Giardia lamblia Infection in Immunosuppressed Animals Causes Severe Alterations to Brush Border Membrane Enzymes

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NMRI mice immunosuppressed with dexamethasone followed by challenge intraesophageally with axenic Giardia lamblia (Portland I) trophozoites had severe infection in terms of the trophozoite counts in the jejunum. Although the immunosuppressive treatment with cortisone itself resulted in a deleterious effect on brush border membrane enzymes, the decline in disaccharidases (sucrase, maltase, and lactase) and alkaline phosphatase was highly significant (P < 0.001) following G. lamblia infection. The alterations in enzymatic activity in immune intact but infected animals demonstrated the potential of the parasite itself to cause damage to the brush border membrane. We believe that individuals with underlying immunodeficiency, upon infection with G. lamblia, may have increased damage of the brush border membrane, leading to severe malabsorption.

KEY WORDS: G. lamblia infection; dexamethasone; trophozoites counts; brush border enzymes; severe malabsorption.

Giardia lamblia is an anaerobic flagellate protozoa and common inhabitant of human intestine. The infection causes diarrhea, abdominal pain, weight loss, and malabsorption, especially in children. Colonization in the small intestine by the parasite is facilitated by attachment of trophozoites to the microvilli of mucosal epithelial cells. The trophozoites, which reproduce by binary fission, evade degradation by the enzymes of the gut through an unknown mechanism. The clinical spectrum of infection with G. lamblia ranges from the asymptomatic passage of cysts (1) to persistent and severe diarrhea with malabsorption (2). The persistence of the infection is more profound in patients with underlying hypogammaglobulinemia (3, 4). Malabsorption in giardiasis may be incapacitating for infants (5) and children (6) with immunodeficiencies. The malabsorption of one or more nutrients in giardiasis may be involved. The nutrients most frequently malabsorbed are carbohydrate (4, 7, 8) and fat (6, 9). It is therefore apparent that malabsorption of nutrients in immunologically intact and in immunosuppressed hosts need to be elucidated.

In the present investigation immune intact and
immunosuppressed animals infected with *Giardia lamblia* were assessed for their intestinal disaccharidases and alkaline phosphatase levels.

**MATERIALS AND METHODS**

**Experimental Animals.** Three- to four-week-old NMRI (Naval Medical Research Institute, U.S.A.) inbred strain mice were used in all experiments. The mice were bred at our animal facility room and were free and kept free from *G. muris* infection (10).

**Groups of Animals.** All the animals were broadly divided into four groups, ie, immune intact and immunosuppressed animals, as follows: group I (18 animals), immune intact *Giardia lamblia*-infected animals; group 2 (18 animals), immune intact uninfected animals; group 3 (18 animals), cortisone-treated *G. lamblia*-infected animals; and group 4 (18 animals), cortisone-treated uninfected animals.

**Preparation and Inoculation of *G. lamblia* Trophozoites.** *G. lamblia* trophozoites (Portland I strain) were axenically cultured in TPS-1 medium (11). For experimental inoculation, actively growing trophozoites (48- to 72-hr-old culture) were sedimented by centrifugation at 200g for 10 min and finally resuspended in TPS-1 medium to contain 10^7/0.2 ml. Animals of groups 1 and 3 were given 1 x 10^7 trophozoites of *G. lamblia* intraesophaegally via a catheter (10). Animals of groups 2 and 4 were inoculated with an equal volume of TPS-1 medium intraesophaegally.

**Immunosuppression of Animals by Cortisone.** Animals of groups 3 and 4 were given dexamethasone (IDPL, New Delhi, India) as immunosuppressive therapy (12). Briefly, the animals received 0.2 mg of dexamethasone intramuscularly three days prior to and on alternate days after inoculation of the parasite until the end of experiment. The immunosuppression as checked by anti-sheep red blood cells (SRBC) antibodies (12) showed an anti-SRBC titer of 1:16 in dexamethasone-treated animals and 1:16,000 in immune intact animals.

**Follow-up of Animals.** Animals of all groups were sacrificed in batches of six (from each group) on days 3-5, 9-11, and 17-21 postinfection (PI). Trophozoites were counted in the jejunum (13).

**Preparation of Brush Border Membrane.** Brush border membranes from infected and control animals were prepared according to the method of Schmitz et al (14). The purity of the brush border membrane was checked by the marker enzyme sucrase. A 12.6 ± 2.11-fold (61.89 x 10^−3-53.66 x 10^−3 µmol/min/mg protein for brush border membrane and 5.90 x 10^−3-4.06 x 10^−3 µmol/min/mg protein for homogenate) increase in the activity of sucrase was taken as the criterion for brush border membrane purification.

**Enzyme Estimations.** Alkaline phosphatase (EC 3.2.3.1) was estimated by using p-nitrophenyl phosphate as an substrate (15). For disaccharidases, the activity of sucrase (EC 3.2.1.48), maltase (EC 3.2.2.11), and lactase (EC 3.2.1.3) were determined by measuring the D-glucose liberated from the respective sugars using the glucose oxidase-peroxidase system (16).

The linear regression analysis was done to correlate the trophozoite load in the jejunum at various phases of infection with the alterations in levels of brush border membrane enzymes.

**Ultrastructural Studies.** To evaluate the ultrastructural changes at the gut level, a proximal part of the jejunum from animals was fixed in 2.5% buffered glutaraldehyde for scanning electron microscopy. All the tissue samples after dehydration underwent critical point drying with liquid CO2 (Ladd critical point drier). The desiccated samples were mounted on aluminum stubbs, coated with gold-palladium at a thickness of 200 A, and examined under JEOL-JEM 35 Model scanning electron microscope.

**RESULTS**

**Trophozoite Counts.** Oral inoculation of NMRI mice with trophozoites of *Giardia lamblia* (Portland I strain) resulted in establishment of infection as assessed by the number of trophozoites counted in jejunum (Figure 1). The peak trophozoite counts in the small intestine was observed at days 9-11 postinfection. The mean parasite counts in the jejunum in cortisone-treated animals on days 3-5 or 9-11 post infection were significantly (P < 0.01) higher as compared to immune intact infected animals. However, by days 17-21 PI, the parasite load declined in immune intact and immunosuppressed animals (Figure 1).

![Fig 1](image-url)  
Fig 1. Levels of alkaline phosphatase in brush border membranes (B-D) during the course of *G. lamblia* infection (A) in immune intact and immunosuppressed animals: •, immune intact *G. lamblia* infected animals; ○, immune intact uninfected animals; □, cortisone-treated *G. lamblia*-infected animals; ▼, cortisone-treated uninfected animals. Each observation represents mean ± SD of six animals.