GASTROENTEROLOGY IN INDIA

Pathophysiology and Immunobiology of Giardia lamblia Infection

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Introduction

Giardia lamblia is the most frequently encountered flagelated protozoan parasite which colonizes human upper gastrointestinal tract. The infection is more common in children and has been shown to be particularly more severe in immunocompromised subjects.¹ The parasite has been considered to be associated with diarrhea and malabsorption especially in children. The infection by and large remains asymptomatic in chronic status with frequent acute episodes of diarrhea and gastrointestinal disturbances in adults. Although several factors have been proposed, the precise mechanism(s) leading to defective absorption of nutrients from the small intestine remain elusive. Though significant specific antibody response occurs in the host following giardial infection, the immune aberrations especially at the gut level allowing the parasite to colonize, multiply and damage the brush border membrane are ill understood. Local immune factors responsible for the elimination of the parasite are also unclear.² This article will briefly highlight several clinical and experimental investigations that have been carried out by Indian scientists to elucidate some of the above unknowns.

Use of experimental model(s) simulating the human giardial disease has been considered essential since in human disease it is rather difficult to assess a) duration of ongoing giardial infection, b) duration of severity of infection, c) previous silent exposure to G lamblia, or d) the presence of other concomitant asymptomatic infections or underlying disease.¹

Experimental Model

The quest for a suitable rodent model of giardiasis led to successful infection of weanling rats with daily dose of human feces containing G lamblia cysts for three days,³⁴ though 'take' rate of infection was not very high. The infection rate could be improved by one passage of the cysts through rats before inoculation.⁵ However, the most important limitation of this model is that most rodent colonies have natural infection with G muris which is morphologically indistinguishable from G lamblia in unstained fecal preparations. However, differentiation is possible in stained preparations since the median bodies of G muris are oval like and lie behind each other. Even a single G muris infected animal is capable of infecting the whole colony since rodents are habitually coprophagic. Our attempts to infect weanling rats by inoculation with feces containing G lamblia cysts or sensitization of rats with feces from normal human subject followed by a single heavy dose of G lamblia cysts failed to yield desired degree of 'take' of infection. Hence we have developed an alternative and better mouse model of human giardiasis.⁶ ⁷ Increasing the dose of inoculum (G lamblia cysts) from 10⁶ to 10⁷ per animal led to progressive increase in 'take' of infection.⁸ A high dose of 10⁷ or more is essential to achieve a high infection rate.⁹ G lamblia trophozoites excysted from cysts of symptomatic cases were observed to colonize the small intestine better as compared to those from asymptomatic cases;⁹ it is thus difficult to compare the results in different groups of animals inoculated with G lamblia cysts pooled from different human sources. To obviate this problem, Khanna et al,¹⁰ infected inbred NMRI mice free of G muris with 10⁸ asexually cultured G lamblia trophozoites. The post inoculation temporal profile of giardial infection in mice can be classified into establishment phase (3-5 days), peak phase (9-11 days), decline phase (17-21 days) and the clearance phase (between 30-35 days).¹¹ The interplay between the virulence of the parasite and the immune responses of the host appears to determine the pathogenicity of giardial infection. Isolates from asymptomatic and diarrheic subjects can colonize and multiply significantly in experimental animals as compared to isolates from asymptomatic G lamblia carriers.¹² This model provides for differentiation of virulent and avirulent isolates of G lamblia.

Pathophysiology

Various hypotheses forwarded to explain the underlying pathological changes leading to malabsorption of nutrients like carbohydrates, fats and vitamin B₁₂ in giardiasis...
include: i) mechanical blockade of the mucosa by the trophozoites; ii) bile salt deconjugation; iii) bacterial overgrowth; iv) enterotoxin secretion; v) prostaglandin release due to stimulation of mucosal adenyl cyclase; vi) varying degrees of villus atrophy; vii) presence of undifferentiated enterocytes at the villous tips, and viii) brush border injury. During the establishment phase, upper small bowel mucosa shows limited changes in surface epithelium with normal villous height. Under a light microscope, trophozoites have been observed to lie free in the human as discrete parasites. Though active invasion by the trophozoites is difficult to demonstrate, in at least one study, free *G. lamblia* have been demonstrated in the submucosa in human small intestinal biopsies. Changes observed during the peak phase of experimental infection include fuzzy appearance of brush border, marked reduction in villous height and infiltration by intraepithelial lymphocytes. It has also been suggested that lymphocytic infiltration of the gut may itself damage the enterocytes. However, we could not demonstrate the presence of lymphocytes in close contact with the parasite. This is contrary to the earlier observations which suggested that infiltrated intraluminal lymphocytes lying in close contact with parasite had the potential to eliminate the parasite. Scanning and transmission electron microscope observations suggested that the trophozoites preferentially localize in sheltered areas such as crevices of villi which appear to provide the trophozoites mechanical protection from the intraluminal flow. The trophozoites are attached to the mucosa through the ventral or the suction disc. The convexity of the suction disc has been observed to extend over the mucosa like an umbrella. The two curved lateral edges of the disc are apparently embedded in extraneous coat of luminal surface of microvilli. Marks produced by adhesive suction disc indicate the sites of previous attachment of trophozoites. Rarely, instead of ventral disc, the parasite may utilize its dorsal surface for attachment to the gut mucosa. The brush border microvilli get damaged in areas of trophozoite attachment. In addition, severe flattening and blunting of microvilli with occasional loss of intracellular organelar organization of columner cells have also been observed. During the decline/clearance phase of experimental infection, we observed negligible focal damage to microvilli and other cellular components. In immunosuppressed hosts, parasite load is higher and morphological alterations are usually more severe.  

**Brush Border and Cytotoxic Enzymes in Giardiasis**  
*G. lamblia* infection may alter the cellular glycocalyx resulting in alterations in brush border disaccharidase enzymes. During the establishment and acute phases of giardial infection, specific activities of brush border membrane enzymes like alkaline phosphatase, sucrase, maltase and lucine aminopeptidase decline significantly. As the infection declines, these enzyme activities return to normal. However, lactase activity remains unaffected throughout the course of *G. lamblia* infection possibly because trophozoites preferentially reside at the base of microvilli while the lactase activity is maximum at the tips. It has been suggested that the reduction in brush border enzymes is related to the enterocyte damage caused by the trophozoites.

A significant decline in the activity of glucose-6-phosphate dehydrogenase during giardial infection would indicate a structural alteration of enterocytes which in turn may be responsible for alterations in transport of various nutrients. Decreased isocitrate dehydrogenase levels during the establishment and peak phases of experimental giardial infection leading to impairment of energy generating functions (ATP products) of the mucosal cell which in turn results in lowered active transport of glucose and amino acids from the intestine. Reduced activities of isomaltase and glucoamylase impair digestion and absorption of starch, glycogen and isomallose. 

Defects in other cellular enzyme activities may also contribute to defective absorption of nutrients. Besides damage to glycocalyx and cytotoxic enzymes of the gut, gross morphological alterations including enterocyte damage may contribute to reduced absorptive area. Uptake of glucose and amino acids is significantly reduced in severely malnourished *G. lamblia* infected animals. Transport of D-glucose, L-phenylalanine, L-lysine and L-aspartic acid in brush border membrane vesicles has been observed to be low in *G. lamblia* infected animals as compared to uninfected ones. Variations in parasite inoculum size used for infecting protein deficient animals had no significant effect on the uptake of these nutrients.

The clinical malabsorption syndrome, thus, appears to be modulated by various factors viz virologic virulence of the parasite, nutritional status of the host and development of immune response besides damage to intestinal glycocalyx enzymes. The variations in virulence of *G. lamblia* isolates have been shown by isoenzyme analysis. 

**Host Immune Responses**  
Humans and experimental animals develop significant immune responses following infection with *G. lamblia*.  

**GIARDIA LAMBLIA INFECTION - VINAYAK & NAICK**  
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Although specific anti-giardial antibodies have been detected in sera of giardiasis patients, the immune mechanisms by which a host clears *G. lamblia* from the intestine are not fully understood. As membrane antigens form the interface between viable parasites and the host, response to such antigens is considered critical in determining the outcome of infection.1,16

We isolated plasma membrane (PM) from axenically grown *G. lamblia* (Portland 1 strain) by treatment with a membrane stabilizing agent followed by osmotic stress and manual sheering. Two dimensional cross-immunoelectrophoresis identified 6 major PM antigens. Exposure of hyperimmune serum (raised in rabbits to whole parasite antigens) to live *G. lamblia* trophozoites resulted in absorption of antibodies to surface molecules. Use of such absorbed serum in two-dimensional immunoelectrophoresis has led to recognition of two surface associated molecules of *G. lamblia* trophozoites.27 These molecules have molecular masses of 82 and 56 KDa and have, therefore, been named GLSA 82 and GLSA 56 respectively.27 The surface localization of these molecules has been confirmed by agglutination and direct immunofluorescence of live trophozoites.28 Identical surface molecules have been shown to exist on two other axenic *G. lamblia* (WB and ISR) isolates tested. Both GLSA 82 and GLSA 56 have been purified by affinity chromatography from detergent (Trixon X-100) extract of *G. lamblia* trophozoites and shown to be heat labile, pronase and trypsin sensitive and modifiable by sodium metaperiodate oxidation indicating their glycoprotein nature. However, the two antigens differ in their binding specificity. GLSA 82 can bind concanavalin A and pokeweed mitogen thus suggesting that it has alpha- methyl mannoside and N-acetyl beta-D glucosamine as sugar moiety whereas GLSA 56 binds phytohemagglutinin and Ricinus communis thus suggesting that it contains beta-D-galactosamine and N-acetyl beta-D-galactosamine sugar moieties.29 Sera obtained from patients with acute *G. lamblia* infection can recognize GLSA 82 and GLSA 56.31 This suggests that these parasite molecules are recognized by the human immune system and may be important parasite targets. Interestingly, antibodies directed against these cell surface antigens of *G. lamblia* trophozoites have been shown to be capable of killing the parasite in vitro through the activation of classical complement pathway.30 However, agglutination of *G. lamblia* trophozoites has been observed on exposure to anti-GLSA 56 antibodies in the absence of complement. Ultrastructural studies have shown that interactions of anti-GLSA 56/GLSA 82 with trophozoites in the presence of complement result in damage to the ventral sucking disc of the parasite. In addition, 10-15% of anti-GLSA 56/GLSA 82 antibodies pretreated parasites developed rounded bodies at the distal/posterior part resulting eventually in the death of the parasite.30 These studies provide evidence that surface molecules serve as anti-parasite target for the host immune system.

It appears possible to differentiate an active ongoing giardial infection from asymptomatic infection based on isotype specificity of antibodies.32 Antigiardial antibodies can be demonstrated in almost all cases of non-persistent infections and belong to IgM isotype. On the other hand, subjects with asymptomatic or persistent giardial infections develop IgG antibodies against *G. lamblia*.32 Interestingly, persistent or chronic giardiasis patients fail to develop significant levels of antibodies to either GLSA 82 or GLSA 56 cell surface associated antigens while acute/symptomatic cases do.33 The failure to mount optimal IgG anti surface antibody, in persistent giardiasis, may be due to a functional defect of T helper cell (especially switch T cells) which are required for B cell differentiation. Alternatively, B-lymphocytes may fail in these patients to receive maturation signals from T helper cells.34 The chronicity of giardial infection in Balb/C mice has been attributed to the lack of antigen reactive T cells (helper/inducer) which are required for the initiation of immune response to surface antigen.34 It is also possible that those subjects who fail to mount an immune response to either GLSA 82 or GLSA 56 are unable to clear the infection despite adequate anti-giardial chemotherapy. Regulation of *G. lamblia* infection by host immune responses has also been suggested from the following observations: i) development of severe symptoms in hypogammaglobulinemic patients,35,36 ii) acquisition of partial resistance to reinfection by experimental animals on spontaneous clearance of infection,37,38 iii) inability of experimental hosts immunosuppressed by prior treatment with corticosteroids, irradiation or antilymphocytic serum to eradicate the infection18,19 and finally, iv) by passive transfer of immunity by immune cells.39 Some investigators have observed that cytotoxic lymphocytes lie in close contact with Giardia trophozoites. This points to a possible functional role of cytotoxic lymphocytes in elimination of parasites from the gut.16 It appears that destruction precedes phagocytosis of *G. lamblia* by mesocytic macrophages.40 It has also been suggested that the clearance of *G. muris* occurs following activation of helper/inducer T cells.41 This points to involvement of gut associated effector immune mechanisms in regulation of the giardial infection. Therefore, any alteration of mucosal immunity would alter the course and duration of Giardia infection and clinical disease. Earlier investigations have suggested immunologic clearance of Giardia trophozoites from the small intestine via antibody coating of the parasite or due to cytotoxic lymphocytes.41 Immune clearance of the parasite from the gut may thus involve complex interactions of intestine T cells and antibody-producing cells.1,16 Recent investigations reveal that the
establishment and acute phases of *G. lamblia* infection in experimental host are accompanied by an increased influx of Lyr 2.2*"* (suppressor/cytotoxic T cells) in intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) of gut with no significant change in the numbers of helper/inducer T cells in IEL or LPL.32,43 Intestinal lymphocytes isolated during these phases of giardial infections from NMRI mice are incapable of killing G. lamblia trophozoites in vitro either in the presence or absence of *G. lamblia* specific antibodies. Suppressor/cytotoxic T cells in culture as a result of *Giardia* infection appeared not to be cytotoxic T cells in nature but are suppressor T cells.44 The precise reason for an increased influx of suppressor T cells during the establishment and acute phases is unclear. It appears that excretory-secretory factors released by actively multiplying trophozoites or one of the products of dying *G. lamblia* trophozoites may act as suppressor factor by signaling generation and subsequent localization of suppressor T cells in intestinal epithelium. Alternatively, an undefined factor released by the parasite may affect the regulatory mechanisms resulting in loss of check on the generation and functional activity of suppressor T cells.45 An increase in IgM containing cells in the lamina propria in immunocompetent animals during the establishment and acute phases followed by its decline after significant reduction in trophozoite load indicate an early and primary response to the intestinal levels restricted to increased synthesis of IgM following *G. lamblia* infection. In contrast, the establishment and acute phases of *G. lamblia* infection in the jejunum are accompanied by a significant decline in IgA containing cells. This is turn results in reduced availability of local secretory IgA.46 IgA containing plasma cells have been shown to be either significantly reduced or totally absent in small intestinal biopsies of subjects with persistent giardiasis.48 Consequent reduction of secretory IgA (SIgA) levels in the gut permits better colonization and multiplication of the parasite and thus leads to severe giardiasis. This is supported by the clinical observation that children with low IgA levels are more prone to develop severe giardiasis.49 The precise mechanism of decline in IgA bearing plasma cells in acute giardial infection remains obscure; however, an early feedback suppression because of localization of suppressor T-cells in the lamina propria and defective signal from T cells for differentiation and maturation of B cells may be responsible.

The decline phase of *G. lamblia* infection in immunocompetent experimental host involves induction of helper/inducer T cells with significant reduction in suppressor T-cells. This is accompanied by increased number of IgA and IgG containing plasma cells in the lamina propria. It, thus, appears that activation of helper/inducer T cells during this phase is responsible for induction of local antibody dependent effector responses.33,46 Recently, it has also been noticed that the S-IgA bound to the surface of *Giardia* trophozoites results in better clearance from the gut while parasites with negligible surface bound S-IgA persisted during the decline phase of infection.47 The anti-idiotype IgA responses have also been noticed to be altered in mice deplete of T helper lymphocytes.47 Based on these findings it appears that the significant enhancement of T helper inducer T-cells activity along with significant generation of IgA containing plasma cells resulting in enhanced S-IgA are essential for clearance of giardial infection. It, however, remains to be confirmed if identical mechanisms at the gut level are actually operative in patients with giardiasis. Individuals with hypogammaglobulinemia especially those with low IgA bearing plasma cells in lamina propria of gut however, have been observed to have more severe and persistent infection while those who clear the infection have a normal immune responsiveness including the functional levels of IgA bearing plasma cells in gut.35,36 Since it is well known that hypogammaglobulinemic patients as well as immunosuppressed experimental hosts have more severe giardial infection, it appeared worthwhile to analyze effector mechanism at gut level in immunosuppressed experimental hosts. Investigations done in experimental animals revealed that the administration of dexamethasone in mice led to significant decline of helper/inducer T cells in intraepithelial and lamina propria regions and significant decline in IgA and IgG containing plasma cells in lamina propria of the gut.49 Failure of induction of functional activities of helper/inducer T cells at the gut level following administration of dexamethasone might possibly have led to failure of sufficient maturation/generation signals to plasma cells to generate and secrete S-IgA. This in turn may be responsible for significantly higher trophozoite load in the gut. However, it is interesting to know that establishment and acute phase of *G. lamblia* infection in such immunocompromised hosts are also accompanied by influx of suppressor T cells in a fashion identical to immunocompetent cells. Inspite of low number of IgA bearing plasma cells in IEL/LPL, the parasite load declines. This observation suggests that enhanced secretion of IgA may not be mandatory for clearance of parasite and there might be other mechanisms viz generation of other isotypic immunoglobulins at the gut level which may be involved in clearance/decline in the trophozoite load. Dexamethasone itself has no direct cytotoxic effects on *G. lamblia* trophozoites in vitro.44 Treatment of experimental host with dexamethasone, however, has no effect on the IgM containing plasma cells while such treatment abrogates IgA and IgG response during the decline phase of infection. Since the number of IgM containing cells remained unaffected despite dexamethasone treatment,
it is likely that IgM might have taken over the functions of IgA which in turn results in decline/clearance of *G. lamblia* trophozoites from the gut. Similar observations have been made in human subjects suffering from chronic diarrhoea and underlying IgA deficiency. However, further investigations are required in this direction.**

It is pertinent to conclude that activation of inducer T cells at the gut level is important in providing signal to B cells to differentiate into antibodies producing plasma cells. Such immune regulation of diarrhoea may be related to immune stimulation by certain specific antigenic molecules of the parasite especially those residing on the cell surface. Investigations utilizing 56 KDa cell associated immunodominant molecule as immunogen in an experimental host revealed that prior systemic oral immunization of inbred NMRI strain of mice with 56 KDa antigen not only blocked colonization but also resulted in elimination of *G. lamblia* trophozoites. Immunization of host led (i) significant elevation of helper/inducer T cells with no effect on suppressor T cells; (ii) significant enhancement of IgA/IgG containing plasma cells; and (iii) significant development of anti-GLSA 56 antibodies. Such stimulation of immune responses leads to failure of the parasite to colonize and multiply in the gut.** It thus appears that GLSA 56 molecule of the parasite has the potential to immunoregulate the disease process and also has the potential of further development as immunoprophylactic agent. Such an immunoprophylactic agent may have a useful role in protecting vulnerable populations like children or persons from non-endemic areas travelling to endemic areas. Detailed investigations are essential to investigate mechanisms by which the parasite evades the effector immune mechanisms. Nevertheless, the investigations quoted above clearly indicate that although gut associated immune mechanisms are involved in regulating diarrhoeal infection, other effector mechanisms like mucosal mast cells, macrophages and other non-specific intestinal factors such as mucus cannot be ignored.

References


2. Vinayak VK. Immunoregulation by plasma membrane associated antigens of *G. lamblia* in human pediatric parasite. In: Recent advances in Immunology and Biochemistry (Eds RK Chitkara, BR Doo), Vikram University, Ujjain 1990; pp 135-40.


