Purification and characterization of a lytic factor having phospholipase A2 like activity in Trichomonas vaginalis
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Abstract:
T vaginalis produces cytotoxic factors that have been reported to have the ability to damage target cells in vitro. It has been hypothesized that these factors may play a role in the pathogenesis of human trichomoniasis. Parasite extracts were subjected to octyl-Sepharose and gel filtration chromatography to purify a lytic factor (LF) from T. vaginalis, which was further subjected to biochemical tests to determine the molecular characteristics of LF. LF was cytotoxic to WEHI164 cells and bovine red blood cells and inactivation of the LF by treatment with trypsin (130 μg/ml), suggested cytotoxicity was due to a protein. Results of a fluorescence assay in which carboxyfluorescein (CF) labeled liposomes composed of phosphatidylcholine: cholesterol (1:1 ratio) showed that liposomes were hydrolyzed by LF suggesting that LF has phospholipase activity. LF hydrolyzed BODIPY-labeled phosphatidylcholine into components resolved by thin layer chromatography (TLC), providing further evidence of phospholipase A2-like (PLA2) activity. A monoclonal antibody (mAb), 11-5-14, was identified that was specific for an epitope on the 220 kDa protein, which hydrolyzed BODIPY labeled phosphatidylcholine into components resolved by TLC. Flow cytometry analysis using mAb 11-5-14 revealed LF is internally expressed in the parasite and is not expressed on the parasites surface. Flow cytometry and ELISA analysis further revealed increased expression of LF when T. vaginalis was stimulated with fixed WEHI164 cells. T vaginalis appears to store LF in cytoplasmic vesicles that are disrupted when parasites are treated with Brefeldin A (BFA) and BFA-treated T. vaginalis incubated on fixed WEHI164 cells showed a decrease in cytotoxicity when subsequently assayed on live WEHI164 cell targets. These results suggest that LF is a virulence factor of T. vaginalis that may be important in the destruction of host cells contributing to tissue damage and inflammation in trichomoniasis.