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Mini review

The parasitophorous vacuole of *Encephalitozoon cuniculi*: Biogenesis and characteristics of the host cell–pathogen interface

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SUMMARY

Microsporidia are obligate intracellular fungal pathogens of increasing importance in immunocompromised patients. They have developed a unique invasion mechanism, which is based on the explosive discharge of a hollow tubulus, the so-called polar tube. The infectious sporoplasm is subsequently extruded through this flexible tube and injected into the host cell. The model microsporidium *Encephalitozoon cuniculi* is a paradigm of a fungus with an extreme host cell dependency. This human pathogen possesses one of the smallest eukaryotic genomes (<3 MB) identified so far and has reduced its own biosynthetic pathways to a minimum, thus depending on an efficient supply of metabolites from the host cell. *E. cuniculi* spends its entire intracellular life cycle inside a parasitophorous vacuole (PV), which is formed during invasion. We have provided here an overview of the biogenesis and characteristics of this important host cell–pathogen interface and suggest in this context a modified model for *E. cuniculi* invasion. According to the model, the host cell plasma membrane is not pierced by the polar tube, but is pushed at the contact site into the cell interior by the mechanical force of the expelled polar tube. This results in a channel-like invagination of the plasma membrane, from which finally the parasitophorous vacuole is pinched-off.

Introduction

Microsporidia are a diverse group of highly adapted obligate intracellular pathogens, which can infect a broad spectrum of vertebrate and non-vertebrate hosts (Keeling and Fast, 2002). Their phylogenetic origin has been a consistent matter of debate in former decades, since they possess many unique features, which make them difficult to compare to other organisms. Initially, they were thought to lack mitochondria and were considered to be very basal eukaryotes. However, as in most of the former amitochondriate protozoa, small and highly reduced mitochondria, the so-called mitosomes, were identified (Williams et al., 2002; Burri et al., 2006). As in other relic mitochondria, microsporidian mitosomes are the location of iron-sulfur cluster synthesis, a pathway which apparently cannot be translocated into other cellular compartments (Williams and Keeling, 2003; Goldberg et al., 2008). It has now become clear from various genome projects that microsporidia are related to fungi (Hirt et al., 1999; Katinka et al., 2001; Lee et al., 2008; Williams et al., 2008b; Akiyoshi et al., 2009; Corradi et al., 2009). Their exact branching position within the fungal tree is not completely clarified, although there is evidence that their genome contains a sex-related locus and that they share a common ancestor with zygomycetes (Lee et al., 2008; Lee et al., 2009). Approximately 1300 microsporidia species in 160 genera are described, some of them are known as emerging pathogens, particularly for immunocompromised patients (Didier and Weiss, 2006).

Enterozytozoon bieneusi is the most commonly detected microsporidium species in humans, followed by three species of the genus *Encephalitozoon*, namely *E. intestinalis, E. cuniculi* and *E. hellem* (Mathis, 2000; Didier, 2005). *E. bieneusi* causes diarrhea which is normally self limiting, but can get chronic in immunocompromised patients. Research on *E. bieneusi* is extremely hampered by the lack of efficient methods to cultivate this organism in tissue culture. *Encephalitozoon* species, particularly *E. intestinalis* also cause diarrhea, but in contrast to *E. bieneusi,* microsporidia of this genus can be treated with allbendazole (Costa and Weiss, 2000; Gross, 2003). In addition to gut infections, *E. cuniculi* and *E. hellem* are also known to cause disseminated infections with multiple organs being infected (Weber et al., 1994; van Gool and Dankert, 1995).

Infection mode and life cycle of microsporidia

The infectious agent of microsporidia is an environmentally resistant spore, which is surrounded by a rigid spore wall composed of an outer proteinaceous layer and an inner layer that

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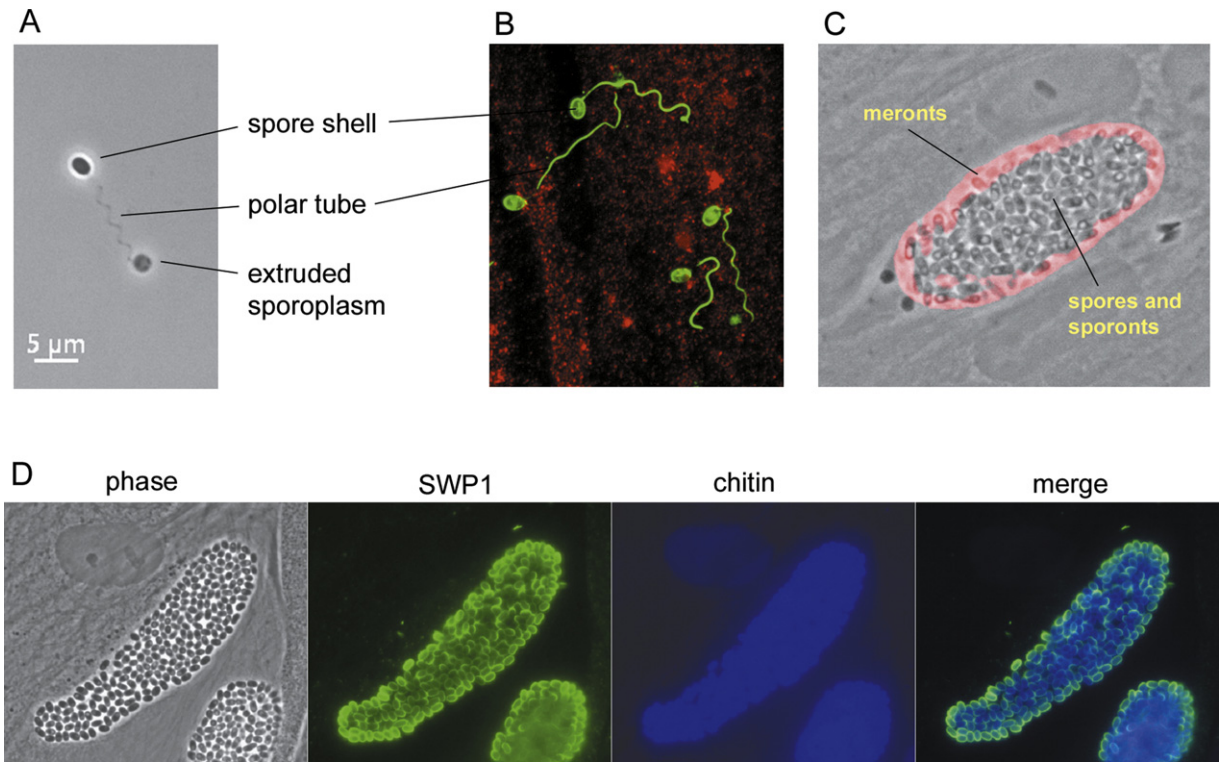

Fig. 1. Developmental stages of *E. cuniculi*. A. Germinated extracellular spore with discharged polar tube. B. Detection of extracellular spores by immunofluorescence staining. C. Intracellular PV with an outer ring of meronts and spores and sporonts in the center. D. Double-immunofluorescence staining of an intracellular PV. Chitin was detected by blankophor staining, the spore wall antigen SWP1 was detected using monoclonal antibody 11A1.

contains chitin (Bigliardi et al., 1996). The rigid spore wall protects extracellular spores against stress situations and permits long-term survival in the environment. As for other fungi, optical brighteners can be used to stain and detect microsporidia in tissue sections or samples by intercalation into the chitin layer (Vavra et al., 1993; Franzen et al., 1995) (Fig. 1).

Microsporidia have evolved a highly specialised invasion mechanism which is unique in nature. The central element for invasion is the polar tube, a filamentous, hollow tube-like structure, composed of proteins (Xu and Weiss, 2005; Delbac and Polonais, 2008). The polar tube is coiled within the interior of the spore and attached to a cellular structure termed the anterior disk. After a trigger, which might involve changes in pH and ion concentrations, a sudden rise of the osmotic pressure occurs, which in some, but not all microsporidia species is mediated by the cleavage of trehalose (Undeen and Frixione, 1990; Keohane and Weiss, 1998). The sudden rise of the osmotic pressure mediates a unidirectional flow of water inside the spore through recently characterised aquaporins (Frixione et al., 1997; Ghosh et al., 2006). This process increases the mechanical pressure inside the spore and finally leads to the breakage of the spore wall at a defined position followed by the explosive discharge of the polar tube, a process known as germination (Bigliardi and Sacchi, 2001) (Fig. 1). In the classical model of microsporidia invasion the tip of the polar tube needs to pierce the host cell plasma membrane during germination. The nucleus-containing sporoplasm is subsequently extruded through the hollow polar tube and injected into the host cell cytoplasm (Williams, 2009).

At the early phase of intracellular development microsporidia undergo a proliferative phase in which morphologically simple structured cells, termed meronts, replicate by binary or multiple fission. Meronts lack typical structures of mature spores, such as the spore wall or the polar tube, and subsequently differentiate into sporonts and later into mature spores (Mathis, 2000; Metenier and Vivares, 2001), the latter being thought to be metabolically dormant. During this development, the morphology is dramatically changed: the organism builds up the rigid spore wall by deposition of electron-dense material at the plasma membrane and acquires new morphological structures such as the polar tube. The transcriptional profile changes during differentiation, and several genes were shown to be stage specifically regulated during meront-to-sporont differentiation, for example genes for spore wall proteins, chitinases, chitin synthases and subtilisin proteases (Bohne et al., 2000; Rönnebaumer et al., 2006).

Beside the extrusion-based active invasion mechanism, microsporidia are also internalized into host cells by phagocytosis. Interestingly, phagocytosis of microsporidia spores is a common event and *Encephalitozoon* species were shown to be efficiently phagocytosed not only by professional phagocytes, but also by non-professional phagocytes such as MRC5 and 293 cells (Couzinet et al., 2000). It was recently proposed that spores might not only germinate from an extracellular location but also after the phagocytic uptake out of phagosomes, thereby escaping phagolysosmal digestion (Franzen, 2004; Franzen et al., 2005). However, when phagocytic uptake was inhibitied in J774 cells by cytochalasin D treatment, the number of established intracellular *E. cuniculi* meronts was identical to untreated controls, suggesting that germination out of phagosomes does not significantly contribute to *E. cuniculi* infection (Orlik et al., 2010).

E. cuniculi – the model microsporidian

In contrast to *E. bieneusi*, the three human pathogens of the genus *Encephalitozoon* can easily be cultivated in tissue culture. Particularly *E. cuniculi* is growing well in many different host–cell types and due to the fact that its genome was the first microsporidian genome which was completely sequenced (Katinka et al., 2001), it has become an accepted model. A fascinating feature of *E. cuni-*