* were lyophilized and subjected to organic solvent extraction with ten volumes of chloroform-methanol-water 1:2:0,8. The pellet was dried, extracted with an aqueous solution of 9% butanol, centrifugated and the supernatant was dried, resuspended in milli-Q water and purified by hydrophobic chromatography, to obtain GPI-anchored proteins. Free GPI anchors were obtained by treating the organic solvent extraction supernatant with ten volumes of water:butanol 1:1 and purified in a octyl-sepharosis chromatography column. GPI anchors and GPI-anchored proteins are promising molecules in the modulation of the inflammatory response in nervous tissue, participating in the immunopathogenesis of neosporosis. Furthermore, there are perspectives of their employment in the diagnosis and prevention of this infection.

Key word: *Neospora caninum*, glial cells, and glycosylphosphatidylinositol