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Short communication

Three-dimensional fine structure of feeder organelle in *Cryptosporidium parvum*

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ABSTRACT

Feeder organelles of *Cryptosporidium* are the convoluted structures located at the host-parasite interface that uptake of nutrients from host cells. Although the ultrastructure of feeder organelles has been summarized as being highly invaginated structure, the three-dimensional form remains uncertain. Osmium-maceration scanning electron microscopy (OS-SEM) allows visualization of the three-dimensional ultrastructure after removing soluble proteins. Here, we assessed *C. parvum* attached to mouse ileal epithelial cells using transmission electron microscopy (TEM) and OS-SEM. Feeder organelles visualized by TEM as aggregated structures of concentrically-, vertically- and randomly-lined bars comprised a complex reticulated network of stacked flat bursiform, ring-shaped bursiform and reticulated tubular membranes on OS-SEM. These findings suggested that the feeder organelles are more complex than was previously thought.

1. Introduction

Cryptosporidium spp. are protozoan parasites of humans and animals, and exposure to *Cryptosporidium* through contact with contaminated feces results in highly contagious, intestinal cryptosporidiosis [1,2]. In addition, *Cryptosporidium* spp. are the leading cause of the outbreaks of waterborne diseases worldwide [3]. Life cycle of *Cryptosporidium* spp. is completed in a single host, and infective sporozoites attach to and invade gastrointestinal epithelial cells to form a unique parasitophorous vacuole on top of the cells [4]. Both the parasite and the apex of the host cell change during invasion, and several specific structures appear at the host-parasite interface such as the feeder organelles [4].

Feeder organelles are convoluted structures that appear in *Cryptosporidium* spp. during invasion, and they are considered to be points of nutrient uptake from host cells [5]. Aldeyarbi and Karanis [6] revealed fine structures of *C. parvum* during *in vitro* axenic development by using transmission electron microscopy (TEM), but it has no feeder organelles during this condition. A *Cryptosporidium*-specific ATP-binding cassette, CpABC1, involved in transportation of various molecules (*e.g.* metabolites, lipids and drugs) across membranes is localized to feeder organelles [7], indicating that these organelles are important for selective nutrient absorption. Feeder organelles are formed in schizont residual bodies and seem to separate from them after merozoite formation has completed [5]. The ultrastructure of feeder organelles in *Cryptosporidium* spp. has examined using TEM in murine models of infection [8–10] and in livestock [11,12], and it has been described as “highly invaginated” [4,5]. However, their three-dimensional form and how they intake and transport molecules at an organelle level from host cells remain uncertain.

Osmium-maceration scanning electron microscopy (OS-SEM) allows visualization of three-dimensional fine structures after removing

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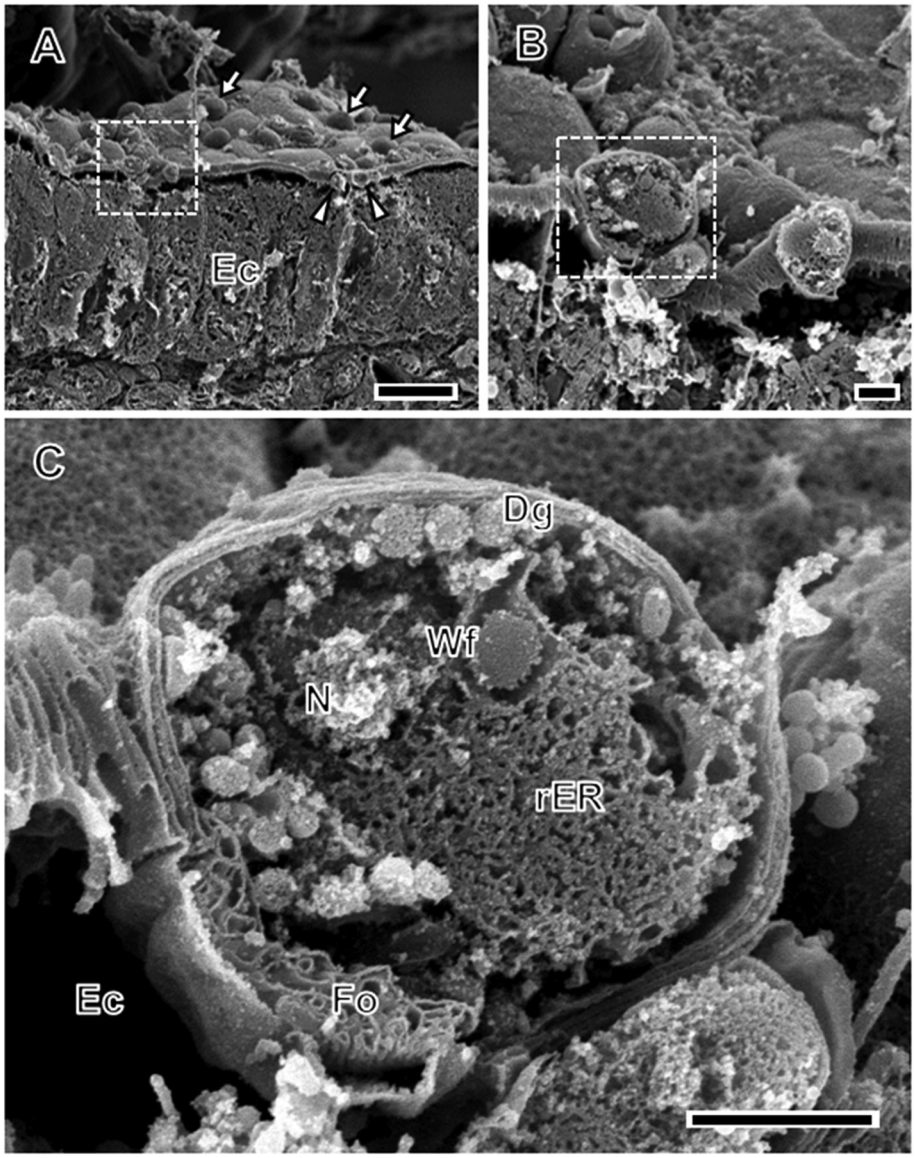

Fig. 1. Osmium-maceration scanning electron microscopic (OS-SEM) images of *Cryptosporidium parvum* attached to mouse ileal cells.
[A] Cross-section of ileal epithelium. Arrows and arrowheads indicate outer surfaces and intracellular images of parasites, respectively. [B] Higher magnified image of epithelial surface. [C] Intracellular structures of *C. parvum* schizont. Areas surrounded by dashed boxes in [A and B] corresponds to panel [B and C], respectively. Dg, dense granule; Ec, epithelial cells; Fo, feeder organelle; N, nucleus; rER, rough endoplasmic reticulum; Wf, wall-forming body. Bar = 10 [A] and 1 [B and C] μm.

soluble proteins. This modality has been applied to determine the intracellular ultrastructural details of some animal cell types [13], but it has never been applied to parasites. The present study used TEM and OS-SEM to determine the three-dimensional features of feeder organelles of *C. parvum* in immunodeficiency mice.

2. Materials and methods

2.1. Animals and tissue sampling

We inoculated SCID mice (Japan SLC Inc., Shizuoka, Japan) with 1.0×10^5*C. parvum* HNJ-1 strain oocysts (a gift from Dr. M. Matsubayashi, Osaka Prefecture University, Japan). Two weeks later, the mice were anesthetized with pentobarbital and euthanized by cervical dislocation. The terminal ileum was excised and trimmed into small blocks that were fixed in 0.1 M phosphate buffer (pH 7.4) containing 0.5% paraformaldehyde and 0.5% glutaraldehyde. This study proceeded according to the Regulations on the Management and Operation of Animal Experiments, and the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine approved the experimental protocol (Approval number 28-38).

2.2. Transmission electron microscopy

Some blocks of the colons were post-fixed with 1% OsO_4 for 30 min, dehydrated and then embedded in LR White resin for TEM. Ultrathin sections (80 nm thick) were examined using an HT7700 transmission electron microscope (Hitachi, Tokyo, Japan) without either uranyl acetate or lead citrate staining.

2.3. Osmium-maceration scanning electron microscopy

Other blocks were immersed in 1% OsO_4 for 6 h followed by dimethyl sulfoxide (25% and 50%), and frozen with a flat aluminum block precooled in liquid nitrogen for OS-SEM. The frozen blocks were broken in two using a screwdriver and a hammer. The specimens were immersed in 0.1% OsO_4 for 96 h at 20 °C, post-fixed in 1% OsO_4, stained

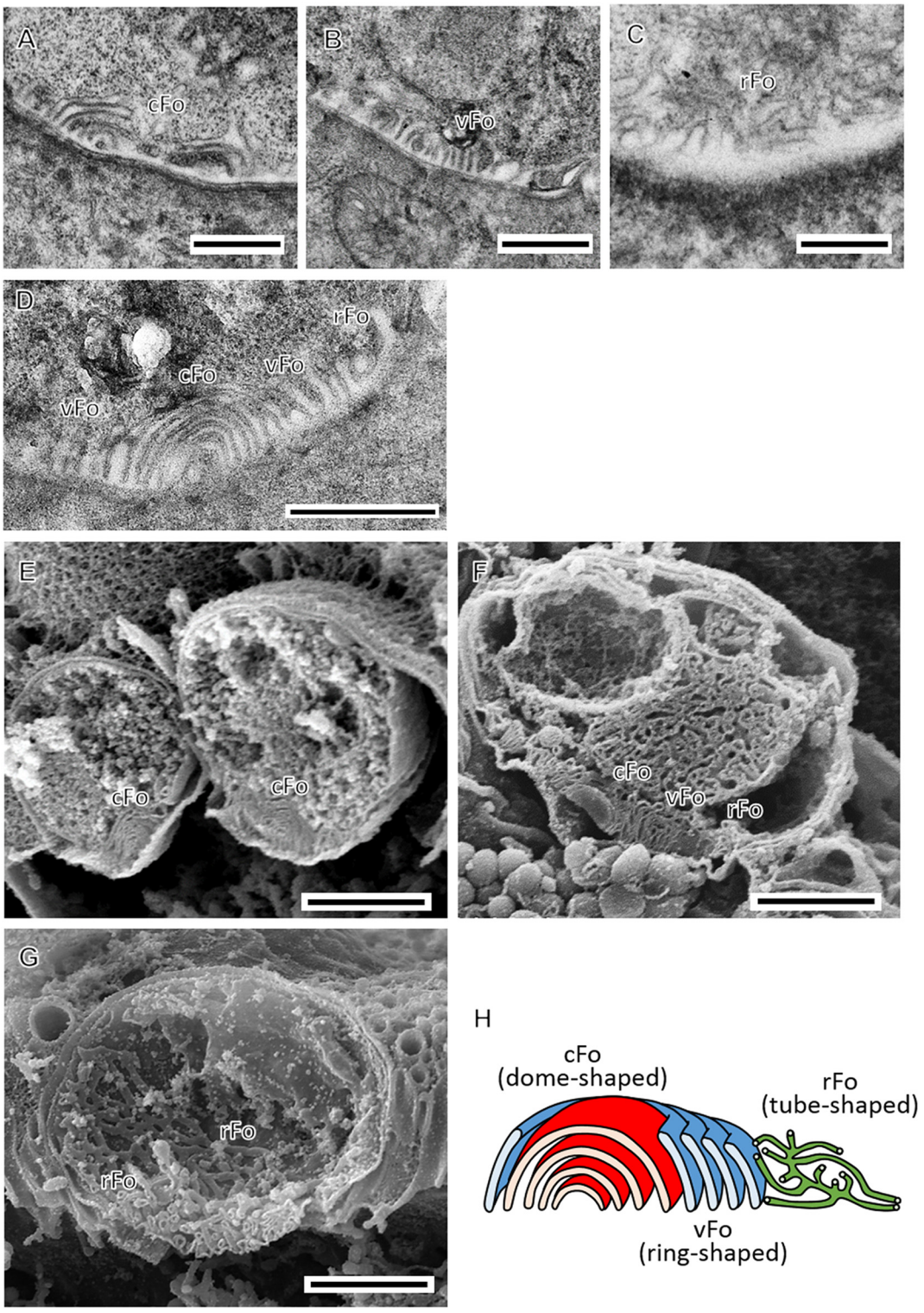

Fig. 2. Transmission electron microscopic (TEM) and OS-SEM images of feeder organelle of *C. parvum* schizont at host-parasite interface.
[A–C] TEM images of feeder organelles composed of concentrically- (cFo), vertically- (vFo) and randomly-lined (rFo) bars. [D] Representative host-parasite interface containing three types of feeder organelles. [*E*–G] OS-SEM images of feeder organelles. "Dome-shaped" bursiform membranes corresponding to cFo [E and F], "ring-shaped" bursiform membranes corresponding to vFo [F] and "tube-shaped" membranes corresponding to rFo [G] are found. [H] Schematic illustration of three-dimensional ultrastructure of feeder organelle. Bars = 500 nm [A–D] and 1 μm [E-G].

with tannic acid and 1% OsO_4, dehydrated, and lyophilized in an ES2030 freeze-dryer (Hitachi) with t-butyl alcohol. The specimens were then mounted onto a metal plate, lightly coated with platinum-palladium using an E1010 ion sputter coater (Hitachi) and evaluated using an S4100 scanning electron microscope (Hitachi). We observed total 29 schizont fractured surfaces, and the feeder organelles were evaluated from 20 schizonts in this study.

3. Results and discussion

First of all, we confirmed that the present OS-SEM visualizes the intracellular structures (Fig. 1A, arrow) as well as surface (Fig. 1A, arrowheads) of *C. parvum* attached to host cells. Parasite organelles including rough endoplasmic reticulum, some types of granules and nuclei were clearly visualized (Fig. 1B and C).

By TEM observation, feeder organelles were recognized as aggregated structures of concentrically-spread (cFo; Fig. 2A), vertically-lined (vFo; Fig. 2B) and randomly-scattered (rFo; Fig. 2C) bars at the host-parasite interface, as previously reported [6–10]. These three types of aggregations were sometimes located even in a single schizont residual body (Fig. 2D).

By OS-SEM observation, reticulated networks of stacked "dome-shaped" bursiform membranes were visualized at the host-parasite interface (Fig. 2E and F) and seemed to correspond to cFo determined by TEM. Networks of "ring-shaped" bursiform membranes concentrically surrounding "dome-shaped" membranes were also found (Fig. 2F), and these structures seemed to correspond to vFo. In addition, reticulated "tube-shaped" membranes that apparently correspond to rFo were visualized by OS-SEM (Fig. 2G), and some parts of the bursiform membranes were continuous with these tubular membranes (Fig. 2E and F). The tubular membranes were more abundant in the middle portion than at the border (Fig. 2G). These findings indicated that feeder organelles of *C. parvum* comprise a three-dimensionally complex network of membrane structures, and Fig. 2H summarizes the fine structure of a *C. parvum* feeder organelle.

Feeder organelles of *Cryptosporidium* spp. have been considered as an invaginated membrane structure that function to secure a large surface area [5]. However, the present study found that feeder organelles are not simple protrusions or lamellae, but reticular networks of tubular and bursiform membranes, suggesting that feeder organelles are more complex than was previously thought.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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