Evidence of plasmotomy in Blastocystis hominis

Tan Tian Chye, University of Malaya

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Evidence of plasmotomy in *Blastocystis hominis*

T. C. Tan • K. G. Suresh

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Abstract *Blastocystis hominis* has been regarded as an enigmatic parasite as many aspects of its basic biology remain uncertain. Many reproductive processes have been suggested for the organism; however, to date, only the binary fission has been proven. Plasmotomy is one of the modes of reproduction previously suggested to be seen in *in vitro* cultures. The present study provides trichrome and acridine orange staining evidence for the existence of nucleic acid suggestive of division of nucleus into multinucleate forms with the respective cytoplasm dividing giving rise to two or three progeny *B. hominis*. Transmission electron micrographs further confirmed that these daughter cells had respective surrounding surface coat, mitochondria, and vacuoles.

Introduction

*Blastocystis hominis* is a highly polymorphic organism with various morphological forms being reported in the literature including vacuolar, granular, amoeboïd, cyst, avacuolar, and multivacuolar forms (Stenzel and Boreham 1996; Tan et al. 2002). The true life cycle has not been conclusively elucidated, and the modes of reproduction are still uncertain (Stenzel and Boreham 1996).

To date, a total of five life cycles have been proposed for *Blastocystis*; however, none of them have been experimentally proven mainly because of the lack of suitable animal models (Alexeieff 1911; Boreham and Stenzel 1993; Singh et al. 1995; Tan 2004). Although several methods for division of *B. hominis* cells such as binary fission, plasmotomy, schizogony, and sporogony (Zierdt 1991; Govind et al. 2002) have been described, binary fission is the only plausible mode of reproduction (Stenzel and Boreham 1996; Tan and Stenzel 2003). We previously described amoeboïd forms isolated from cultures of parasites obtained from symptomatic patients (Tan and Suresh 2006), suggesting its role in contributing toward pathogenicity. In this present study, we further provide evidence for plasmotomy to be one of the reproductive processes involved in *B. hominis* as suggested by others (Zierdt 1991; Zaman 1997; Zhang et al. 2007), whose evidence were only confined to light microscopical observations.

Materials and methods

Source of *B. hominis* isolates

In our previous study (Tan and Suresh 2006), the amoeboïd form of *B. hominis* was seen in ten symptomatic isolates. Three out of the ten isolates, i.e., isolates S1, S8, and S10, were randomly selected for detailed study to assess if plasmotomy occurs in *B. hominis*.

In *in vitro* culture of *B. hominis* isolates

The parasites were isolated from patients by *in vitro* cultivation using Jones’s (1946) medium supplemented with 10% horse serum (Suresh et al. 1994a; Zaman 1997). Subsequently, after isolation, the parasites were maintained in Jones’ medium by consecutive subculture every 3–4 days.
for at least 1 month before the morphological and ultrastructural studies.

Trichrome staining

The trichrome technique of Wheatley (1951) was used. Briefly, a direct smear was prepared from days-2 to -6 cultures of each isolate. The smears were air-dried at room temperature and subsequently fixed in Schaudinn’s fixative for at least 1 h at room temperature. The fixed smears were dipped through three changes of 70% ethanol for 1 min each (iodine was added to the first change of 70% ethanol) and followed by staining in trichrome stain for 2–8 min. The stained smears were differentiated in 90% ethanol containing 0.005% glacial acetic acid for 10–20 s followed by a brief rinse in 95% ethanol. The smears were transferred into 100% ethanol (1 min) and then xylene (1 min). The stained smears were eventually mounted with DePeX mounting medium.

Acridine orange staining

Parasites from days-2 to -10 culture of each isolate were stained with acridine orange solution according to the method previously described by Suresh et al. (1994b). Briefly, 5 ml of 0.1% acridine orange stock solution was diluted with 45 ml of phosphate buffered saline pH 7.4 before use. A drop of culture sediment containing parasites was mixed thoroughly on a clean glass slide with a drop of diluted acridine orange. The preparation was viewed with a fluorescence microscope (Leitz Wetzlar, Germany) using incident light transmission at ×400 magnification.

TEM study

The contents from the day-5 culture were washed three times using PBS, pH 7.4, and centrifuged at 500×g for 5 min. The pelleted cells were resuspended overnight in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 at 4°C, washed thoroughly with cacodylate buffer, and postfixed for 30 min in 1% osmium tetroxide in cacodylate buffer. The fixed cells were dehydrated in ascending series of ethanols and embedded in epoxy resin. Semithin sections were stained with toluidine blue. Ultrathin sections were cut using an ultramicrotome, contrasted with uranyl acetate and lead citrate and viewed using a TEM (LEO Libra 120).

Results

The amoeboid form of *B. hominis* was irregular in shape with a prominent nucleus at the central zone and multiple extended pseudopodia at the periphery (Fig. 1a). Phase
contrast microscopy revealed the pinching of cytoplasm from the body of the irregular amoebic forms (Fig. 1a). Ultrastructural studies using TEM showed that a long irregular mass of cytoplasm was seen to be pinched off from the mother cell with electron dense surface coat surrounding the prospective progenies of \textit{B. hominis}. The body of the mother cell showed intense electron dense material. These progenies further showed multiple vacuoles and mitochondrion (Fig. 1b).

Under trichrome staining, the cell body of the vacuolar form of \textit{B. hominis} is stained green, while the nuclei at the peripheral region is stained reddish brown (Fig. 2a).

\textbf{Fig. 2} Trichrome staining of \textit{B. hominis}. \textbf{a} The central zone of the vacuolar form of \textit{B. hominis} is stained green, while the peripheral nuclei is stained reddish brown. \textbf{b} The central zone of the amoeboid form is stained with intense reddish brown, while the peripheral region is stained green. Note: Three prospective daughter cells appear to pinch out from the mother cell. Reddish brown material is seen in the periphery of one of the prospective daughter cells (arrow). Bar, 20 \( \mu \text{m} \)

\textbf{Fig. 3} Acridine orange staining of \textit{B. hominis}. \textbf{a} The nuclei and central body of the vacuolar form of \textit{B. hominis} is stained bright and dull yellow-green, respectively. \textbf{b} Three daughter cells (arrow) appear to pinch out from the amoeboid form by plasmotomy. Note: The entire cell body is stained flaming red-orange. \textbf{c} Progeny cell with a prominent bright yellow stained nuclei (arrow) at the periphery pinched from mother cell. Note the thin band of cytoplasm connecting the progeny to the mother cell. Bar, 10 \( \mu \text{m} \)
However, the central body of the amoeboid form showed intense reddish brown staining (Fig. 2b). Acridine orange stained the nuclei and the central body of vacuolar forms bright and dull yellow green, respectively (Fig. 3a). The entire body of the amoeboid form is stained with intense flaming red-orange (Fig. 3b,c).

Discussion

Reproductive processes in Blastocystis has always been in controversy especially so when the proposed binary fission as the only mode of reproduction accepted cannot account for the high growth rate of parasites seen in in vitro cultures of B. hominis. In the present study, plasmotomy was observed only in the amoeboid form of B. hominis which concurs with findings from previous studies (Zierdt 1991; Zaman 1997). However, the report of plasmotomy by Zhang et al. (2007) in vacuolar forms needs further substantial evidence to provide support for this reproductive mechanism.

In the present study, using trichrome staining, the amoeboid forms undergoing plasmotomy showed intense reddish brown staining in the central body, which is suggestive of high nucleic acid level, which could lead to active protein synthesis before the progeny being pinched off from the mother cell. This was further confirmed with acridine orange staining, which showed intense yellow orange coloration in the central body indicative that the organism is in an active state (Villar et al. 1998) and that there is high level of proteins and RNA synthesis before the cell division (Suresh et al. 1994b).

‘Plasmotomy’ is defined as the multinucleate body that divides into two or more small, multinucleate individuals, the cytoplasmic division taking place independently of nuclear division (Kormos and Kormos 1958). This typical mechanism of plasmotomy in Pelomyxa gruberi was described by Frolov et al. (2006), who showed that uninnucleate individuals and mass appearance of multinucleate stages lead to micropopulations. In the present study, we observed the accumulation of the nucleic acid at the central zone of the amoeboid form of B. hominis as evidenced by the reddish brown coloration and intense flaming red-orange in both trichrome and acridine orange staining, respectively, indicating high levels of nucleic acid (Fig. 2b). This is suggestive that this process is leading to the formation of multiple nuclei during plasmotomy.

The amoeboid form, which is generally shown to be bigger in its size (5–65 μm; Tan and Suresh 2006) than the vacuolar forms, undergoes plasmotomy to produce multiple smaller progenies for the purposes of propagation of its species to survive in adverse conditions as evidenced by observing the occurrence of this reproductive process in older cultures, which are known to be deprived of essential nutrients.

In our previous study (Tan and Suresh 2006), we provided evidence for the existence of amoeboid forms seen only in cultures of B. hominis isolated from symptomatic patients. It is tempting to speculate that plasmotomy could be a reproductive mechanism seen only in isolates that cause symptoms and may represent a particular subtype of the organism; however, this would need further studies to confirm.

Recent phylogenetic studies have shown that B. hominis could be placed within the Stramenopiles (Silberman et al. 1996). Nevertheless, the Stramenopiles is a diverse group of organism includes slime nets, water moulds, and brown algae. It is rather difficult to find any morphological similarity between B. hominis and other members of the Stramenopiles. However, the ability of B. hominis to reproduce by plasmotomy may possibly indicate its phylogenetic affinity to the Stramenopiles. Protoopalina intestinalis, an Opalinid (a member of the Stramenopile group), which undergoes plasmotomy, was recently confirmed to be the closest sister group of B. hominis in the Stramenopiles (Kostka et al. 2004).

Our present findings show that plasmotomy in B. hominis confirm our previous suggestions (Govind et al. 2002) that Blastocystis does reproduce by method other than binary fission.

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