Analysis of humoral immune response in human blastocystosis by Western Blotting

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Abstract

Blastocystis hominis can be pathogenic but asymptomaric carriers are often seen. As part of the immunological studies on the infection, we carried out immunoblot analysis of sera from 20 blastocystosis patients using SDS-PAGE separated polypeptides of two axenic and 12 xenic *B. hominis, Giardia lamblia, Entamoeba histolytica, Endolimax nana* and *Escherichia coli.*

B. hominis isolates showed two types of polypeptide patterns in SDS-PAGE. Axenic isolates had prominent bands at 55, 63, 66.4 and 97.2 kD, while common bands of xenic isolates were at 34, 38, 55 and 68 kD. Prominent bands at 8.1, 12, 15, 21.5, 23.5, 26.6 and 32 kD were present in these *B. hominis* isolates as well as *E. coli.* The differences in pattern between the axenic and xenic isolates are mainly due to *E. coli* polypeptides.

Sera from all the blastocystosis patients did not react with antigens of *G. lamblia, E. histolytica,* and *E. nana.* Twelve sera showed reactive bands while 8 others had very weak or no reaction against *B. hominis* antigens. Eight showed reactivity against axenic isolates C and H; and another three sera cross-reacted against at least nine *B. hominis* isolates tested. We believe that anti-*B. hominis* antibodies in patients' sera are heterogeneous, and the important *B. hominis* polypeptides recognised are those at 43, 55 and 66.4 kD. Negative reaction seen with eight of the above sera may be due to antigenic differences in the *B. hominis* strains involved. Immunoblot reactive band patterns due to antibodies against *B. hominis* can be differentiated from those against *E. coli* polypeptides.

Key words: *B. hominis,* sera, SDS-PAGE, immunological

Introduction

Blastocystis hominis (Brumpt, 1912) is now intensely studied in relation to its pathogenicity. As with *Entamoeba histolytica,* the asymptomatic carrier srare is often seen. It was previously thought to be harmless but is now recognised to be pathogenic in both immunocompetent and immunocompromised humans. The most common clinical presentation of the infection is gastroinresrinal disturbance lasting from a week to more than a year (Giacia *et al.*, 1984; Telalbasic *et al.*, 1991; Lambert *et al.*, 1992).

The vacuolated and amoeboid forms of this parasite are commonly found in stool samples, while the granulated form is normally seen in culrure. The biochemical profile of these three morphological forms is uncertain. The differences in protein profile among *B. hominis* isolates were first reported by Boreham *et al.* (1992). However, there are very few immunological studies on this infection and Zierdt & Tan (1976), using the immunofluorescence rest, reported a negative antibody response in infected patients. Detection of antibodies in sera of symptomatic parients was first reported by Zierdt *et al.* (1995), using an enzyme-linked immunosorbent assay (ELISA). Chen *et al.* (1987) did not detect any reactivity with sera of four blastocysrosis patients, using immunoblot analysis. In this study, we used several isolates of *B. hominis* as well as *Giardia lamblia* (7404), *E. histolytica* (HK9) and *Endolimax nana* (EN1) to detect and characterise antibody reactivity to SDS-PAGE separated polypeptides of these parasites.

Materials and Methods

Preparation of B. hominis antigens

Axenic isolates (C and H) were grown in IMDM with 10% horse serum while xenic isolates (H2, H4, H6, H7, 6105, 10203(1), 27B05(1), KP1, Y51, RN, INDO, M12) were grown in Jone's medium supplemented with 10% horse serum. *Escherichia coli* (Bac-1 and Bac-2) isolates from *B. hominis* cultures of KP1 and RN respectively, were also grown in the latter medium. The parasite isolates were harvested when their growth was in the log phase. An isolate each of *G. lamblia* (7404), *E. histolytica* (HK9), and *E. nana* was also used in rhis study.

The parasites were washed five rimes with normal saline to remove horse serum. The washed pellet was then resuspended in an equal volume of 0.85% normal saline and the suspension kept at -70°C overnight (or until used). These frozen parasites were thawed slowly at 4°C for 2 hours, and then centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant (crude antigen extract) was filter-sterilised, aliquoted into smaller volumes, and kept at -20°C for use within a week of preparation. *B. hominis* parasites are very fragile and easily

lysed by freezing and thawing. Sonication of parasites was nor used so as to reduce contamination with *E. coli* antigens.

Crude somatic antigen from bacteria was similarly prepared by sonicating the suspended pellet and keeping the suspension overnight at 4°C. The mixture was then centrifuged, filter-sterilised, and aliquoted as above. A similar method was used to prepare crude antigens of *G. lamblia, E. histolytica* and *E. nana.*

Human blastocystosis sera
Blood samples from 20 patients and animal handlers infected with *B. hominis* were collected. The samples were centrifuged, the sera aliquoted, and kept at -20°C until used.

Analysis of parasite antigens by Western Blotting
The discontinuous system of Laemmli (1970) was used to separate polypeptides of all the above organisms on SDS-PAGE. Polypeptides separated on the gels were transferred electrophoretically to nitrocellulose paper (NCP), pore size 0.45 µm (MSI, Westboro, MA) by the method of Towbin *et al.* (1979), using a Semidry Blotter II (KEMENTEC) at 0.8 mA/cm gel for one hour. A NCP blot with the standard molecular weight markers was stained with amido black and the remaining NCP was soaked with quenching solution (5% skim milk in PBS pH 7.4) to saturate non-specific binding sites. It was then reacted with diluted human blastocystosis sera (1:100), followed by horseradish peroxidase goat anti-mouse immunoglobulins (KPL, USA). The blot was then immersed in the substrate 4-chloro-1-naphthol (ICN, Biomedicals) solution, rinsed in distilled water, air-dried and photographed.

Results

Polypeptide patterns from SDS-PAGE
Distinct polypeptide patterns were produced by two groups of *B. hominis* isolates on SDS-PAGE analysis (Fig. 1). The first pattern given by the group consisting of axenic isolates C and H, was characterised by major bands at molecular weights 55, 63, 66.4, and 97.2 kD.

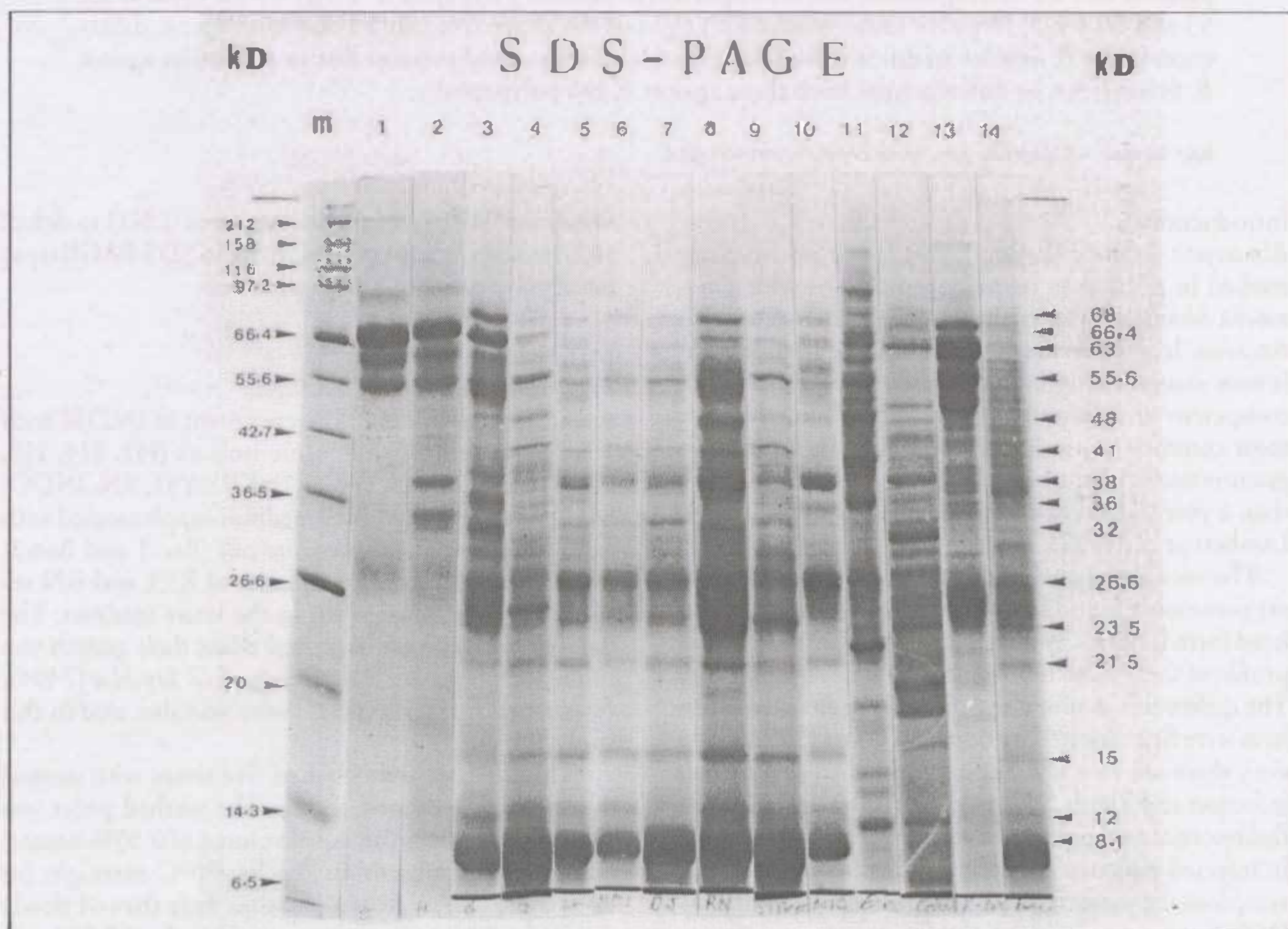

Fig. 1. SDS-PAGE polypeptide patterns of *B. hominis* isolates and other organisms.
Standard protein marker (lane m), *B. hominis* isolate C (lane 1), isolate H (lane 2), isolate H6 (lane 3), isolate H7 (lane 4), isolate KP1 (lane 5), isolate Y51(lane 6), isolate DJ1 (lane 7), isolate RN (lane 8), isolate 27B05(1) (lane 9), isolate M12 (lane 10), *Entamoeba histolytica*, HK9 (lane 11), *Giardia lamblia*, 7404 (lane 12), *Endolimax nana*, EN1(lane 13), *E. coli* isolated from culture medium of KP1, Bac-1(lane 14).

Two other bands at 34 and 38 kD were also present in isolate H. Except for the 97.2 kD band, these bands were also present as weak bands in *E. coli*. The second pattern was shown by another group consisting of xenic isolates H6, H7, KP1, Y51, DJ, RN, 27B05(1), and M12. At least 15 polypeptides ranging from 6.5 to 68 kD could be seen. Those at 8.1, 12, 15, 21.5, 24, 26.6, 32, 41, 63 and 66.4 kD were also present in *E. coli* (Bac-1). The prominent bands that were only presenr in this group of *B. hominis* isolates but not *E. coli* were at 34, 38, 55 and 68 kD. Certain isolates showed less intense or absent polypeptide bands e.g. the 41 kD band was absent in isolate M12. Other local isolates (H2, H4, 6105, 7105, INDO, BANG and 10203(1)) (not shown in Fig. 1) also had a similar pattern as the above local isolates.

The protein patterns of *E. histolytica* (HK9) and *G. lamblia* in SDS-PAGE were different from *B. hominis*. These parasites showed more than 15 prominent bands ranging from 12 to 158 kD and 6.5 to 97.2 kD respectively. The polypeptides of *E. nana* had molecular weights ranging from 12 to 158 kD while *E. coli* (Bac-1), isolated from KP1 culture, and Bac-2 isolated from RN culture (not shown in Fig. 1) had many polypeptide bands which were similar ro those of the local *B. hominis* isolates. The presence of at least 10 unique bands between 36 to 63 kD differentiated them from the local *B. hominis* isolates. Other protozoa such as *E. histolytica, G. lamblia, E. nana* and bacteria mentioned above showed their own distinctive polypeptide patterns.

Reactivity patterns of human blastocystosis sera

Twenty sera from blastocystosis patients were reacred against the 14 *B. hominis* isolates, one isolate each of *E. histolytica, G. lamblia, E. nana* and Bac-2 antigens respectively. All these sera did not react with *E. histolytica, G. lamblia* and *E. nana* anrigens. Twelve showed strong reactive bands against *B. hominis* isolates (Figs. 2-6) and 8 other sera had very weak or no reaction.

Serum from patient P1 infected with *B. hominis* (isolate INDO), only reacted against INDO and Bac-2 antigens (Fig. 2). It gave a number of bands between 43 to 55 kD against INDO and also recognised polypeptides at 58 to 70 kD in both INDO and Bac-2 antigens. No reactivity was shown against all other isolates of *B. hominis*.

Sera from patients P2, P3, P4, P5, P6, P7, P8 and P9 reacted against *B. hominis* (isolates C and/or H) (Fig. 3). There was weak or no reactivity against other *B. hominis* isolates and Bac-2 antigens (not shown in Fig. 3). Sera P3, P4, P6, P7 and P8 reacted strongly against isolate C. P2 and P5 showed strong reaction against polypeptides of isolates C and H at higher molecular weight while P9 recognised low molecular weight polypeptides of isolates C and H. Major bands recognised by most of these sera were at 43, 55, and 66.4 kD.

Human anti-RN serum, from a patient with diarrhoea, stomach discomfort and joint pains, reacted with all *B. hominis* isolates as well as Bac-2 antigen (Fig. 4). Most reactive bands were common to both *B. hominis* and Bac-2.

Serum P11, from an asymptomatic patient, reacted strongly against isolates C, H, H4, H6, 6105, INDO, 27B05(1), 10203(1), M12 and Bac-2. Reactive bands were seen at 42-55, 66.4 and 116 kD with these antigens. Ir reacted weakly against isolates KP1 and RN, and had no reaction against *B. hominis* isolate (Fig. 5).

Serum P12, from another asymptomatic patient did not react against isolates C, H and INDO (Fig. 6). Re-

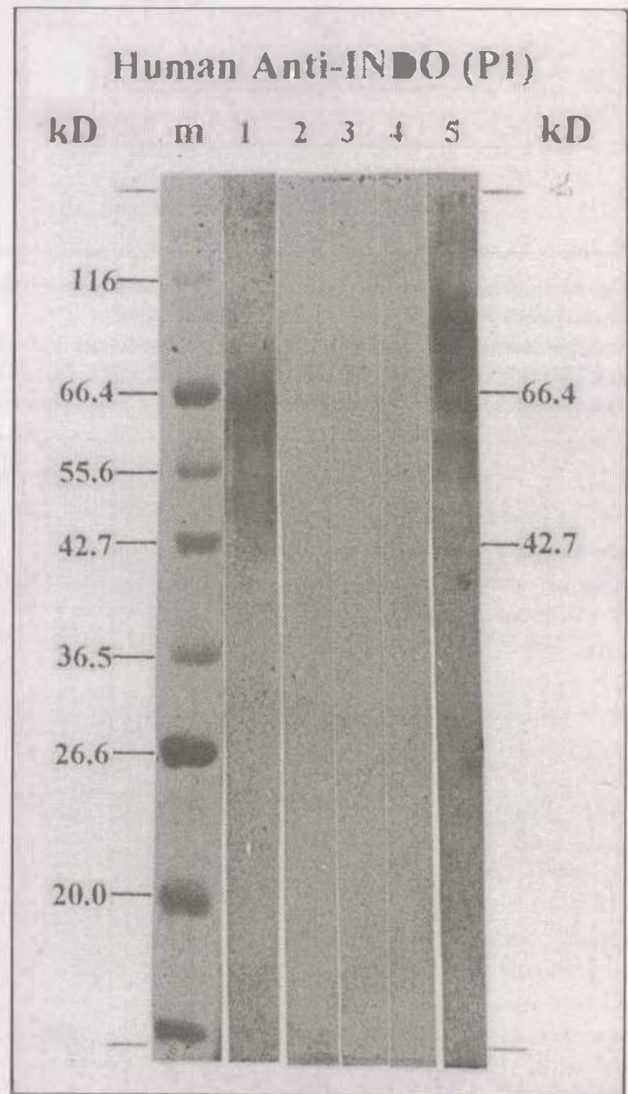

Fig. 2. Immunoblot parterns of SDS-PAGE separated polypeptides of *B. hominis* and other organisms on analysis with patient serum infected with isolate INDO (P1).
Standard protein marker (lane m), *B. hominis* isolate INDO (lane 1), No reaction partern against *B. hominis* isolates; H, H2, H4, H6, H7, 6105, 7106, KP1, Y51, DJ1, RN, 27B05(1) and M12 (lane 2), *E. histolytica*, HK9 (lane 3), *G. lamblia*, 7404 (lane 4), *E. coli* isolated from culture medium of RN, Bac-2 (lane 5).

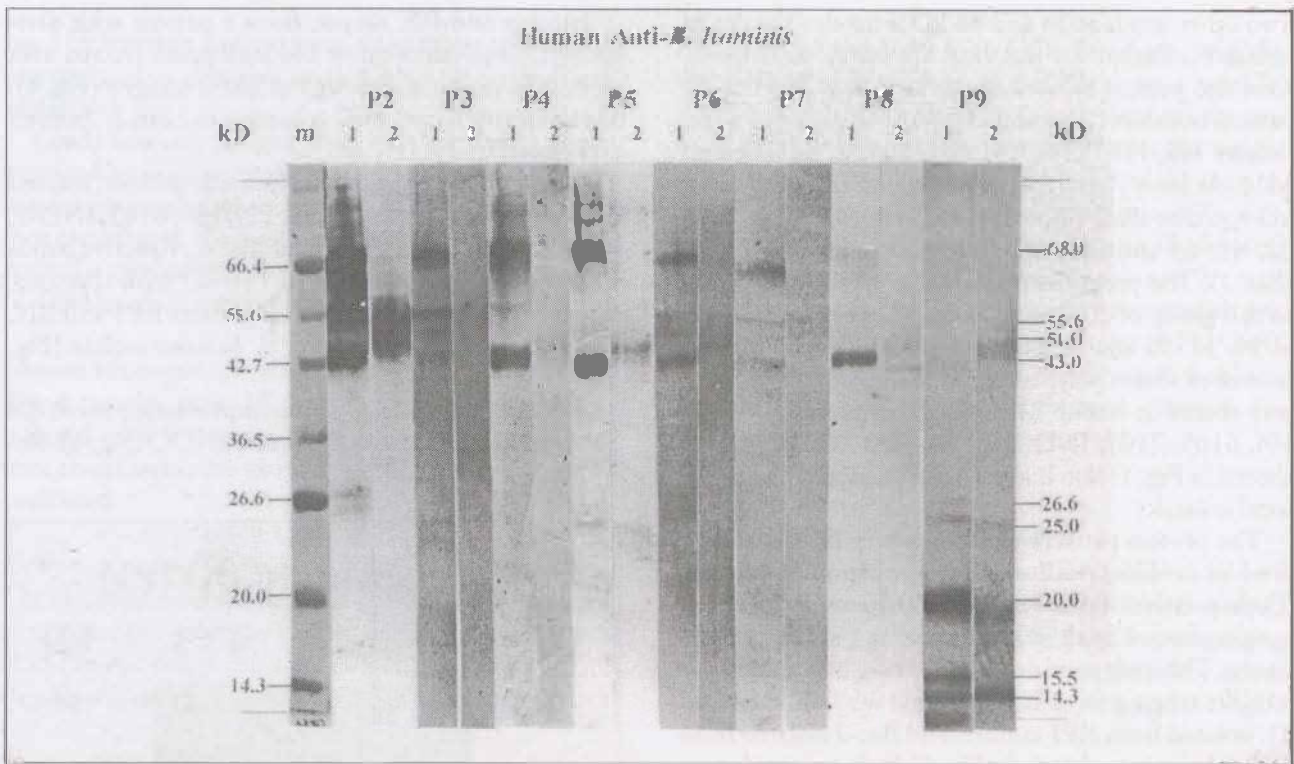

Fig. 3. Immunoblot patterns of SDS-PAGE separated polypeptides of *B. hominis* isolates and other organisms on analysis with human blastocystosis sera (P2, P3, P4, P5, P6, P7, P8 and P9).
Standard protein marker (lane m), *B. hominis* isolate C (lane 1), isolate H (lane 2). All above human antisera did not react with isolates H2, H4, H6, H7, 6105, 7106, 10203(1), 27B05(1), KP1,Y51, DJ1, RN, *E. histolytica* (HK9); *G. lamblia* (7404); and *E. coli* (Bac-2) antigens (not shown in Fig.).

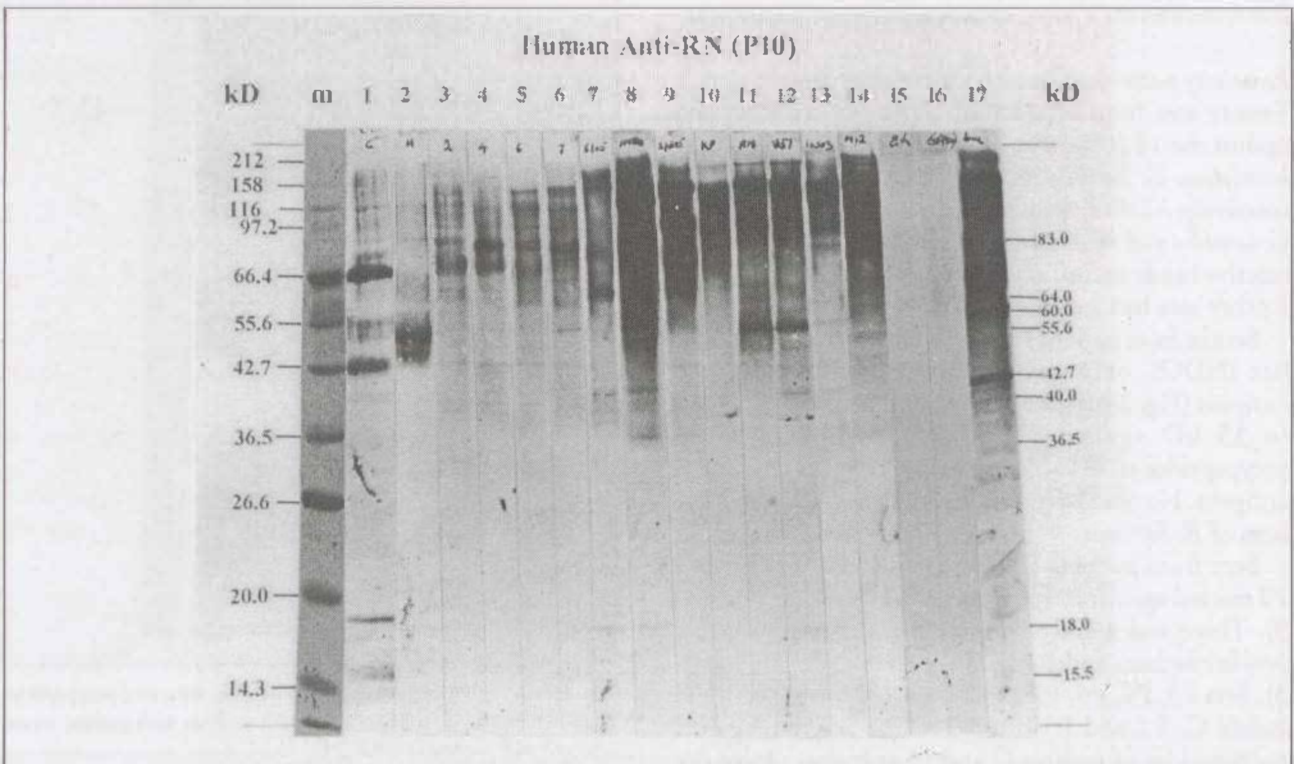

Fig. 4. Immunoblot patterns of SDS-PAGE separated polypeptides of *B. hominis* and other organisms on analysis with serum from a patient (P10) infected with RN.
Standard protein marker (lane m), *B. hominis* isolate C (lane 1), isolate H (lane 2), isolate H2 (lane 3), isolate H4 (lane 4), isolate H6 (lane 5), isolate H7 (lane 6), isolate 6105 (lane 7), isolate INDO (lane 8), isolate 27B05(1) (lane 9), isolate KP1 (lane 10), isolate RN (lane 11), isolate Y51 (lane 12), isolate 10203(1) (lane 13), isolate M12 (lane 14), *E. histolytica*, HK9 (lane 15), *G. lamblia*, 7404 (lane 16), *E. coli* isolated from culture medium of RN, Bac-2 (lane 17).

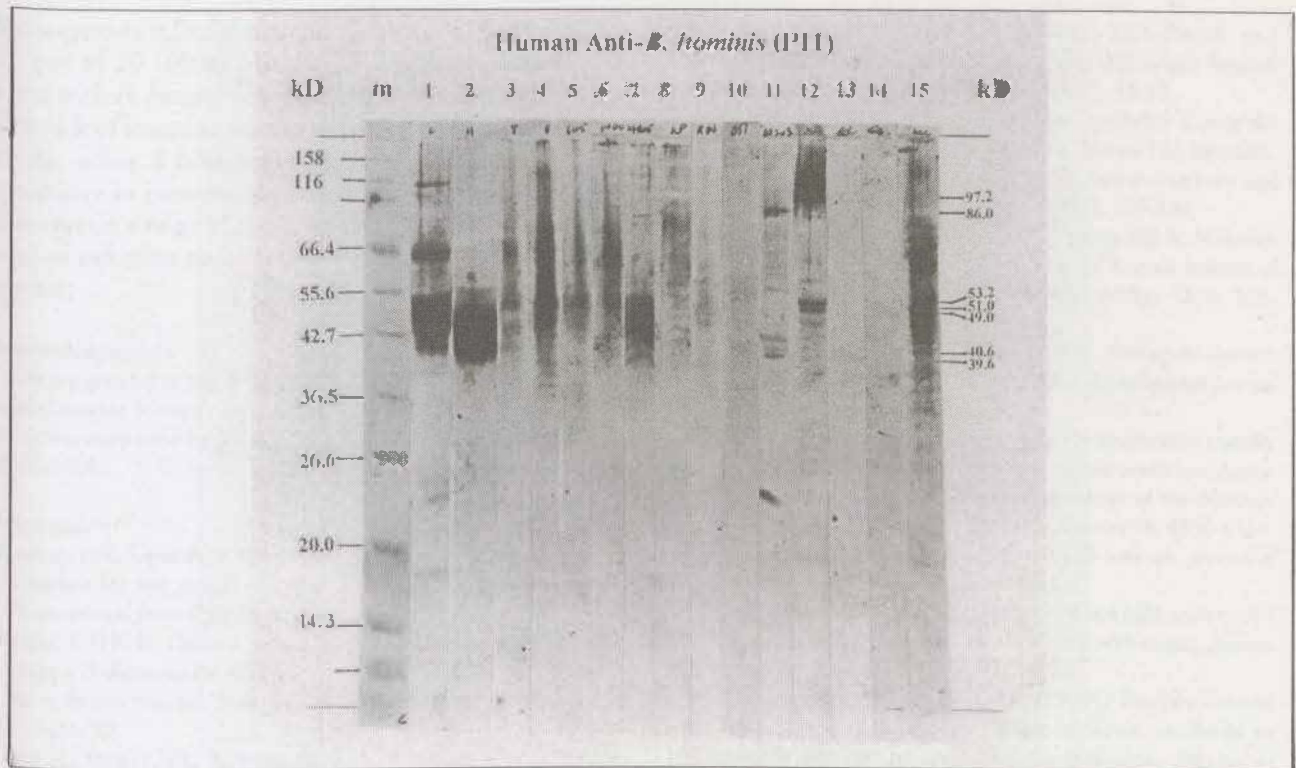

Fig. 5. Immunoblot patterns of SDS-PAGE separated polypeptides of *B. hominis* and other organisms on analysis with serum from blastocystosis patient (P11).
Standard protein marker (lane m), *B. hominis* isolate C (lane 1), isolate H (lane 2), isolate H4 (lane 3), isolate H6 (lane 4), isolate 6105 (lane 5), isolate INDO, (lane 6), isolate 27B05(1) (lane 7), isolate KP1 (lane 8), isolate RN (lane 9), isolate Y51 (lane 10), isolate 10203(1) (lane 11), isolate M12 (lane 12), *E. histolytica*, HK9 (lane 13), *G. lamblia*, 7404 (lane 14), *E. coli* isolated from culture medium of RN, Bac-2 (lane 15).

active bands at 43, 55, 66.4 kD were obtained against isolates H4, H6, H7, 6105, Y51, RN 27B05(1), 10203(1), M12 and KP1, as well as Bac-2. However, the latter antigen also produced prominent reactive bands between 36.5 to 43 kD, which were not seen with the parasite isolates.

Discussion

The differences in morphology of various isolates of *B. hominis* may reflect variations in the biochemistry, pathogenicity and general cell biology of the organism. There are very few reports on the biochemical or protein profiles of *B. hominis* even though its pathogenicity in humans has been reported (Zierdt, 1991). Kukoschke & Muller (1991) showed that there were consistent major differences by SDS-PAGE, Western blotting and Ouchterlony immunodiffusion in four axenic and microscopically indistinguishable strains obtained from different sources. They postulated that at least two variants with different polypeptide patterns and antigens existed, implying the existence of pathogenic and non-pathogenic strains. A study by Mansour *et al.* (1995) also found more than one pattern of polypeptides among 11 *B. hominis* isolates tested.

In the present study, SDS-PAGE followed by Coomasie blue gel staining was used to obtain the polypeptide profile of our local isolates as well as two isolates from Singapore. Two prominent polypeptide patterns were obtained. The first pattern shown by the axenic isolates C and H (isolated from Singaporean) had major bands at 55, 63, 66.4 and 97.2 kD. Two other bands at 34 and 38 kD were also present in isolate H. Except for the 97.2 kD band, these bands were also present as weak bands in *E. coli.* The second pattern, given by local xenic isolates H6, H7, KP1, Y51, DJ, RN, 27B05(1), M12 as well as H2, H4, 6105, 7105, INDO, BANG & 10203(1) (not shown in Fig.1), had prominent bands ranging from 8.1 - 68 kD. Those at 8.1, 12, 15, 21.5, 24, 26.6, 32, 41, 63 and 66.4 kD were also present in *E. coli* (Bac-1). The prominent bands that were only present in this group of *B. hominis* isolates but not *E. coli* were at 34, 38, 55.6 and 68 kD. Certain isolates showed less intense or absent polypeptide bands e.g. the 41 kD band was absent in isolate M12. Other local isolates (H2, H4, 6105, 7105, INDO, BANG and 10203(1)) (not shown in Fig. 1) also showed a similar pattern as the above local isolates. Polypeptides at 63, 66.4, and 97.2 were present only as extremely weak bands in *E. coli.* The differences in polypeptide profile between the axenic and xenic isolates are probably mainly due to bacterial polypeptides.

In the immunoblot analysis, of the 20 sera samples

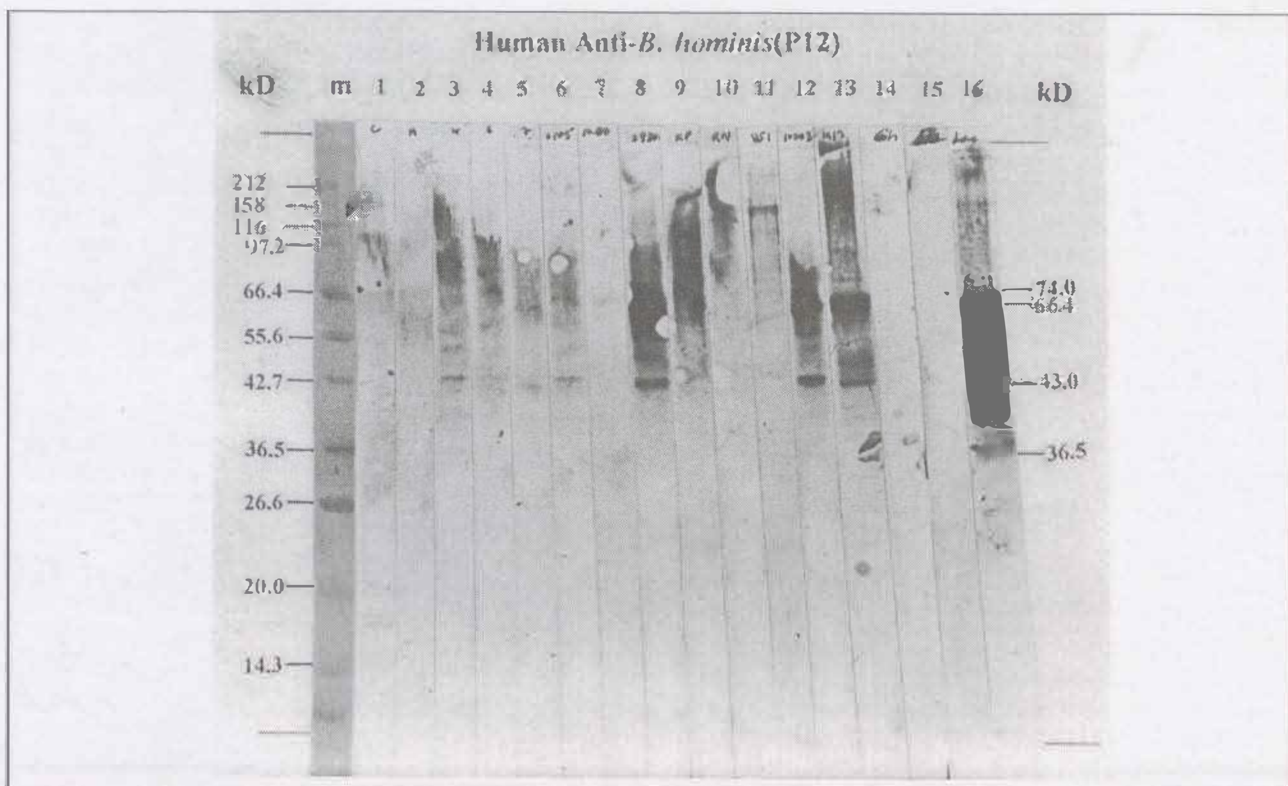

Fig. 6. Immunoblot patterns of SDS-PAGEseparated polypeptides of *B. hominis* and other organisms on analysis with serum from blastocystosis patient (P12).

Standard protein marker (lane m), *B. hominis* isolate C (lane 1), isolate H (lane 2), isolate H4 (lane 3), isolate H6 (lane 4), isolate H7 (lane 5), isolate 6105 (lane 6), isolate INDO (lane 7), isolate 27B05(1) (lane 8), isolate KP1 (lane 9), isolate RN (lane 10), isolate Y51 (lane 11), isolate 10203(1) (lane 12), isolate M12 (lane 13), *E. histolytica*, HK9 (lane 14), *G. lamblia*, 7404 (lane 15), *E. coli* isolated from culture medium of RN, Bac-2 (lane 16).

tested, one was from a patient infected with *B. hominis* isolate INDO (P1) and the other with isolate RN (P10). Serum anti-INDO only recognized isolate INDO. This suggests that isolate INDO can only induce specific antibody response against its antigens. Specific response was also shown in five other blastocystosis samples (P3, P4, P6, P7, P8). These sera only recognized isolate C while three other blastocystosis sera (P2, P5, P9) recognized isolate C and H (Fig. 2). There are differences in the recognition patterns of blastocystosis sera against isolates C and H. Serum P2, P3, P4, P5, P6 and P7 had reactive bands at 43 and 66.4 kD with isolate C. With the exception of serum P5, all these sera also reacted with a 55 kD polypeptide in this isolate. Serum P2, P5, P6 and P9 recognised a 50 kD polypeptide of isolate H. Serum P9 also reacted with the 14.3, 20 and 25 kD polypeptides in isolates C and H. This serum was from a person who has been working with *B. hominis* isolate C for about three years, and has probably been infected with it. We also suspect that P2, P5 and P9 were sera from patients infected with isolate C which cross-reacted with isolate H.

Serum P10 (infected with isolate RN; Fig. 4) showed cross-reactivity to all *B. hominis* isolates tested, showing prominent recognition bands against all the suspected pathogenic isolates and Bac-2, but weak bands to all suspected nonpathogenic isolates. It also showed strong reaction against Bac-2 which may be due to host immune response against bacteria in the patient as she was the original source of Bac-2.

We believe that serum P11 (Fig. 5) was from a patient infected with C or H isolates; as the most prominent reactivity was against isolates C and H, even though there was cross-reaction against other *B. hominis* isolates tested. Serum P12 was from a patient infected with one or more pathogenic strains of *B. hominis* (Fig. 6). Prominent reaction bands against suspected pathogenic isolates (27B05(1), KP1, 10203(1), M12) and weak reactivity bands against local (H4, H6, H7, 6105) and Singapore (C and H) nonpathogenic isolates were seen.

The above immunoblot characteristics may be due to host immune response, the duration of infection, the parasite load, parasite changes (whether vacuolared, granulated, ameoboid or cystic), and differences in toxins or excretory-secretory contents released in the host.

In a study by Chen *et al.* (1987) reactivity was not seen on immunoblot analysis with sera from four

blastocystosis infected patients. In this study, we found 12 out of 20 (60%) blastocystosis antisera tested reacted with *B. hominis* antigens on immunblot analysis. The lack of immune reactivity of human blastocystosis in the other 8 blastocystosis sera could be due to specificity in immune response. Another possibility is that certain strains of this parasite may need a long period of infection to induce host humoral immune response.

Acknowledgements

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