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Antigenic Differences Between *Blastocystis hominis* and *Blastocystis* sp. Revealed by Polyclonal and Monoclonal Antibodies

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ABSTRACT

The antigenic differences between *Blastocystis hominis* and *Blastocystis* sp. (isolated from a human and a chicken sources, respectively) were investigated by using polyclonal and monoclonal antibodies. Polyclonal antibodies did not reveal distinct antigenic differences between the strains by Western blot analysis, whereas monoclonal antibodies (MAbs) produced against *B. hominis* did detect antigenic polymorphism between *B. hominis* and *Blastocystis* sp. Six out of 8 MAbs were specific to *B. hominis* antigens and 2 MAbs reacted with both *B. hominis* and *Blastocystis* sp. antigens. Immunoelectron microscopy using these MAbs, revealed labeling on the filamentous layer or the central vacuole. Interestingly, the 6 MAbs reacted only with *B. hominis* antigens on the filamentous layer, whereas the other 2 MAbs labeled on the central vacuole. Therefore, the antigenic components of the filamentous layer may be strain or species specific. In contrast, the antigenic components of the central vacuole are shared by both *B. hominis* and *Blastocystis* sp. suggesting the metabolic substances in the central vacuole are antigenically common.

INTRODUCTION

Since *Blastocystis hominis* was first reported in stool samples of inhabitants of the tropics (Brumpt 1912), many *B. hominis*-like organisms have been found in a

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variety of vertebrates (Burden et al. 1978, 1979; Yamada et al. 1987; Teow et al. 1991, 1992; Boreham and Stenzel 1993; Stenzel et al. 1994). Most of these organisms were indistinguishable from *B. hominis* by light and electron microscopy because they lacked consistent morphological characteristics. The great morphological variations among individual isolates (Yamada et al. 1987; Dunn et al. 1989; Teow et al. 1992), left the questions of whether or not these *Blastocystis* organisms were the same or different species. Practically, *B. hominis*-like organisms are collectively designated as *Blastocystis* sp. except for those of human origin (Yamada et al. 1987; Boreham and Stenzel 1993; Stenzel et al. 1994). However, a new species, *B. lapemi*, which was isolated from the sea snake, was recently reported based on differences in optimal growth temperature and in the electrophoretic karyotype (Teow et al. 1991). Therefore, it is possible that more than one *Blastocystis* species is involved in a variety of hosts.

On the other hand, the pathogenicity of *B. hominis* is now controversial (Editorial 1991) and there is no convincing information as to the transmission route of *Blastocystis*. It is clear that what is critically needed, is a reagent that can distinguish possible new species of *Blastocystis* and/or zoonotic strains. Antigenic polymorphism between human isolates revealed by immuno blotting suggested the presence of possible variants of *B. hominis* species (Kukoschke and Müller 1991; Boreham et al. 1992; Müller 1994). These studies had some limitations in that, the resolution required to detect precise differences relied on polyclonal antibodies. In this study, we developed 8 lines of MAbs against *B. hominis* isolated from a human. The antigenic polymorphisms between human and chicken isolates, which were previously indistinguishable by morphological observations (Yamada et al. 1987), were compared by Western blotting and immunoelectron microscopy.

MATERIALS AND METHODS

Strains and culture

The culture and strains of HE87-1 of *B. hominis* and of CK86-1 of *Blastocystis* sp. isolated from a chicken were described previously (Yoshikawa et al. 1995a, 1995b).

Antigen preparation

Blastocystis and coexisting bacteria in the culture medium were purified as described previously (Yoshikawa et al. in press). After 4 days of cultivation, *Blastocystis* settled on the bottom of the culture tubes and most of the culture medium was carefully removed. The sediments were slowly pipetted with siliconized Pasteur pipets and transferred to sterile centrifuge tubes. These were centrifuged at 50*g* for 1 min to remove large sediments and the supernatant fractions were transferred to clean tubes. The suspension was centrifuged at 350*g*

for 5 min. The supernatant fractions were filtered through 5 μm sterile filters to collect bacterial antigens from the culture medium. The sediments were resuspended in Ringer solution and recentrifuged as mentioned above. This process was repeated five times. After the final centrifugation, the packed cells were appropriately resuspended in Ringer solution, counted by using a haematocytometer, and stored at -30°C until used as *Blastocystis* antigens. The bacterial contamination was monitored by light microscopy and the contaminations of protein and genomic DNA were checked again when bacterial contamination was eliminated more than 99% (Yoshikawa et al. in press). On the other hand, bacterial fractions were centrifuged at 750*g* for 5 min and the sediments were resuspended in 0.5 ml Ringer solution, and stored as bacterial antigens at -30°C. Samples showing no contaminations of proteins and genomic DNA were used in this study.

Production of monoclonal and polyclonal antibodies

Monoclonal antibodies were produced as described previously (Graves et al. 1986). Briefly, female BALB/c mice (6 week-old) were injected subcutaneously with sonicated 300 µl antigen containing $1x10^6$ *B. hominis* or 5-10 μg protein of bacterial antigens emulsified in the same volume of the Freund's complete adjuvant (Difco Laboratories). Two weeks after the primary injection, a booster of the same amount of antigen mixed in the Freund's incomplete adjuvant (1:1, v/v) was injected subcutaneously once every 14 days for 42 days. Three days after the final boost, mice were sacrificed under ether anesthesia and spleen cells were fused with X63 Ag 8.653 mouse myeloma cells (Galfre and Milstein 1981). For polyclonal antibodies, blood from sensitized mice was collected by cardiac puncture. The collected blood was allowed to clot for several hours at room temperature and then stored over-night at 4°C. The clot was centrifuged at 1,200*g* for 10 min at 4°C. The serum was then removed and stored at -30°C. Hybridomas producing MAbs against *Blastocystis* antigens were screened by enzyme-linked immunosorbent assay (ELISA) using the positive control polyclonal serum. Isotyping of the monoclonal antibodies was assayed by using a commercial ELISA kit (Zymed Lab., Inc.).

ELISA for detection of Blastocystis-specific antibodies

The indirect ELISA for antibody detections was performed as described previously (Graves et al. 1986). Briefly, the antigens were prepared by sonicating a preparation containing $2\text{-}6x10^7$ cells per ml and 0.5 μg protein per well was bound to the solid phase of the Nunc Immunoplate polystyrene 96-well microtiter plates. These plates were incubation at 37°C for 2 hrs in humid chamber and then washed three times with phosphate buffered-saline containing 0.05% Tween 20 (PBS-T). To avoid non-specific reactions, the wells were incubated with 200 µl 5% nonfat dry milk for 1 hr at room temperature. After washing two times in PBS-T, 50 µl of the undiluted culture fluids from the original fused hybridoma cells or diluted sera as a

positive control (1:500 with PBS-T) were added to each well and incubated for 2 hrs at room temperature. For the negative control, diluted sera were added to uncoated wells. The plates were washed four times with PBS-T and 50 µl of anti-mouse IgG or IgM conjugated with alkaline phosphatase (Sigma) diluted 1:1,000 with the same buffer. After incubation for 2 hrs at room temperature, the conjugate was discarded and the plates were washed four times with PBS-T, and 50 µl of freshly prepared p-nitrophenyl phosphate substrate solution was added. The plates were incubated at room temperature for 30 min with shaking and optical density was measured at 405 nm.

Western blotting procedure

Antigens were solubilized with sample buffer [0.1% sodium dodecyl sulfate (SDS), 1.25% 2-mercaptoethanol, 0.02% bromophenol blue, 0.02 M Trishydrochloride, and 7% glycerol, pH 6.8] for 5 min at 100°C. Antigen samples containing 100 µg protein for a preparatory gel or otherwise 15 to 20 µg protein per well were electrophoresed through 4.5% stacking gel and 10% SDS polyacrylamide running gels (Laemmli 1970). For the bacterial antigens, 2 µg of protein were used. Electrophoresis was performed under a constant voltage of 80 V per gel (Bio-Rad, Mini-PROTEAN II cell) or under a constant current at 40 mA (ATTO, AE-6200) for 1 hr at room temperature. Molecular weight protein standards (14 to 116 or 17 to 200 kDa) were included in each run. Proteins separated by SDS gel electrophoresis were transferred to nitrocellulose paper by a method similar to that of Towbin et al. (1979). Briefly, the proteins were electrophoretically transferred to nitrocellulose paper (0.45 µm pore size, Schleicher & Schuell) under a constant voltage of 100 V for 1 hr (Bio Rad, Trans-Blot cell) or under a constant current (1.5 mA/cm^2) for 90 min (ATTO, AE-6675 P/N). A portion of the membrane containing the molecular weight standards was stained with amido black. The other portion of the membrane was incubated with 1% Tween 20 in PBS for 1 hr at room temperature or over-night at 4°C. After washing once with PBS-T, the membrane sheets were cut into strips or without cutting, and subsequently incubated 2 hrs at room temperature or over-night at 4°C with either polyclonal sera (diluted 1:500 in PBS-T) as positive control or with culture fluids from various hybridomas. After the strips or sheets were washed four times with PBS-T, they were incubated for 2 hrs at room temperature with alkaline phosphatase-conjugated anti-mouse IgG or IgM (Sigma) diluted 1:1,000 in PBS-T. These were subsequently washed five times and the antigenic bands were developed by the addition of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium in the dark. The antigenic reactive bands were allowed to develop for 5 to 7 min. The reaction was terminated by washing the blots in a solution of 0.4 M Tris buffer containing 0.1M EDTA (pH 2.9) followed by a final rinse in distilled water.

Immunoelectron microscopy

After 4 days cultivation of the HE87-1 strain, most of the supernatant fractions of culture tubes were carefully removed and discarded. Immediately thereafter, 1/2 Karnovsky fixative (2% paraformaldehyde and 2.5% glutaraldehyde) in 0.04 M phosphate buffer, pH 7.4, was slowly introduced into the tubes, and the *B. hominis* organisms were fixed for 2 hrs at room temperature (Yoshikawa et al. 1995a). After fixation, all of the supernatant fraction was transferred to clean tubes and centrifuged at 180*g* for 5 min. The packed sediments were resuspended into 0.1 M phosphate buffered saline (PBS), pH 7.4. After washing with the same buffer at 4°C, the samples were dehydrated with a graded series of ethanol and embedded into L-R-White resin. Ultrathin sections collected on collodion coated nickel grids were immuno-labeled as described previously (Yoshikawa et al. 1991). Briefly, the grids were first placed on a drop of 0.1 M PBS containing 1% bovine serum albumin with 0.01% Tween 20 (PBS-BSA-T) and transferred to drops of culture fluids of MAbs. After incubation for 30 min at room temperature, the grids were washed with PBS-BSA-T and placed on drops of 5 μg/ml biotinylated anti-mouse IgG or IgM (Vector Laboratories) for 30 min. After washing with PBS-BSA-T, the grids were incubated for 30 min with streptoavidin-gold conjugates (Yoshikawa et al. 1988) diluted 1:200 with PBS-BSA-T. Finally, grids were washed with distilled water, stained with uranyl acetate and lead citrate, and examined with Hitachi H-7000 electronmicroscope at 100 kV. To demonstrate the specificity of the immunolabelling, control grids were incubated with the secondary antibodies, followed by streptoavidin-gold conjugate, or with conjugate alone .

RESULTS AND DISCUSSION

Western blotting with polyclonal antibodies

Contamination with bacteria in the isolated antigens of *Blastocystis* was monitored by means of Western blotting with anti-bacterial antibodies. No distinct band was observed in the lanes of HE87-1 and CK86-1 antigens, whereas many reactive bands were observed in the lanes of bacterial antigens (Fig. 1). These results indicated that bacterial antigens were no longer detectable in the *Blastocystis* antigen preparation by immuno blotting. Therefore, the repetitive washes were effective enough to eliminate the bacterial contamination from *Blastocystis* cultures. The same was true for DNA levels; namely, PCR products of bacteria-specific bands tended to fade after washing the culture samples with the Ringer solution, while the rest of *Blastocystis*-specific bands tended to increase the intensity (Yoshikawa et al. in press). Antigenic differences between HE87-1 and CK86-1 were examined by Western blotting using polyclonal sera produced against HE87-1 or CK86-1. No major differences were observed between the different

antisera produced to *B. hominis* (HE87-1) or *Blastocystis* sp. (CK86-1) (Fig. 2). With regard to specific antigens, only a few different bands were observed. A single band at 33 kDa was evident in lanes containing HE87-1 antigens, whereas a broad band of between 20-32 kDa was observed in the lanes containing CK86-1 antigens (Fig. 2). Since both antisera showed the same band patterns with both antigens, it was concluded that antigenic differences between *B. hominis* and *Blastocystis* sp. can not be detect by use of polyclonal antibodies.

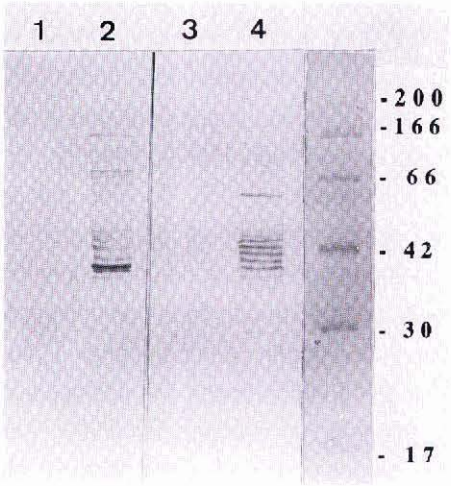

Figure 1. Western immunoblot analysis of contamination of bacterial antigens in purified *B. hominis* (HE87-1) and *Blastocystis* sp. (CK86-1) antigens with polyclonal antibodies against bacterial antigens. Many reactive bands were seen in bacterial antigens (lanes 2, 4), whereas no distinct band was observed in HE87-1 antigens (lane 1) or in CK86-1 antigens (lane 3). The numbers on the right indicate the size of molecular markers in kilodalton.

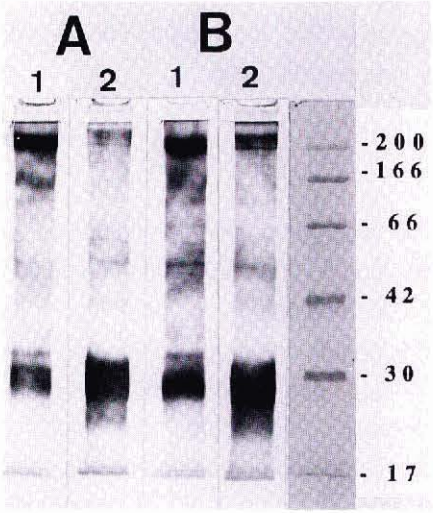

Figure 2. Western immunoblot analysis of *B. hominis* (HE87-1) antigens (lane 1) and *Blastocystis* sp. (CK86-1) antigens (lane 2) with polyclonal antibodies against HE87-1 (A) or CK86-1 (B). The reactive bands observed in HE87-1 antigens and CK86-1 antigens were quite similar in both polyclonal antibodies. Note the difference of banding pattern; a single band of 33 kDa was evident in HE87-1 antigens, whereas a broad band was seen in 20-32 kDa of CK86-1 antigens. The numbers on the right indicate the size of molecular markers in kilodalton.

Western blotting with monoclonal antibodies

Eight hybridomas specific to *B. hominis* were selected by using the ELISA. The epitope reactivity of the MAbs was examined by Western blotting against both HE87-1 and CK86-1 antigens. Six out of 8 MAbs were specific to *B. hominis* which reacted with HE87-1 antigens (Fig. 3A, lanes 1-6). However, the same MAbs

showed weak or no reactivity with CK86-1 antigens (Fig. 3B, lanes 1-6). One MAb produced 3 reactive bands with HE87-1 antigens, whereas with CK86-1 antigens, upper two reactive bands were absent (Figs. 3A and B, lane 7). Another MAb reacted with both antigens (Figs. 3A and B, lane 8). With regard to the antigenic differences, specific antigenic reactive bands were observed in antigens greater than

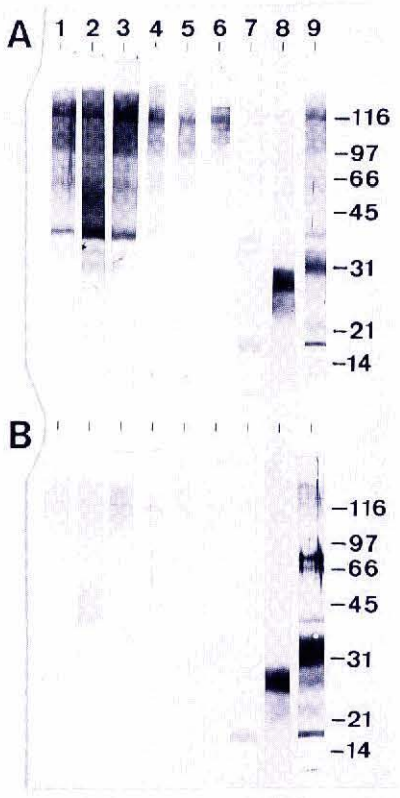

Figure 3. Western immunoblot analysis of *B. hominis* antigens (A) and *Blastocystis* sp. antigens (B) with monoclonal antibodies (MAb). Both panels were reacted with MAbs indicated in Table I. Lanes 1-6 were reacted with *B. hominis* antigens, whereas no or weak reactions were observed with *Blastocystis* sp. antigens. Lane 7 showed three reactive bands with *B. hominis* antigens, but with *Blastocystis* sp. antigens, two bands were absent. Lane 8 was reacted with both antigens. Lane 1, 2gA8A11; lane 2, 2hB2A2; lane 3, 2fA3A9; lane 4, 2hC4A5; lane 5, 2fA3C9; lane 6, 2hC4D5; lane 7, 2iE1C10; lane 8, 2hA5A1; lane 9, molecular markers in kilodalton.

Table I. General properties of monoclonal antibodies against *Blastocystis hominis*.

Antibodies	Immunoglobulin Isotype	Epitopes	Major reactive components(kDa)
2gA8A11	IgM	FL	200 - 40
2hB2A2	IgM	FL	200 - 30
2fA3A9	IgM	FL	200 -40
2hC4A5	ND	ND	130 - 95
2fA3C9	IgM	FL	130 - 95
2hC4D5	IgG1	FL	130 - 100
2iE1C10	IgG1	CV	40, 35, 17
2hA5A1	IgG1	CV	30 - 25

FL: filamentous layer, CV: central vacuole, ND; Not determined,

33 kDa in *B. hominis* (Fig. 3, lanes 1-6), whereas antigens under 33 kDa were reactive to both *B. hominis* and *Blastocystis* sp. (Fig. 3, lanes 7 and 8). These results indicated that the antigenic differences between *B. hominis* and *Blastocystis* sp. can be detected with 6 MAbs (2gA8A11, 2hB2A2, 2fA3A9, 2hC4A5, 2fA3C9, 2hC4D5). Since one MAb, 2hA5A1, was reactive with both *B. hominis* and *Blastocystis* sp. antigens under 33 kDa (lane 8), it is concluded that this antigenic determinant is shared between both HE87-1 and CK86-1. Interestingly, one MAb, 2iE1C10, reacted with both antigens (lane 7), but upper two reactive bands, 40 and 35 kDa, were not recognized in CK86-1 antigens. These results indicate the same antigenic epitope is shared by different proteins. The major reactive components and isotypes of these MAbs were summarized in Table 1.

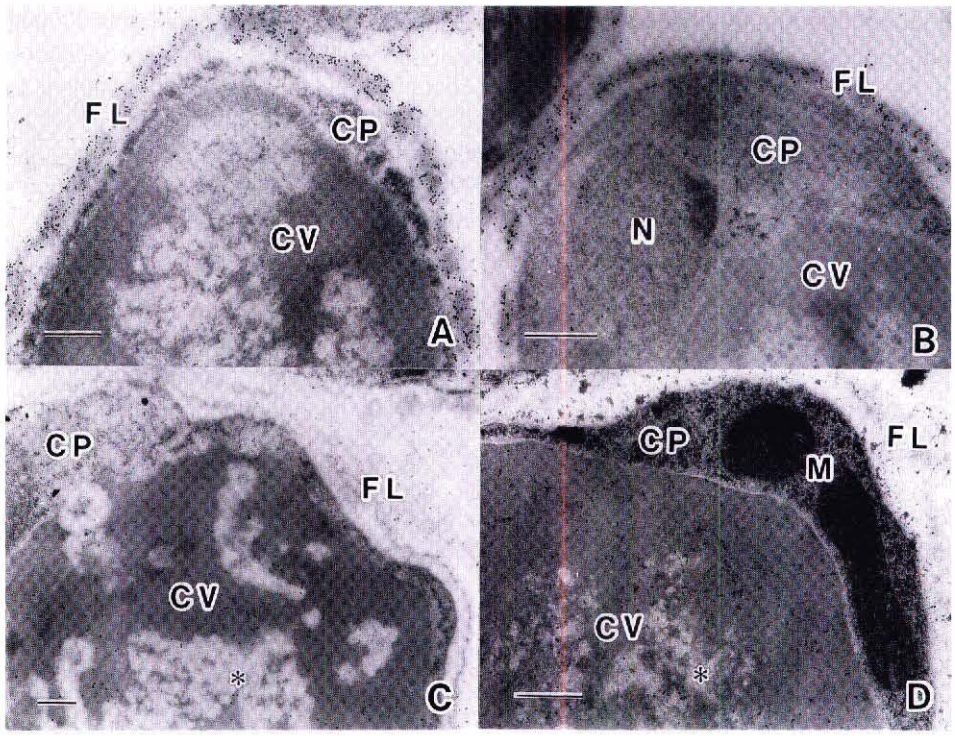

Figure 4. Immunogold labeling of *B. hominis* with monoclonal antibodies (MAb). The surface of *B. hominis* was covered by the filamentous layer (FL) which was labeled with gold particles by MAb 2gA8A11 (A) and MAb 2hC4D5 (B). Since the central vacuole (CV) was occupied at the center of the cell, the nucleus (N) and cytoplasm (CP) were displaced peripherally. No labeling was observed in the central vacuole and cytoplasm. The central vacuole showed a great variation of the electron density and distribution suggesting different substances in the contents. Immunogold labeling with MAb 2iE1C10 (C) or MAb 2hA5A1 (D). Gold particles were only observed on the electron-dense materials in the central vacuole, while the electron-lucent regions in the central vacuole (asterisks) and the filamentous layer (FL) were not labeled. A, x19,000. B, x24,000. C, x13,000. D, x22,000 Bars= 0.5 μm.

Immunoelectron microscopy

Seven MAbs tested in this study were divided into two groups based on the localizations of epitopes (Table 1). One group of MAbs (2gA8A11, 2hB2A2, 2fA3A9, 2fA3C9, 2hC4D5) recognized the filamentous layer of *B. hominis* (Figs. 4A and 4B). Another group (2iE1C10 and 2hA5A1) recognized the so-called central vacuole (Figs. 4C and 4D). The central vacuole showed a great variation of the electron density and distribution suggesting different substances in the contents. The positive labeling with the latter two MAbs was only observed on the electron-dense parts of the central vacuole. Recently, we had confirmed the accumulation of carbohydrates and lipids in the central vacuole of *B. hominis* by histochemical staining methods (Yoshikawa et al. 1995a, 1995b). Histochemical reactions with carbohydrates or lipids in the central vacuole showed great variations in the staining intensity at light and electron microscopic levels. However, the distribution of the carbohydrates were quite similar to the electron-dense parts observed in this study. Therefore, some antigenic substances may be carbohydrates.

The immunoelectron microscopy revealed that all of the MAbs reacted only with HE87-1 antigens by immunoblotting labeled on the filamentous layer of *B. hominis* (Table 1). Therefore, the antigenic components of the filamentous layer may be strain and/or species specific, because CK86-1 strain recently designated as *B. hominis* judging from the PCR banding pattern (Yoshikawa et al. in press). However, the antigenic components of the central vacuole which were shared with HE87-1 and CK86-1, suggested the metabolic substances of *Blastocystis* were antigenically common.

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REFERENCES

Boreham, P. F. L., Upcroft, J. A. & Dunn, L. A. 1992. Protein and DNA evidence for two demes of *Blastocystis hominis* from humans. *Int. J. Parasitol. 22:* 49-53.

Boreham, P. F. L. & Stenzel, D. J. 1993. *Blastocystis* in humans and animals: morphology, biology, and epizootiology. *Adv. Parasitol. 32:* 1-70.

Brumpt, E. 1912. *Blastocystis hominis* n. sp. et formes voisines. *Bull. Soc. Pathol. Exot. 5:* 725-730.

Burden, D. J., Anger, H. S. & Hammet, N. C. 1978/1979. *Blastocystis* sp. infections in pigs. *Vet. Microbiol. 3:* 227-234.

Dunn, L. A., Boreham, P. F. L. & Stenzel, D. J. 1989. Ultrastructural variation of *Blastocystis hominis* stocks in culture. *Int. J. Parasitol. 19:* 43-56.

Editorial 1991. Blastocystis hominis: commensal or pathogen. *Lancet 337:* 521-522.

Galfre, G. & Milstein, C. 1981. Preparation of monoclonal antibodies: strategies and procedure, pp. 3-46. *In:* Methods in Enzymology, Langone, J. J. & Van Vunakis, H. (Ed.), Vol. 73. Academic Press, New York.

Graves, D. C., McNabb, S. J. N., Ivey, M. H. & Worley, M. A. 1986. Development and characterization of monoclonal antibodies to *Pneumocystis carinii. Infect. Immun. 51:* 125-133.

Kukoschke, K. G. & Müller, H. E. 1991. SDS-PAGE and immunological analysis of different axenic *Blastocystis hominis* strains. *J. Med. Microbiol. 35:* 35-39.

Laemmli, U. K. 1970. Cleavage of structure proteins during assembly of the head of bacteriophage T4. *Nature (London) 227:* 680-685.

Müller, H. S. 1994. Four serologically different groups within the species *Blastocystis hominis. Zbl. Bakt. 280:* 403-408.

Stenzel, D. J., Cassidy, M. F. & Boreham, P. F. L. 1994. Morphology of *Blastocystis* sp. from domestic birds. *Parasitol. Res. 80:* 131-137.

Teow, W. L., Zaman, V., Ng, G. C., Chan, Y. C., Yap, E. H., Howe, J., Gopalakrishnakone, P. & Singh, M. 1991. A *Blastocystis* species from the sea-snake,*Lapemis hardwickii* (Serpentes: Hydrophiidae). *Int. J. Parasitol. 21:* 723-726.

Teow, W. L., Ng, G. C., Chan, P. P., Chan, Y. C., Yap, E. H., Zaman, V. & Singh, M. 1992. A survey of Blastocystis in reptiles. *Parasitol. Res. 78:* 453-455

Towbin, H., Stahelin, T. & Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA 76:* 4350-4354.

Yamada, M., Yoshikawa, H., Tegoshi, T., Matsumoto, Y., Yoshikawa, T., Shiota, T. & Yoshida, Y. 1987. Light microscopical study of *Blastocystis* spp. in monkeys and fowls. *Parasitol. Res. 73:* 527-531.

Yoshikawa, H., Morioka, H. & Yoshida, Y. 1988. Ultrastructural detection of carbohydrates in the pellicle of *Pneumocystis carinii. Parasitol. Res. 74:* 537-543.

Yoshikawa, H., DeBault, L. E. & Graves, D. C. 1991. Ultrastructural localization of epitopes recognized by monoclonal antibodies produced to rat *Pneumocystis carinii. J. Protozool. 36:* 18S-20S.

Yoshikawa, H., Kuwayama, N. & Enose, Y. 1995a. Histochemical detection carbohydrates of *Blastocystis hominis. J. Euk. Microbiol. 42:* 70-74.

Yoshikawa, H., Satoh, J. & Enose, Y. 1995b. Light and electron microscpcopic localization of lipids in *Blastocystis hominis*. *J. Electron Microsc. 44:* 100-103.

Yoshikawa, H., Nagano, 1., Yap, E. H., Singh, M. & Takahashi, Y. 1996. DNA polymorphism revealed by arbitrary primers polymerase chain reaction among *Blastocystis* strains isolated from humans, a chicken, and a reptile. *J. Euk. Microbiol.* in press