Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice.

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Biotherapeutic Effects of Probiotic Bacteria on Candidiasis in Immunodeficient Mice

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Four species of probiotic bacteria were assessed for their capacities to protect athymic bg/bg-nu/nu and euthymic bg/bg-nu/+ mice from mucosal and systemic candidiasis. Each bacterial species and Candida albicans colonized the gastrointestinal tracts of both strains of mice. The presence of probiotic bacteria (Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus casei GG, or Bifidobacterium animalis) in the gastrointestinal tracts prolonged the survival of adult and neonatal bg/bg-nu/nmu mice compared to that of isogenic mice colonized with C. albicans alone. The incidence of systemic candidiasis in bg/bg-nu/nmu mice was significantly reduced by each of the four probiotic bacterial species. The numbers of C. albicans present in the alimentary tracts of euthymic bg/bg-nu/+ mice were significantly reduced by L. casei GG and B. animalis. None of the probiotic bacteria species completely prevented mucosal candidiasis, but B. animalis reduced its incidence and severity. Probiotic bacteria also modulated antibody- and cell-mediated immune responses to C. albicans. The prolonged survival of mice, decreased severity of mucosal and systemic candidiasis, modulation of immune responses, decreased number of C. albicans in the alimentary tract, and reduced numbers of orogastric infections demonstrated not only that probiotic bacteria have biotherapeutic potential for prophylaxis against and therapy of this fungal disease but also that probiotic bacteria protect mice from candidiasis by a variety of immunologic (thymic and extrathymic) and nonimmunologic mechanisms in this model.

Both species of lactic acid-producing bacteria are being promoted as probiotics, i.e., live organisms that are ingested to produce beneficial effects on health. Several biotherapeutic effects have been attributed to lactic acid-producing bacteria, including ameliorating lactose intolerance (17, 21), enhancing recovery of a commensal flora after oral antibiotic therapy (28), prophylaxis against and treatment of infant diarrhea (7, 30), and reduction of recurrent urinary tract infections (29).

Candidiasis of oral and vaginal mucosal tissues is very common. For example, nearly 90% of AIDS patients are infected with Candida albicans (22). Several studies have assessed the efficacy of probiotics for prophylaxis against and therapy of C. albicans infections (2, 6, 12, 32). Vaginitis in apparently healthy women can be caused by C. albicans, and the ingestion of yogurt containing Lactobacillus acidophilus has been reported to reduce the occurrence of recurrent vaginal candidiasis (12). Laboratory animal studies also suggest that probiotics may be useful for the prevention of candidiasis. Mice immunosuppressed with corticoid drugs recovered more quickly from orogastic candidiasis when they were fed cultures of L. acidophilus, Lactobacillus casei, and Lactobacillus delbrueckii prior to oral C. albicans challenge (6). Oral administration of heat-killed Enterococcus faecalis prior to oral and systemic infection of cyclophosphamide-treated mice with C. albicans prolonged their survival (32).

In this study, we assessed the ability of four probiotic bacterial species, L. acidophilus, Lactobacillus reuteri, L. casei GG, and Bifidobacterium animalis, to protect immunodeficient bg/bg-nu/nu and bg/bg-nu/+ mice from mucosal candidiasis and systemic candidiasis of endogenous (alimentary tract) origin.

MATERIALS AND METHODS

Microorganisms. Commercial starter cultures of probiotic bacteria L. acidophilus, L. reuteri, and Bifidobacterium infantis were obtained from BioGaia Biologics, Inc., Raleigh, N.C. B. infantis has subsequently been determined by ribosomal DNA typing to closely resemble B. animalis (20). L. casei GG was obtained from Valio, Ltd., Helsinki, Finland. All bacteria were grown overnight in deMan-Rogosa-Sharpe (MRS) medium (Difco, Detroit, Mich.) or on plates of MRS medium with 1.5% agar in anaerobic jars (GasPak; BBL, Cockeysville, Md.) containing anaerobic generators (AnaeroPack System; Cari-Scarborough Microbiologies, Decatur, Ga.) at 37°C. C. albicans was cultured on Sabouraud’s dextrose agar (SDA; BBL). Microbiological identification and characterization was conducted with the API 50CH biochemical identification system (BioMerieux Vitek, St. Louis, Mo.) and fatty acid analysis by gas-liquid chromatography (Microbial ID, Inc., Newark, Del.).

Mice. C57BL/6 bg/bg-nu/nmu mice, which are susceptible to lethal candidiasis (4), and bg/bg-nu/+ mice, which are resistant to lethal candidiasis (after oral challenge with the pathogenic yeast), were obtained from breeding stocks maintained at the University of Wisconsin Gnotobiote Laboratory, Madison (http://www.biostat.wisc.edu/gnotoblab/gnotolab.html). Germfree (GF) male bg/bg-nu/nmu and female bg/bg-nu/+ mice were mated to obtain litters of approximately equal numbers of nude and heterozygous mice. Groups of breeder mice, their progeny, and all adult mice were housed in sterile flexible film isolators and colonized with pure cultures of C. albicans or with one of the probiotic species by inoculating their oral and anal orifices with 1 ml (107 CFU/ml) of inoculum. Mice colonized with a probiotic species were also inoculated with C. albicans (107 CFU/ml) for assessment of the effects of probiotics on colonization and infection by C. albicans. The microbial colonizations were monitored by quantitative cultures of the alimentary tract, and reduced numbers of orogastric infections demonstrated not only that probiotic bacteria have biotherapeutic potential for prophylaxis against and therapy of this fungal disease but also that probiotic bacteria protect mice from candidiasis by a variety of immunologic (thymic and extrathymic) and nonimmunologic mechanisms in this model.

Survival and growth of immunodeficient mice colonized with probiotics. Survival of mice born to gnotobiote mothers was assessed at 4 and at 12 weeks of age. Survival of adult mice was assessed at 4 and at 12 weeks after colonization with a probiotic bacterium species and C. albicans.

Body weights were measured on a Sartorius balance (Brinkman Instruments, 

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Probiotic bacteria inhibit *C. albicans* in the gastrointestinal tracts of gnotobiotic mice

<table>
<thead>
<tr>
<th>Microbial status*</th>
<th>CFU of <em>C. albicans</em>/g (dry wt) (log₁₀ mean ± SEM)</th>
<th>CFU of probiotic bacteria/g (dry wt) (log₁₀ mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stool</td>
<td>small intestine</td>
</tr>
<tr>
<td><em>C. albicans</em> alone</td>
<td>8.0 ± 0.3</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td><em>C. albicans</em> plus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>7.5 ± 0.3*</td>
<td>7.3 ± 0.4*</td>
</tr>
<tr>
<td>L. reuteri</td>
<td>9.2±</td>
<td>7.9±</td>
</tr>
<tr>
<td>L. casei GG</td>
<td>7.6 ± 0.4</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td><em>B. animalis</em></td>
<td>6.4 ± 0.7*</td>
<td>7.8 ± 0.3</td>
</tr>
</tbody>
</table>

* Significantly fewer CFU than in *C. albicans-*monoassociated mice (*P* < 0.05).
* Significantly fewer CFU than in *C. albicans-* monoassociated mice (*P* = 0.01).
* Only one mouse was analyzed due to rapid mortality in this group.
* Significantly fewer CFU than in *C. albicans-* monoassociated mice (*P* < 0.001).
* For groups of 4 to 24 mice 4 to 8 weeks after colonization.

**RESULTS**

Probiotic suppression of *C. albicans* colonization. Weekly cultures of feces from *bg/bg-nu/nu* and *bg/bg-nu/+* mice housed in gnotobiotic isolators were used to verify that each group of mice was colonized with *C. albicans*, and that the difference in *C. albicans* colonization between the two groups was statistically significant. Differences in *C. albicans* colonization were observed between the survival of *bg/bg-nu/nu* mice colonized with *C. albicans* and that of the *bg/bg-nu/+* mice. The differences in colonization were determined to be significant by using a statistical test (ANOVA). Two-way ANOVA was used to test for differences in the number of viable *C. albicans* in the intestinal tracts or internal organs of mice from the various treatment groups. The data were log transformed to better meet the assumptions of ANOVA. Two-way ANOVA, with factors of treatment and sex, was employed to detect significant differences in the body weights of probiotic-colonized adult and neonatal mice and to assess significant differences between histopathology severity scores for tissue sections from mice with mucosal candidiasis.
mice was continuously colonized with either \textit{C. albicans} alone or with \textit{C. albicans} and one of the probiotic bacteria species. In euthyemic \textit{bg/bg-nu/+} mice, \textit{L. casei} GG and \textit{B. animalis} significantly inhibited \textit{C. albicans} throughout the alimentary tract. We recovered as much as 100-fold-fewer CFU of \textit{C. albicans} in disassociated mice than in \textit{C. albicans}-monoaosociated mice (Table 1). As shown in Table 1, the number of CFU of \textit{C. albicans} in the stomachs, small intestines, and colons of \textit{bg/bg-nu/nu} mice disassociated with \textit{L. acidophilus} and \textit{C. albicans} was significantly decreased compared with the number in \textit{C. albicans}-monoaosociated \textit{bg/bg-nu/nu} mice. The number of viable \textit{C. albicans} was reduced by \textit{L. casei} GG in the ceca, colons, and feces and by \textit{B. animalis} in the stomachs, ceca, colons, and feces of \textit{bg/bg-nu/nu} mice. Neither \textit{C. albicans} nor any of the probiotic bacteria species was eliminated from the alimentary tract of the mice over the 12-week study. \textit{C. albicans} did not appear to affect the capacity of probiotic bacteria to colonize \textit{bg/bg-nu/nu} or \textit{bg/bg-nu/+} mice, because the numbers of the probiotic bacteria cultured from \textit{C. albicans}- and probiotic bacteria-disassociated mice were very similar to the number cultured from mice monoassociated with a pure culture of the probiotic bacteria (35).

### TABLE 2. Inhibition of systemic candidiasis of endogenous (gastrointestinal tract) origin by probiotic bacteria

<table>
<thead>
<tr>
<th>Microbial status</th>
<th>\textit{bg/bg-nu/nu} mice</th>
<th>\textit{bg/bg-nu/+} mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissemination(^a) (%)</td>
<td>No. of \textit{C. albicans}(^b)</td>
</tr>
<tr>
<td>\textit{C. albicans} alone</td>
<td>75</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>\textit{C. albicans} plus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{L. acidophilus}</td>
<td>–</td>
<td>NG(^c)</td>
</tr>
<tr>
<td>\textit{L. reuteri}</td>
<td>–</td>
<td>NG(^c)</td>
</tr>
<tr>
<td>\textit{L. casei} GG</td>
<td>0(^e)</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>\textit{B. animalis}</td>
<td>14(^g)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Percentage of mice with disseminated candidiasis (4 to 27 mice/group), euthanized at 4 to 12 weeks after colonization.

\(^{b}\)Values are expressed as mean ± SEM log10 CFU \textit{C. albicans}/gram of homogenized tissues (spleen, liver, and kidney).

\(^{c}\)Significantly less than the result for the \textit{C. albicans}-monoaosociated control (\(P<0.05\)).

\(^{d}\)NG, no growth.

\(^{e}\)–, data not available due to early mortality.

### TABLE 3. Incidence and severity of orogastric candidiasis in mice disassociated with \textit{C. albicans} and probiotic bacteria

<table>
<thead>
<tr>
<th>Microbial status</th>
<th>\textit{bg/bg-nu/nu} mice</th>
<th>\textit{bg/bg-nu/+} mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mucosal infection(^a) (%)</td>
<td>Severity score(^b)</td>
</tr>
<tr>
<td>\textit{C. albicans} alone</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>\textit{C. albicans} plus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{L. acidophilus}</td>
<td>87(^c)</td>
<td>3</td>
</tr>
<tr>
<td>\textit{L. reuteri}</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>\textit{L. casei} GG</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>\textit{B. animalis}</td>
<td>100</td>
<td>37(^e)</td>
</tr>
</tbody>
</table>

\(^{a}\)Percentage of mice with histopathologically confirmed candidiasis of tongue, esophagus, stomach, or hard palate 4 to 12 weeks after colonization (4 to 26 mice/group).

\(^{b}\)Mean severity score for mucosal candidiasis. Histopathology score in infected tissues was scored as follows: 1, 1 to 10 microorganisms/HPF (magnification, \(×100\); 2, 10 to 50 microorganisms/HPF; 3, 50 to 100 microorganisms/HPF; yeast cells and hyphae of \textit{C. albicans}; 4, confluent microorganisms/HPF (yeast cells and hyphae of \textit{C. albicans}); 5, confluent microorganisms/HPF with hyphal penetration of viable tissues (yeast cells and hyphae of \textit{C. albicans}).

\(^{c}\)Significantly decreased from result for \textit{C. albicans}-monoaosociated mice, \(P<0.05\) by repeated measures ANOVA.
C. albicans; only bg/bg-nu/+ males diassociated with C. albicans and L. acidophilus had significantly smaller body weights.

Due to their early deaths, the body weights of bg/bg-1nu/nu mice born to dams colonized with a pure culture of C. albicans, were not compared with the weights of pups born to dams colonized with C. albicans and a probiotic. Euthymic bg/bg-1nu/+ mice born to dams monoassociated with C. albicans weighed significantly less at 4 and 8 weeks of age than comparable GF mice (Table 6). The euthymic bg/bg-1nu/+ pups born to dams diassociated with C. albicans and a probiotic bacterium species weighed significantly less than GF pups at 4 weeks of age, but by 8 weeks their body weights were comparable to those of GF controls.

Modulation of host immune responses to C. albicans by probiotic bacteria. Ig isotypes (IgG, IgA, and IgM) were quantified in sera from mice colonized for 4 weeks with C. albicans or with C. albicans and a probiotic bacterium species (Table 7). Athymic bg/bg-1nu/nu mice did not produce significant levels of serum IgA except when colonized with C. albicans and B. animalis; however, serum IgG and IgM were also significantly increased in bg/bg-1nu/nu mice colonized with C. albicans and B. animalis. Only IgM was increased in L. casei GG- and C. albicans-colonized bg/bg-1nu/nu mice. Interestingly, we observed that the presence of L. acidophilus or L. casei GG prevented the C. albicans-induced increase of serum IgG in bg/bg-1nu/nu mice. Alimentary tract colonization by C. albicans or by probiotic bacteria and C. albicans significantly increased IgG, IgA, and IgM in sera from bg/bg-1nu/+ mice over levels in sera from GF mice. (Table 7).

The induction of specific serum Ig (IgG, IgA, or IgM) to C. albicans antigens was further investigated by Western blotting analyses. As shown in Fig. 2, sera from C. albicans-colo-
C. albicans (Fig. 2, lane 11). The recovery of their humoral immune responses to C. albicans antigens was greater with splenocytes from C. albicans GG or C. albicans-associated control; bg/bg-nu/nu C. albicans mice diassociated with P bg/bg-nu/nu associated mice (Table 8). The latter protective effect was evident in athymic (bg/bg-nu/nu) and eugenic (bg/bg-nu/+ ) mice. Consistent with the latter protective effect, inhibition of systemic dissemination of gastrointestinal pathogens has been described as an attribute of probiotic microorganisms (2, 16).

Few studies have addressed the ability of probiotics to protect immunodeficient hosts from candidiasis. In a previous study, researchers reported that feeding heat-killed E. faecalis to mice with cyclophosphamide-induced leukopenia enhanced the recovery of their humoral immune responses to C. albicans antigens (32). In another study, L. acidophilus and Streptococcus thermophilus protected corticosteroid-immunosuppressed mice from systemic (intraperitoneal challenge) candidiasis (6). The latter study involved the use of immunocompetent mice that were treated with immunosuppressive agents to enhance the efficacy of probiotic protection from candidiasis of endogenous (alimentary tract) origin in congenitally immunodeficient mice.

In vitro lymphocyte proliferation assays showed that splenocytes from mice diassociated with C. albicans and either L. casei GG or B. animalis had less of a lymphocyte proliferative (mitogenic) response to LPS than C. albicans-monoassociated mice (Table 8). Conversely, lymphocyte proliferation to C. albicans antigens was greater with splenocytes from bg/bg-nu/+ mice diassociated with C. albicans and either L. casei GG or B. animalis than with lymphocytes from C. albicans-monoassociated mice (Table 8).

DISCUSSION

All four of the probiotic bacteria species we tested not only prolonged the survival of bg/bg-nu/nu mice after oral colonization with C. albicans compared with that of C. albicans (pure culture)-colonized mice but also decreased the incidence of disseminated candidiasis in both strains (bg/bg-nu/nu and bg/bg-nu/+ ) of mice. The presence of a functional thymus was not necessary for the probiotic bacteria to enhance survival and decrease the dissemination of candidiasis in these mice, since the latter protective effect was evident in athymic (bg/bg-nu/nu) and eugenic (bg/bg-nu/+ ) mice. Consistent with the latter protective effect, inhibition of systemic dissemination of gastrointestinal pathogens has been described as an attribute of probiotic microorganisms (2, 16).

Few studies have addressed the ability of probiotics to protect immunodeficient hosts from candidiasis. In a previous study, researchers reported that feeding heat-killed E. faecalis to mice with cyclophosphamide-induced leukopenia enhanced the recovery of their humoral immune responses to C. albicans antigens (32). In another study, L. acidophilus and Streptococcus thermophilus protected corticosteroid-immunosuppressed mice from systemic (intraperitoneal challenge) candidiasis (6). The latter study involved the use of immunocompetent mice that were treated with immunosuppressive agents to enhance the efficacy of probiotic protection from candidiasis of endogenous (alimentary tract) origin in congenitally immunodeficient mice.
Our results show that probiotic bacteria can partially protect congenitally immunodeficient mice from lethal candidiasis. The four probiotic bacterial species that we studied differed in their biotherapeutic effects on candidiasis. The best overall biotherapeutic effects were observed with *B. animalis*. *B. animalis* prolonged survival compared with that of *C. albicans*-monoaosociated controls, decreased systemic dissemination, inhibited *C. albicans* in the alimentary tract, stimulated antibody- and cell-mediated immunity and, in bg/bg-nu/+ mice, significantly increased the incidence and severity of orogastric candidiasis. *B. animalis* was more effective as a biotherapeutic agent in mice with a functional thymus than in bg/bg-nu/nu (athymic) mice. Our data not only support the importance of a functional thymus in protecting mice against orogastric candidiasis but also demonstrate that *B. animalis* enhanced the resistance of bg/bg-nu/+ mice to candidiasis to a greater extent than the other three probiotic bacterial species we studied. The role of thymus-matured T cells in resistance to orogastric candidiasis has been well documented (8, 25, 34). Further research is needed to delineate the immune and inhibitory mechanism(s) that enable *B. animalis* to enhance resistance of mice to mucosal and systemic candidiasis.

None of the probiotic strains we tested provided complete protection against candidiasis. It was evident from our studies that suppression of *C. albicans* growth in the intestinal tract by probiotic bacteria was not always associated with enhanced resistance to orogastric candidiasis. We observed that some of the probiotic bacteria inhibited the growth of *C. albicans* in the intestinal tract to some degree; however, the inhibition of *C. albicans* did not always correlate with a reduction in the overall severity of orogastric candidiasis. Two of the probiotics (*L. reuteri* and *L. casei*) are known to produce broad-spectrum antimicrobial compounds, reuterin and caseicin, respectively (1, 24). Volatile fatty acids, such as lactic and propionic acids, and reactive oxygen species, such as H2O2, are also produced (1, 24). *L. casei* and *L. acidophilus* respectively suppressed the growth of *C. albicans* in vivo better than *L. reuteri* and *L. casei* did suggests that they can produce candida-inhibitory compounds in vivo. Our observation that probiotic inhibition of *C. albicans* growth in the alimentary tract did not always correlate with protection from orogastric candidiasis suggests that probiotic stimulation of host defense (innate and acquired) mechanisms may be more important than bacterial inhibition of *C. albicans* in the intestinal tract in the protection of mice from orogastric or systemic candidiasis.

Our results showed that two strains of probiotic bacteria (*L. acidophilus* and *B. animalis*) enhanced the inflammatory response (consisting of polymorphonuclear leukocytes, macrophages, and lymphocytes) in infected mucosal tissues of bg/bg-nu/nu mice. Very little inflammatory cell infiltration was observed in the stomachs of bg/bg-nu/nu mice colonized with *C. albicans* (pure culture) or dissociated with *L. casei* GG or *L. reuteri* and *C. albicans*. Thus, *L. acidophilus* and *B. animalis* enhanced the recruitment of inflammatory cells to a *C. albicans*-infected mucosal tissue without the involvement of thymus-matured T cells. The capacity of probiotic bacteria to enhance inflammatory responses likely contributed to the prolonged survival and decreased dissemination of candidiasis we observed in these mice. We are unaware of any other reports on the enhancement of inflammatory cell infiltration by probiotic bacteria in response to an infectious agent.

We observed that either *B. animalis* or *C. albicans* could induce IgA production in bg/bg-nu/+ mice; however, only

### Table 7. Serum Ig (IgG, IgA, and IgM) responses in gnotobiotic mice colonized with probiotic bacteria and/or *C. albicans*

<table>
<thead>
<tr>
<th>Microbial status</th>
<th>bg/bg-nu/nu mice</th>
<th>Mean ± SEM (µg/ml)</th>
<th>bg/bg-nu/+ mice</th>
<th>Mean ± SEM (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgA</td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>GF</td>
<td>293 ± 51</td>
<td>&lt;200</td>
<td>28 ± 2</td>
<td>301 ± 123</td>
</tr>
<tr>
<td><em>C. albicans</em> alone</td>
<td>1,936 ± 1,049</td>
<td>229 ± 29</td>
<td>32 ± 7</td>
<td>2,257 ± 121*</td>
</tr>
<tr>
<td><em>C. albicans</em> plus:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>244 ± 25</td>
<td>&lt;200</td>
<td>48 ± 24</td>
<td>1,285 ± 292*</td>
</tr>
<tr>
<td><em>L. reuteri</em></td>
<td>&lt;b&gt;244 ± 25&lt;/b&gt;</td>
<td>&lt;b&gt;200&lt;/b&gt;</td>
<td>&lt;b&gt;48 ± 24&lt;/b&gt;</td>
<td>&lt;b&gt;1,285 ± 292*&lt;/b&gt;</td>
</tr>
<tr>
<td><em>L. casei</em> GG</td>
<td>233 ± 64</td>
<td>&lt;200</td>
<td>66 ± 8*</td>
<td>4,751 ± 1,474*</td>
</tr>
<tr>
<td><em>B. animalis</em></td>
<td>2,179 ± 367*</td>
<td>1,106 ± 39*</td>
<td>108 ± 26*</td>
<td>3,269 ± 418*</td>
</tr>
</tbody>
</table>

*Significantly greater than result for GF control (P < 0.05 by ANOVA). Each group contained five mice.

*-, data not available due to early deaths and cannibalism.

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**FIG. 2.** Antibodies (IgG, IgM, and IgA) to *C. albicans* antigens in mouse sera. Each panel contains a 4 to 20% gradient polyacrylamide denaturing gel electrophoresis of *C. albicans* antigens. Lanes across *C. albicans* antigen separations were immunoblotted with pooled antiserum (three mice per pool) from GF bg/bg-nu/+ mice (lane 1); bg/bg-nu/+ mice colonized with *C. albicans* (lane 2) or dissociated with *C. albicans* and *L. casei* GG (lane 3), *B. animalis* (lane 4), *L. reuteri* (lane 5), or *L. acidophilus* (lane 6); GF bg/bg-nu/nu mice (lane 7); *C. albicans*-monoaosociated bg/bg-nu/nu mice (lane 8); and bg/bg-nu/nu mice dissociated with *C. albicans* and *L. casei* GG (lane 9), *B. animalis* (lane 10), or *L. casei* GG (lane 11). This blot is representative of two experiments with different serum pools. MW, molecular weight (in thousands).
C. albicans in combination with B. animalis (dissociated) induced IgA production in bg/bg-nu/nu mice. IgA production is generally considered to be thymus dependent (26); however, athymic mice are capable of T cell-dependent processes via mucosal T cells of extrathymic origin and maturation (10, 13). Probiotic bacteria are known to enhance antibody responses to pathogens in mice (3). For example, in one previous study, increased antibody production in mice that were fed Bifidobacterium breve and infected with rotavirus was reported (36). In other studies, increased resistance and elevated serum antibodies to Salmonella typhi were induced by feeding humans L. acidophilus (19) and increased resistance and elevated serum antibodies to Salmonella typhimurium and Escherichia coli were induced by feeding mice L. casei (27). Transient increases in IgA (26) and IgG and IgM (18) have also been reported after mice were colonized with L. acidophilus or L. casei. Our study strongly suggests that B. animalis, but not the other three probiotic bacterial species we tested, has the unique capacity to stimulate T cell-dependent IgA and IgG antibody responses in athymic mice, possibly via extrathymic-matured T cells that are present in mucosal tissues.

Our study also showed that in pure culture, C. albicans inhibited the growth of bg/bg-nu/nu mice. The weight loss appears to be related to the severity of the orogastric infection. B. animalis was the most effective probiotic in mice of the four we studied and provided the best overall protection against orogastric and systemic candidiasis; however, we observed that L. casei GG and L. reuteri were better than B. animalis to counteract the growth-inhibitory effects of C. albicans on mice. Thus, L. casei GG and L. reuteri appeared to produce biotherapeutic effects via nutrient utilization, supplementation, and/or availability. Further study is needed to determine how probiotic bacteria prevent C. albicans-induced weight loss.

Overall, this study demonstrated that probiotic bacteria can protect immunodeficient mice from candidiasis; however, none of the probiotic bacteria we studied eliminated C. albicans from the alimentary tract or provided complete protection against orogastric and systemic candidiasis. The probiotic bacteria we studied differed in their capacities to prolong survival, inhibit C. albicans in the intestinal tract, stimulate antibody- and cell-mediated immunity, and affect the growth rate of guttobiotic mice. Our data indicate that the probiotic bacteria produced biotherapeutic effects by inhibition of C. albicans growth, stimulation of the mucosal and systemic immune systems and possibly by nutritional and competitive means. Of the four probiotic bacterial species that we studied, B. animalis was the most biotherapeutic and provided the best overall protection against mucosal and systemic candidiasis. B. animalis apparently stimulated host resistance to candidiasis via thymus- and mucosal tissue-associated lymphoid tissues. Overall, thymus and mucosal tissue stimulation by probiotic bacteria strains such as B. animalis likely plays a very important role in the enhancement of resistance to infectious agents. More research is needed to elucidate the basic mechanisms utilized by probiotic bacteria so that their beneficial biotherapeutic effects can be optimized.

ACKNOWLEDGMENTS

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Radial immunodiffusion assays of serum Ig isotypes were run by Lisa Roberts and Jeff Farmer at Abbott Laboratories, Abbott Park, Ill. We thank JoAnne Croft and Barb Reese for maintenance of the gnotobiotic mice at the University of Wisconsin Medical School Gnotobiotic Laboratory. We also appreciate the statistical analyses conducted by Dennis Heisey of the University of Wisconsin Medical School Department of Surgery, and thank Donna Brackett for her assistance in the preparation of the manuscript.

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