John C. Samuelson

Grant Title: Roles for β-1,3-glucan and acid-fast lipids in oocyst walls of *Toxoplasma*, *Eimeria*, and *Cryptosporidium*

Abstract

This was the final collaboration between the principle investigator John Samuelson and Phillips Robbins, who recently retired after >50 year career as a glycobiologist.

Objectives: Coccidian parasites cause life threatening diarrhea in infants (*Cryptosporidium parvum*) and disseminated infections in fetuses and immunosuppressed adults (*Toxoplasma gondii*). Oocysts (walled forms) are spread by the fecal-oral route. Our goal was to determine the structure and composition of the oocyst wall of each parasite.

Methods: *Toxoplasma* oocysts were isolated from infected cats, while *Cryptosporidium* oocysts (from calves) were obtained from a commercial supplier. Transmission electron microscopy of sectioned parasites and negative stains of isolated walls were used to determine the structure of oocyst walls of each parasite. Fluorescence microscopy was used to identify fibrils of β-1,3-glucan (anti-glucan antibody or macrophage dectin-1), acid-fast lipids (auramine-O), and proteins (antibodies to glucan hyrolases or to abundant wall proteins). Mass spectrometry was used to identify glucans, acid-fast lipids, and proteins from oocyst walls.

Results: We showed that β-1,3-glucan, the major sugar polymer of fungal walls, is present in *Toxoplasma* oocyst walls. A glucan hydrolase of *Toxoplasma* has a unique glucan-binding domain and is present in the oocyst wall. Fibrils of β-1,3-glucan form a porous scaffold on the inner layer of the *Toxoplasma* oocyst wall Glucan synthase inhibitors (echinocandins) kill fungi but arrest the development of oocysts. Deletion of the glucan synthase gene of *Toxoplasma* has no effect on tissue cyst formation *in vitro*, suggesting echinocandins cannot be used to treat human infections. *Cryptosporidium* lacks β-1,3-glucan but appears to have fibrils of a novel sugar polymer on the inside of its oocyst wall.

We showed that the oocyst walls are acid-fast, as are mycobacteria walls. Organic solvents disrupt a rigid lipid bilayer on the surface of the *Cryptosporidium* oocyst wall and the outer acid-fast layer of *Toxoplasma* oocyst wall. Oocyst wall lipids are triglycerides that contain long chain fatty acyl chains like mycobacteria (*Cryptosporidium*) or polyhydroxy fatty acyl chains like the cuticle of plants (*Toxoplasma*).

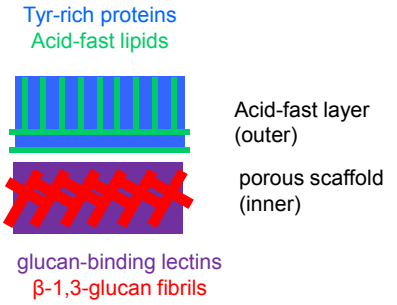

Fig. 1. Model of the *Toxoplasma* oocyst wall.

We propose a two-layer model for oocyst wall, in which the outer layer contains acid-fast lipids, while the inner layer contains fibrils of β-1,3-glucan (*Toxoplasma*) or of a novel sugar polymer (*Cryptosporidium*) (Fig. 1).

Publications:

1: Samuelson J, Bushkin GG, Chatterjee A, Robbins PW. 2013. Strategies to discover the structural components of cyst and oocyst walls. Eukaryot Cell 12:1578-87.

2: Bushkin GG, Motari E, Carpentieri A, Dubey JP, Costello CE, Robbins PW, Samuelson J. 2013. Evidence for a structural role for acid-fast lipids in oocyst walls of *Cryptosporidium*, *Toxoplasma*, and *Eimeria*. MBio 4:e00387.