The Structural Analysis of Oocyst Walls of Cryptosporidium, Toxoplasma, and Eimeria with Mass Spectrometry and Microscopy

View Presentation Detail

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Institutes

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Introduction

Toxoplasma gondii is a protozoan parasite that is infectious to humans and other animals, with the cat being the primary host. The oocysts of *Toxoplasma* gondii are shed in cat feces and after ingestion can cause severe infections in fetuses and in patients with AIDS. *Cryptosporidium* spp. cause diarrhea in humans and animals. *Eimeria* is a major pathogen of commercial chickens. Although numerous proteins have been identified in oocyst walls, there is no model for the wall structure of these organisms.

Methods

Enter MethUnsporulated oocysts of *Toxoplasma* and *Eimeria* were prepared from infected cats and chickens, respectively. Oocysts of *Cryptosporidium*, which had been passaged through newborn calves, were purchased. Oocysts of each species were purified with a discontinuous CsCl2 gradient. Oocysts walls were broken with glass beads and washed with HPLC-grade H2O. For analysis of sugar polymers, one fraction was treated with NaOH to remove the proteins, ?-glucans were digested with zymolyase, and released sugars were examined by MALDI-TOF MS and GC/MS. For lipid analysis, another wall fraction was extracted in 2:1 chloroform/methanol overnight, and analyzed with a Bruker 12-T solariX hybrid Qq-FTICR MS. For protein analysis, a third wall fraction was digested with trypsin and analyzed with a Thermo-Fisher LTQ-orbitrap MS.ods

Preliminary Results/Abstract

Using mass spectrometry, fluorescence microscopy and electron microscopy, we showed that *Toxoplasma* and *Eimeria* oocyst walls have an inner layer that is a porous scaffold of fibrils of ?-1,3-glucan, the sugar polymer present in fungal walls. The ?-1,3-glucan stained with dectin and anti-?-glucan antibody. *Cryptosporidium* oocysts do not have ?-1,3-glucan and their walls did not stain with these reagents. Glucan synthase inhibitors (echinocandins), which kill fungi, arrested the development of oocyst walls of *Eimeria* and blocked the organism's release into the intestinal lumen.

We showed that the outer layer of the oocyst walls of *Toxoplasma* and *Eimeria* contain acid-fast lipids like those that cover the surface of mycobacteria. Oocyst walls these parasites and *Cryptosporidium* labeled with the fluorescent acid-fast stain auramine-O. The oocyst walls of all three parasites disintegrated when they were treated with chloroform/methanol, although these solvents have little effect on walls of fungi. FTICR-MS showed that the oocyst wall lipids are predominantly triglycerides which contain acyl chains with 18 to 22 carbons and up to five double bonds (*Toxoplasma* and *Cryptosporidium*) or are polyhydroxylated (*Eimeria*). The fatty acyl chains may be made by the polyketide synthases that are extraordinarily abundant in the oocysts and resemble polyketide synthases of mycobacteria.

Ooocyst wall proteins of *Toxoplasma* and *Eimeria* include a glucan hydrolase which contains a novel glucan-binding domain at its N-terminus. Their oocyst walls also have Cys-rich and His-rich proteins belonging to classes which were first identified in *Cryptosporidium* (COWPs) as well as Tyr-rich proteins that can form dityrosines that are autofluorescent under UV irradiation.

Our characterization of ?-1,3-glucan and acid-fast lipids in oocyst walls could represent a major breakthrough in the understanding of how these parasites make their walls, which are essential for transmission of the parasite.

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Novel Aspect

Mass spectrometry showed that Toxoplasma oocyst walls contain ?-glucans (like fungi) and acid fast lipids (like mycobacteria).