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Evaluation of Specific and Non-Specific Cellular Immune Responses in Amoebiasis Patients

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Abstract

Specific and non- specific cellular immune responses were studied in 20 amoebiasis cases and 10 controls. All the study cases and controls were Indians living in Varanasi, India. Ten amoebic liver abscess cases were patients admitted to University hospital. Ten non-invasive intestinal amoebiasis cases constituted the second study group. Non-specific immune responses were studied using E-rosette technique. Specific cellular immune response was evaluated by measuring tritiated thymidine uptake by transformed lymphocytes using crude amoebic extract prepared from axenically grown *Entamoeba histolytica* NIH: 200 and two of its chromatographed fractions, i.e. fraction I (FI) & fraction II (FII) as well as a mitogen, i.e. Phyto Haemagglutinin-A (PHA). Results show those crude antigens followed by FI & FII are able to induce blastoid transformation of lymphocytes prepared from different cases. Moreover, it was shown that specific cellular immune response was greatly depressed in amoebic liver abscess cases (P<0.001). While the CPM (count per minute) of different groups on using PHA for stimulation did not vary significantly.

Keywords: *Thymidine uptake, Specific cellular immune response, Non- specific cellular immune response, Amoebiasis*

Introduction

The intestinal protozoan parasite *Entamoeba histolytica* is capable of invading and destroying human tissues, leading to potentially life-threatening diseases such as haemorrhagic colitis and extra-intestinal amoebiasis(1). It is estimated that *Entamoeba histolytica* is responsible for about 50 million cases of invasive amoebiasis annually, resulting in approximately 100,000 deaths, and thus rates among the leading parasitic causes of death, surpassed only by malaria and schistosomiasis(2).

Pathogenic effect of the parasites depends on the parasite interactions (3).It is often difficult to draw clear-cut distinction between infection and disease (4). The conditions leading to the occurrence and subsequent resolution of invasive disease are multifactorial and probably, relates to a balance between pathogenic mechanisms and host defense mechanisms (5, 6). The present study was undertaken with an aim to study the alterations in cellular immune responses, specific and non- specific, in human amoebiasis cases and controls.

Materials and Methods

In order to study the cellular immune responses, a total of 30 individuals were included for the study design. Out of these, 10 were amoebic liver abscess patients. These patients were admitted to gastroenterology ward, B.H.U (Banaras Hindu University) hospital. They were diagnosed on the bases of clinical and ultrasonography findings and confirmed by aspiration of typical anchovy sauce pus from the liver abscess. Parasitological investigations like microscopic investigation of stool and aspirated pus were carried out.

Second group, with 10 patients, were named as non-invasive intestinal amoebiasis that showed bowel pain but no dysentery. Three consecutive examinations of their stool showed amoebic cysts. Another 10 cases were grouped as control subjects who were healthy persons with no signs and symptoms related to amoebiasis.

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Three consecutive examinations of their stool by direct microscopy and after employing formol-ether concentration technique (7) did not reveal any amoebic cyst.

a) Evaluation of non specific cellular immune responses by E-rosettes i) Preparation of sheep erythrocytes. Sheep blood was collected aseptically into equal volumes of Alsever′ s solution 0.5% (8). Suspension of SRBC (Sheep Red Blood Cells) in Hank′ s balanced salt solution was prepared with 10% faecal calf serum (Hi Media, Bombay).

ii) Preparation of rosettes. Rosettes were prepared as described earlier (9).

iii) Microscopic examination of cell suspension. A coverslip preparation was made and 200 lymphocytes were counted to calculate the percentage of rosettes forming lymphocytes (RFC %). Lymphocytes covered with three or more sheep red blood cells were taken as rosettes.

b) Evaluation of specific cellular immune response by transformation of lymphocytes: Once lymphocytes were prepared their count was adjusted to 1*10 and transformed into RPMI 1640 medium and divided into following tubes: Tube I (control tube) which contained lymphocytes from healthy individuals. To tube II, 0.11 ml of PHA was added. To tube III, 0.11 ml of ultrasonicated axenically grown *Entamoeba histolytica* (NIH: 200) crude extract containing 400 µg of protein was added. Protein content of the antigen was measured according to Lowry method (10). To tube IV, 0.15 ml of chromatographed fraction (FI) of amoebic antigen containing 400 µg of FI protein was added. To tube V, 289 ml of chromatographed fraction (FII) of amoebic antigen containing 400 µg of FII protein was added.

Processing of the tubes Tubes were processed as described earlier (11). The stimulation index and transformation index induced by each individual antigen of *Entamoeba histolytica* for the blastogenic response of the cases and controls were compared. The statistical analysis of the results (calculating student t-test and estimating the P values) was carried out to evaluate the antigen (s) specifically inducing maximum blast transformation for cell mediated immunity.

Results

i) Evaluation of rosette forming cells (RFCs) After counting 200 rosette forming cells, it was found out that in invasive amoebiasis cases (lymphocyte suspensions were prepared from amoebic liver abscess patients) mean percentage of rosette forming cells was 47.2% while in non invasive amoebiasis cases, the mean percentage was 49.3% (Table 1). In control group, the mean percent of RBCs was found to be 54.8%. Significant difference was noticed when mean percentage of rosette forming cells of invasive amoebiasis cases was compared with that of control group (P<0.01). On comparing the mean values of non invasive amoebiasis with control group no significant difference was observed.

ii) Blastoid transformation of lymphocytes The result of uptake of tritiated thymidine by lymphocytes is presented in (Table 2). Further analysis of the blastogenic response of the lymphocytes to PHA and amoebic antigen in different study groups revealed that cell mediated immune response was altered significantly in invasive amoebiasis group. Significant difference was observed when cultures of invasive amoebiasis group stimulated with different amoebic antigens were compared with mean CPM of control group and with the mean CPM of unstimulated culture of amoebiasis cases (P<0.001). Significant difference was also observed when mean CPM (count per minute) of non invasive amoebiasis cultures stimulated with different amoebic antigens was compared with mean CPM of control cultures stimulated with the respective antigens (P<0.001). The mean stimulation index of the lymphocytes of different cases by different antigens is calculated and is presented in (Table2).

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Table 1: Rosette forming cells in amoebiasis and controls

Clinical Categories	Absolute lymphocyte count	Mean percent RFC +S.D
Invasive amoebiasis	2854	47.2 +7.8
Non-invasive amoebiasis	2735	49.3 +7.1
Control individuals	2486	54.8 +3.5

Table 2: Result of CPM in amoebiasis and control groups on stimulation with different antigens

Clinical Categories	Mean CPM of unstimulated culture	Mean CPM of crude stimulated culture	Mean CPM of FI stimulated culture	Mean CPM of FII stimulated culture
Invasive amoebiasis	598 +173.20	18807+1373.24	17812+1872.30	13606 +374.36
Non-invasive amoebiasis	602 +186.24	17165+1391.37	16258+1165.40	11854+1837.35
Control individuals	493 +172.57	1501+313.15	1135 +218.27	1066 +312.79

t =1.58 P (1:2) NS t =2.9 P (1:2) 0.01 t =2.33 P (1:2)0.05
t =1.45 P (1:4) NS t =38.92 P (1:4) 0.001 t =27.84 P (1:4)0.001
t =0.07 P (2:4) NS t =34.73 P (2:4) 0.001 t =40.33 P (2:4)0.001

Discussion

Non specific cellular immune response was assayed by percentage of rosettes forming cells in different amoebiasis cases and controls. In this study, marked difference was observed in invasive amoebiasis group compared to control group. Similar findings have been reported by Segovia et al (12), Acharya et al (13) and Ahluwalia et al (14). The blast transformation of lymphocytes in invasive amoebiasis group was found to be significantly depressed as compared to healthy controls when stimulated by crude antigen followed by FI and FII. The results suggest that both specific and non specific cell mediated immunity is involved but, specific cell mediated immune response is depressed to a greater extent. Our finding correlates well with the findings of the Ortiz-ortiz et al (15) who showed that specific cell mediated immunity to amoebic antigen is reduced in amoebic liver abscess cases. In the present study non specific response was also depressed in invasive amoebiasis group though to lesser extent than that of antigen stimulation. Vinayak et al (16) also published similar findings that non specific cellular immunity in acute phase of hepatic amoebiasis had been depressed. Both specific and non specific cell mediated immunity appears to be normal in non invasive amoebiasis patients as no significant difference could be observed when lymphocytes were stimulated by PHA.When FI and FII antigens were used for stimulation of lymphocytes significant difference was observed (P<0.001) as compared to healthy controls. Vinayak et al (16) also reported that in contrast to hepatic amoebiasis, colitis is not associated in a significant way with the development of blastogenic response, as in the latter, the antigenic stimulus is not strong enough to sensitize the lymphocytes. The lowered thymidine incorporation in control group compared to amoebiasis cases is due to the fact that there is no amoebic manifestation in these persons. To conclude, both specific and non specific cell mediated immunity are involved in amoebiasis and crude antigen followed by FI induce stronger immune responses.

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