Serologic Response to *Blastocystis hominis* Infection in Asymptomatic Individuals

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The pathogenic potential of *Blastocystis hominis* in the human intestine is subject to controversy because the organism has been found in both symptomatic and asymptomatic individuals. To help clarify this issue, we monitored the serologic response to the organism in *B. hominis*-infected individuals free of gastroenterologic disorders.

1) Serum antibodies to *B. hominis* were detected in 70% of infected asymptomatic individuals by an indirect immunofluorescence (IFA) test.

2) IFA and immunoelectron microscopy revealed that the antibody response was directed against a surface antigen(s) of the organism.

3) Analysis by immunoblotting implicated a 12 kDa protein of *B. hominis*.

4) The strongest positive reaction was obtained in an individual chronically infected for more than 2 years. It may be that long exposure to the parasite is necessary for a serologic response.

Key words : *Blastocystis hominis*, Serum antibody, Asymptomatic carrier

INTRODUCTION

The first reports of *Blastocystis hominis* infection were by Perroncito in 1899 [15] and Lynch in 1917 [11]. Recently, case reports concerning *B. hominis* have increased in number [7, 17, 21]. Even though some investigators consider *B. hominis* a potential pathogen, it is still an open and, at times, controversial issue, although asymptomatic infections are an established fact. To date, there have been few studies on the immune response to *B. hominis* infection. The humoral antibody response of individuals with symptoms has been investigated primarily to aid in the diagnosis of blastocystosis [1, 5, 8, 22, 24]. The organism has never been found to invade human tissues nor has a specific anatomic site for its presence in the gut been uncovered. It seemed unlikely that serum antibodies would develop in symptom-free infected individuals, because tissue invasion appeared prerequisite for a pronounced humoral response. We recently detected numbers of asymptomatic infected individuals in Japan during an epidemiologic survey [6]. Previous epidemiologic surveys have detected asymptomatic individuals [9, 12, 13, 14, 16, 18], but little data was presented concerning serology.

To help clarify the putative relationship between *B. hominis* infection and the host's humoral response, we decided to monitor the serologic (antibody) response of asymptomatic infected individuals by an indirect fluorescent antibody test (IFA), and to characterize the epitope determinants of *B. hominis* by Western immunoblotting and immunohistochemistry.

MATERIAL AND METHODS

Organisms and sera: The *B. hominis* pool consisted of 19 isolates from 19 apparently healthy individuals who came to the Health Screening Center of St. Luke's International hospital in Tokyo for a regular annual physical check-up, during the period October 1994 to October 1995, and in whom the infection was detected by routine fecal examination. A blood sample was with-

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drawn from each individual with informed consent, and the serum collected and frozen until used. As our *Blastocystis* reference, we obtained a Nand Ⅱ strain from the American Type Culture Collection (ATCC Number: 50177). All organisms were anaerobically cultured in biphasic egg slant medium (Medium 1671) at 35℃, as described by Zierdt *et al.* [23].

IFA test: The IFA test was performed using both formalin-fixed and live, intact organisms of the reference strain of *B. hominis* as the antigenic substrate. In addition, organisms isolated from asymptomatic individuals were also used as antigen. After reaction of antigen with a serum sample, fluorescein isothiocyanate-conjugated goat anti-human IgG $F(ab')_2$ was used as the second, labeled antibody. Positive reactions on the antigenic substrate were visualized by fluorescence microscopy.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot analysis: *B. hominis* organisms cultured in Medium 1671 were sedimented by centrifugation, washed twice, and solubilized with an equal volume of sample buffer [10], containing 2mM phenylmethyl-sulfonyl fluoride, 2mM Nα-ρ-tosyl-L-lysine chloromethyl ketone, 2mM ρ-hydroxymercuriphenyl sulfonic acid, and 4 μM leupeptin, for 5 min at 95℃. The supernatant obtained following centrifugation was subjected to SDS-PAGE. As a molecular mass marker, "DAIICHI"・Ⅱ (Daiichi Pure Chemicals Co., Tokyo, Japan) was used. Western immunoblot analysis was performed as described previously [19]. Horseradish peroxidase-labeled goat anti-mouse IgG (Organon Teknica Co., Durham, N.C.) was used as the second antibody. Color development was with a Konica Immunostaining HRP-1000 color developer (Konica Co., Tokyo, Japan). To search for the presence of carbohydrate residues on the epitope, periodate oxidation was carried out by the method of Woodward, Young & Bloodgood [20]. Briefly, the blotted membrane was cut into strips and rinsed with 50 mM sodium acetate buffer (pH 4.5). One strip was exposed to 10 mM sodium metaperiodate in 50 mM acetate buffer for 1 hr, in the dark, at 23℃. Another strip, as a control, was incubated with buffer only. The strips were then washed with the acetate buffer and exposed to 50mM sodium borohydride in PBS for 30 min. After 3 washes with PBS containing 0.05% Tween 20, the strips were immuno-analyzed as described above.

Immunoelectron microscopy: Cultured organisms were sedimented by centrifugation and the pellet fixed overnight in cold 4% paraformaldehyde (pH 7.2), then washed in sucrose containing cold PBS. After embedding in OCT compound (Sakura Co., Torrance, CA), the specimens were frozen in dry ice-hexane, sectioned at 5 μm on a cryostat, and placed on silane-coated slides. The slides were incubated overnight with the serum of an infected individual, diluted 1:100, at 4℃. After rinsing with PBS, anti-human IgG horseradish peroxidase-labeled $F(ab')_2$, diluted 1:200 (Amesham International, England) was applied for 1 hr, at room temperature. The slides were rinsed with PBS, then reacted with DAB (3,3'-diaminobenzidine. 4HCl 25mg/dl; H_2O_2 0.003%) for 5 min. After post-fixation in 2 % glutaraldehyde for 10 min, the sections were refixed with 1 % OsO_4 at room temperature for 1 hr, dehydrated in a graded ethanol series, and embedded in Quetol 812. Ultrathin sections were cut by an Ultratome (LKB, Sweden), counterstained lightly with lead citrate, and examined on a JOEL 2000EX electron microscope.

RESULTS AND DISCUSSION

The sera of 19 individuals, infected with *B. hominis* but free of clinical signs, were examined by IFA. Of the 19 serum samples, 13 (70 %) were positive. The results of IFA using the reference strain did not differ from those using the isolates as antigenic substrate. Fluorescence was located on the surface of the organisms, as shown in Figure 1. Furthermore, we confirmed that the positive sera were non-reactive against bacteria associated with *B. hominis*. In the asymptomatic individuals, antibody titers were very low and serum dilutions greater than 1 : 60 failed to elicit a reaction. Sera from 4 non-infected individuals were negative by IFA. These observations indicate that antibodies against *B. hominis* are detectable in the serum, although Chen *et al.* [1] reported that a specific antibody response was lacking in patients with intermittent diarrhoea caused by *B. hominis*. It seems that *B. hominis* does not induce a strong antibody response in

humans. Reports stating that *B. hominis* does not invade human tissue [2, 3, 4, 9], may account for the lack of a vigorous antibody response. Therefore, it appears that the presently available serum antibody tests for *B. hominis* are unreliable, although serum antibodies have been reported in patients with clinical signs [5, 8, 17, 22, 24].

To identify the antigenic component(s) of the organism that reacted with antibody, western immunoblot analysis was performed, using the Nand Ⅱ strain as antigen. As shown in Fig. 2, several bands of reaction were observed in the blotted strips, although all reactions were weak. A band common to all the strips was not found. However, 1 serum (case No. 1) did react strongly, yielding a distinct band to a 12 kDa protein of

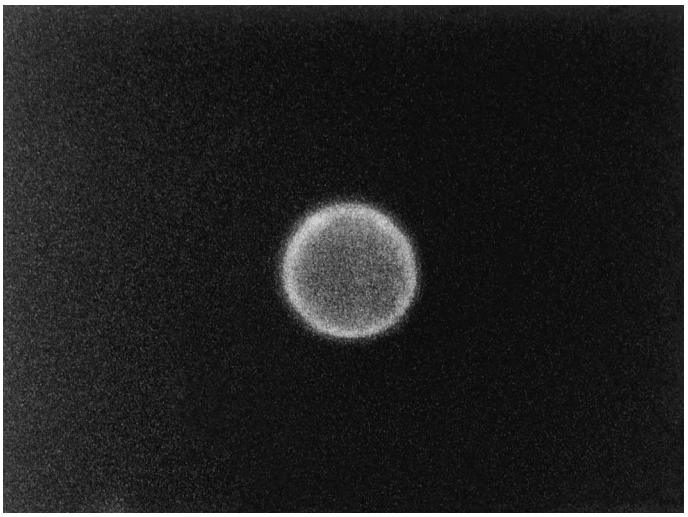

Fig. 1 IFA of Case No. 1 (serum dilution 1/60). Note fluorescence on the surface of the organism.

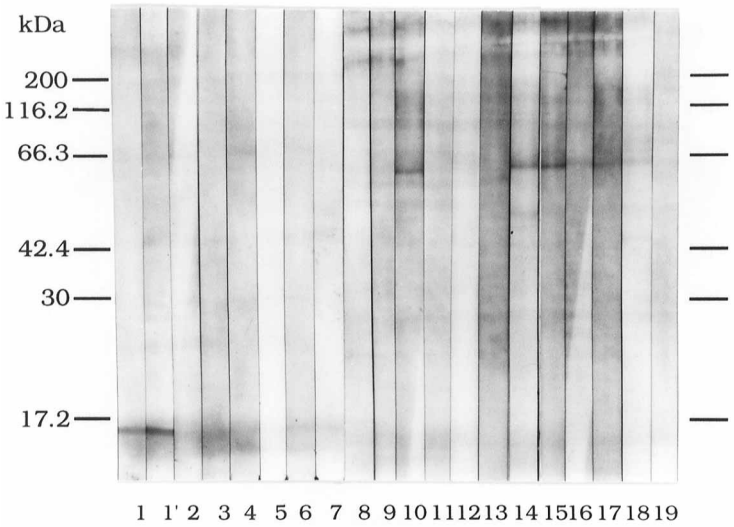

Fig. 2 Western immunoblotting patterns of the reactions between 19 test sera and the ATCC reference strain of *B. hominis*. Lanes 1 and 1' were sera drawn from the same individual (case No. 1) but at an interval of several months. Note that the serum of case No. 1 strongly reacted with the 12 kDa protein.

the ATCC reference strain (Lanes 1 and 1' in Fig. 2). The other 18 serum samples did not exhibit this strong reaction. The serum of case No. 1 was also positive by IFA, whereas the other positive sera did not react well. No relation between seropositivity and the immunoblotting patterns was detected. When we tested the serum of case No. 1 with the other 18 *B. hominis* isolates, it was seen to react with the 12 kDa protein of almost every isolate, even though each reaction exhibited differences in intensity (Fig. 3). A comparison between the protein composition of the 19 isolates and the ATCC reference strain was done by SDS-PAGE. Differences among the patterns were not observed (Fig. 4); all isolates and the reference strain had the 12 kDa protein. It seems, therefore, that although all 19 individuals were infected with *B. hominis*, only 1 individual responded strongly to the 12 kDa protein, whereas the other individuals had either a weak or no response. The 70% seropositivity recorded by IFA likely is a reflection of a humoral response to other protein constituents of *B. hominis*.

As to why only 1 of the 19 infected persons evinced a "strong" humoral response, we suggest that this individual's chronic infection for over 2 years, perhaps in conjunction with the presence of intestinal diverticula (Table 1), may have combined to

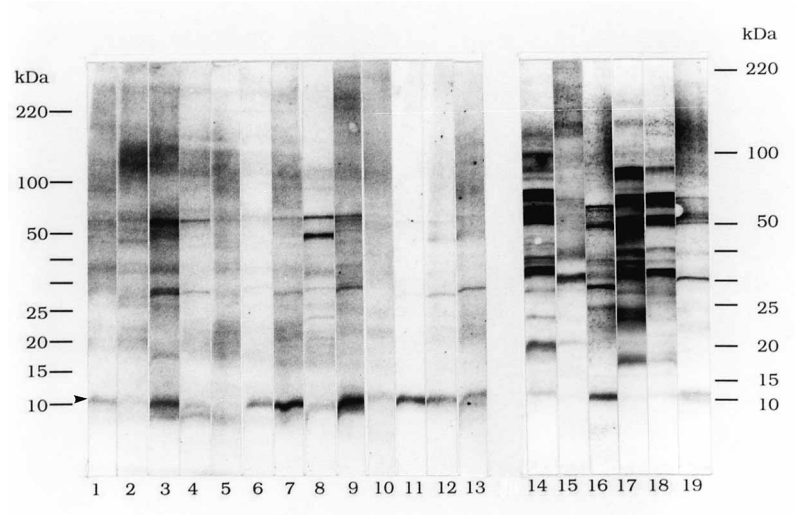

Fig. 3 Western immunoblotting patterns of reactions between the serum of case No. 1 and 19 *B. hominis* isolates from infected, asymptomatic individuals. Note that the 12 kDa protein (arrowhead) of every isolate except No. 17 was recognized by the serum.

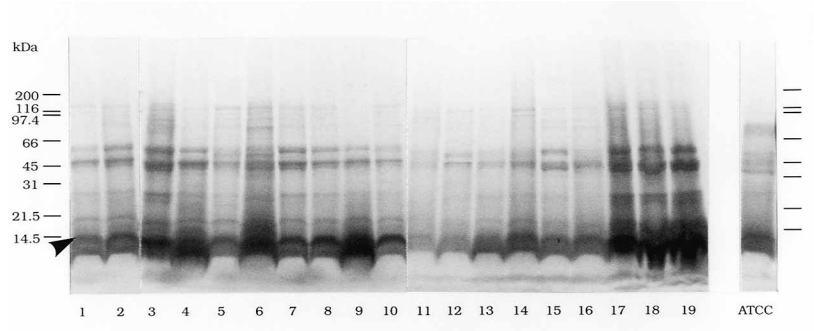

Fig. 4 SDS-PAGE patterns of the 19 *B. hominis* isolates and the ATCC reference strain. All isolates exhibited similar protein patterns; note especially the band for the 12 kDa protein (arrowhead).

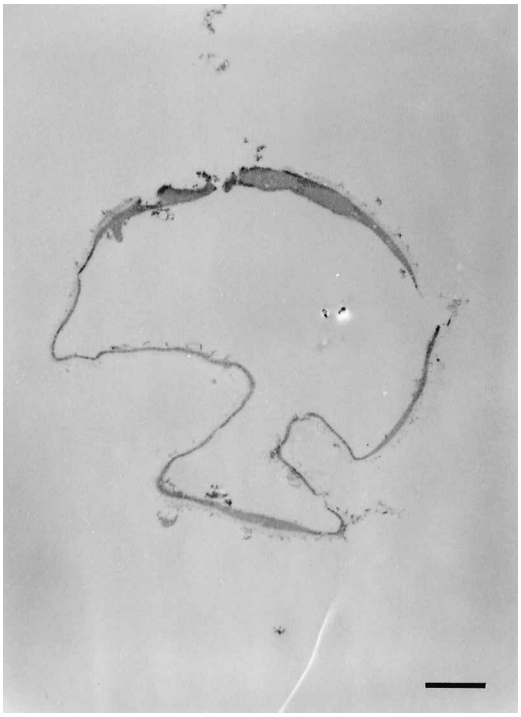

Fig. 5 Immunoelectron micrograph. Cultured *B. hominis* organism exposed to the serum of case No. 1. The electron microscopic photograph shows labeling on the surface of the organism. Bar indicates 2.5 μm.

Table 1 Clinical Course of Case No. 1

A healthy Japanese male, 55 years old, visited the St. Luke's International Hospital Health Screening Center in Tokyo for a routine health check-up in August, 1995. He had no complaints and no history of hospitalization.
All hematological and blood chemistry findings were consistently normal, at every health check-up during the following 2 years.
Stool examinations: *B. hominis* was detected on August 1995. Seven additional stool examinations were performed during the next 2 years, and *B. hominis* was detected in the stool on every occasion.
Stool cultures were consistently negative for *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia* and enteropathogenic *Escherichia coli* organisms.
Blood samples were drawn at every health check-up, and used in serological analyses.
Colonoscopy on May, 1997 revealed diverticula in the ascending colon, without evidence of inflammation.

magnify the antigenic stimulus, leading to the enhanced antibody response. It was observed that the reactive features of the sera remained constant during the individual's repeated health check-ups, as shown in Figure 2.

Location of the 12 kDa antigen reactive with serum antibodies was determined by immunoelectron microscopy. As shown in Figure 5, it is apparent that the site of the 12 kDa antigen is on the surface of *B. hominis*.

On the basis of these findings, it is suggested that a long-term persistent infection is needed to establish an immune response against the surface antigens of *B. hominis*.

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