

1: Structure and Development of Oocyst and Sporocyst Walls - John Samuelson

Cryptosporidium SpringerLink - analysis of the *Cryptosporidium* oocyst wall infectivity of *Cryptosporidium parvum* oocyst in Structural physiology of the.

Costello Find articles by Catherine E. Robbins Find articles by Phillips W. This is an open-access article distributed under the terms of the Creative Commons Attribution-Noncommercial-ShareAlike 3. This article has been cited by other articles in PMC. Eimeria is a major pathogen of commercial chickens. Oocysts, which are the infectious form of *Cryptosporidium* and *Eimeria* and one of two infectious forms of *Toxoplasma* the other is tissue cysts in undercooked meat, have a multilayered wall. Here we present evidence for a structural role for lipids in the oocyst walls of *Cryptosporidium*, *Toxoplasma*, and *Eimeria*. Briefly, oocyst walls of each organism label with acid-fast stains that bind to lipids in the walls of mycobacteria. Polyketide synthases similar to those that make mycobacterial wall lipids are abundant in oocysts of *Toxoplasma* and *Eimeria* and are predicted in *Cryptosporidium*. The outer layer of oocyst wall of *Eimeria* and the entire oocyst wall of *Cryptosporidium* are dissolved by organic solvents. Oocyst wall lipids are complex mixtures of triglycerides, some of which contain polyhydroxy fatty acyl chains like those present in plant cutin or elongated fatty acyl chains like mycolic acids. We propose a two-layered model of the oocyst wall glucan and acid-fast lipids that resembles the two-layered walls of mycobacteria peptidoglycan and acid-fast lipids and plants cellulose and cutin. **IMPORTANCE** Oocysts, which are essential for the fecal-oral spread of coccidia, have a wall that is thought responsible for their survival in the environment and for their transit through the stomach and small intestine. We show here that all oocyst walls are acid fast, have a rigid bilayer, dissolve in organic solvents, and contain a complex set of triglycerides rich in polyhydroxy and long fatty acyl chains that might be synthesized by an abundant polyketide synthase. These results suggest the possibility that coccidia build a waxy coat of acid-fast lipids in the oocyst wall that makes them resistant to environmental stress.

Introduction

Coccidian parasites make infectious walled oocysts that are spread by the fecal-oral route 1. *Toxoplasma gondii*, a zoonotic coccidian of worldwide distribution, makes oocysts with a double-layered wall that are shed by cats. Once shed in the environment, *Toxoplasma* makes a sporulated oocyst that contains two-walled sporocysts, each of which contains four sporozoites that infect humans and other warm-blooded animals 2. In immunocompetent persons, acute *Toxoplasma* infections are controlled, but the parasite remains within cysts in brain and muscle, which are not symptomatic. In contrast, *Toxoplasma* causes disseminated infections in fetuses and in AIDS patients who lack cellular immunity 3. However, *Eimeria* is limited to a specific animal and specific region of the gut. For example, *Eimeria tenella* is confined to ceca of chickens, where it causes dysentery and costs billions of dollars worldwide 5. *Cryptosporidium parvum* causes diarrhea in people and in livestock. Recently *Cryptosporidium* has been found to be among the four most important causes of moderate to severe diarrhea in children in the developing world 6. *Cryptosporidium* makes a different oocyst than those of *Toxoplasma* and *Eimeria*, which does not contain sporocysts and has a simpler wall 7. A parasite glucan hydrolase has a unique glucan-binding domain and is present in the inner layer of the oocyst wall. Echinocandins, which are inhibitors of fungal glucan synthases, arrest development of the *Eimeria* oocyst wall and inhibit release of oocysts into the intestinal lumen of chickens. Prior to the identification of the human immunodeficiency virus HIV, AIDS was diagnosed by the presence of opportunistic infections, such as *Cryptosporidium*, which was detected in stools by an acid-fast stains Fig. The goal here was to determine the structural role, if any, of acid-fast lipids in oocyst walls of *Cryptosporidium*, *Toxoplasma*, and *Eimeria*. As background, the cell walls of mycobacteria are acid-fast i. S1 in the supplemental material 12, Among the best-characterized mycobacterial wall lipids are mycolic acids, which are synthesized in part by polyketide synthases

2: Structural Physiology of the Cryptosporidium Oocyst Wall : H. Ward :

This project develops probes to identify and assess the relative roles of proteins, carbohydrates, and lipids in the overall integrity and resistance of the Cryptosporidium oocyst wall for the assessment of disinfection of the oocyst. The experiments use a panel of lectins with varying sugar.

Methods Smears of formalin-fixed feces containing Cryptosporidium oocysts were examined with U2B and 3 CFW products under a variety of conditions of concentrations, pH, staining times, and temperatures. Results Under the conditions that a wide range of fungal and parasite structures, including spores of the microsporidia, fluoresce well with CFW and U2B ie, 0. Conclusions Any of the FB reagents tested could be used as a simple and rapid stain procedure for Cryptosporidium oocysts in fecal smears. Gastrointestinal cryptosporidiosis is an infection caused by the protozoan parasite Cryptosporidium. Infection in immunocompetent persons may range from asymptomatic to clinical illness with watery diarrhea, abdominal cramping, low-grade fever, nausea, and vomiting. In patients infected with the human immunodeficiency virus HIV , infection may result in a life-threatening diarrhea. Transmission is fecal-oral, with the infectious dose being as low as 10 to 30 oocysts in immunocompetent persons. It has been reported that the oocysts do stain with a modified trichrome stain procedure used for microsporidial spores. In a study of the use of the fluorescent brightener Uvitex 2B Fluorescent Brightener to detect microsporidial spores in stool samples, van Gool and colleagues found the presence of formalin as a fixative in the specimens led to a considerable reduction in the fluorescence intensity of stained spores. Material and Methods Fecal Specimens A total of 15 fecal specimens were examined. The most recent specimen had been in formalin for 9 days; the oldest specimen had been in formalin for several years. All FB solutions were stored in the dark at RT when not in use. Staining Procedures The procedure of van Gool and colleagues: The 30-second staining with 0. An advantage of covering the smears over the stain solution is that, providing the preparations are not allowed to dry, staining times can be extended indefinitely if no staining is found on initial examination of the preparations. Same as procedure 2 except the FB concentration was 1. Same procedure as 4 except the FB concentration was 1. Slides with air-dried smears were placed in the stain solution for 5 minutes. Excess stain solution was drained off and the smears covered. Same procedure as 6 except the staining solution was equal volumes of 0. Slides were covered with a Petri dish lid to reduce evaporation. All 15 fecal specimens were then tested with those staining procedures that resulted in oocyst fluorescence. To investigate the reported adverse effect of formalin on the fluorescence of U2B, 0. Both of these formalin solutions are stabilized with methanol. Both were tested immediately after preparation and then again after storage at RT in the dark for 6 weeks. For silver staining for mucopolysaccharides, smears were made from specimen ODH and a random selection of 7 of the other 14 specimens, air dried, and stained with the Grocott methenamine silver GMS stain. Smears were examined with an epifluorescence Zeiss microscope with a 50-watt mercury lamp and excitation filter of 365 to 405 nm band pass violet light with 365 nm and 405 nm barrier filters. Staining Controls For positive controls, the following were used: In addition to these, some of the fecal specimens contained yeasts and various plant cells and structures that also served as positive staining controls and reference material for fluorescence intensity. No reduction of the staining and fluorescence intensity was found even after these 2 solutions were 6 weeks old. Image 1 Oocysts of Cryptosporidium. Image 2 Oocysts of Cryptosporidium, several of which show only 1 or 2 speckles or granules A. The brightly fluorescing oval structure Y is a yeast. Image 3 Oocysts of Cryptosporidium showing sausage-shaped internal structures and bright speckles or granules. Image 4 Oocysts of Cryptosporidium showing a range of fluorescence intensity brightness from a few speckles A to very bright, solid fluorescence B. Image 5 Oocysts of Cryptosporidium showing a few large, bright speckles or granules. Modified Kinyoun acid-fast stain x.

STRUCTURAL PHYSIOLOGY OF THE CRYPTOSPORIDIUM OOCYST WALL

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