Effects of probiotic bacteria on humoral immunity to *Candida albicans* in immunodeficient *bg/bg-nu/nu* and *bg/bg-nu/+* mice

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Summary

Germfree beige-nude (*bg/bg-nu/nu*) and beige-heterozygous (*bg/bg-nu/+*) mice were colonized with a pure culture of *Candida albicans* or with a probiotic bacterium (*Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus casei*, or *Bifidobacterium infantis*). Probiotic-colonized mice were subsequently challenged orally with *C. albicans*. The effect of prior colonization with probiotic bacteria on the antibody responses of the immunodeficient mice to alimentary tract colonization with *C. albicans* was compared to the antibody responses of the gnotobiotic mice colonized only with *C. albicans*. This study demonstrated that, although the probiotic bacteria did not induce a vigorous antibody response to their own antigens, they altered the antibody responses of mice to *C. albicans*. In T cell competent *bg/bg-nu/+* mice, *B. infantis* enhanced and focused IgG1, $IgG2_A$, and IgA responses to *C. albicans* antigens. Some of the probiotic bacteria also enhanced the IgG1 and $IgG2_A$ antibody responses of *bg/bg-nu/nu* mice to *C. albicans* antigens. This study not only shows the value of gnotobiotic animal models in demonstrating that probiotic bacteria can affect the capacity of mice to form antibodies to *C. albicans*, but it also points out their usefulness in comparing the capacity of different probiotic bacteria to produce beneficial health effects in mice.

Key words Probiotic, Antibodies, Immunodeficiency, *Candida albicans*

Efectos de las bacterias probióticas en la inmunidad humoral frente a *Candida albicans* en ratones inmunodeficientes *bg/bg-nu/nu* y *bg/bg-nu/+*

Resumen

Se colonizaron ratones libres de gérmenes (*germfree*) *beige-nude* (*bg/bg-nu/nu*) y *beige-heterozygous* (*bg/bg-nu/+*) con un cultivo puro de *Candida albicans* o con bacterias probióticas (*Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus casei*, o *Bifidobacterium infantis*). Los ratones colonizados con bacterias probióticas fueron posteriormente inoculados por vía oral con *C. albicans*. El efecto de la colonización previa con bacterias probióticas sobre las respuestas de anticuerpos en ratones inmunodeficientes con colonización digestiva por *C. albicans* se comparó con las respuestas de anticuerpos en ratones gnotobióticos colonizados únicamente por *C. albicans*. Este estudio ha demostrado que a pesar de que las bacterias probióticas no inducen una respuesta humoral enérgica contra sus propios antígenos, alteran las respuestas de anticuerpos contra *C. albicans* en estos ratones. En ratones *bg/bg-nu/+* T-competentes, *B. infantis* mejora y dirige las respuestas IgG1, $IgG2_A$, e IgA contra antígenos de *Candida*. Algunas de las bacterias probióticas también mejoraban las respuestas de anticuerpos IgG1 e $IgG2_A$ de los ratones *bg/bg-nu/nu* frente a antígenos candidiásicos. Este estudio no sólo muestra el valor de los modelos con animales gnotobióticos para comprobar que las bacterias probióticas pueden afectar la

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capacidad de producción de anticuerpos frente a *C. albicans* en los ratones, si no que también resalta su utilidad para comparar la capacidad de diferentes bacterias probióticas para producir efectos beneficiosos para la salud en ratones.

Palabras clave Probiótico, Anticuerpos, Inmunodeficiencia, *Candida albicans*

A previous study reported that *bg/bg-nu/nu* and *bg/bg-nu/+* mice, colonized with either a *Lactobacillus* species, or a *Bifidobacterium* species and then orally challenged with *Candida albicans*, had better cell-mediated (CMI) and antibody-mediated immune (AMI) responses to *Candida* antigens than mice monoassociated with *C. albicans* [1]. In the latter study, the improvement in the antibody responses of mice to *C. albicans* antigens by probiotic bacteria correlated with their increased resistance to candidiasis [1]. The role of AMI in murine resistance to candidiasis is still controversial [2]. We reported in a previous study, that B cell-knockout mice were resistant to mucosal candidiasis and systemic candidiasis of endogenous origin, but were more susceptible to experimental (intravenous [i.v] inoculation) systemic candidiasis than B cell-competent controls [3]. Since host resistance to *C. albicans* probably relies on several immune mechanisms, the resistance of B cell-deficient mice to mucosal and systemic candidiasis of endogenous origin does not rule out a protective role for AMI in other forms of candidiasis, especially acute systemic candidiasis after i.v. challenge. Several others have proposed that specific antibodies may protect against systemic candidiasis [2,4,5]. Thus, in this study, we investigated the capacity of probiotic bacteria to affect the antibody response to *C. albicans*.

Probiotic bacteria may affect host antibody responses to pathogens by causing increased production of total immunoglobulins or by selective enhancement of specific immunoglobulin isotypes. Previously, we observed that total serum immunoglobulin (Ig) levels were elevated after germfree mice were monoassociated with *C. albicans* and the antibody levels were not further increased in mice diassociated with a probiotic bacterium and *C. albicans* [1]. In contrast, specific anti-*C. albicans* antibodies in the sera of the mice diassociated with the probiotic bacteria and *C. albicans* bound to different *C. albicans* antigens than antibodies in sera from *C. albicans*-monoassociated mice. Only weak cross-reactive antibody responses were directed to antigens of the probiotic bacteria, suggesting that the mice were quickly tolerized or were largely unresponsive to the probiotic bacteria in the monoassociated mice [6].

Production of certain immunoglobulin classes is related to the type of T cell regulation promoting the antibody response. A T helper 1 (Th1) response is defined by the production of interferon-gamma (IFN-γ) and interleukin-2 (IL-2), whereas the Th2 type of regulatory T cell produces IL-4, IL-10 and other cytokines, but not IL-2 and IFN-γ [7]. It has been suggested that the Th1 response, which promotes the production of predominantly $IgG2_A$ and IgA, is important for protective immunity against candidiasis [8]. A Th2 regulated antibody profile, consisting of IgG1 and IgG3 has been associated with susceptibility to candidiasis [7,8]. In the present study, we evaluated the capacity of probiotic bacteria to induce alterations in the pattern of antibody isotypes that mice produced after oral challenge with *C. albicans.*

MATERIALS AND METHODS

Bacteria. This study focused on commercial starter cultures of lactic acid bacteria (LAB) that were readily available for use as human probiotics. *Lactobacillus acidophilus* NCFM, *Bifidobacterium infantis*, *Lactobacillus casei* GG, and *Lactobacillus acidophilus* LA-1, and *L. reuteri* were cultured, maintained, and handled as previously described [1].

Mice. Germfree C57BL/6 *bg/bg-nu/nu* mice, which die (5 to 10 weeks) after oral colonization with *C. albicans* [1], and GF *bg/bg-nu/+* mice, which survive *C. albicans* colonization, were obtained from breeding stocks maintained at the University of Wisconsin Gnotobiote Laboratory, Madison, Wis. The mice were associated with probiotic bacteria and *C. albicans* as previously described [1].

Western blot. Immunoblot assays of serum antibodies to specific antigens were conducted with modifications of our previous protocols [1,6]. Antigens were prepared from 48-h aerobic cultures of *C. albicans* and used for Western blot assays [1,6]. Antigens were also prepared from 48-h anaerobic cultures of *L. acidophilus*, *L. reuteri*, *L. casei*, and *B. infantis*. Briefly, the entire volume of a 500 ml culture was centrifuged at 2000 x g for 15 min. The fungal or bacterial pellets were washed three times with an equal volume of phosphate-buffered saline and re-centrifuged. The final fungal or bacterial pellet was resuspended in 10 ml of phosphate-buffered saline and passed through a French pressure cell (SLM/AMINCO, Urbana, Ill.) at 15,000 lb/in^2 to disrupt the fungi or bacteria. The disrupted fungi or bacteria were centrifuged at 2,000 x g and the protein content of the supernatant was determined by the bicinchoninic acid BCA protein assay (Pierce Chemical Co., Rockford, Ill.) and diluted to 1 mg/ml concentrations for use as the antigens in Western blot assays.

Antigen preparations (200 mg) from *C. albicans* were applied to a single gel-wide lane of a denaturing 4 to 20% polyacrylamide mini-gel (Novex, San Diego, Calif.) and electrophoresed at 35 mA until the bromphenol blue tracking dye reached the end of the gel. The separated antigens were electroblotted from the gel onto a nitrocellulose membrane, which was incubated in TBS-Tween buffer (0.01 M Tris, 0.15 M NaCl, 0.2% Tween [polyoxyethylene sorbitan monolaurate; Sigma Chemical, St. Louis, Mo.]), and 5% powdered milk for 30 min to block nonspecific antibody binding sites. Pooled serum samples from mice colonized with *C. albicans* and probiotic bacteria for 4 to 8 weeks were diluted 1:20 in TBS-Tween buffer and 1% powdered milk and incubated in lanes on blots with a miniblotter-16 manifold (Immunetics, Cambridge, Mass.) for 2 h. The blots were washed with TBS-Tween buffer and incubated for 1 h with alkaline phosphatase-conjugated goat antiserum to mouse IgG, IgA, and IgM (Zymed) diluted 1:1000. The nitrocellulose membranes

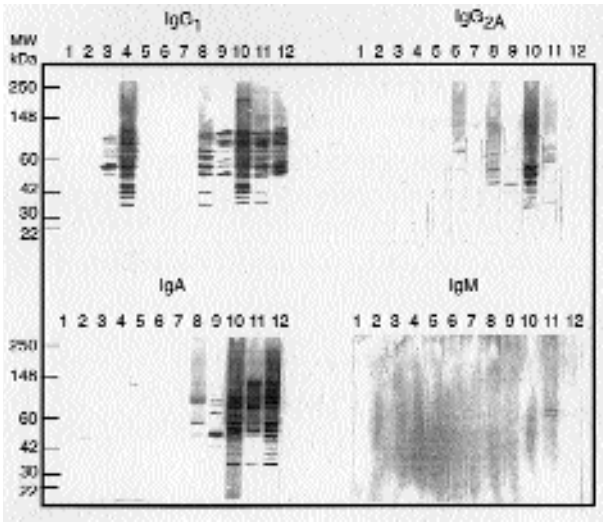

Figure 1. Effects of probiotic bacteria on specific antibody isotype production against *C. albicans* antigens. This series of Western blots show IgA, IgM, IgG1, and IgG2A antibodies in sera pooled from three to six mice colonized as indicated with *C. albicans* and/or a probiotic bacterium for 4 to 8 weeks. These blots are representative of 2 to 3 pools of sera for each experimental group. *C. albicans* antigens were separated on denaturing 4-20% polyacrylamide gels. The antisera were from *bg/bg-nu/nu* mice (lanes 1 through 6) or *bg/bg-nu/+* mice (lanes 7 through 12), either germfree (lanes 1 and 7), *C. albicans* monoassociated (lanes 2 and 8), or diassociated with *C. albicans* and *L. acidophilus* NCFM (lanes 3 and 9), *C. albicans* and *L. reuteri* (lanes 4 and 10), *C. albicans* and *L. casei* GG (lanes 5 and 11), or *C. albicans* and *B. infantis* (lanes 6 and 12).

were incubated with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) substrate solutions (Sigma) until bands appeared. Molecular weights were determined by linear estimation with an HP-20S calculator (Hewlett-Packard, Corvallis, OR) referenced to MultiMark molecular weight standards (Novex).

All blots were run simultaneously to avoid run-to-run variations. Although quantitative comparisons were avoided, some general comparisons of band intensities were made when visible differences were apparent.

RESULTS

Antibody isotype responses of bg/bg-nu/nu mice to C. albicans. In a previous study, we reported that the presence of probiotic bacteria in the alimentary tracts of gnotobiotic mice altered their serum antibody responses to an oral challenge with *C. albicans* [1]. The latter study also showed that probiotic bacteria could induce antibody production in *bg/bg-nu/nu* mice, in spite of the immunodeficiencies.

In this study, we assessed whether probiotic bacteria affected the capacity of *bg/bg-nu/nu* mice to produce individual antibody isotypes to *C. albicans* after an oral challenge with the opportunistic yeast. Due to their athymic status, *bg/bg-nu/nu* mice produce little serum antibody. We were unable to detect IgA or IgM antibodies to *C. albicans* antigens by Western blot with sera from *bg/bg-nu/nu* mice that were either colonized (monoassociated) with a probiotic bacterium or *C. albicans* or diassociated with a probiotic bacterium and *C. albicans* (Figure 1: IgA and IgM, lanes 1 through 6). No *bg/bg-nu/nu* mice monoassociated with a probiotic bacterium had antibodies to *C. albicans* antigens (data not shown). The *bg/bg-nu/nu* mice monoassociated with *C. albicans* were also unable to produce detectable IgG1 or IgG2A antibodies to *C. albicans* antigens (Figure 1: IgG1 and IgG2A, lane 2); however, *bg/bg-nu/nu* mice colonized with a probiotic bacterium and *C. albicans* produced IgG1 antibodies to a number of *C. albicans* antigens (Figure 1: IgG1, lanes 3 through 6). The strongest IgG1 responses to *C. albicans* antigens were evoked in *bg/bg-nu/nu* mice that were diassociated with *C. albicans* and either *L. reuteri* or *B. infantis* (Figure 1: IgG1, lanes 4 and 6).

Table 1 lists the molecular weights (estimated) of *C. albicans* antigens that reacted with IgA, IgM, IgG1, and IgG2A antibodies in sera from gnotobiotic *bg/bg-nu/nu* mice that were diassociated with the probiotic bacteria and *C. albicans*, as compared with antibody responses detected in sera from gnotobiotic *bg/bg-nu/nu* mice that were monoassociated with *C. albicans*. Sera from the probiotic bacteria and *C. albicans*-diassociated *bg/bg-nu/nu* mice had IgG1 antibody responses to antigens of *C. albicans* that were not induced by colonization of *bg/bg-nu/nu* mice with *C. albicans* alone (Table 1). Diassociation of *bg/bg-nu/nu* mice with *C. albicans* and *L. acidophilus* or *B. infantis*, but not with *L. reuteri* or *L. casei*, also induced IgG2A antibodies to *C. albicans* antigens that were not induced by *C. albicans* alone (Table 1). The probiotic bacteria did not induce detectable (by Western blot) serum IgA or IgM antibodies to *C. albicans* antigens in *bg/bg-nu/nu* mice (Table 1).

Antibody isotype responses of bg/bg-nu/+ mice to C. albicans. The *bg/bg-nu/+* mice that were monoassociated with *C. albicans* produced IgA, IgM, IgG1 and IgG2A antibodies to numerous *C. albicans* antigens (Figure 1, lanes 8). The presence of probiotic bacteria in the gut

Table 1. Probiotic bacteria induce specific immunoglobulin isotype responses to *C. albicans* antigens in gnotobiotic mice.

Microbial Status	Molecular weight (kDa)[a]							
	bg/bg-nu/nu				*bg/bg-nu/+*			
C. albicans +	IgA	IgM	IgG1	IgG2A	IgA	IgM	IgG1	IgG2A
L. acidophilus NCFM	[b]	[b]	67,60,47,12	53,42	42,17	[b]	75,67,60,39, 30	30
L. reuteri	[b]	[b]	67,60,53,42, 39, 30,17	[b]	60,42,30,27, 25	70,63,47,42 30,27,25,17, 12	67,47,27	92,87,84,53, 47,42,30,27, 25,17,12
L. casei GG	[b]	[b]	75,67,60,47, 39,27	[b]	84,67,60,47, 12	[b]	67,27,25	25,17
B. infantis	[b]	[b]	75,70,67,60, 53,47,30,27, 25,17,12	110,78,75, 70,53	84,75,70,60, 42,25,17,12	[b]	67,60,47, 30,27	[b]

[a]: Numbers represent the molecular weights (kDa) of *C. albicans* antigens that reacted more strongly with antibodies of the specified isotypes in sera from *bg/bg-nu/nu* or *bg/bg-nu/+* mice diassociated with *C. albicans* and *L. acidophilus* NCFM, *L. reuteri*, *L. casei*, or *B. infantis*, compared with *C. albicans*-monoassociated mice. Sera were collected from 6 mice/group and pooled 4 to 8 weeks after the probiotic-colonized mice (8 to 12 weeks old) were orally-challenged with *C. albicans*.
[b]: No change from antibodies observed in sera from *C. albicans*-monoassociated mice.

Table 2. Suppression of specific immunoglobulin isotype responses to *C. albicans* antigens by probiotic bacteria in *bg/bg-nu/+* mice.

Microbial status:	Molecular weights (kDa)[a]			
C. albicans +	IgA	IgM	IgG1	IgG2A
L. acidophilus NCFM	75,67,47,25	70,60,42	70,53,47	78,75
L. reuteri			75	
L. casei GG	42	[b]	75,70,53,39	53,57,42
B. infantis		[b]		47,30

[a]: Numbers represent the molecular weights (kDa) of *C. albicans* antigens that did not react with antibodies of the specified isotypes in sera from *bg/bg-nu/+* mice that were diassociated with *C. albicans* and *L. acidophilus* NCFM, *L. reuteri*, *L. casei*, or *B. infantis*, compared with antibodies that were present in sera from *C. albicans*-monoassociated mice. Sera were collected 4 to 8 weeks after *C. albicans* was given orally to the probiotic-colonized mice, 6 mice/group.
[b]: No change from antibodies observed in sera from *C. albicans*-monoassociated mice.

appeared to enhance (number and intensity of bands) antibody responses to *C. albicans* antigens in *bg/bg-nu/+* mice; however, different spectra of antibodies to *C. albicans* antigens were apparent, on the Western blots, with sera from gnotobiotic *bg/bg-nu/+* mice that were diassociated with *C. albicans* and each of the four probiotic bacteria we tested (Figure 1, lanes 9 through 12). No probiotic bacteria monoassociated *bg/bg-nu/+* mice had cross-reactive antibodies to *C. albicans* antigens (data not shown).

Many isotype-specific antibody responses of *bg/bg-nu/+* mice to *C. albicans* antigens were altered, either induced (Table 1) or suppressed (Table 2), by the probiotic bacteria, in comparison to antibody responses we detected in sera from *C. albicans*-monoassociated *bg/bg-nu/+* mice. As shown in Tables 1 and 2, *L. acidophilus* did not induce IgA responses to *C. albicans* antigens, as well as the other probiotic bacteria (Table 1) and it appeared to suppress the IgA response of *bg/bg-nu/+* mice to *C. albicans* antigens (Table 2). The IgA responses, to *C. albicans* antigens of several molecular weights, were generally induced by *L. reuteri*, *L. casei*, or *B. infantis*, when compared with IgA in sera from *C. albicans*-monoassociated mice (Table 1). IgM responses to *C. albicans'* antigens in *bg/bg-nu/+* mice were suppressed by *L. acidophilus* NCFM (Table 2), induced by *L. reuteri* (Table 1), and appeared to be unaffected by *L. casei* or *B. infantis* (Table 1). No distinct pattern of induction or suppression of IgG1 or IgG2A antibody responses to *C. albicans* antigens by probiotic bacteria were apparent. Some IgG1 antibodies to individual *C. albicans* antigens were induced by all four probiotic bacteria (Table 1), while other IgG1 antibody responses were suppressed in the diassociated *bg/bg-nu/+* mice (Table 2). *L. reuteri*-induced IgG2A responses to individual *C. albicans* antigens better than the other probiotic bacteria tested (Table 1) and did not appear to suppress IgG2A responses to *C. albicans* antigens in *bg/bg-nu/+* mice (Table 2).

DISCUSSION

Recently we reported that viable probiotic bacteria altered the serum antibody responses of mice to an oral challenge (colonization) with *C. albicans* [1]. We now report that viable probiotic bacteria can not only alter the production of individual isotypes of *C. albicans*-specific antibodies in mice, but that different strains of probiotic bacteria apparently can stimulate or interfere with the formation of different isotypes of antibodies, even in immunodeficient *bg/bg-nu/nu* mice which lack natural killer and thymus-matured T-cell functions.

A recent study [9] reported that obligate anaerobic gut bacteria (*Peptostreptococcus* sp.) could prime specific antibody production against *E. coli* antigens, much the same as we have observed with lactobacilli and bifidobacteria priming antibody production to *C. albicans* antigens in the present study. The immunostimulatory and immunosuppressive mechanisms of probiotic bacteria have not been elucidated; however, our results with *bg/bg-nu/nu* and *bg/bg-nu/+* mice suggest that probiotic bacteria can affect both T cell-independent and T cell-dependent AMI. Several types of helper T cells are involved in the regulation of T-dependent antibody production. Isotypes of antibodies elaborated against antigens of a pathogen can reveal the types of T cells involved in regulating the antibody response [7,8], at least in beige-euthymic mice. The Th1 type of helper T cells activate predominantly CMI responses [7] but they also influence the production of IgG2A antibodies [8]. The Th2 cell predominantly activates AMI via the production of IgG1 and IgA [8,10,11]. In this study, we observed that colonization of *bg/bg*-nu/nu-mice with *C. albicans* induced IgG1, but little IgG2A or IgA, suggesting that regulation of the antibody response to the fungus is either T-independent (neither Th1 nor Th2 regulated) or dependent on another class of T cells. In contrast, IgG1 and IgG2A, but not IgA antibodies specific for *C. albicans* antigens were activated in *bg/bg-nu/nu* mice diassociated with *C. albicans* and *L. acidophilus* NCFM or *B. infantis*. Therefore, the presence of viable probiotic bacteria in the alimentary tract appeared to induce the host to produce more of a Th1-dependent immune response to *C. albicans*. The shift from a mostly T-independent IgG1 antibody response to a Th1-dependent IgG2A antibody response against *C. albicans* could account for the protection that the probiotic bacteria afforded immunodeficient mice against this opportunistic agent [1]. Probiotic activation of IgG2A antibodies to *C. albicans* antigens in *bg/bg-nu/nu* mice likely occurred through the stimulation of extrathymically-matured T cells known to reside in the intestines [12,13].

A more prominent antibody response to *C. albicans* antigens was observed in *bg/bg-nu/+* mice

Table 3. Beneficial effects of probiotic bacteria used in this study[a].

	bg/bg-nu/nu				*bg/bg-nu/+*			
Probiotic Effect	La	Lr	Lc	Bi	La	Lr	Lc	Bi
1. Prolonged survival of *bg/bg-nu/nu*	+	+	+	+				
2. Suppressed *C. albicans* induced weight loss in *bg/bg-nu/nu*	+	+	-	-				
3. Increased inflammatory cell migration in *bg/bg-nu/nu* stomach	-	-	+	+				
4. Reduced disseminated candidiasis	+	+	+	+	+	+	+	+
5. Decreased fungal dimorphism	-	-	-	-	+	-	-	-
6. Reduced *C. albicans* infection of the alimentary tract	+	-	+	+	-	-	+	+
7. Increased total serum IgM	-	-	+	+	+	-	+	+
8. Increased total serum IgG	-	-	-	-	+	-	+	+
9. Increased total serum IgA	-	-	-	-	-	-	+	+

[a]These results were reported in references 1,16,17.
[b]La = *L. acidophilus*, Lr = *L. reuteri*, Lc = *L. casei*, Bi = *Bifidobacterium infantis.*

than in *bg/bg-nu/nu* mice. The latter data suggest that the antibody response to *C. albicans* antigens is primarily dependent on thymus-matured T cells; however, the immunostimulatory effects of probiotic bacteria on *bg/bg-nu/nu* mice diassociated with *C. albicans* and *L. acidophilus* or *Bifidobacterium spp*. correlated with enhanced survival of these mice [1]. While we have not found any other studies on the effect of probiotics on candidiasis in athymic mice, euthymic mice immunosuppressed with corticosteroids have been protected against systemic candidiasis by probiotic treatments [14]. The latter studies suggest that immunostimulation played a role in protecting the mice; however, we demonstrated that probiotic bacteria can induce specific antibody responses in athymic mice (presumably via a T-independent or extrathymic T cell-dependent mechanism), which may have played a role in the prolonged survival of these mice colonized with *C. albicans*.

In the present study we observed that probiotic bacteria induced antibody responses to some *C. albicans* antigens but inhibited antibody responses to other *C. albicans* antigens. We are not the only ones to have observed mixed immunomodulatory effects of probiotic bacteria. A recent study using SJL mice, orally treated with various strains of lactobacilli, demonstrated both enhanced and reduced antibody responses in experimental autoimmune encephalitis that was dependent upon the strain of bacteria used to colonize the mice [15]. The latter study implicated different cytokine expression profiles in the regulation of these complicated effects. Further study will be needed to work out the complex immunoregulatory effects of probiotic anaerobes.

In our previous reports we have described numerous beneficial effects of the four probiotic bacteria presented in the present study [1,16,17]. A thorough interpretation of the antibody isotype data presented here is difficult because of the number of probiotic effects. We have summarized the probiotic effects we reported in previous studies in Table 3 to assist the reader in interpreting the role of AMI in protection of mice from candidiasis.

Overall, this study shows that probiotic bacteria can affect the host's antibody response to *C. albicans* from predominantly T-independent (IgG1 and IgM) to Th1-dependent ($IgG2_A$ and IgA) isotype production. Our study also demonstrates that probiotic bacteria can enhance thymus-independent (IgG1 and IgM) or extrathymic T cell-dependent ($IgG2_A$) antibody responses to *C. albicans* antigens in *bg/bg-nu/nu* mice. Although we are uncertain of the protective efficacy of antibody-mediated immunity to *C. albicans* [2,13], our data indicate that probiotic bacteria, which effectively prolong survival of beige-athymic mice colonized with *C. albicans* [1] (e.g., *L. acidophilus* NCFM and *B. infantis*), also provided a strong stimulation of antibody production to *C. albicans* antigens in these mice. These results suggest that commensal bacterial flora should be considered an important component of the humoral immune system in protection against candidiasis. They also demonstrate that the presence of certain probiotic bacteria can enhance or suppress antibody responses to antigens administered via the mucosal surfaces of the alimentary tract.

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References

1. Wagner RD, Pierson C, Warner T, *et al.* Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. Infect Immun 1997; 65: 4165-4172.
2. Casadevall A. Antibody immunity and invasive fungal infections. Infect Immun 1995; 63: 4211-4218.
3. Wagner RD, Vazquez-Torres A, Jones-Carson J, Warner T, Balish E. B cell "knockout" mice are resistant to mucosal and systemic candidiasis of endogenous origin, but susceptible to experimental systemic candidiasis. J Infect Dis 1996; 174: 589-597.
4. Han Y, Cutler JE. Antibody response that protects against disseminated candidiasis. Infect Immun 1995; 63: 2714-2719.
5. Matthews RC, Burnie JP, Howat D, Rowland T, Walton F. Autoantibody to heat-shock protein 90 can mediate protection against systemic candidosis. Immunology 1991; 74: 20-24.
6. Wagner RD, Warner T, Roberts L, Farmer J, Balish E. Colonization of congenitally immunodeficient mice with probiotic bacteria. Infect Immun 1997; 65: 3345-3351.
7. Coffman RL, Varkila K, Scott P, Chatelain R. Role of cytokines in the differentiation of $CD4^+$ T-cell subsets *in vivo*. Immunol Rev 1991; 123: 189-207.
8. Bistoni F, Cenci E, Mencacci A, *et al.* Mucosal and systemic T helper cell function after intragastric colonization of adult mice with *Candida albicans*. J Infect Dis 1993; 168: 1449-1457.
9. Herias MV, Midtvedt T, Hanson LA, Wold AE. Increased antibody production against gut-colonizing *Escherichia coli* in the presence of the anaerobic bacterium *Peptostreptococcus*. Scand J Immunol 1998; 48: 277-282.
10. Howard M, Miyajima A, Coffman R. T-cell derived cytokines and their receptors. In: Paul W (Ed.) Fundamentals of Immunology. 3rd Ed. New York, Raven Press, 1993: 777-778.
11. Snapper CM, Finkelman FD. Immunoglobulin class switching. In: Paul W (Ed.) Fundamental Immunology. 3rd Ed. New York, NY: Raven Press, 1993: 845-846.
12. Hamad M, Whetsell M, Klein JR. T cell precursors in the spleen give rise to complex T cell repertoires in the thymus and the intestines. J Immunol 1995; 155: 2866-2876.
13. Kennedy MJ, Pierce CW, Lake JP. Extrathymic T cell maturation. Phenotypic analysis of T cell subsets in nude mice as a function of age. J Immunol 1992; 148: 1620-1629.
14. DePetrino SF, DeJorrat MEBB, Meson O, Perdigon G. Protective ability of certain lactic acid bacteria against an infection with *Candida albicans* in a mouse immunosuppression model by corticoid. Food Agric Immunol 1995; 7: 365-373.
15. Maassen CB, van Holten JC, Balk F, *et al.* Orally administered *Lactobacillus* strains differentially affect the direction and efficacy of the immune response. Vet Q 1998; 20: S81-S83.
16. Wagner RD, Warner T, Dohnalek M, Hilty M, Balish E. Variable biotherapeutic effects of *Lactobacillus acidophilus* isolates on orogastric and systemic candidiasis in immunodeficient mice. Rev Iberoam Micol 1998, 15: 271-276.
17. Wagner RD, Warner T, Pierson C, *et al.* Biotherapeutic effects of *Bifidobacterium* spp. on orogastric and systemic candidiasis in immunodeficient mice. Rev Iberoam Micol 1998, 15: 265-270.