Extracellular vesicles from *Toxoplasma gondii* and interaction with host immune response

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Toxoplasmosis is a cosmopolitan infection acquired via ingestion of oocysts produced by protozoan *Toxoplasma gondii*. This infection can cause severe neonatal malformations, ocular complications, and encephalitis. Parasites can modulate the host immune response using tools for molecular invasion as mechanisms for persistence of the infection such as excretory/secretory antigens. These antigens are released by tachyzoites and possibly can be inside nanoparticles. Extracellular vesicles (EV) are nanoparticles highly conserved and stable. They are involved in an important role such as gene expression and regulation. This study was aimed to investigate whether tachyzoites are able to release EV and characterize part of the molecules that comprise EV. Tachyzoites (RH strain) were grown and maintained in VERO cells. Aliquots of 1 x 10^9 tachyzoites were used for EV release in culture medium. After 5 washes with PBS, parasites were incubated with RPMI medium for 24 hours at 37º C in CO_2 for EV release. The recovery EVs were filtered and isolated to analyses in Nanosight Nanoparticle Tracking. The results showed high concentration of particles/mL with size 133 nm. The size of these nanoparticles was similar than those EVs excreted by other organisms. EV purification was done in gel-exclusion chromatography (Sepharose Colum). Fractions containing tachyzoite EVs were screened by ELISA using as antibody, a pool of 5 seropositive human sera for toxoplasmosis. As negative control, in the same reaction, fractions were tested with a pool of 5 seronegative sera for toxoplasmosis. Analyses in transmission electronic microscopy of reactive fractions confirmed the presence of numerous vesicles, with typical size, features and morphology of EVs from other mammalian and non-mammalian cells. The analyses using Bioanalyzer equipment detected the presence of small RNAs/miRNA in tachyzoite EVs with a ratio of around 54% (small RNA/miRNA). Similar electrophoretic profile was produced when total RNA extraction was performed with entire tachyzoites (1 x 10^8) and this RNA were diluted 200 times: 357.50 pg/µL and 195 pg/µL (55%) for small RNAs and miRNA respectively. Pooled EVs were loaded on 10% SDS-PAGE gel, transferred onto membranes, and used as antigen for immunoblot. A total of 4 sera was used as antibodies. A human seropositive serum (chronic infection) and 3 pooled serum from mice infected with RH, VEG and ME-49 strains. The results showed that antibodies recognize different epitopes in EVs, according to the stage of the disease and the infected strain. These results indicate the presence and release of EVs by tachyzoites. Initial

findings suggest that these nanoparticles contain miRNAs and probably participate in parasite internalization excreting/secreting different antigens that modulate the host immune response as described in EVs produced by other parasite models.

Keywords: *Toxoplasma gondii*, miRNA, tachyzoites, extracellular vesicles, immune response