Saccharomyces boulardii Stimulates Intestinal Immunoglobulin A Immune Response to *Clostridium difficile* Toxin A in Mice

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***Saccharomyces boulardii* is a nonpathogenic yeast that protects against antibiotic-associated diarrhea and recurrent *Clostridium difficile* colitis. The administration of *C. difficile* toxoid A by gavage to *S. boulardii*-fed BALB/c mice caused a 1.8-fold increase in total small intestinal immunoglobulin A levels ($P = 0.003$) and a 4.4-fold increase in specific intestinal anti-toxin A levels ($P < 0.001$). Enhancing host intestinal immune responses may be an important mechanism for *S. boulardii*-mediated protection against diarrheal illnesses.**

Clostridium difficile is the most common known cause of nosocomial infectious diarrhea in the developed world (18, 19, 27, 28, 37). Pathogenic strains of *C. difficile* produce two large protein exotoxins, toxin A and toxin B (41). Toxin A is a 308-kDa cytotoxin and enterotoxin that induces marked intestinal inflammation, fluid secretion, and mucosal injury (12, 23, 41). Toxin B, a 270-kDa protein, stimulates the release of inflammatory cytokines from monocytes and is cytotoxic to mammalian cells (2, 21, 22, 41). Toxin A appears to be the main cause of intestinal injury and inflammation in animal models of *C. difficile* ileocolitis (25, 41). However, toxin B may also cause injury to human colon (43).

Saccharomyces boulardii is a nonpathogenic yeast used to prevent or treat infectious diarrhea of many etiologies (14). In animal studies, *S. boulardii* protects against diarrhea and enterocolitis induced by a variety of enteric pathogens, including *C. difficile* (4, 5, 7–10, 13, 36, 42, 48, 49). In human studies, *S. boulardii* treatment significantly reduced the incidence of simple antibiotic-associated diarrhea (38, 46). *S. boulardii* also reduced the risk of subsequent relapse in patients with a history of multiple episodes of *C. difficile* diarrhea (20, 30, 39, 47).

The host's immune response to *C. difficile* toxins is now known to play a major role in determining disease expression (24, 26, 29, 31, 32, 33, 50). High titers of serum or intestinal antibodies against toxin A have been associated with asymptomatic carriage of toxigenic *C. difficile* and with shorter and less severe episodes of *C. difficile* diarrhea (32, 33, 34, 40, 45, 51, 52). Buts and colleagues found that *S. boulardii* significantly increased the secretion of immunoglobulin A (IgA) and secretory component in rat small intestine, but they did not study the specificity of the secretory IgA response (3). Based on these findings, we hypothesized that one mechanism whereby *S. boulardii* may protect against infection by *C. difficile* and other enteric pathogens is through a stimulation of the host's intestinal mucosal immune response (1, 14). The aim of this

study was to examine this hypothesis by determining whether *S. boulardii* treatment altered serum or intestinal anti-toxin A antibody production in mice exposed to *C. difficile* toxin A.

C. difficile toxin A was purified from culture supernatants of strain VPI 10463 (American Type Culture Collection, Rockville, Md.) and inactivated by overnight incubation with 1% formaldehyde followed by ultrafiltration (5, 6, 42). For most experiments, BALB/c mice were immunized with formalin-inactivated toxoid A (100 μ g) administered by gavage on days 0 and 7, and animals were sacrificed on day 21. *S. boulardii* (Biocodex Laboratories, Montrouge, France) was administered in the drinking water (3×10^8 CFU per ml) from the time of the first oral immunization until the time of sacrifice. In experiments that compared the mucosal adjuvant effects of *S. boulardii* to those of *C. difficile* toxoid A and/or cholera toxin (10 μ g; Calbiochem, San Diego, Calif.) was administered on days 0, 7, 14, and 21, and the animals were sacrificed on day 35 (15–17, 53). After sacrificing the animals, the small intestine from the pylorus to the cecum was immediately excised and the intestinal contents were harvested by gently wrapping the small intestine around a Pasteur pipette. An equal volume of phosphate-buffered saline containing protease inhibitors was added (Protease Inhibitors-Complete; Boehringer, Mannheim, Germany), the samples were centrifuged, and the supernatants were collected and stored at -80°C .

Antibodies against *C. difficile* toxin A were measured by enzyme-linked immunosorbent assay (ELISA) as described previously (29, 30, 34, 52). Briefly, microtiter plates (Polysorp; Nunc, Roskilde, Denmark) were coated with purified toxin A (0.5 μ g/ml). Intestinal and serum samples were assayed at a 1:50 dilution. Peroxidase-labeled goat anti-mouse IgA (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was used to determine intestinal and serum IgA anti-toxin A. Peroxidase-labeled anti-mouse IgM (Kirkegaard and Perry) and biotinylated goat anti-mouse IgG (Sigma, St. Louis, Mo.) were used to determine serum IgM and IgG anti-toxin A, respectively. Antibody levels are reported as the mean optical density of triplicate samples. To measure total intestinal IgA, microtiter plates (Immunosorp; Nunc) were coated with purified anti-mouse IgA (0.5 μ g/ml; Sigma) and intestinal samples were

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FIG. 1. *S. bouardii* increases total IgA levels in small intestinal secretions. Mice were immunized with formalin-inactivated toxoid A administered by gavage on days 0 and 7 and were sacrificed on day 21. *S. bouardii* was administered in the drinking water (3×10^8 CFU per ml). Small intestinal total IgA levels were measured by ELISA. Data are shown as mean and standard error (control, $n = 33$; toxoid A, $n = 13$; *S. bouardii* alone, $n = 15$; *S. bouardii* plus toxoid A, $n = 27$). Total IgA levels were significantly higher in each of the two *S. bouardii*-fed groups versus the control ($P = 0.003$ by ANOVA).

assayed at a 1:50,000 dilution. Purified mouse IgA (Pharmin-gen, San Diego, Calif.) was used as the standard.

Statistical analyses were performed using SigmaStat for Windows (version 2.0; Jandel Scientific Software, San Rafael, Calif.). Analysis of variance (ANOVA) on ranks and pairwise intergroup comparisons by Dunn's method were used. A P value of <0.05 was considered statistically significant.

BALB/c mice treated with *S. bouardii* had a small but statistically significant increase ($P = 0.003$) in total IgA levels in their small intestine secretions (Fig. 1). In the *S. bouardii*-fed group, mean IgA levels were 1.9-fold higher than in controls. A similar, 1.8-fold, increase in mean total IgA levels was seen in mice who received both *S. bouardii* and toxoid A. We next examined whether *S. bouardii* treatment could stimulate an intestinal immune response to *C. difficile* toxin A. Small intestinal IgA anti-toxin A levels were low in control mice, in mice that were immunized orally with toxoid A in the absence of a mucosal adjuvant, and in mice that were treated with *S. bouardii* but not exposed to toxoid A (Fig. 2). However, when mice were immunized orally with toxoid A during treatment with *S. bouardii*, there was a marked (4.4-fold) and highly significant ($P < 0.001$) increase in specific small intestinal IgA anti-toxin A levels.

We next asked whether the increase in intestinal IgA anti-toxin A levels in *S. bouardii*-treated mice could be the result of a nonspecific stimulation of small intestinal IgA production. We therefore determined the ratio of specific IgA anti-toxin A to total IgA levels in the different groups of mice. As illustrated in Fig. 3, the median ratios of specific to total IgA were similar in the control (0.23) and toxoid A-treated (0.25) mice. The median ratio was somewhat lower (0.16; $P > 0.05$) in mice that were treated with *S. bouardii* but not immunized with toxoid A. This decrease in the ratio results from an increase in total IgA levels in this treatment group that is not associated with a corresponding increase in specific IgA anti-toxin A levels. In

FIG. 2. *S. bouardii* increases small intestinal IgA anti-toxin A levels following oral immunization with *C. difficile* toxoid A. Mice were immunized with formalin-inactivated toxoid A administered by gavage on days 0 and 7 and were sacrificed on day 21. *S. bouardii* was administered in the drinking water (3×10^8 CFU per ml). Small intestinal IgA anti-toxin A antibody levels were measured by ELISA. Results are expressed as mean and standard error of optical density (O.D.) units ($\times 1,000$) (control, $n = 32$; toxoid A, $n = 13$; *S. bouardii* alone, $n = 15$; *S. bouardii* and toxoid A, $n = 15$). Small intestinal IgA anti-toxin A antibody levels were significantly higher in the *S. bouardii* plus toxoid A group compared to each of the other three treatment groups ($P < 0.001$ by ANOVA).

contrast, there was a 4.7-fold increase in the ratio of specific to total IgA (1.09; $P = 0.01$) in mice that were exposed to toxoid A during *S. bouardii* treatment. Thus, *S. bouardii* specifically increases intestinal IgA anti-toxin A levels during mucosal immunization against *C. difficile* toxin A.

These findings suggested that *S. bouardii* may act as a mucosal adjuvant. The mucosal adjuvant activities of cholera toxin

FIG. 3. *S. bouardii* specifically increases small intestinal IgA anti-toxin A levels following oral immunization. Mice were immunized with formalin-inactivated toxoid A administered by gavage on days 0 and 7 and were sacrificed on day 21. *S. bouardii* was administered in the drinking water (3×10^8 CFU per ml). The ratios of specific anti-toxin A IgA to total IgA in small intestine secretions (mean and standard error) are shown (control, $n = 32$; toxoid A, $n = 13$; *S. bouardii* alone, $n = 15$; *S. bouardii* and toxoid A, $n = 15$). The ratio of specific anti-toxin A IgA to total IgA was higher in the *S. bouardii* plus toxoid A group compared to the *S. bouardii* group ($P = 0.01$ by ANOVA).

FIG. 4. *S. boulardii* is more effective than cholera toxin in increasing small intestinal IgA anti-toxin A levels following oral immunization. Mice were immunized with formalin-inactivated toxoid A (TxA) administered by gavage on days 0, 7, 14, and 21 and were sacrificed on day 35. Other mice received cholera toxin (CT; 10 μ g) administered by gavage either alone or with toxoid A. Other mice received toxoid A by gavage and *S. boulardii* (Sb) in their drinking water (3×10^8 CFU per ml). Small intestinal IgA anti-toxin A levels were measured by ELISA. Results are expressed as optical density (O.D.) units ($\times 1,000$) (mean and standard error; $n = 9$ for each treatment group). IgA anti-toxin A levels were significantly higher in the TxA plus Sb group compared to the control, CT, or TxA plus CT groups ($P = 0.003$ by ANOVA).

are well recognized (15–17, 53). Therefore, we compared the intestinal IgA anti-toxin A responses following mucosal immunization with toxoid A during treatment with either *S. boulardii* or cholera toxin. As illustrated in Fig. 4, a significant increase in intestinal IgA anti-toxin A was observed only in the *S. boulardii*- and toxoid A-immunized group (3.0-fold increase; $P = 0.003$). In these experiments we also examined serum antibody levels against *C. difficile* toxin A. As shown in Fig. 5, serum IgA anti-toxin A was highest in the cholera toxin plus toxoid A group, but the differences were not statistically significant ($P = 0.35$). Serum IgG anti-toxin A was also highest in the cholera toxin plus toxoid A group, but again the differences did not reach statistical significance ($P = 0.34$). Serum IgM anti-toxin A was highest in the *S. boulardii* plus toxoid A group and in this instance was significantly higher than the control group. Serum IgM anti-toxin A was also somewhat increased in the cholera toxin and toxoid A group, but the increase did not reach statistical significance.

The main finding of this study is that feeding with *S. boulardii* during oral immunization of BALB/c mice with *C. difficile* toxin A stimulates a specific immune response to toxin A. As reported previously, *S. boulardii* caused a twofold increase in IgA levels in small intestine secretions (3). However, previous studies did not examine the effect of *S. boulardii* treatment on specific immune responses to luminal antigens. We now find that *S. boulardii* induces a specific IgA immune response to toxin A when both are coadministered. The relative increase in specific IgA anti-toxin A production is greater than the observed increase in total IgA levels. This finding is consistent with antigen-specific stimulation of the intestinal mucosal immune system by *S. boulardii*. The mechanisms whereby *S. boulardii* can stimulate intestinal immune responses to coadministered antigens are poorly understood. However, stimulation

FIG. 5. Serum anti-toxin A antibody levels following oral immunization against *C. difficile* toxoid A. Mice were immunized with formalin-inactivated toxoid A (TxA) administered by gavage on days 0, 7, 14, and 21 and were sacrificed on day 35. Some mice received cholera toxin (CT; 10 μ g) administered by gavage with toxoid A. Other mice received toxoid A by gavage and *S. boulardii* (Sb) in their drinking water (3×10^8 CFU per ml). Serum IgA, IgG, and IgM anti-toxin A levels were measured by ELISA. Results are expressed as optical density (O.D.) units ($\times 1,000$ for IgA and $\times 100$ for IgG and IgM) (mean and standard error; $n = 9$ for each treatment group). Serum IgM anti-toxin A levels were higher in the Sb plus TxA group compared to the control ($P = 0.002$ by ANOVA). There were no other statistically significant differences between any of the treatment groups.

of a more effective host mucosal immune response to prevalent antigens may be a general mechanism for the efficacy of *S. boulardii* in protecting against a wide variety of enteric disorders (4–11, 13, 35, 36, 42, 44, 48, 49).

There is increasing evidence that the immune response to *C. difficile* toxin A plays an important role in determining the clinical outcome of *C. difficile* infection in humans (24, 31). We recently reported a strong association between high levels of serum IgG anti-toxin A and asymptomatic carriage of *C. difficile* (32). Other clinical studies have shown that a low serum and/or intestinal antibody response to *C. difficile* toxin A is also associated with severe, prolonged, and recurrent *C. difficile* diarrhea (33, 34, 40, 45, 51, 52). Taken together, these studies indicate that an adequate antibody response to toxin A is an important element in asymptomatic carriage of *C. difficile* and in clinical recovery from *C. difficile* diarrhea. Thus, our finding of increased intestinal anti-toxin A levels in *S. boulardii*-treated mice may be directly relevant to the yeast's protective effects in recurrent *C. difficile* diarrhea and colitis.

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REFERENCES

1. Arnheim, K. 1994. Intestine-associated immune system stimulation. *Saccharomyces boulardii*—mechanism of action. *Fortschr. Med.* **112**:52–53. (In German.)
2. Barroso, L. A., S. Z. Wang, C. J. Phelps, J. L. Johnson, and T. D. Wilkins. 1990. Nucleotide sequence of *Clostridium difficile* toxin B gene. *Nucleic Acids Res.* **18**:4004.
3. Buts, J. P., P. Bernasconi, J. P. Vaerman, and C. Dive. 1990. Stimulation of secretory IgA and secretory component of immunoglobulins in small intestine of rats treated with *Saccharomyces boulardii*. *Dig. Dis. Sci.* **35**:251–256.
4. Buts, J. P., G. Corthier, and M. Delmee. 1993. *Saccharomyces boulardii* for *Clostridium difficile*-associated enteropathies in infants. *J. Pediatr. Gastroenterol. Nutr.* **16**:419–425.
5. Castagliuolo, I., J. T. LaMont, S. T. Nikulasson, and C. Pothoulakis. 1996.

- Saccharomyces boulardii* protease inhibits *Clostridium difficile* toxin A effects in the rat ileum. Infect. Immun. **64**:5225–5232.
6. Castagliuolo, I., M. F. Riegler, L. Valenick, J. T. LaMont, and C. Pothoulakis. 1999. *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. Infect. Immun. **67**:302–307.
 7. Castex, F., G. Corthier, S. Jouvert, G. W. Elmer, F. Lucas, and M. Bastide. 1990. Prevention of *Clostridium difficile*-induced experimental pseudomembranous colitis by *Saccharomyces boulardii*: a scanning electron microscopic and microbiological study. J. Gen. Microbiol. **136**:1085–1089.
 8. Corthier, G., F. Dubos, and R. Ducluzeau. 1986. Prevention of *Clostridium difficile* induced mortality in gnotobiotic mice by *Saccharomyces boulardii*. Can. J. Microbiol. **32**:894–896.
 9. Corthier, G., F. Lucas, S. Jouvert, and F. Castex. 1992. Effect of oral *Saccharomyces boulardii* treatment on the activity of *Clostridium difficile* toxins in mouse digestive tract. Toxicon **30**:1583–1589.
 10. Dias, R. S., E. A. Bambirra, M. E. Silva, and J. R. Nicoli. 1991. Protective effect of *Saccharomyces boulardii* against the cholera toxin in rats. Can. J. Microbiol. **37**:315–317.
 11. Dias, R. S., E. A. Bambirra, M. E. Silva, and J. R. Nicoli. 1995. Protective effect of *Saccharomyces boulardii* against the cholera toxin in rats. Braz. J. Med. Biol. Res. **28**:323–325.
 12. Dove, C. H., S. Z. Wang, S. B. Price, C. J. Phelps, D. M. Lyster, T. D. Wilkins, and J. L. Johnson. 1990. Molecular characterization of the *Clostridium difficile* toxin A gene. Infect. Immun. **58**:480–488.
 13. Elmer, G. W., and L. V. McFarland. 1987. Suppression by *Saccharomyces boulardii* of toxigenic *Clostridium difficile* overgrowth after vancomycin treatment in hamsters. Antimicrob. Agents Chemother. **31**:129–131.
 14. Elmer, G. W., C. M. Surawicz, and L. V. McFarland. 1996. Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. JAMA **275**:870–876.
 15. Elson, C. O., and W. Ealading. 1984. Generalized systemic and mucosal immunity in mice after mucosal stimulation with cholera toxin. J. Immunol. **132**:2736–2741.
 16. Elson, C. O., and W. Ealading. 1984. Cholera toxin feeding did not induce oral tolerance in mice and abrogated oral tolerance to an unrelated protein antigen. J. Immunol. **133**:2892–2897.
 17. Elson, C. O., S. Woogen, M. Gaspari, and W. Ealading. 1987. Induction of secretory IgA responses to protein antigens. Adv. Exp. Med. Biol. **B 216**:877–885.
 18. Fekety, R. 1997. Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis. American College of Gastroenterology, Practice Parameters Committee. Am. J. Gastroenterol. **92**:739–750.
 19. Fekety, R., and A. B. Shah. 1993. Diagnosis and treatment of *Clostridium difficile* colitis. JAMA **269**:71–75.
 20. Hassett, J., S. Meyers, L. McFarland, and M. E. Mulligan. 1995. Recurrent *Clostridium difficile* infection in a patient with selective IgG1 deficiency treated with intravenous immune globulin and *Saccharomyces boulardii*. Clin. Infect. Dis. **20**(Suppl. 2): S266–S268.
 21. Hofmann, F., C. Busch, U. Prepens, I. Just, and K. Aktories. 1997. Localization of the glucosyltransferase activity of *Clostridium difficile* toxin B to the N-terminal part of the holotoxin. J. Biol. Chem. **272**:11074–11078.
 22. Just, I., J. Selzer, M. Wilm, C. von Eichel-Streiber, M. Mann, and K. Aktories. 1995. Glucosylation of Rho proteins by *Clostridium difficile* toxin B. Nature **375**:500–503.
 23. Just, I., M. Wilm, J. Selzer, G. Rex, C. von Eichel-Streiber, M. Mann, and K. Aktories. 1995. The enterotoxin from *Clostridium difficile* (ToxA) monoglucosylates the Rho proteins. J. Biol. Chem. **270**:13932–13936.
 24. Kelly, C. P. 1996. Immune response to *Clostridium difficile* infection. Eur. J. Gastroenterol. Hepatol. **8**:1048–1053.
 25. Kelly, C. P., S. Becker, J. K. Linevsky, M. A. Joshi, J. C. O'Keane, B. F. Dickey, J. T. LaMont, and C. Pothoulakis. 1994. Neutrophil recruitment in *Clostridium difficile* toxin A enteritis in the rabbit. J. Clin. Investig. **93**:1257–1265.
 26. Kelly, C. P., and J. T. LaMont. 1993. Treatment of *Clostridium difficile* diarrhea and colitis, p. 199–212. In M. M. Wolfe (ed.), Gastrointestinal pharmacotherapy. W. B. Saunders, Philadelphia, Pa.
 27. Kelly, C. P., and J. T. LaMont. 1998. *Clostridium difficile* infection. Annu. Rev. Med. **49**:375–390.
 28. Kelly, C. P., C. Pothoulakis, and J. T. LaMont. 1994. *Clostridium difficile* colitis. N. Engl. J. Med. **330**:257–262.
 29. Kelly, C. P., C. Pothoulakis, J. Orellana, and J. T. LaMont. 1992. Human colonic aspirates containing immunoglobulin A antibody to *Clostridium difficile* toxin A inhibit toxin A-receptor binding. Gastroenterology **102**:35–40.
 30. Kimmey, M. B., G. W. Elmer, C. M. Surawicz, and L. V. McFarland. 1990. Prevention of further recurrences of *Clostridium difficile* colitis with *Saccharomyces boulardii*. Dig. Dis. Sci. **35**:897–901.
 31. Kyne, L., and C. P. Kelly. 1998. Prospect for a vaccine for *Clostridium difficile*. BioDrugs **10**:173–181.
 32. Kyne, L., M. Warny, A. Qamar, and C. P. Kelly. 2000. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N. Engl. J. Med. **340**:390–397.
 33. Kyne, L., M. Warny, A. Qamar, and C. P. Kelly. 2001. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. Lancet **357**:189–193.
 34. Leung, D. Y., C. P. Kelly, M. Boguniewicz, C. Pothoulakis, J. T. LaMont, and A. Flores. 1991. Treatment with intravenously administered gamma globulin of chronic relapsing colitis induced by *Clostridium difficile* toxin. J. Pediatr. **118**:633–637.
 35. Massot, J., M. Desconclois, and J. Astoin. 1983. Protection by *Saccharomyces boulardii* against *Escherichia coli* diarrhea in newborn mice. Ann. Pharm. Fr. **40**:445–449. (In French.)
 36. Massot, J., O. Sanchez, R. Couchy, J. Astoin, and A. L. Parodi. 1984. Bacterio-pharmacological activity of *Saccharomyces boulardii* in clindamycin-induced colitis in the hamster. Arzneim.-Forsch. **34**:794–797.
 37. McFarland, L. V., M. E. Mulligan, R. Y. Kwok, and W. E. Stamm. 1989. Nosocomial acquisition of *Clostridium difficile* infection. N. Engl. J. Med. **320**:204–210.
 38. McFarland, L. V., C. M. Surawicz, R. N. Greenberg, G. W. Elmer, K. A. Moyer, S. A. Melcher, K. E. Bowen, and J. L. Cox. 1995. Prevention of beta-lactam-associated diarrhea by *Saccharomyces boulardii* compared with placebo. Am. J. Gastroenterol. **90**:439–448.
 39. McFarland, L. V., C. M. Surawicz, R. N. Greenberg, R. Fekety, G. W. Elmer, K. A. Moyer, S. A. Melcher, K. E. Bowen, J. L. Cox, and Z. Noorani. 1994. A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. JAMA **271**:1913–1918.
 40. Mulligan, M. E., S. D. Miller, L. V. McFarland, H. C. Fung, and R. Y. Kwok. 1993. Elevated levels of serum immunoglobulins in asymptomatic carriers of *Clostridium difficile*. Clin. Infect. Dis. **16**(Suppl. 4):S239–S244.
 41. Pothoulakis, C. 1996. Pathogenesis of *Clostridium difficile*-associated diarrhoea. Eur. J. Gastroenterol. Hepatol. **8**:1041–1047.
 42. Pothoulakis, C., C. P. Kelly, M. A. Joshi, N. Gao, C. J. O'Keane, I. Castagliuolo, and J. T. LaMont. 1993. *Saccharomyces boulardii* inhibits *Clostridium difficile* toxin A binding and enterotoxicity in rat ileum. Gastroenterology **104**:1108–1115.
 43. Riegler, M., R. Sedivy, C. Pothoulakis, G. Hamilton, J. Zacherl, G. Bischof, E. Cosentini, W. Feil, R. Schiessel, and J. T. LaMont. 1995. *Clostridium difficile* toxin B is more potent than toxin A in damaging human colonic epithelium in vitro. J. Clin. Investig. **95**:2004–2011.
 44. Rodrigues, A. C., R. M. Nardi, E. A. Bambirra, E. C. Vieira, and J. R. Nicoli. 1996. Effect of *Saccharomyces boulardii* against experimental oral infection with *Salmonella typhimurium* and *Shigella flexneri* in conventional and gnotobiotic mice. J. Appl. Bacteriol. **81**:251–256.
 45. Salcedo, J., S. Keates, C. Pothoulakis, I. Castagliuolo, J. T. LaMont, and C. P. Kelly. 1997. Intravenous immunoglobulin therapy for severe *Clostridium difficile* colitis. Gut **41**:366–370.
 46. Surawicz, C. M., G. W. Elmer, P. Speelman, L. V. McFarland, J. Chinn, and G. van Belle. 1989. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*: a prospective study. Gastroenterology **96**:981–988.
 47. Surawicz, C. M., L. V. McFarland, G. Elmer, and J. Chinn. 1989. Treatment of recurrent *Clostridium difficile* colitis with vancomycin and *Saccharomyces boulardii*. Am. J. Gastroenterol. **84**:1285–1287.
 48. Toothaker, R. D., and G. W. Elmer. 1984. Prevention of clindamycin-induced mortality in hamsters by *Saccharomyces boulardii*. Antimicrob. Agents Chemother. **26**:552–556.
 49. Vidon, N., B. Huchet, and J. C. Rambaud. 1986. Influence of *Saccharomyces boulardii* on jejunal secretion in rats induced by cholera toxin. Gastroenterol. Clin. Biol. **10**:13–16. (In French.)
 50. Viscidi, R., B. E. Laughon, R. Yolken, P. Bo-Linn, T. Moench, R. W. Ryder, and J. G. Bartlett. 1983. Serum antibody response to toxins A and B of *Clostridium difficile*. J. Infect. Dis. **148**:93–100.
 51. Warny, M., C. Denie, M. Delmee, and C. Lefebvre. 1995. Gamma globulin administration in relapsing *Clostridium difficile*-induced pseudomembranous colitis with a defective antibody response to toxin A. Acta Clin. Belg. **50**:36–39.
 52. Warny, M., J. P. Vaerman, V. Avesani, and M. Delmee. 1994. Human antibody response to *Clostridium difficile* toxin A in relation to clinical course of infection. Infect. Immun. **62**:384–389.
 53. Wilson, A. D., C. R. Stokes, and F. J. Bourne. 1989. Adjuvant effect of cholera toxin on the mucosal immune response to soluble proteins. Differences between mouse strains and protein antigens. Scand. J. Immunol. **29**:739–745.