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Three-dimensional Imaging of *Toxoplasma gondii*- Host Cell Membrane Interactions Reveals Numerous Bridges and Fission Pore With High Resolution Low Voltage Field Emission Scanning Electron Microscopy on De-embedded Thick Sections

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The protozoan parasite *Toxoplasma gondii* invades host cells through an unconventional motility system and becomes engulfed in a membrane system that forms the parasitophorous vacuole after host cell invasion. The interactions between the host cell and parasite membranes are complex. Previous studies had shown that the parasitophorous vacuole is formed from the host cell membrane and pinches off via a fission pore. Recent studies have revealed that both, the host cell and the parasite membrane contribute to the formation of the parasitophorous vacuole.

By using our a new specimen preparation technique [1,2] which allows imaging of thick sections of internal cell structures with high resolution field emission scanning electron microscopy (FESEM), we were able to visualize the interactions of the host cell membrane with the parasite within the parasitophorous vacuole. Fibrous material extends from the host cell membrane toward the parasite and connects to parasite membrane components while shorter protrusions are also elaborated from the parasite. A number of these shorter fine protrusions connect to the fibrous material of the host cell membrane. The interactions between parasite and host cells become dissociated with time and only a fission pore is left that connects the parasite with the host cell. The fission pore is anchored in the host cell by thick structural components of unknown nature. We present this technique as a tool to investigate the molecular interactions between host cell and parasite membranes. Previous studies have shown that antigenicity is maintained which will allow further studies using immunogold labeling techniques.

The images in Figures 1 and 2 are of parasites residing in the parasitophorous vacuole. Figure 1 (top) depicts the fibrous network between the host cell and parasite membranes. Figure 2 (bottom) depicts a parasite in the parasitophorous vacuole with only a fission pore left that connects the parasite to the host cell membrane. The images were obtained as follows. Human fibroblasts and *Toxoplasma* parasites were cultured on coverslips, fixed with 1% glutaraldehyde in 0.1M HEPES buffer (pH 7.2) plus 0.05% saponin and 0.2% tannic acid. After washing in buffer, they were postfixed in 0.1% osmium tetroxide and 1% uranyl acetate, and embedded in Epon. 200nm thick sections were cut orthogonally to the cell substrate. Ribbons were collected on cover glass strips fitting into the Hitachi S-900 stage. The Epon was extracted from the sections with the Polysciences Epoxy Resin Removal Kit [1,2]. The extracted sections were critical point dried, and Argon ion sputter coated with a thin layer of Pt. Micrographs were obtained with the Hitachi S-900 FESEM in Madison, WI, operating at 1.5kV [3].

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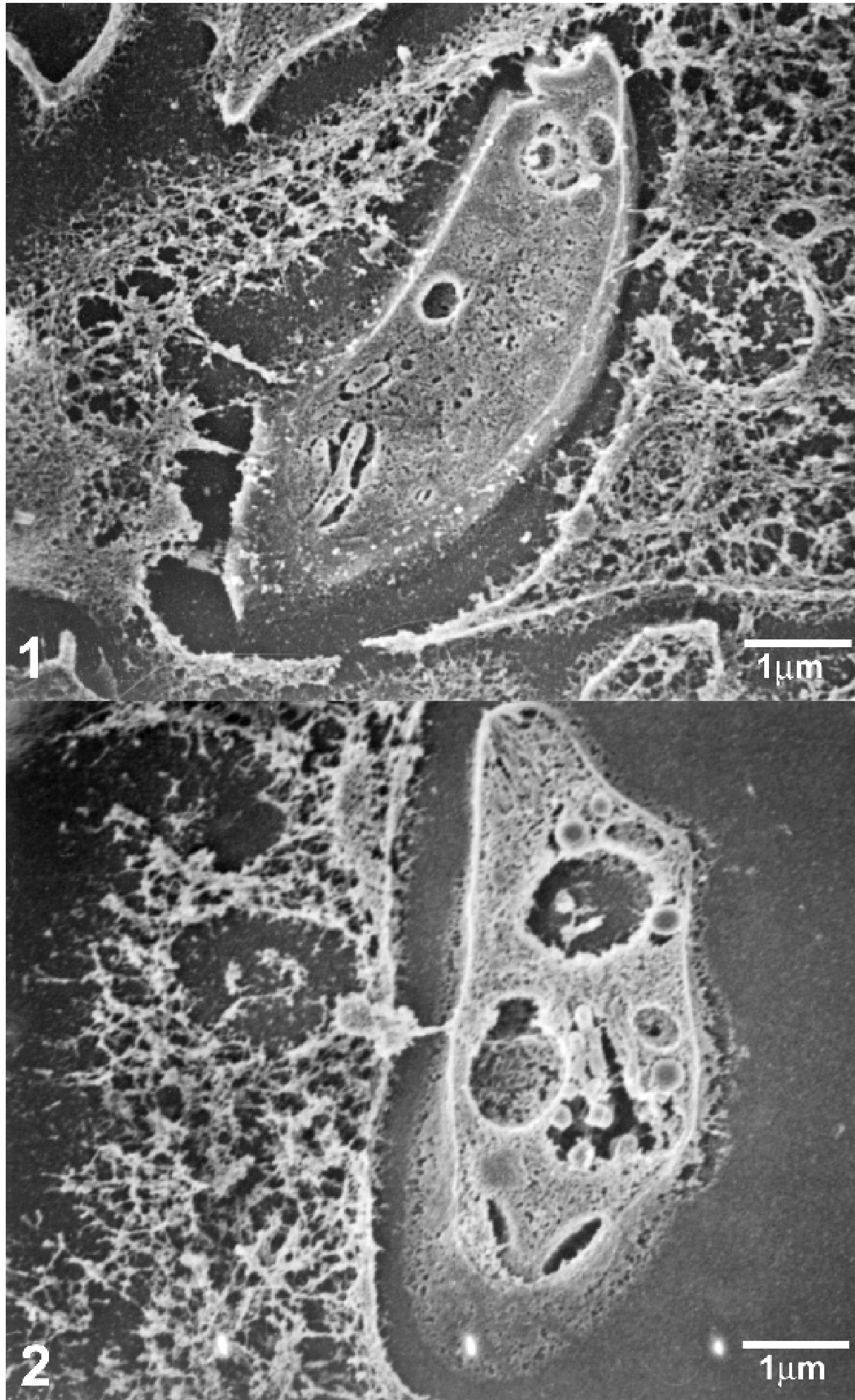

Figure 1. High resolution field emission scanning electron micrograph of Epon deembedded section. Oblique section of a parasite in the process of host cell invasion. A fibrous network connects the parasite membrane with the membrane of the host cell.

Figure 2. High resolution field emission scanning electron micrograph of Epon deembedded section. The photograph displays a parasite in the parasitophorous vacuole with only a fission pore left that connects the parasite to the host cell membrane.

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