The Journal of International Medical Research
2007; 35: 201 – 212

Effect of *Saccharomyces boulardii* in Children with Acute Gastroenteritis and Its Relationship to the Immune Response

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We evaluated the effect of *Saccharomyces boulardii* administration in otherwise healthy children aged between 6 months and 10 years who were admitted for acute diarrhoea (15 males, 12 females). The patients were randomized into two groups: group 1 (*n* = 16) received 250 mg *S. boulardii* dissolved in 5 ml of water orally twice daily for 7 days and group 2 (*n* = 11) received placebo. Clinical and laboratory assessments were performed on admission and on day 7 of follow-up. Both groups experienced reduced daily stool frequency, the decrease being significantly greater in group 1 on days 3 and 4 compared with group 2. Group 1 demonstrated significant increases in serum immunoglobulin A and decreases in C-reactive protein levels on day 7. The percentage of CD8 lymphocytes on day 7 was significantly higher in group 1 than group 2. This study confirmed the efficacy of *S. boulardii* in paediatric acute gastroenteritis and the findings suggest that *S. boulardii* treatment enhances the immune response.

KEY WORDS: Probiotics; *Saccharomyces boulardii*; Gastroenteritis; Children; Breast-feeding; Immune response; Paediatrics

Introduction

Diarrhoea is defined as frequent and watery stools due to impaired fluid and electrolyte absorption in the intestinal lumen, and it is usually caused by pathogenic micro-organisms.[1] It is a serious health issue with a high mortality rate in developing countries. In Turkey, according to data from the National Institute of Statistics, diarrhoea is the sixth most common cause of child mortality.[2]

The aetiology of acute diarrhoea in children includes infections of the gastrointestinal tract (i.e. bacteria, viruses, protozoa), intoxications, systemic infections, malabsorption disorders, nutritional deficiency and allergy or intolerance to food or drugs (i.e. antibiotics, laxatives).[3] The negative effects of these conditions on the protective and/or antagonistic role of intestinal flora on pathogenic micro-organisms results in the development of diarrhoea. The intestinal flora of infants fed with breast milk, which is known to contain prebiotics, consists mainly of *Bifidobacterium,* whereas anaerobic bacteria such as *Bacteroides* and *Clostridium* are the main species found in the intestinal flora of babies

fed with milk formula, which does not contain probiotics and prebiotics.[4]

In experimental studies, it has been established that intestinal flora bacteria adhere to the intestinal epithelium in competition with pathogenic micro-organisms and act in concert with local immune factors to prevent pathogenic invasion of the mucosa.[5]

Probiotics are a particular group of micro-organisms, usually bacterial and fungal species, which are defined as 'live micro-organisms which, when administered in adequate amounts, confer a beneficial health effect on the host'.[6] Recently, many investigations have focused on the therapeutic effect of probiotics on intestinal infections. *Lactobacillus* strains such as *Lactobacillus* GG, *L. reuteri*, *L. rhamnosus*, yoghurt, brewer's yeast and *Saccharomyces boulardii* have demonstrated beneficial effects on the course of acute diarrhoea in children.[7 – 13] Several studies and meta-analyses have confirmed the efficacy of *S. boulardii* in antibiotic-associated diarrhoea and in the prevention of relapses of *Clostridium difficile* infections.[14 – 20]

This randomized, double-blind, placebo-controlled study evaluated the effect of *S. boulardii* administration on acute paediatric diarrhoea. The study also investigated the relationship between *S. boulardii* administration and changes in the immune response and the impact of breast-feeding.

Patients and methods

PATIENT POPULATION

The study was conducted prospectively between October 2004 and March 2005 in the Paediatric Department of Uludag University, Bursa, Turkey, and was approved by the Research Ethics Committee of the Faculty of Medicine, Uludag University. Written informed consent was obtained from the parents of each patient. The study included otherwise healthy children aged between 6 months and 10 years who were admitted to our paediatric clinic with acute diarrhoea. Patients with severe systemic infection or sepsis, those who had a chronic disease, those who had previously received antibiotics, anti-diarrhoeal or other drugs that influence intestinal motility, and those with previously diagnosed primary or secondary immune deficiency were excluded. Patients were randomly allocated to one of two treatment groups: group 1 received 250 mg *S. boulardii* dissolved in 5 ml of water orally twice daily for 7 days; and the control group (group 2) was given a placebo treatment that had identical characteristics and appearance. In addition, all patients were given oral rehydration therapy and a lactose-free diet.

CLINICAL AND LABORATORY ASSESSMENTS

Clinical and laboratory assessments were performed on all patients at baseline on admission and on day 7 of follow-up. For the clinical assessment, the need for hospitalization and the nutritional status of the patient were documented. In addition, mothers described the history and duration of breast-feeding. A physical examination of each patient was carried out on admission and on days 1, 2, 3 and 4 of therapy. The number and characteristics of the stools were recorded at the same time.

Laboratory tests included a complete blood count (CBC), C-reactive protein (CRP) levels, a blood smear, serum complement C3 and C4 levels, serum immunoglobulin (Ig) levels, lymphocyte subsets (LS) analysis, and serum cytokine (tumour necrosis factor-alpha [TNF-α] and transforming growth factor-beta [TGF-β]) levels. For cytokine measurements, blood

samples from the patients were centrifuged at 1500 ***g*** for 5 min, the serum was then separated and stored frozen at –20 °C until evaluation. Serum lymphocyte subsets were studied using flow cytometry (EPICS® X L-MCL™, Beckman Coulter, Inc., Foullerton, Calif., USA). The Ig levels and serum complement levels were measured by the nephelometric method (BN™ II, Dade Behring, Inc., Marburg, Germany) and cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA; Diaclone, Besançon, France). The nature of any intestinal infection was determined using stool microscopy, culture for intestinal pathogens, and a faecal *Clostridium difficile* toxin A test. The identification of erythrocytes and leukocytes in stool samples under microscopic examination suggested a bacterial infection. Stool samples were incubated under both aerobic and anaerobic conditions.

STATISTICAL ANALYSIS

Statistical analysis was performed with the SPSS® 13.0 software program (SPSS, Chicago, Illinois, USA). Parametric data were examined with the χ^2 test and Fisher's exact test. The Mann Whitney *U*-test was used for comparison between groups and Wilcoxon's signed rank test was used for the comparison within each group. A *P*-value < 0.05 were considered to be statistically significant.

Results

A total of 27 children were enrolled in the study (15 males, 12 females). Group 1 included 16 patients (9 males, 7 females) and group 2 included 11 patients (6 males, 5 females). The mean ages (± SD) of the two treatment groups were comparable (Table 1).

TABLE 1:
Baseline clinical characteristics of the children (*n* = 27) with acute diarrhoea enrolled in the study

	Group 1 (*n* = 16)	Group 2 (*n* = 11)	*P*-value[a]
Age (months) (mean ± SD)	23.4 ± 6.6	17.6 ± 4.6	NS
Gender (male/female)	9/7	6/5	NS
Duration of diarrhoea prior to admission (days) (mean ± SD)	3.4 ± 1.3	2.3 ± 1.1	< 0.05
Hospitalization required *n* (%)	6 (37.5)	2 (18.2)	NS
Outpatient follow-up *n* (%)	4 (25.0)	7 (63.6)	NS
Malnutrition *n* (%)	2 (12.5)	1 (9.1)	NS
Metabolic disease *n* (%)	0 (0)	0 (0)	NA
Dehydration *n* (%)	7 (43.8)	2 (18.2)	NS
< 5% mild	3 (18.8)	0 (0)	NA
5 – 10% moderate	3 (18.8)	2 (18.2)	NS
> 10% severe	1 (6.3)	0 (0)	NA
Fever (> 38 °C) *n* (%)	11 (68.8)	7 (63.6)	NS
Vomiting *n* (%)	8 (50.0)	6 (54.5)	NS

[a]χ^2 and Fisher's exact test.
NA, not available; NS, not significant.

Evaluation of the baseline characteristics of the two groups revealed that the duration of watery diarrhoea before admission was significantly longer in group 1 than group 2 ($P < 0.05$) (Table 1). Clinical symptoms such as fever and dehydration were resolved on the second day, with no significant difference between the two groups. Furthermore, the mean stool frequency during admission was similar in both groups (Table 2). During follow-up, daily stool frequency significantly decreased in both groups compared with baseline ($P < 0.001$). A statistical difference that favoured group 1 was observed on days 3 and 4 ($P < 0.05$) (Table 2, Fig. 1). No adverse reaction related to *S. boulardii* therapy was observed during the study.

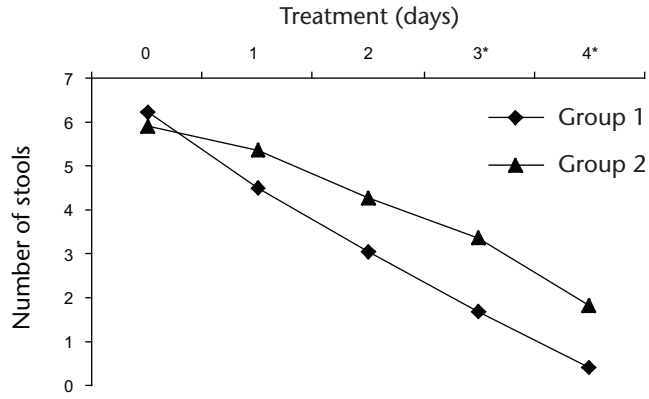

FIGURE 1: Mean number of stools produced during each day of treatment in children with acute diarrhoea treated with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily for 7 days (group 1, $n = 16$) or placebo (group 2, $n = 11$). *$P < 0.05$ between the two groups (Mann-Whitney *U*-test) at days 3 and 4

TABLE 2:
Mean number of stools produced during each day of treatment in children ($n = 27$) with acute diarrhoea treated with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily for 7 days (group 1) or placebo (group 2)

	Group 1 ($n = 16$)		Group 2 ($n = 11$)		*P*-value[b]
	Number of stools	*P*-value[a]	Number of stools	*P*-value[a]	
Baseline	6.23 ± 0.53		5.92 ± 0.44		NS
Day 1	4.50 ± 0.36	< 0.001	5.36 ± 0.38	< 0.001	NS
Day 2	3.06 ± 0.33	< 0.001	4.27 ± 0.38	< 0.001	NS
Day 3	1.68 ± 0.23	< 0.001	3.36 ± 0.38	< 0.001	< 0.05
Day 4	0.43 ± 0.22	< 0.001	1.81 ± 0.42	< 0.001	< 0.05

Values are mean ± SD.
NS, not significant.
[a]*P*-value compared with the baseline value on admission (Wilcoxon's signed rank test).
[b]*P*-value between the two groups (Mann-Whitney *U*-test).

Laboratory tests were performed in both groups twice, at baseline on admission and on day 7 of follow-up. The baseline results were similar in both groups (Table 3). Although leukocytes and erythrocytes were observed in the stool samples of seven patients in each group following microscopic examination, the difference in the proportion of patients between the two groups was not statistically significant. *C. difficile* toxin A was found in one patient in group 1 together with a Gram-positive anaerobic micro-organism in the stool culture. No other bacterial growth was found in other stool cultures (Table 3).

Serum IgG and IgM levels on day 7 did not increase significantly in either group compared with baseline levels (Table 4). In group 1, the increase in serum IgA and the decrease in CRP levels on day 7 were significantly different compared with baseline ($P < 0.05$). Similar changes were observed in group 2, but they were not significant. There was no difference between groups 1 and 2 for serum CRP and IgA levels on day 7.

In group 1, the proportion of CD3 and CD4 lymphocytes and natural killer (NK) cells remained unchanged during *S. boulardii* treatment, whereas the percentage of CD8 cells was significantly higher on day 7 compared with baseline ($P < 0.05$) (Table 5, Fig. 2). There was also a statistically significant increase in the proportion of CD8 cells in group 1 compared with group 2 on day 7 ($P < 0.05$). The CD4/CD8 ratio decreased significantly in group 1 on day 7 compared with baseline ($P < 0.05$) (Table 5, Fig. 3).

In both groups, there was a non-significant increase in serum TGF-β and a non-significant decrease in TNF-α levels. The evaluation of serum complement levels showed a significant decrease in C4 in both groups on day 7 ($P < 0.05$). However, the decrease in serum C3 levels was negligible in both groups.

Both groups contained six infants less than 2 years of age with a history of breast-feeding for more than 4 months; there was no significant difference between the groups

TABLE 3:
Baseline laboratory test results for children ($n = 27$) with acute diarrhoea on admission to the paediatric clinic randomized to be treated with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily for 7 days (group 1) or placebo (group 2)

	Group 1 ($n = 16$)	Group 2 ($n = 11$)	*P*-value[a]
Leukocytosis	12 (75.0)	6 (54.5)	NS
CRP positivity	11 (68.8)	6 (54.5)	NS
Mucus in the stool sample	14 (87.5)	8 (72.7)	NS
Macroscopic blood in the stool sample	1 (6.25)	2 (18.2)	NS
Microscopic pathology in the stool sample (erythrocytes, leukocytes)	7 (43.8)	7 (63.6)	NS
Pathogen positivity in stool culture	1 (6.25)	0 (0)	NA
Clostridium difficile toxin A positivity	1 (6.25)	0 (0)	NA

Values are number (%).
[a]χ^2 and Fisher's exact test.
CRP, C-reactive protein; NA, not available; NS, not significant.

TABLE 4:
Comparison of serological parameters between children (*n* = 27) with acute diarrhoea on admission to the paediatric clinic (baseline) and after 7 days of treatment with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily (group 1) or placebo (group 2)

Serological parameters	Group 1 (*n* = 16)			Group 2 (*n* = 11)			
	Baseline	Day 7	*P*-value[a]	Baseline	Day 7	*P*-value[a]	*P*-value[b]
CRP (mg/dl)	2.40 ± 0.78	0.30 ± 0.21	< 0.05	1.20 ± 0.50	0.35 ± 0.20	NS	NS
IgA (mg/dl)	40.0 ± 5.16	55.25 ± 7.0	< 0.05	31.14 ± 4.09	35.80 ± 6.18	NS	NS
IgG (mg/dl)	804.87 ± 83.12	908.06 ± 78.39	NS	671.54 ± 66.55	719.45 ± 72.2	NS	NS
IgM (mg/dl)	117.8 ± 14.64	134.01 ± 9.5	NS	87.18 ± 13.18	118.7 ± 21.88	NS	NS

Values are mean ± SD.
CRP, C-reactive protein; Ig, immunoglobulin; NS, not significant.
[a]Within-group comparison of day 7 and baseline (Wilcoxon's signed rank test).
[b]Between-group comparison of day 7 and baseline (Mann-Whitney *U*-test).

TABLE 5:
Comparison of immunological parameters between children (n = 27) with acute diarrhoea on admission to the paediatric clinic (baseline) and after 7 days of treatment with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily (group 1) or placebo (group 2)

Immunological parameters	Group 1 (n = 16)			Group 2 (n = 11)			
	Baseline	Day 7	*P*-value[a]	Baseline	Day 7	*P*-value[a]	*P*-value[b]
CD3 (%)	61.31 ± 3.14	65.23 ± 3.36	NS	62.01 ± 3.47	66.60 ± 3.77	NS	NS
CD4 (%)	37.03 ± 2.55	38.43 ± 2.22	NS	37.87 ± 3.77	42.20 ± 2.75	NS	NS
CD8 (%)	18.57 ± 1.65	24.41 ± 1.56	< 0.05	20.65 ± 2.77	22.18 ± 2.61	NS	< 0.05
CD4/CD8 ratio	2.18 ± 0.20	1.72 ± 0.14	< 0.05	2.17 ± 0.36	2.14 ± 0.24	NS	NS
NK cells (%)	14.01 ± 1.60	15.17 ± 1.78	NS	12.32 ± 2.23	9.99 ± 1.38	NS	NS
TNF-α (ng/ml)	7.06 ± 3.29	3.78 ± 2.62	NS	5.07 ± 2.34	4.01 ± 1.98	NS	NS
TGF-β (pg/ml)	4.03 ± 0.81	4.42 ± 0.72	NS	3.55 ± 0.64	3.60 ± 0.86	NS	NS
C3 (mg/dl)	125.96 ± 7.65	112.20 ± 6.45	NS	136.06 ± 12.4	120.0 ± 12.09	NS	NS
C4 (mg/dl)	24.99 ± 2.83	17.16 ± 2.13	< 0.05	26.12 ± 2.96	20.14 ± 2.41	< 0.05	NS

Values are mean ± SD.
NK, natural killer; TNF-α, tumour necrosis factor-alpha; TGF-β, transforming growth factor-beta; NS, not significant.
[a]Within-group comparison of day 7 and baseline (Wilcoxon's signed rank test).
[b]Between-group comparison of day 7 and baseline (Mann-Whitney *U*-test).

TABLE 6:
Comparison of the proportion of CD4 and CD8 lymphocytes and the CD4/CD8 ratios between infants who were breast-fed for more than 4 months in both treatment groups (250 mg *S. boulardii* dissolved in 5 ml of water orally twice daily for 7 days [group 1] or placebo [group 2])

Lymphocytes	Group 1 (n = 6)		Group 2 (n = 6)		P-value[a]
	Baseline	Day 7	Baseline	Day 7	
CD4 (%)	22.85 ± 11.92	15.30 ± 9.02	22.40 ± 5.43	17.16 ± 6.60	0.041
CD8 (%)	13.91 ± 2.53	21.93 ± 6.29	19.36 ± 4.38	21.93 ± 6.29	0.015
CD4/CD8 ratio	2.73 ± 0.76	1.83 ± 0.53	1.72 ± 0.65	2.09 ± 0.64	0.015

Values are mean ± SD.
[a]Between-group comparison of day 7 and baseline (Mann-Whitney *U*-test).

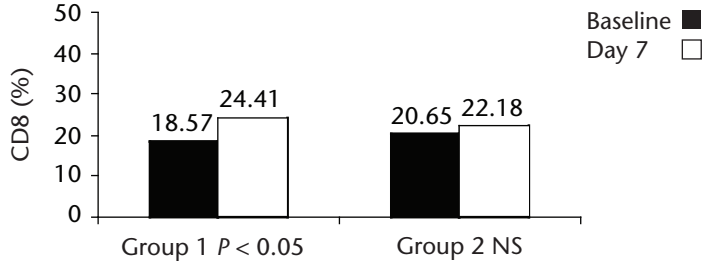

FIGURE 2: Changes in serum CD8 lymphocyte levels (%) from baseline to day 7 in children with acute diarrhoea treated with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily for 7 days (group 1, n = 16) or placebo (group 2, n = 11) (Wilcoxon's signed rank test: group 1, $P < 0.05$; group 2, NS, not significant)

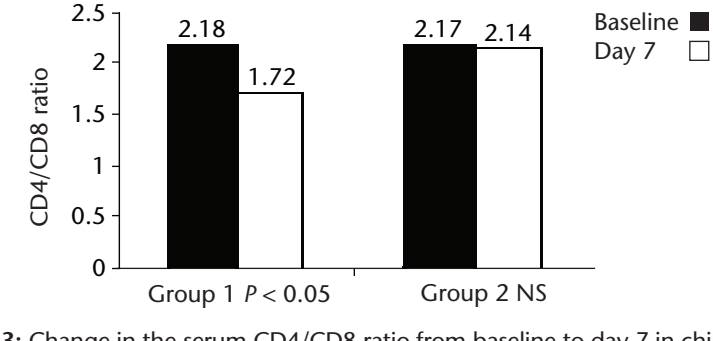

FIGURE 3: Change in the serum CD4/CD8 ratio from baseline to day 7 in children with acute diarrhoea treated with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily for 7 days (group 1, n = 16) or placebo (group 2, n = 11) (Wilcoxon's signed rank test: group 1, $P < 0.05$; group 2, NS, not significant)

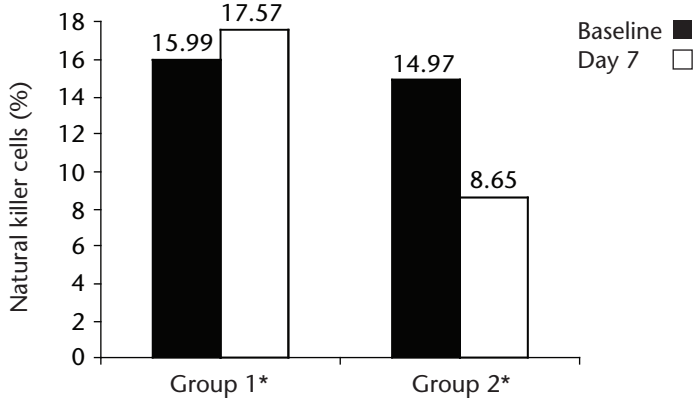

FIGURE 4: Change in the percentage of serum natural killer cells from baseline to day 7 in children with acute diarrhoea breast-fed for more than 4 months treated with either 250 mg *Saccharomyces boulardii* dissolved in 5 ml of water orally twice daily for 7 days (group 1, $n = 6$) or placebo (group 2, $n = 6$). *$P < 0.05$ between the two groups at day 7 (Mann-Whitney *U*-test)

in terms of the proportion of breast-fed infants. Infants who were breast-fed for more than 4 months and treated with *S. boulardii* demonstrated significantly greater changes in their lymphocyte proportions (decreased CD4 and increased CD8) and a lower CD4/CD8 ratio compared with placebo-treated breast-fed infants ($P = 0.041$, $P = 0.015$ and $P = 0.015$, respectively; Mann-Whitney *U*-test) (Table 6). Moreover, babies who received breast milk for more than 4 months demonstrated a significant increase in the percentage of NK cells on day 7 of *S. Boulardii* therapy compared with breast-fed infants given placebo who had a decreased proportion of NK cells ($r = 0.719$, $P < 0.05$; Mann-Whitney *U*-test (Fig. 4).

Discussion

In recent years, clinical investigations have focused on understanding the efficacy of *S. boulardii* in the treatment of acute gastroenteritis in both adult and paediatric populations and many studies have addressed the action of probiotics *in vivo*.[9, 12, 13]

A study by Chapoy *et al.*[10] demonstrated an improvement in stool frequency in patients with acute gastroenteritis who were given oral rehydration therapy and *S. boulardii* in comparison with patients given oral rehydration therapy alone. Other studies have also confirmed the efficacy of *S. boulardii* in the clinical improvement of acute gastroenteritis.[11 – 13] The effect of *S. boulardii* appears to be comparable with other previously described probiotics such as *Lactobacillus* and *Bifidobacterium* strains.[10] In the present study, *S. boulardii* did not appear to have any therapeutic effect on fever and vomiting but it did contribute to a general improvement in well-being and stool consistency. In the study group, there was a significant decrease in the stool frequency after day 3 of treatment, whereas in recent studies, this effect was observed by day 3.[11 – 13] Studies on a larger patient population might clarify this point.

Qamar *et al.*[21] demonstrated that *S. boulardii* stimulates intestinal IgA secretion against *C. difficile* toxin A in mice. In our study,

C. difficile toxin A was detected in one patient. Although the clinical course did not resemble a severe pseudomembraneous colitis, symptoms improved with *S. boulardii* and the patient was asymptomatic by day 5 of treatment, which was earlier than expected. This might indicate an antigen-specific stimulation of the intestinal mucosal immunity. In the present study, intestinal IgA was not evaluated but serum IgA levels increased both inversely and proportionally to serum CRP levels ($P < 0.05$ for both). The decrease in CRP levels during clinical recovery may be related to the synergistic effect of *S. boulardii* on the intestinal flora.

In a study by Jahn *et al.*,[22] an increase in CD25-expressing T lymphocytes was detected in healthy individuals after *S. boulardii* treatment for 3 weeks. In contrast, administration of *Lactobacillus* GG to children with a food allergy resulted in a decrease in CD4 lymphocytes and an increase in serum TGF-β levels, suggesting a positive effect in the treatment of atopic diseases.[23,24] In the present study, the percentage of CD4 lymphocytes did not differ between the two groups; this may be related to the timing of the evaluation of lymphocyte subset changes.

Clinical studies investigating the impact of probiotics on inflammatory responses demonstrated that *Lactobacillus* spp. stimulates T helper 1 (T_h1) lymphocytes by enhancing the secretion of interleukin (IL)-12 and IL-18 from myeloid dendritic cells; similarly, the stimulation of T_h1 cells by another *Bifidobacterium* probiotic was shown to increase IL-10 and decrease interferon-gamma secretion by dendritic cells.[25,26]

The evaluation of lymphocyte subsets in the present study demonstrated a significant increase in CD8 cells in group 1 on day 7 compared with baseline cell counts; this increase was significantly higher than that observed for group 2 on day 7 ($P < 0.05$). This may indicate late pro-inflammatory activity of *S. boulardii,* resulting in cytokine release and CD8 cell activation, which would help to limit the infection. Consistent with our findings, other studies have reported a slight elevation in TGF-β and decreasing levels of TNF-α late in the course of infection.[27–29] The negative feedback of CD8-induced pro-inflammatory cytokines on TNF-α levels is still controversial but could be established by the monitoring of cytokine levels during the inflammatory period.

Rodrigues *et al.*[30] evaluated the effect of *S. boulardii* on the clearance of *Escherichia coli* B41 in mice. They observed increased levels of TNF-α and IL-12, 45 and 90 min after intravenous administration of *E. coli*. The increased release of cytokines is related to the immunomodulatory effect of *S. boulardii,* as mentioned above. Alternatively, the variation in cytokine levels observed during infections might be associated with the time of sampling and individual patient variations.

Caetano *et al.*[31] demonstrated that *in vitro* complement activation, granulocyte and monocyte migration were all triggered by *S. boulardii*. In our study, the lowered levels of complement C4 in group 1 might be explained by Caetano's findings,[31] although lowered levels of C4 were also found in the control group.

The importance of breast-feeding on native immunity was demonstrated by Tarcan *et al.*[32] in a study comparing the percentage of NK cells between breast-fed and formula-fed infants; NK cell counts were significantly higher in breast-fed infants.[32] Similarly, Hawkes *et al.*[33] demonstrated an increased CD8 lymphocyte count and decreased CD4/CD8 ratio in breast-fed infants. In concordance with these studies, we demonstrated that different diets (milk

formula or breast-feeding) might result in different immune profiles, confirming the synergism between breast milk and probiotics against infections and their combined effect on the consistency of intestinal flora during the early infantile period.

The safety of probiotics during pregnancy was discussed in a study that demonstrated the beneficial effects of probiotics on the development of intestinal flora.[34] According to the results of this present study, breast milk, in synergy with probiotics, seems to have a long-lasting effect, even after the cessation of breast-feeding.

In conclusion, this study confirmed the efficacy of *S. boulardii* on acute gastroenteritis in children. The significant changes in the serum levels of IgA, CD8 and CRP may be related to enhancement of the immune response by *S. boulardii* as suggested in recent studies. Further research on cytokine levels in stools and the effects of breast-feeding are required.

Acknowledgement

We thank Sanofi-Aventis (Paris, France) for providing laboratory materials and a commercial preparation of *S. boulardii* (Reflor®, Sanofi-Synthelabo, Istanbul, Turkey) for this study.

Conflicts of interest

No conflicts of interest were declared in relation to this article.

• Received for publication 10 October 2006 • Accepted subject to revision 27 October 2006
• Revised accepted 16 January 2007

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