Research Note

Detection of 64 and 57 kDa immunogenic epitopes in experimental infections of *Blastocystis hominis* in Wistar rats

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There are few immunological studies on the intestinal human protozoan parasite, *Blastocystis hominis*, Brumpt (1912). Zierdt & Tan (1976) using infected patient's sera on fixed *B. hominis* cells did not show antibodies to be present with the immunofluorescence test (IFA). Chen *et al.* (1987) investigated four patients with *Blastocystis* for the presence of humoral antibody by immunoblot analysis and did not detect any serum antibody response to *B. hominis* proteins, even after exposure of autoradiographs for 6 days.

An experimental infection in Wistar rats with cysts of *B. hominis* was recently established (Suresh *et al.*, 1993). The present study reports antibody response in these rats as detected by the immunoblot analysis.

A local axenic isolate of *B. hominis* (isolate B) was maintained in minimum essential medium (GIBCO) under anaerobic conditions at 37°C according to the method of Upcroft *et al.* (1989). A non-axenic human isolate (MS) of *Blastocystis* was grown anerobically in Jones' medium at 37°C.

Cultures of isolate B were concentrated by centrifugation at 600 g for 10 minutes. The pellet of *Blastocystis* was washed six times, and subjected to ultrasonication (using a MSE sonicator with a titanium probe) with 30 seconds pulses at 30-second intervals for 10 min. Microscopic examination of the suspension at this stage showed that no intact cells remained. After centrifugation (Beckman JS-21 centrifuge, rotor JA-20.1) at 18,000 rpm (25,000 g) the supernatant was carefully collected, aliquoted and stored at -20°C until required. The protein concentration of the soluble extract using the Bio-Rad assay was estimated to be 1.11 µg/ml.

Ten laboratory-bred Wistar rats were screened for *Blastocystis* infection prior to their use. Rats were infected with the parasite following the procedure described previously (Suresh *et al.*, 1993).

Eight of these rats were divided into 4 groups of 2 rats each. Rats in groups 1 and 2 were infected through oral intubation with 10^4 and 10^5 cystic stages (isolate B) from 3 day-old cultures in encystation medium respectively. Animals in groups 3 and 4 were similarly infected with 10^4 and 10^5 cystic stages of the non-axenic isolate MS respectively. Blood was collected in eppendorf tubes at week 0,1,2,3,4,6,8,10,12 and 24 post-infection from the tail of the rats. Sera were stored at -20°C until tested.

The remaining two rats were immunized with the soluble extract of isolate B in Freund's complete adjuvant via the subcutaneous route once every two weeks. At 8 weeks post-immunization the rats were killed and the sera obtained via cardiac puncture.

SDS-PAGE was performed by the method of Laemmli (1970) using a 10% acrylamide resolution gel. A total of 50 µl of soluble antigen was applied to each lane of the gel (Bio Rad). After electrophoresis the separated proteins were transferred to nitrocellulose paper (0.2 µm) for 3 hours at 0.75 A according to the method of Towbin *et al.* (1979). After reacting overnight with sera (1:50) of cyst-infected rats the bands were visualized after reacting with 1:2000 dilution of peroxidase-conjugated rabbit anti-rat immunoglobulin (Dakopatts) followed by development in H_2O_2/4-chloro-1-naphthol.

Rats infected with 10^5 cysts of both isolates showed bands whereas rats infected with 10^4 cysts of both isolates were negative. Sera from control rats showed no bands. Discrete bands at 64 and 57 kDa were seen in rats from group 2 (Fig. 1) while rats from group 4 showed only a distinct band at 64 kDa. The band at 57 kDa became more intense with increasing weeks of post-infection. The bands from sera of all rats became faint by week 24 post-infection. Serum from immunized rat at week 8 showed distinct bands at 98, 68, 61, 57 and 41kDa.

The antigen used in the study was a soluble extract of cultured cells of *B. hominis* of axenic isolate B. Antigen was not prepared from the non-axenic isolate MS and this could be the reason for the different reactions.

There are increasing reports that the parasite is a diarrhoea causing pathogen but however our knowledge on the biology and the immunology of the parasite continue to remain an enigma. Other reports of an antibody response against *B. hominis*

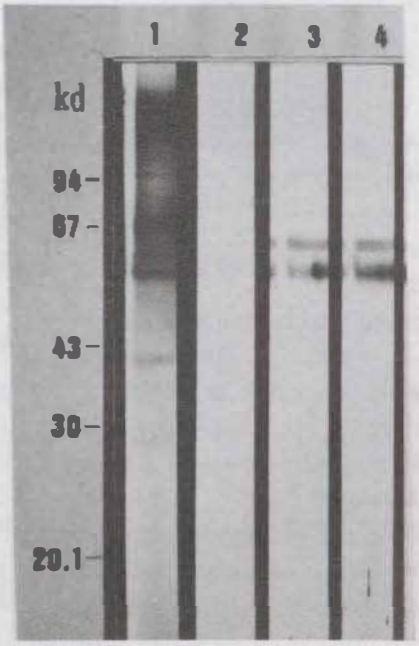

Fig. 1. Immunoblots with sera from rats immunized or infected with *Blastocystis hominis*. Lane 1: Serum from immunized rat at week 8. Note prominent 98, 68, 61, 57 and 41 kDa bands; Lane 2: Serum from uninfected rat without prior *Blastocystis* infection. No bands were seen; Lane 3: Distinct bands at 64 and 57 kDa reaction with serum from one of the rats in group 2 at week 8 post-infection; Lane 4: Similar bands were seen in the same rat at week 10 postinfection. Note that the 57 kDa band is more intense

were studies of Zierdt (unpublished observation) and that of Kukoschke & Muller (1991). The former used rabbit antisera to *B. hominis* antigen and demonstrated immunofluorescence with the central body, amoeboid and granular forms from both culture and faeces while the latter using western blot analysis and Ouchterlony immunodifusion, provided immunological evidence for the existence of two different strains of *B. hominis*. Zierdt *et al.* (1995) showed that serum antibody to *B. hominis* could be detected in symptomatic patients using the enzyme-linked immunosorbent assay. Seventeen patients who showed clinical symptoms and had only *B. hominis* as the sole pathogen showed elevated levels of IgG immunoglobulin .

In the present study, we have demonstrated that a immune response was evoked during acute infection of *B. hominis*. A protein of MW 57 kDa appears to be the common dominant *B. hominis* antigen with weaker reactivity against the 64kDa protein.

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Received 2 January 1998; revised 2 May 1998; accepted for publication 1 July 1998.