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Stimulation of Secretory IgA and Secretory Component of Immunoglobulins in Small Intestine of Rats Treated with *Saccharomyces boulardii*

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Saccharomyces boulardii (S.b.) *is largely used in Western European countries for the treatment of acute infectious enteritis and antibiotic-induced gastrointestinal disorders. To study the mechanisms of the protective effect of* S.b. *against enteral pathogen infection, we assessed the response of the intestinal secretion of secretory IgA (s-IgA) and of the secretory component of immunoglobulins (SC) to oral administration of high doses (0.5 mg/g body weight, three times per day) of* S.b. *cells in growing rats.* S.b. *cells (biological activity: 2.8 × 10^9 viable cells/100 mg) were administered daily by gastric intubation to weanling rats from day 14 until day 22 postpartum. Control groups received either 0.9% saline or ovalbumin following the same schedule. Expressed per milligram of cell protein, SC content was significantly increased in crypt cells isolated from the jejunum (48.5% vs saline controls, $P < 0.05$) as it was in the duodenal fluid (62.8% vs saline controls, $P < 0.01$) of rats treated with* S.b. *Oral treatment with* S.b. *had no effect on the secretion of SC by the liver. In the duodenal fluid of rats treated with* S.b. *cells, the mean concentration of s-IgA was increased by 56.9% ($P < 0.01$) over the concentration of s-IgA measured in saline controls. Compared to control rats treated from day 14 until day 22 postpartum with an antigenic load of ovalbumin equivalent to the total protein load provided by Sb cells (0.05 mg protein/g body weight, three times per day),* S.b.*-treated rats also exhibited a significantly higher intestinal concentration of SC (69% in villus cells, $P < 0.025$ and 80% in crypt cells, $P < 0.01$). These changes in intestinal SC and s-IgA concentration appeared not to be due to an increase in enterocyte turnover rate, since the mucosal mass parameters and the incorporation rate of [^{3}H]thymidine into DNA measured in the jejunum, ileum, and colon remained unchanged in* S.b.*-treated rats. Our findings suggest that one of the mechanisms by which* S.b. *exerts its immunoprotective effect in the gastrointestinal tract is a stimulation of the intestinal secretion of s-IgA and of the secretory component of immunoglobulins.*

KEY WORDS: *Saccharomyces boulardii;* secretory component; secretory IgA; intestinal mucosal mass; growing rat.

Saccharomyces boulardii (*S.b.*) is a thermophilic, nontoxic yeast presently used in many countries for the treatment of acute infectious gastroenteritis and antibiotic-induced gastrointestinal disturbances. Clinical trials (1, 2) and experimental studies (3, 5) have demonstrated that oral treatment with a lyophilized preparation of *S.b.* (Ultra-Levure, Bioco-

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dex, Montrouge, France) has beneficial effects in preventing the occurrence of complications linked to changes in the normal gut flora such as toxigenic bacterial overgrowth and experimental pseudomembranous colitis. However, the physiological impact of the yeast on the gastrointestinal tract of mammals remains largely unknown. In a recent study (6), we showed that oral treatment of human volunteers and rats with *S.b.* is associated with a marked increase in the activity of brush border disaccharidases without morphological alteration of the intestinal mucosa. The exact mechanism of enzyme stimulation by the yeast is not elucidated, but polysaccharides of the external cell wall could play a role since the increase in disaccharidase activity was observed after treatment with either viable or killed *S.b.* cells. It also remains to be established whether treatment with *S.b.* cells may influence the expression of other glycoproteins synthesized by the intestinal mucosa, such as the secretory component of immunoglobulins and, in turn, the intestinal concentration of secretory IgA.

The objective of the present study is to further characterize the response of the intestinal mucosa of growing rats to oral administration of *S.b.* cells, regarding their effects on the mucosal mass parameters and mucosal DNA synthesis rate and the intestinal secretion of secretory component of immunoglobulins (SC) and secretory IgA (s-IgA).

MATERIALS AND METHODS

Animal Studies. We have chosen the weanling period of the rat to perform our experiments because during this period the intestinal mucosa is still immature and the production of SC is very low compared to the adult period (8). Furthermore, it was necessary to avoid the suckling period because rat milk is an exogenous source of s-IgA. Therefore, litters (eight rats each) of 14-day-old Wistar rats, weighing about 30 g were used. All animals were kept in polystyrene cages in an air-conditioned room at 21 ± 1° C with a 12-hr light–dark cycle. Suckling rats remained with their mothers from the day of birth and had free access to the nipples. At the time of weaning (day 14), they were fed *ad libitum* a solid standard stock diet (N 103, UAR, Villemoisson-sur-Orge, France). Each litter was divided at random into experimental and control groups of rats. The experimental groups received either 0.9% sterile saline or ovalbumin in 0.9% saline. Ovalbumin was given at the dose of 0.05 mg/g body weight three times per day. This antigenic load of ovalbumin was equivalent to the daily protein load provided by *S.b.* cells (0.1 mg protein/mg *S.b.* cells). *S.b.* (Ultra-Levure) was prepared in a lyophilized form (100 mg/flask having a biological activity of 2.8×10^9 viable cells) by the manufacturer (Biocodex Laboratories). Each flask was identical in composition and stored at +4° C. A dose of 0.5 mg/g body weight of lyophilized *S.b.* (suspended in 0.5 ml of saline) was administered by gastric intubation to the experimental animals three times per day, from the age of 14 days up to the age of 22 days postpartum. The control groups were treated according to the same schedule as the experimental groups.

Preparation of Tissues. On the day of sacrifice (day 22 postpartum) at 0800 hr, the rats were killed rapidly by decapitation and the small intestine from the pylorus to the ileocecal valve was immediately excised. After rinsing it in 0.9% cold saline, the total length was measured as described (9) and divided into two equal segments. The proximal half was defined as the jejunum and the distal half as the ileum. The mucosa of each intestinal segment was scraped off between glass slides and weighed. Except for mucosal separation, the same procedure was applied to the colon. The liver was trimmed of fat and weighed. Tissues were thereafter wrapped in parafilm and frozen immediately in liquid nitrogen until use. Tissues, mucosa, and cells from each rat were separately sonicated (Branson Sonifier B12) and were prepared for the determination of protein, DNA, SC, and IgA.

Isolation of Enterocytes. The collection of epithelial cells from the villous tip to the crypt base was performed as previously described (8). We used the Weiser's procedure (9) modified by Raul et al (10). Briefly, rat jejunal segments (10 cm distant from the pylorus) were removed, everted, and submitted to successive incubations of 10 min at 37° C in phosphate-buffered saline (PBS, pH = 7.4) containing 1.5 mM EDTA and 0.5 mM dithiothreitol (no Ca^{2+} or Mg^{2+}) under agitation at 170 rpm in a waterbath shaker. By successive incubations, sequential fractions of isolated cells were obtained. The released cells were collected (8) and washed twice as described (8, 10). Villous and crypt cell fractions were pooled separately (villous cells: fractions 1–5, crypt cells: fractions 5–10) and stored at −20° C until use.

Quantitation of Secretory Component and of Secretory IgA. The concentrations of total IgA and of total SC (SC free and bound to immunoglobulins) were measured in rat duodenal fluid and jejunal villous and crypt cells by a sensitive double-antibody immunoradiometric assay (IRMA). The goat anti-IgA and anti-SC rat antisera were described earlier (11). The successive steps of the IRMA techniques have been reported previously in detail (12, 14). For SC measurement, a standard reference curve was made with purified bile s-IgA (15) at the following concentrations in 20% horse serum: 0, 12.5, 25, 50, 100, 200, 300, and 400 ng/ml (measured by optical density at 280 nm, using E_{280} 1%, 1 cm = 13.8). The IRMA measured all forms of SC, and results were calculated as equivalents of s-IgA concentration, which was the standard used. The limit of sensitivity of the assay (+4 SD above radioactivity of the zero standard) was 4 ng/ml.

For measurement of total IgA, the standard curve was made with purified rat IgA (11) in 10% goat serum at the following concentrations: 0, 3.9, 7.8, 15.6, 31.2, 62.5, 125, and 250 ng/ml. The final IgA and SC amounts in duodenal fluid and jejunal and crypt cells were expressed in nanograms per milligram of protein.

Biochemical Determinations. For the determination of DNA synthesis rates, 2 μCi/g body weight of [^{3}H]thymidine (specific activity 23 Ci/mM, Amersham, England) was injected intraperitoneally to each animal. The injections were all given at the same hour in the morning to circumvent circadian periodicity in DNA synthesis. The animals were sacrificed 2 hr after the injection, and tissue samples (100 mg) of the liver, colon, and duodenojejunal and ileal mucosa were homogenized in 8 ml of 0.25 N cold perchloric acid as reported (14) and assayed for DNA by the diphenylamine method of Burton (16) as modified by Giles and Meyers (17). Polymerized calf thymus DNA (Sigma) was used as standard. The amount of [^{3}H]thymidine incorporated into DNA was determined by counting 0.5 ml of the DNA extract in a Beckman scintillation system using Aqualuma as vehicle. DNA extracts were counted in duplicate. Protein contents in mucosa and in tissues were measured by the Lowry method (18).

Calculations and Statistics. All results were expressed as mean ± SE. DNA was calculated in milligrams per gram tissue and per centimeter of intestinal length and DNA synthesis rates in cpm per milligram DNA and per centimeter of intestinal length. SC was expressed in nanograms per milligram protein and in micrograms per gram of mucosa. Statistical analysis was performed by using a two-way (treatments/litters) analysis of variance with cross classification.

RESULTS

Suckling and weanling rats treated orally with viable *S.b.* cells suspended in 0.9% saline and control animals receiving saline alone or ovalbumin appeared healthy. During the experimental period (eight days), the growth rate was similar in all the groups.

In Table 1 are compared the results of intestinal mucosal mass parameters and of the incorporation rate of [^{3}H]thymidine into DNA expressed per centimeter of intestinal length for the jejunum, ileum, and colon of *S.b.*-treated rats and saline controls. Compared to the saline controls, these parameters remained unchanged in *S.b.*-treated rats. Surprisingly, the determination of mucosal DNA content whether expressed per gram of mucosa or per centimeter of mucosal length was significantly higher in the jejunum (19–22%, $P < 0.05$ vs saline controls) and in the ileum (16%, $P < 0.05$ vs saline controls) of *S.b.*-treated rats. DNA concentration measured in the colon and in the liver was similar in both groups. These changes in mucosal DNA content of the jejunum and ileum in response to *S.b.* treatment were likely due to an exogenous supply of DNA provided by the yeast itself since subsequent *in vitro* experiments on

TABLE 1. MUCOSAL MASS PARAMETERS AND DNA SYNTHESIS (INCORPORATION OF [^{3}H]THYMIDINE IN JEJUNUM, ILEUM, AND COLON OF SALINE CONTROL RATS AND RATS TREATED WITH *S.b.*

	Saline controls	S.b.-*treated*
Intestinal length (cm)	72.3 ± 1.6	70.1 ± 1.5
Jejunum		
Mucosal weight (mg/cm)	39.7 ± 2.2	39.3 ± 1.4
DNA synthesis [^{3}H]thymidine incorp. (cpm/cm)	129.900 ± 19.329	100.356 ± 15.120
Ileum		
Mucosal weight (mg/cm)	17.3 ± 1.1	19.3 ± 1.2
DNA synthesis [^{3}H]thymidine incorp. (cpm/cm)	125.254 ± 19.235	100.285 ± 17.108
Colon		
Total weight (mg/cm)	79.7 ± 4.9	70.1 ± 2.0
DNA synthesis [^{3}H]thymidine incorp. (cpm/cm)	284.029 ± 35.141	278.099 ± 42.122

suspensions of viable *S.b.* cells revealed that the yeast contained 4.49 mg of DNA per gram of *S.b.*

The changes in SC content measured in isolated villous and crypt cells and in the duodenal fluid are depicted in Figure 1. Expressed per milligram of protein, SC concentration was significantly increased in isolated crypt cells (48.5% vs saline controls, $P < 0.05$) and in the duodenal fluid (62.8% vs saline controls, $P < 0.01$) of rats treated with *S.b.* In the villous cells, the increase in SC concentration was very slight and not significant (3.4% vs

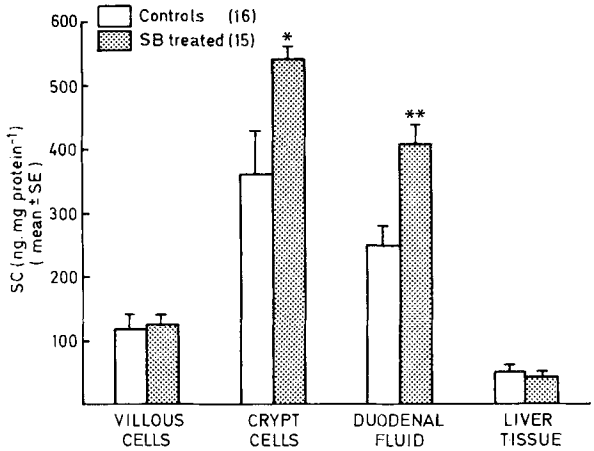

Fig 1. Changes in SC content measured in jejunal villous and crypt cells, in the duodenal fluid, and in the liver tissue of *S.b.*-treated rats ($N = 15$) and saline controls ($N = 16$). Values are means ± SEM. *$P < 0.05$; ** $P < 0.01$.

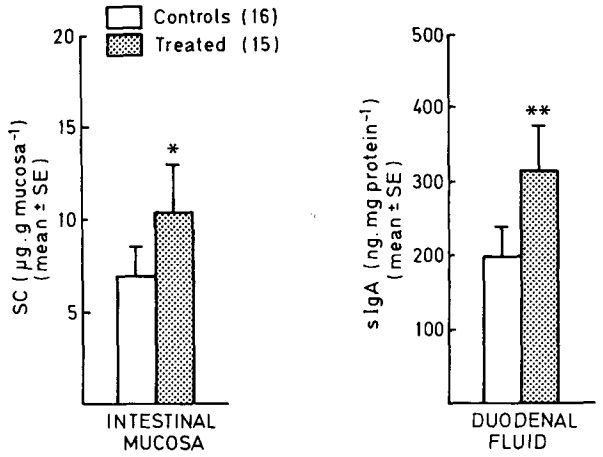

Fig 2. Changes in SC content measured in the intestinal mucosa of *S.b.*-treated rats ($N = 15$) and saline controls ($N = 16$) (left). Note increase (right) in IgA in the duodenal fluid of *S.b.*-treated rats ($N = 15$) compared to saline controls ($N = 16$). Values are means ± SEM. * $P < 0.05$; ** $P < 0.01$.

saline controls). Oral treatment of rats with *S.b.* had no effect on the concentration of SC in the liver.

Expressed per gram of intestinal mucosa (Figure 2), SC concentration was also significantly enhanced in the jejunum of treated rats (48.7% vs saline controls, $P < 0.05$). To document a possible effect of oral treatment with *S.b.* cells on the secretion of IgA by the intestinal cells, we measured s-IgA concentration by a specific immunoradiometric assay in the duodenal fluid. Figure 2 shows that the mean s-IgA concentration in the *S.b.*-treated group was increased by 56.9% ($P < 0.01$ vs saline controls) over the concentration measured in the saline control group.

Details of the results of SC concentration in villus and crypt cells of *S.b.*-treated rats and of ovalbumin controls are shown in Table 2. Compared to control rats treated from day 14 until day 22 postpartum with an antigenic load of ovalbumin equivalent to the total protein load provided by *S.b.* cells (0.1 mg protein/mg *S.b.* cells), *S.b.*-treated rats also exhibited a significantly higher production of SC (69% in villus cells, $P < 0.025$ and 80% in crypt cells, $P < 0.01$).

DISCUSSION

Saccharomyces boulardii is widely used for the prevention of diarrhea and other intestinal disturbances caused by the administration of antibiotics. This yeast can remain viable all along in the gastrointestinal tract (6, 19) and is nontoxic (6). A double-blind controlled clinical trial (1) has shown the efficacy of *S.b.* against antibiotic-associated diarrhea in humans. Recent studies (4, 5) also have confirmed its efficacy in animal experimental models of pseudomembranous colitis. When oral administration of *S.b.* was initiated in hamsters before clindamycin exposure, the authors (4) noted a significant decrease of clindamycin-induced mortality and of the cytotoxic effects of *Clostridium difficile* toxigenic strains on the colon and cecum. However, the mechanism(s) of the protective action of *S.b.* against diarrhea resulting from pathogenic strains is far from well understood. On the basis of preliminary experimental studies in animals, two possible mechanisms have been suggested: (1) a direct antagonism of *S.b.* cells against the overgrowth of various enteral pathogens (20, 21) and (2) an inhibition of either the cytotoxic effects (4, 22) or the production of bacterial toxins (23). In human volunteers (24), *S.b.* cells and their by-products such

TABLE 2. CONCENTRATION OF SECRETORY COMPONENT (SC) IN VILLUS CELLS AND CRYPT CELLS OF RATS TREATED WITH (*S.b.*) (DAYS 14–22 POSTPARTUM) AND CONTROLS TREATED WITH EQUIVALENT ANTIGENIC LOAD OF OVALBUMIN

	Controls (ovalbumin)	S.b.-*treated*	P*	*Increase (%)*
Intestinal cell fractions (*N*)	16†	16		
Animals treated (*N*)	4	4		
Villus cell fractions	8	8		
SC (ng/mg protein)	25.0 ± 3.4‡	42.4 ± 6.4	<0.025	69
Protein (mg/ml)	4.36 ± 0.1	4.61 ± 0.1	NS	
Crypt cell fractions	8	8		
SC (ng/mg protein)	263.0 ± 55.3	473.7 ± 52.1	<0.01	80
Protein (mg/ml)	3.54 ± 0.1	3.44 ± 0.08	NS	

†Number of villus and crypt cell fractions derived from four animals. NS = not significant.
*Probability versus controls treated with ovalbumin.
‡Each value represents mean ± SEM.

as glucans potentiate host resistance by activating the reticuloendothelial system and the complement system (alternate pathway). *In vitro*, *S.b.* is able to activate the complement directly and to fix C3b, its phagocytosis by mononuclear cells being complement-dependent (24). However, until now, the effect of *S.b.* cells on the immune defenses of the host intestinal tract has not yet been studied.

The main finding of the present study is that the oral administration of *S.b.* cells to growing animals significantly increased SC and s-IgA concentration in the small intestine even at time of incomplete maturity of its immune system. In external secretions s-IgA represents the main humoral component of the immune system. By binding to external antigens before they enter the body, s-IgA appears at the first line of immune defenses against external pathogens (25, 26). The significant increase of s-IgA in duodenal fluid of *S.b.*-treated rats was associated with a parallel increase in the cellular content of SC.

Like the disaccharidases, SC is a surface membrane glycoprotein synthesized by the intestinal crypt cell (8, 27) and acts as a receptor of p-IgA at the basolateral membrane of the cell (28). The receptor–ligand complex formed (p-IgA–SC) is internalized within the epithelial cell, actively transported to the apical membrane, and released into the intestinal fluid (29–31). During this receptor-mediated endocytotic transport, SC is not recycled by the cell. In the intestinal fluid, s-IgA antibodies bind to bacterial and viral antigens, reducing the uptake of antigens across the epithelium (32). They also impair the attachment of microorganisms to epithelial cells and prevent their proliferation (33).

Although we did not assess the specificity of the s-Iga antibodies against *S.b.* antigens, convincing evidence indicates that the s-IgA antibodies measured were almost exclusively natural antibodies. Indeed, in our study protocol, a last dose of lyophilized *S.b.* was administered to the rats 2 hr before sacrifice and collection of duodenal fluid. This allowed *S.b.* antigens to complex in the gut lumen specific anti-Sb antibodies, which usually do not exceed 5–10% of the total amount of s-IgA secreted. The determination of the final s-IgA concentration was performed in the supernatant after precipitation of the immune complexes formed by centrifugation. In normal rats, natural s-IgA antibodies detected in bile, intestinal fluid, and gut-associated lymphoid tissues have been found to be directed against a large variety of antigens, including the resident microbial flora as well as *Staphylococcus aureus*, *Vibrio cholerae*, *Lactobacillus*, and *Salmonella* antigens (34–36). Thus, if the present findings could be extrapolated to man, they suggest that *S.b.* cells exert an immunoprotective effect against pathogen infection of the gastrointestinal tract at least in part by the stimulation of the intestinal production of natural s-IgA. The exact mechanism if immunoglobulin stimulation remains unknown. Since bacterial polysaccharides are more potent immunogens that are proteins, glucan (β-1, 3-polyglucopyranose) and mannans of the yeast external capside could play an immunogenic role.

Supporting evidence is provided by the fact that *S.b.* cells exert their effect on brush border membrane glycoproteins (disaccharidases) whether they are viable or killed (6). The possibility that the increase in s-IgA and SC concentration could be the result of an increase in enterocyte turnover rate has to be considered but appears unlikely since mucosal DNA synthesis measured by the incorporation rate of [^{3}H]thymidine into DNA was similar in *S.b.*-treated rats and controls. Alternately, the changes in s-IgA and in SC concentration cannot be accounted for by a retention of mature enterocytes along the villi because SC concentration was significantly enhanced in isolated immature crypt cells, which are the main site of s-IgA production (8, 27). Experiments are in progress to characterize further the mechanisms of the protective effect of *S.b.* cells in man and animals.

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