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T Lymphocyte Subsets in BALB/c Mice Infected with Different Strains of *Giardia lambilia*

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ABSTRACT

The precise reason for variability of disease spectrum in *Giardia lamblia* infected persons is not understood, but host immunity (especially local immunity) appears to be an important contributory factor. To study the interaction between host and the parasite in the small intestine, it is important to study the local cell mediated immune response to understand the pathophysiology of giardiasis. For this, in the present study the T-lymphocyte subsets were studied in intestines of BALB/c mice infected with different *G. lamblia* strains isolated from asymptomatic and symptomatic patients. An increase in percentages of $CD3^+$ cells (Thy 1^+) and B cells was observed, with a peak on 12th day post inoculation . There was also an increase in $CD4^+$ cells ($L3T4^+$) which reached a peak on 12th and 18th day post inoculation in mice infected with strains isolated from asymptomatic and symptomatic patients respectively. $CD8^+$ ($Lyt2^+$) cells were also slightly elevated and peaked at 12th and 24th day post inoculation in mice infected with asymptomatic and symptomatic strains respectively. It is therefore suggested that the increase in $CD4^+$ cells may be beneficial to the host as it correlated directly with the elimination of the parasite and thus these cells might play an important role in pathogenesis of diarrhoea caused by this parasite.

INTRODUCTION

Symptomatic or asymptomatic infection due to *Giardia lamblia* in man has been related to the immunological status of the host (Ament and Rubin 1972). A higher incidence of malabsorption has been reported in persons with immunoglobulin deficiency(Logalbo et al 1982) and in malnourished children (Gracey 1973). Studies carried out in experimental animals have shown that intestinal lesions and a local immunologic response helps in elimination of the parasite from the gut (Snider and Underdown 1986).

In *G. lamblia* infected mice, increase in intra-epithelial lymphocytes has been reported (Upadhyay et al 1986). This increase is similar to that observed in coeliac disease where these cells have been implicated in causation of tissue damage (Davidson and Bridges 1987). The role of T lymphocyte subsets in immunity has been elegantly demonstrated by

studies in *G.muris* infected mice (Heyworth et al 1987). Mice depleted of T helper cells develop chronic infection whereas those depleted of T suppressor cells or deficient in natural killer cells clear their infection (Heyworth et al 1986, Heyworth et al 1987) indicating that T helper cells have an important role in clearing the infection. However, no such information on *G. lamblia* infection in experimental animals is available. It is also not clear whether strains of *G. lamblia* isolated from either asymptomatic or symptomatic patients elicit similar or different immune responses. In the present study, we have investigated the immune response of mice infected with different strains of *G. lamblia* and correlated this with clearence of the parasite and hence with the pathogenesis of diarrhoea caused by the parasite.

MATERIALS AND METHODS

ANIMALS : BALB/c mice,3-4 weeks old, and weighing 15-20 gms each were used throughout the study. Three consecutive stool specimens of these animals were checked for any parasite by direct smear examination and formal ether concentration method and only parasite free animals were used.

STRAINS OF *GIARDIA LAMBLIA* AND THEIR PURIFICATION : *Giardia lamblia* strains were isolated from stool samples of infected patients admitted to the Nehru Hospital attached to the Postgraduate Institute of Medical Education and Research,Chandigarh. The strains were isolated from two types of patients.

Symptomatic patients: Where giardia infection was associated with one or more symptoms consistent with giardiasis (diarrhoea,abdominal pain, vomiting, flatulence and abdominal distension) which persisted for more than 72 h and which were not attributed to any other infection or illness

Asymptomatic patients: Who were positive for *Giardia lamblia* cysts but there was absence of any symptoms mentioned above. 10 patients were studied in each group . Cysts were purified from stool samples by sucrose density gradient according to the method described by Sheffield and Bjorvatn (1977).

INFECTION OF MICE WITH *G. LAMBLIA* CYSTS : Each mouse was infected intra oesophageally with 10,000 cysts in 0.2 ml of normal saline (Roberts-Thomson et al 1976). A minimum of 6 animals were sacrificed on 6th, 9th, 12th, 18th, 24th and 30th day post inoculation (d.p.i). The trophozoites were counted in intestinal perfusate of infected animals (Vasudeva et al 1982). Uninfected animals sacrificed on same days served as controls.

ANALYSIS OF GUT LYMPHOCYTES : Lymphocytes were separated from the small intestine (Davies and Parrott 1981). The immunofluorescent staining of the gut lymphocytes was done by the method described elsewhere (Wangoo et al 1990). Lymphocytes were incubated with 100 µl of appropriate monoclonal antibody (MAb) (Sera Lab, Sussex Ltd, England) diluted to 1:100 in phosphate buffered saline (pH- 7.2) containing 0.1% sodium azide, 0.5% bovine serum albumin and 5% inactivated rabbit serum (to block Fc binding). Different MAb's used were anti mouse Lyt 2^+ for CD8+ cells, anti mouse L3 $T4^+$ for $CD4^+$ cells and anti mouse $Thy1^+$ for total T cells. The tubes were then agitated and incubated on ice for 30 min. The cell-MAb suspension was layered over 0.5 ml of foetal bovine serum (FBS) in small test tubes and centrifuged at 200xg for 5 min. at $4^{O}C$. The supernatant was decanted and 100 µl of diluted FITC mouse monoclonal anti-rat IgG 2b(Sera Lab) was added. The mixture was agitated and incubated on ice for 30 min. After this , the cell-antibody suspension was again overlaid onto 0.2 ml FBS and centrifuged as described above. The fluorescein labelled conjugate was carefully removed with a Pasteur pipette. The cells were suspended in buffered glycerol and observed under a fluorescent microscope (Carl Zeiss). The percentage of fluorescent cells was calculated.

B lymphocytes (Ig^+) were stained with 100 µl of swine anti-mouse immunoglobulins (FITC-conjugated) by the method described elsewhere (Ganguly et al 1981).

STATISTICAL ANALYSIS : Data was expressed as mean ±S.D. of percentages obtained from a set of six animals. Significance was calculated by student's t test.

RESULTS AND DISCUSSION

The overall pattern of infection in both groups of animals i.e. those infected with strains of *G. lamblia* isolated from asymptomatic and others infected with strains from symptomatic patients was similar. The trophozoite count on 6th and 9th dpi was significantly higher ($P<0.05$) in strains isolated from symptomatic cases as compared to those infected with strains isolated from asymptomatic patients (Fig. 1), but on 12 dpi, the difference was not statistically significant ($P>0.01$). The prepatent period of infection in both groups was 5-6 d and peak infection was observed on 9th dpi. Following that there was a spontaneous decline in the trophozoite counts in both the groups.

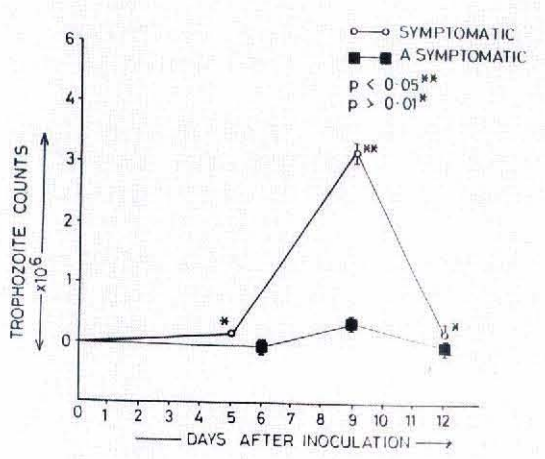

Fig. 1. Trophozoite counts on different postinoculation days in animals infected with asymptomatic and symptomatic strains.

Similar observations have been reported by Kanwar et al (1984) in Swiss albino mice where prepatent period was about 6th d, peak infection on 9th dpi and infection was almost eliminated on 30th dpi. Visvesvara et al (1988) also reported the infectivity of human strains of *Giardia lamblia* from symptomatic and asymptomatic patients in gerbils.

The present study suggests that strains from symptomatic patients leads to a more vigorous colonisation by the parasite as compared to the response elicited by strains isolated from asymptomatic patients thereby resulting in increased number of trophozoite counts in mice infected with strains from symptomatic patients as compared to asymptomatic.

As regards the changes in levels of cells, there was an early increase in percentages of $CD3^+$ cells in mice infected with strains isolated from asymptomatic patients. The percentages of $CD3^+$ cells reached at its peak on 12th dpi in mice infected with both types of strains. Subsequently the levels showed a greater decrease in mice infected with strains isolated from asymptomatic patients (Fig. 2).

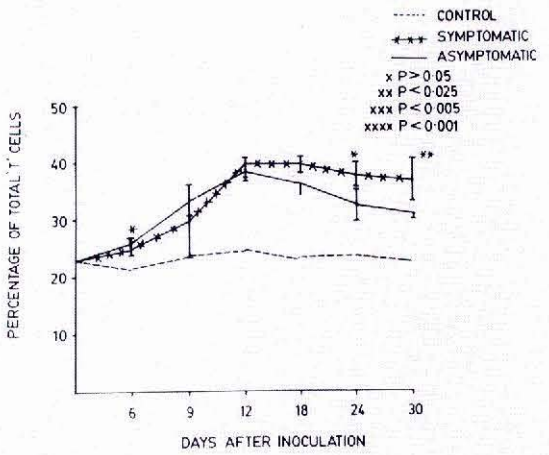

Fig. 2. Percentages of total T cells on different days after inoculation in animals infected with symptomatic and asymptomatic strains.

There was also a significant increase in the percentages of B cells in mice inoculated with strains isolated from symptomatic patients on 18 dpi (Fig. 3). The percentages of B cells remained elevated till the end of the study period in the mice infected with strains isolated from symptomatic patients, while they declined to the baseline values by 24 dpi in the other group.

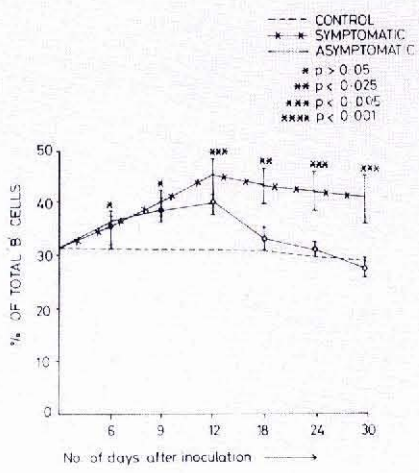

Fig. 3. Percentages of total B cells on different days after inoculation in animals infected with symptomatic and asymptomatic strains.

Increase in intestinal lymphocytes has been reported earlier (Mac Donald and Ferguson 1978). Heyworth et al (1985) characterised these intraluminal lymphocytes by staining with biotin conjugated antibodies to Thy 1.2 antigen, followed by rhodamine avidin conjugate and found that these were T lymphocytes and the peak lymphocyte count was associated with parasite clearance. Direct and antibody dependent cytotoxicity by intraepithelial and lamina propria lymphocytes against *G. lamblia* trophozoites has also been reported (Kanwar et al 1986).

A significant increase in $CD4^+$ cells in *G. lamblia* infected mice was observed which reached a peak on 12th dpi in animals infected with strains isolated from asymptomatic patients and on 18th dpi in mice infected with strains from symptomatic patients (Fig. 4). The $CD4^+$ cells remained elevated till 30th dpi but there was a general decline after 18 dpi $CD4^+$ cells have been implicated in clearing the *G.muris* infection in mice (Heyworth et al 1986 and 1987) as their depletion by MAb's leads to a chronic infection. This depletion also causes an impaired intestinal response against the parasite (Heyworth 1989).

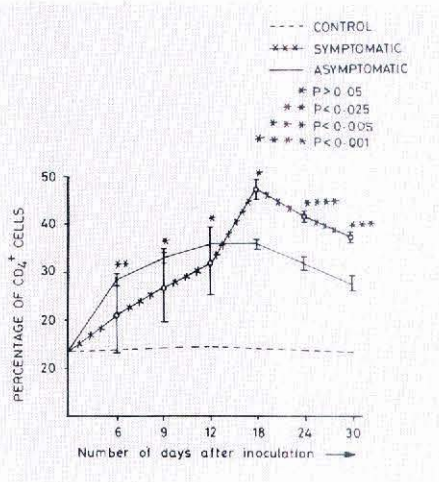

Fig. 4. Percentages of $CD4^+$ cells on different days after inoculation in animals infected with symptomatic and asymptomatic strains.

$CD8^+$ cells were also slightly elevated in mice infected with strains from asymptomatic patients. They were maximum on 12th dpi and subsequently declined to baseline on 18th dpi nd remained at this level till 30th dpi. In case of mice infected with strains from symptomatic cases the counts were maximum on 24th dpi and decline was obseved on 30th dpi (Fig. 5).

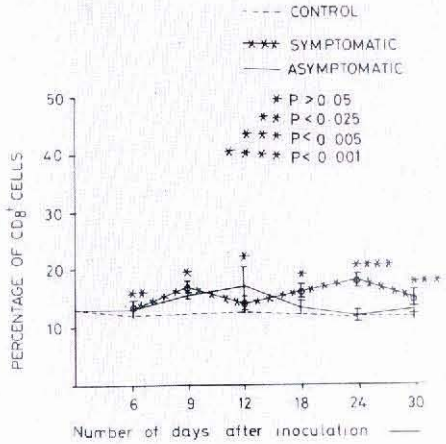

Fig. 5. Percentages of $CD8^+$ cells on different days after inoculation in animals infected with symptomatic and asymptomatic strains.

In the present study an increase in T helper cells ($CD4^+$) with slight elevation of T suppessor cells ($CD8^+$) in *G. lamblia* infected mice was observed. Increase in $CD4^+$ cells was observed till 30th dpi, when infection was almost eliminated . It was observed that although strains from asymptomatic and symptomatic patients led to a vigorous response, it was more so in animals infected with strains isolated from symptomatic patients as compared to others isolated from asymptomatic patients. An increase in CD4+ cells appears to be beneficial as it relates very well with elimination of *G. lamblia* infection, where it has been seen that when the local immunologic response is at its peak, *G. lamblia* is eliminated from the gut.

Persistence of $CD4^+$ cell response at a higher level in mice infected with symptomatic strains raises the possibility about the role of these cells in immune mediated damage in animals infected with these strains. Both these aspects of host-parasite relationship as the causative factor of symptomatology in giardiasis need further evaluation to clarify which factors interplay to produce either asymptomatic or symptomatic infection in humans.

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