HETEROLOGOUS EXPRESSION OF TOXOPLASMA GONDII DENSE GRANULE PROTEIN 2 AND 5

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Abstract. Toxoplasmosis is a foodborne disease caused by Toxoplasma gondii, an obligate intracellular parasite. The parasite remains protected within a parasitophorous vacuole (PV), a specialized compartment formed within the infected host cell during and after invasion. Dense granules (GRA) are T. gondii specialized secretory organelles involved in PV development. GRA2 contributes to the formation of intravacuolar network in the PV, allowing nutrients transportation to nourish the parasites. GRA5 helps to inhibit apoptosis of the infected cells thereby protecting the parasites. As such, these two essential antigens have been selected as the target subjects. Heterologous expression in E. coli BL21 pLysS (DE3) of GRA2 and GRA5 fragment was achieved by transfecting with recombinant expression GRA2- and GRA5-pRSET B plasmid, respectively. His-tagged recombinant proteins were affinity purified using a Nickel-nitrilotriacetic acid column. The identities of recombinant rGRA2 (30 kDa) and 5 (20 kDa) proteins were confirmed by western blotting using immune serum from a patient with toxoplasmosis and by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. The purified T. gondii antigens provide candidates for future development of diagnostic kits of human infection as well as vaccines.

Keywords: Toxoplasma gondii, GRA2, GRA5, heterologous expression, western blot