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Gut homeostasis is never static and can be altered by oral administration of health promoting live microorganisms, namely probiotics. Clinical and experimental studies have provided evidence for the use of probiotics in prevention of intestinal diseases and various inflammatory conditions (Erickson and Hubbard 2000; Gill 2003; Benyacoub et al., 2005; Shukla et al., 2008). Therefore, for a healthy life, supplementation of millions of live bacteria to boost and replenish levels of good bugs in the digestive tract is being employed. Keeping these interests in mind, the present study was designed to delineate an effective probiotic and its underlying modulatory mechanisms in *Giardia intestinalis* infected BALB/c mice.

The four standard *Lactobacillus* strains namely *L. casei, L.acidophilus, L.plantarum* and *L.rhamnosus* GG were monitored for their probiotic characteristics. Since gastrointestinal systems have varying concentrations of bile ranging from 0.5% to 2.5% in the first hour of digestion and thereafter, decreases further in subsequent hours. Therefore, these probiotic strains were screened for bile tolerance as bile salts are considered to be a main prerequisite for growth, colonization and metabolic activity of bacteria in the host's gut (Liong 2005). It was found that all the four *Lactobacillus* strains employed in the present study well tolerated the varying concentrations of bile salts. However, the beneficial aspects of probiotic strains can be expected only when they are able to survive passage through the human stomach and colonize the human gut. As it is reported that some probiotic strains are more tolerant to acidic conditions than others either due to high cytoplasmic buffering capacity (pH 3.72–7.74) or membrane ATPases, that in turn may resist changes in the cytoplasmic pH and gain stability under acidic conditions (Ritus 1994). In the present study all the four probiotic strains were also found to survive both the acidic and alkaline pH. Normally bacteria and yeast used as probiotic adjuncts are commonly delivered in a food system, therefore, these organisms should be resistant to the enzymes in the oral cavity (e.g. lysozyme) and in the intestine (α-amylase, lysozyme and trypsin). It was observed that all the probiotic
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strains grew equally well in the presence of either of these enzymes and suggests that these enzymes neither interfere with the survival nor with the colonization of these organisms in the gut. Thus, all the four *Lactobacillus* strains tolerated well the conditions simulating *in vivo* stresses encountered in the gastrointestinal tract stress *in vitro* and corroborates with earlier studies (Usman 1999; Kheadr 2006; Preet et al., 2009; Shukla et al., 2010).

The data of present study demonstrated that oral administration of *Giardia* trophozoites to BALB/c mice led to transient colonization of the intestine with actively multiplying trophozoites and infection reached peak on day 7 PI. Thereafter, the *Giardia* parasite was cleared naturally by day 25 PI and is in accordance with earlier studies (Benyacoub et al., 2005; Humen et al., 2005; Shukla et al., 2008). Further, attempts were made to find out an effective probiotic for murine giardiasis and it was observed that LGG was the most efficient in clearing *Giardia* infection in mice. Most notably, the protective effect of LGG against *Giardia* infection was measured in terms of reduced number of cysts in feces and duration of infection compared with other three probiotic strains. This could be due to the better survival and colonization of LGG in the small intestine that improved the microenvironment and inhibited the adhesion of *Giardia* trophozoites to the intestinal epithelium compared with other three probiotic strains. Along with these, other factors that may also be involved are bacterial extracellular factors (Lievin et al., 2002), interference with parasite-enterocyte interactions (Bibiloni et al., 1999) and modulation of the immune response (Haller et al., 2000; Benyacoub et al., 2005). The present observation is also supported by various researchers who have employed different probiotics and found that probiotics were able to clear *Giardia* infection from the mice intestine (Benyacoub et al., 2005; Shukla et al., 2008; Shukla et al., 2011). Since the probiotic LGG exhibited better elimination response for the *Giardia* parasite in mice, for further experiments LGG was used as an effective probiotic and its underlying protective mechanism in murine giardiasis was elucidated.
The ability of LGG to persist in the gastrointestinal tract in vivo was monitored by counting their number in mice feces. It was observed that mice fed with LGG had significantly higher lactobacilli count in feces that led to reduced colonization of *Giardia* trophozoites to enterocytes compared with *Giardia* infected mice. This observation supports the fact that LGG was able to colonize the intestine efficiently showing better interaction with the enterocytes that altered the intestinal microbiota or endogenous microbiota and is in agreement with previous studies (Cano et al., 2002; Humen et al., 2005; Shukla et al., 2008). However, modifications of other key components of the intestinal microbiota cannot be ruled out.

The presence of viable *Giardia* trophozoites in the small intestine is a recognized marker of *Giardia* infection. It is interesting to note that oral administration of LGG dramatically reduced the number of viable trophozoites in the gut and led to early resolution of *Giardia* infection compared with *Giardia*-infected mice. This could be either due to efficient colonization of enterocytes with LGG or better priming of immune system by LGG that led to early elimination of *Giardia* trophozoites from the small intestines of LGG fed *Giardia*-infected mice. The present observation of reduced trophozoite count in the small intestine of LGG fed mice is in accordance with earlier study where it has been demonstrated that administration of *L. johnsonii* La 1 dramatically reduced the trophozoite count in probiotic fed *Giardia* infected gerbils and led to resolution of giardiasis within 14 days of post infection (Martin et al., 2005).

Lipid peroxidation is the process of oxidative damage of polyunsaturated fatty acids, a feature of many types of cell injury, resulting in excessive production of free radical intermediates (Slater, 1984; Comporti, 1985). These radicals can lead to molecular disfiguring of lipids, proteins, carbohydrates and DNA (Reiter et al., 2002). In the present study lipid peroxidation was measured indirectly by assessing the levels of MDA (Draper and Hadley, 1990). Moreover, the development of tissue injury depends on the balance between the generation of toxic radicals and the tissue antioxidant state (Winrow et al., 1993). Host cells are
The discussion focuses on protecting against oxygen-derived radical injury through naturally occurring free radical scavengers and antioxidant pathways. This includes non-enzymatic components such as vitamins A, C, and E, as well as three basic enzymes: SOD, catalase, and glutathione reductase (GSH), which act as the body's first line of defense against reactive oxygen species (ROS; Mate, 2000). However, the antioxidant defense mechanism fails due to overproduction of free radicals or decreased activities of scavenging enzymes, leading to lipid peroxidation (Fantone and Ward, 1982). In the present study, administration of probiotic LGG to *Giardia* infected mice either seven days prior to or simultaneously with *Giardia* infection reduced oxidative stress-mediated intestinal tissue damage, as evidenced by decreased lipid peroxidation and increased levels of antioxidants SOD and GSH compared to increased lipid peroxidation and decreased levels of antioxidants in *Giardia*-infected mice. Oxidative stress-mediated tissue damage in the small intestine of *Giardia* infected mice aligns with earlier findings (Shant et al., 2005; El Taweel et al., 2007). These observations were consistent with studies by various scientists, including Archibald and Fridovich (1981) who noted that LAB can deal with oxygen radicals due to higher SOD levels. Lin et al. (1998) also observed that lactobacilli released intracellular antioxidative constituents that likely reduced tissue injury and protected the host.

Brush border enzymes, such as alkaline phosphatase, lactase, and sucrase, are biomarkers for intestinal damage. Their expression levels are indicative of intestinal epithelial cell health. Alkaline phosphatase, lactase, and sucrase are predominantly expressed in the brush border (microvilli) of intestinal epithelial cells.
in the upper villous while maltase activity is present towards the base of villous (James et al., 1987). It has been documented that *G. intestinalis* induces both structural and functional derangement in the small intestine due to strong adherence with the enterocytes via ventral disc leading to damaged brush border microvilli and impaired activities of brush border membrane enzymes (Khanna et al., 1988; Ceu Sausa et al., 2001). In giardiasis, disaccharidase deficiencies have been consistently identified as one of various abnormalities of the small intestinal pathology (Jennings et al., 1976). Data of the present study indicated that probiotic feeding to mice either seven days prior or simultaneously with *Giardia* infection increased the activities of brush border enzymes alkaline phosphatase, sucrase and lactase compared with decreased activities of these enzymes in *Giardia* infected mice. More specifically, it can be said that supplementation of probiotic LGG prevented brush border injury leading to restored activities of brush border enzymes in *Giardia* infected mice. This observation is also supported by the better colonizing and antioxidative ability of LGG as found in the present study and is in accordance with earlier studies (Humen et al., 2005 and Southcott 2008). Southcott (2008) has reported increased activities of brush border enzymes sucrase and lactase in methotrexate induced rat mucositis, following supplementation with sheep yoghurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* whereas Humen et al. (2005) have found only increased sucrase activity in *Lactobacilli jonsonii* lal treated rats. However, the decreased activities of sucrase and lactase in *Giardia*-infected mice could be due to increased oxidative stress mediated tissue injury and altered gut morphology that led to reduced activities of brush border membrane associated enzymes (MacDonald and Ferguson, 1978). Studies by Belosevic et al. (1989) have also found that *G. intestinalis* induced a transient decrease in disaccharidase activity during the acute phase of a primary infection in gerbils due to parasitic effect on the brush border membrane of the small intestine.

We have also observed that *Giardia intestinalis* infection in mice had a profound effect on the morphological and cellular alterations in the small intestinal mucosa as evident by the histopathological and scanning electron microscopic studies.
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This observation could be explained by the fact that attachment of excessive number of trophozoites on the epithelial surface of the small intestine creates a barrier and blocks the absorptive surface of the small intestine which ultimately causes epithelial cell damage and inflammatory reactions. Similar observations have also been observed by other scientists in the jejuna mucosa of mice infected with *Giardia* (Daniel and Belosevic 1995; Khanna et al., 1988). However, oral supplementation of probiotic LGG either prior or simultaneously with *Giardia* infection in mice altered the *Giardia* induced gut morphology that is further supported by the present observation of restored brush border enzyme activities in LGG fed *Giardia*-infected mice. Further, scanning electron microscopic studies also revealed reduced epithelial cell damage and inflammation in the small intestine of LGG fed *Giardia*-infected mice compared with altered gut morphology in *Giardia*-infected mice and is in concordance with earlier studies (Shukla et al., 2008, 2010 and 2011). They have described the ameliorating potential of probiotic *L.casei* and *L.acidophilus* in restoring the normal gut morphology in *Giardia* infected mice as well as in malnourished *Giardia*-infected mice.

Giardiasis is a self limiting disease, with spontaneous resolution of the acute phase in individuals with fully developed immune system (Belosevic et al., 1989). Various scientists have documented the role of both innate and acquired immunity in experimental giardiasis (Hecht, 1999; Heyman and Menard 2002; Harish and Varghese, 2006; Singer and Nash, 2000). As *Giardia* trophozoites reside in the lumen of small intestine, suggesting the role of effective molecules such as IgA antibody that reach the lumen and helps in clearing the intestinal parasite (Eckmann 2003; Langford et al. 2002). Moreover, it has been shown that different probiotic strains have different immunomodulatory effects with varied *in-vivo* potentials (Isolauri 2001; Tamboli, 2003), therefore, immunomodulatory potential of the probiotic LGG in murine giardiasis was assessed. It was interesting to note that probiotic administration to mice either seven days prior or simultaneously with *Giardia* infection accentuated both the humoral and cell mediated immune response suggesting the immunomodulatory potential of LGG. Moreover, quantification of secretory IgA antibody and IgA+ cells showed marked increase in
the levels of both secretory IgA and IgA+ cells in the probiotic fed *Giardia*-infected mice compared with *Giardia*-infected mice and is in concordance with earlier study (Benyacoub et al., 2005). They have reported a significant increase in the levels of IgA in *Giardia* infected C57BL/6 mice fed with probiotic *Enterococcus fecalis*.

This enhanced humoral immune response might be due to the increased priming of gut associated lymphoid tissue which is mediated by the interaction of probiotic LGG with dendritic cells. Such interaction may have modulated both, the maturation and functioning of the dendritic cells thereby, enhancing the local immune response (Isolauri et al., 2001; Christensen 2002). However, in another study, LGG has been found to enhance the antibody-secreting cells in patients with Crohn's disease and fecal IgA in individuals allergic to cow's milk (Malin et al., 1996; Viljanen 2005).

The production and delivery of effector molecules against *Giardia* is likely to be regulated by network of cells and mediators that may not be directly involved in killing of the pathogen (Singer and Nash 2000; Scott et al., 2004). We have observed an increased percentage of CD4+T cells and decreased percentage of CD8+T cells in probiotic fed *Giardia*-infected mice compared with *Giardia*-infected mice and corroborates with earlier studies (Benyacoub et al., 2005; Feleszko 2007). These scientists have also discussed the immunomodulatory potentials of LGG in inflammatory diseases and have reported a significant increase in the number of CD4+T cells in *Giardia* infected C57BL/6 mice fed with probiotic *Enterococcus fecalis*.

It has been observed that specific probiotic strains stimulate the secretion of specific cytokines and facilitate the development of naïve T cells towards a particular immune pathway (Neurath et al., 2002). In the present study *Giardia* infected mice had enhanced levels of both TNF-α and INF-γ, the pro-inflammatory cytokines whereas probiotic LGG fed *Giardia*-infected mice had decreased levels of proinflammatory cytokines particularly INF-γ and increased levels of anti-inflammatory cytokines IL-6 and IL-10. These results clearly show the protective potentials of LGG to modulate the expression of both pro-inflammatory and anti-
inflammatory cytokines. This observation is supported by in vitro study performed by Borruel et al., 2002 who have suggested that probiotic strains have the ability to attenuate the release of proinflammatory cytokines and can induce the secretion of anti-inflammatory cytokines which reduces the Th1 associated inflammatory response. The increased levels of secretory IgA and IgA + cells in the probiotic fed *Giardia*-infected mice could also be correlated with the enhanced levels of IL-6 as it is instrumental to IgA secretion and increased clonal expansion of IgA+ cells (Zhou et al., 2003). However, we found that probiotic feeding to *Giardia* infected mice did not alter the expression of IL-4 and TNF-α supporting the fact that immunomodulatory mechanism of probiotics is both species and strain specific.

The intestinal inflammation observed in *Giardia*-infected mice may be mediated by proinflammatory cytokines TNF-α and INF-γ. Scott et al. 2004 have also indicated that in murine giardiasis the brush border injury and malfunction are mediated by CD8+ T lymphocytes.

Based on these present observations, it can be proposed that the immunomodulatory potential of LGG is mediated via modulation of both humoral and cellular immune response. It is suggested that colonization of intestinal epithelial cells with probiotic LGG, might be sufficient to trigger the signaling cascades that would ultimately activate both the innate and acquired immune response. It is believed that innate immunity may be enhanced due to the association of the effective probiotic LGG with enterocytes that may have augmented the production of mucin and nitric oxide thus reducing the severity and duration of *Giardia*-infection. On the other hand, for acquired immune responses the probiotic might have entered either via mucosal (M) cells or toll like receptors (TLR) in the intestinal mucosa where they cross talk with the antigen presenting cells resulting into the clonal expansion of intraepithelial lymphocytes, germinal centres, thereby sensitizing the lymphocytes between enterocytes and lamina propria. This interaction resulted into the increased number of IgA secreting cells in the follicles and lamina propria along with the transmission of signals to the underlying mucosal cells resulting into the expression of various cytokines that, in turn stimulated the secretion of intestinal IgA and balancing of the T cell response.
Findings of the present study prompted us to carry out the *in vitro* studies highlighting the adhesion and colonizing property of the effective probiotic LGG viz-a-viz its ability to inhibit the adherence of active trophozoites to murine enterocytes. It was interesting to note that both the *Giardia* trophozoites and probiotic LGG adhered to the isolated mouse enterocytes and the adherence of *Giardia* trophozoites was maximum at pH 7.8 after 1h of incubation at 37°C in the presence of 0.3% bile salt. This observation is in accordance with earlier studies where rat intestinal epithelial cells and human intestinal cell line were used as models to assess the adherence of *Giardia* trophozoites *in vitro* (Inge et al., 1988 and Katelaris et al., 1995). Scanning electron microscopy studies demonstrated that the co-incubation of mouse enterocytes with LGG either 30 min. prior or simultaneously with *Giardia* trophozoites led to significant decrease in the percent adhered trophozoites to the murine enterocytes due to the colonization of murine enterocytes with LGG that displaced the *Giardia* trophozoites. This displacement could be due to the display of various specific adhesions and other surface determinants by the probiotic LGG that are involved in their interaction with intestinal epithelial cells (Bernet 1994; Reid et al. 2001; Servin and Coconnier, 2003). The present observation is in accordance with Collado et al., 2007 who have also shown that treatment of pig intestinal mucus with *Bifidobacterium lactis* Bb12 and LGG, alone or in combination, significantly reduced (P<0.05) the adhesion of pathogens *Salmonella*, *Clostridium* and *Escherichia coli*. Thus, it can be suggested that the probiotic LGG is able to displace *Giardia* trophozoites due to its ability to adhere and colonize the isolated murine enterocytes.

Taken together, the observations of the present study suggest that oral supplementation of the effective probiotic LGG in mice either prior or simultaneously with *Giardia* infection elicited both the non-immunological and immunological responses. The underlying protective mechanism of the probiotic LGG in murine giardiasis appears to be multifactorial and is due to better colonization of the enterocytes, modification the intestinal microbiota, restoration of gut morphology and impaired activities of brush border membrane enzymes namely alkaline phosphatase, sucrase and lactase. It was further observed that
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LGG has the ability to scavenge the oxygen free radicals and to release antioxidants in the small intestine along with the modulation of mucosal humoral and cell mediated immune responses. The data of our study also suggests that probiotics can be applied as functional foods in our daily life for the prevention of intestinal infections. The novelty of such application is that simple home made products can be used to prevent such infections that are safe and cost effective. The study also supports the old saying by Hippocrates "Let food be the medicine and medicine be the food".