Probiotic stool secretory immunoglobulin A modulation in children with gastroenteritis: a randomized clinical trial

Stephen B Freedman,[1,2] Rachael Horne,[3] Kathene Johnson-Henry,[3] Jianling Xie,[4] Sarah Williamson-Urquhart,[4] Linda Chui,[5] Xiao-Li Pang,[5] Bonita Lee,[6] Suzanne Schuh,[7] Yaron Finkelstein,[8] Serge Gouin,[9] Ken J Farion,[10] Naveen Poonai,[11] Katrina Hurley,[12] David Schnadower,[13] and Philip M Sherman,[14] on behalf of the Pediatric Emergency Research Canada Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment (PROGUT) Trial Group

[1]Sections of Pediatric Emergency Medicine and Gastroenterology, Department of Pediatrics, Alberta Children's Hospital, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Canada; [2]Department of Emergency Medicine, Cumming School of Medicine, University of Calgary, Calgary, Canada; [3]Cell Biology Program, Research Institute, Hospital for Sick Children, Toronto, Canada; [4]Section of Pediatric Emergency Medicine, Department of Pediatrics, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, Canada; [5]Alberta Precision Laboratories - ProvLab, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada; [6]Department of Pediatrics, Faculty of Medicine & Dentistry, Women and Children's Health Research Institute, University of Alberta, Edmonton, Canada; [7]Division of Emergency Medicine, Department of Paediatrics, Hospital for Sick Children, Toronto, Canada; [8]Divisions of Emergency Medicine and Clinical Pharmacology and Toxicology, Research Institute, Hospital for Sick Children, University of Toronto, Toronto, Canada; [9]Departments of Pediatric Emergency Medicine & Pediatrics, CHU Sainte-Justine, Université de Montréal, Montreal, Canada; [10]Departments of Pediatrics and Emergency Medicine, and Pediatric Emergency Department, Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa, Canada; [11]Division of Pediatric Emergency Medicine, Departments of Pediatrics, Internal Medicine, Epidemiology & Biostatistics, Schulich School of Medicine and Dentistry, London, Canada; [12]Division of Paediatric Emergency Medicine, Dalhousie University, Halifax, Canada; [13]Division of Emergency Medicine, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, OH, USA; and [14]Division of Gastroenterology, Hepatology, and Nutrition, Department of Paediatrics, and the Cell Biology Program, Research Institute, Hospital for Sick Children, Toronto, Canada

ABSTRACT

Background: We previously conducted the Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment (PROGUT) study, which identified no improvements in children with acute gastroenteritis (AGE) administered a probiotic. However, the aforementioned study did not evaluate immunomodulatory benefits.

Objectives: The object of this study was to determine if stool secretory immunoglobulin A (sIgA) concentrations in children with AGE increase more among participants administered a *Lactobacillus rhamnosus/helveticus* probiotic compared with those administered placebo.

Methods: This *a priori* planned multicenter, randomized, double-blinded, placebo-controlled ancillary study enrolled children presenting for emergency care who received a 5-d probiotic or placebo course. Participants submitted stool specimens on days 0, 5, and 28. The primary endpoint was the change in stool sIgA concentrations on day 5 compared with baseline.

Results: A total of 133 (n = 66 probiotic, 67 placebo) of 886 PROGUT participants (15.0%) provided all 3 specimens. Median stool sIgA concentrations did not differ between the probiotic and placebo groups at any of the study time points: day 0 median (IQR): 1999 (768, 4071) compared with 2198 (702, 5278) (P = 0.27, Cohen's d = 0.17); day 5: 2505 (1111, 5310) compared with 3207 (982, 7080) (P = 0.19, Cohen's d = 0.16); and day 28: 1377 (697, 2248) compared with 1779 (660, 3977) (P = 0.27, Cohen's d = 0.19), respectively. When comparing measured sIgA concentrations between days 0 and 5, we found no treatment allocation effects [β: −0.24 (−0.65, 0.18); P = 0.26] or interaction between treatment and specimen collection day [β: −0.003 (−0.09, 0.09); P = 0.95]. Although stool sIgA decreased between day 5 and day 28 within both groups (P < 0.001), there were no differences between the probiotic and placebo groups in the median changes in sIgA concentrations when comparing day 0 to day 5 median (IQR) [500 (−1135, 2362) compared with 362 (−1122, 4256); P = 0.77, Cohen's d = 0.075] and day 5 to day 28 [−1035 (−3130, 499) compared with −1260 (−4437, 843); P = 0.70, Cohen's d = 0.067], respectively.

Conclusions: We found no effect of an *L. rhamnosus/helveticus* probiotic, relative to placebo, on stool IgA concentrations. This trial was registered at clinicaltrials.gov as NCT01853124. *Am J Clin Nutr* 2021;113:905–914.

Keywords: probiotic, immunoglobulin A, pediatric, gastroenteritis, clinical trial

Introduction

The secretion of immunoglobulin A (IgA) antibody is one of the primary defense mechanisms protecting against enteric infections in both human and animal models (1, 2). IgA provides

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protective effects by binding to microbial antigens, toxins, and food proteins, and it inhibits adherence and penetration of the intestinal epithelium (3–5). Animal studies report a substantial increase in intestinal commensal anaerobic bacteria in the absence of normal intestinal secretory IgA (sIgA) concentrations, with intestinal microbiota returning to baseline composition following normalization of sIgA production (6).

The use of probiotics is increasing rapidly despite questionable evidence of efficacy (7), particularly as it relates to pediatric acute gastroenteritis (AGE) (8). However, the interest in probiotics is founded on how they may contribute to immune homeostasis by altering the microbial balance, interacting with the host immune system (9), regulating inflammatory cytokines (10), and increasing sIgA production (11). In mouse models, probiotics protect against enteric infections by inducing intestinal IgA secretion (12, 13), which has been correlated with survival in mice (14). In healthy humans, probiotics increase stool sIgA concentrations across the age spectrum (15–18). However, in children with AGE, conflicting results have been reported (19–21), and no trials have correlated stool sIgA concentrations with clinical outcomes. The possibility that probiotics enhance the protective immune response in the host intestinal tract is a crucial topic requiring clarification (21).

In 2017, we completed the Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment (PROGUT) clinical trial (22), which randomized children with AGE to receive either a *Lactobacillus rhamnosus/helveticus* probiotic mixture or identical-appearing placebo twice daily for 5 d. Although this trial identified no significant benefits associated with administration of the *L. rhamnosus/helveticus* product (23), we sought to explore the effect of its administration on stool sIgA concentrations by determining if supplementation promotes sIgA secretion as reflected by higher sIgA concentrations in stool specimens relative to those administered a placebo. We also sought to determine if higher day 5 sIgA concentrations would promote pathogen clearance and hence result in less severe disease.

Methods

Study design

This was an *a priori* planned ancillary study designed within the investigator-initiated, multicenter, PROGUT trial (22). Children with acute diarrhea received a 5-d course of a combination *L. rhamnosus/helveticus* probiotic or placebo. Research ethics boards at each of the 6 participating Canadian, tertiary care, university-affiliated sites approved the trial. Caregivers provided written informed consent for their children to participate.

Eligibility criteria

Children aged 3–48 mo were eligible to participate in the PROGUT study if they presented for emergency department (ED) care, experienced ≥3 episodes of watery stools in a 24-h period, had diarrhea or vomiting for <72 h, and were diagnosed as having AGE. Children were excluded if they or a person living in their household had an indwelling vascular-access catheter or if they were immunocompromised, receiving immunosuppressive therapy, or had structural heart disease. Additional exclusion criteria were bilious vomiting, hematochezia, pancreatic dysfunction or insufficiency, a chronic gastrointestinal disorder (e.g., celiac disease, milk allergy), an allergy to soy, probiotic use during the preceding 14 d, and an inability to complete follow-up. Children who had undergone gastrointestinal or oral surgery within the preceding 7 d or previously participated in the trial were also excluded. For this ancillary study, participants submitted stool specimens for sIgA analysis on the day of enrollment (day 0), day 5 (i.e., last day of probiotic/placebo administration), and day 28 following enrollment.

Randomization and masking

Random-number–generating software, accessed through a Web-based randomization system (https://www.randomize.net), which used random block sizes of 4 and 6 and a 1:1 trial group assignment ratio stratified according to site, was used to sequentially assign children to the intervention or placebo. Knowledge of the assignment sequence was restricted to the research pharmacy at the coordinating center and Randomize.net until the databases were locked. Participants and their caregivers, trial and clinical staff, and specimen and data analysts were unaware of trial group assignments.

Illness severity

Gastroenteritis severity following randomization was quantified using the Modified Vesikari Scale (MVS) score, which ranges from 0 to 20, with higher scores indicating more severe disease (**Supplemental Table 1**) (24, 25). Scores of 0–8, 9–10, and ≥11 denote mild, moderate, and severe gastroenteritis episodes, respectively. Each participant's score was assigned based on clinical data collected during the follow-up period. Baseline symptoms that preceded the visit to the ED were not included in the outcome measure.

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Supplemental Tables 1–12 and Supplemental Methods are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to SBF (e-mail: Stephen.freedman@ahs.ca).

Abbreviations used: AGE, acute gastroenteritis; ED, emergency department; IgA, immunoglobulin A; MVS, Modified Vesikari Scale; sIgA, secretory immunoglobulin A.

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Primary and secondary outcomes

The following primary and secondary outcomes were neither altered during the course of this study nor conducted as post hoc analyses. The primary endpoint was the change in stool sIgA concentrations on day 5 compared with baseline (i.e., day 0) concentrations. Secondary endpoints included *1*) comparison of the change in stool sIgA concentrations on day 28 compared with day 5; *2*) correlation of day 0 stool sIgA concentrations with the baseline MVS score and of days 5 and 28 stool sIgA concentrations with the postrandomization, follow-up MVS scores; and *3*) correlation of days 0, 5, and 28 stool sIgA concentrations with identified pathogens in both group (i.e., virus and bacteria) and virus-specific agents (i.e., adenovirus, norovirus, and rotavirus).

Procedures

Rectal swabs, stool specimens, or both were obtained during the enrollment visit (26). If stool was not provided prior to ED discharge, the first stool sample produced following enrollment was collected at home. Additional samples were collected from all children at home on days 5 and 28 postrandomization. Stools collected at home were stored at room temperature for up to 12 h and then retrieved by a study-funded courier and transported to the laboratory on ice packs. Specimens were stored at −80°C until they were thawed for sIgA analyses. Processing was performed as previously described (27).

The investigational preparation employed was a lyophilized powder containing 4.0×10^9 CFUs of 2 bacterial strains—*L. rhamnosus* R0011 and *L. helveticus* R0052—in a 95:5 ratio. These strains have been studied (28–33) and demonstrated to consist of live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [i.e., a probiotic (34)]. Sachets containing placebo and probiotics were identical in appearance, smell, and weight. The contents of 1 sachet of probiotics or placebo, maintained at a temperature between 0°C and 25°C, were sprinkled into 30 mL of the child's preferred liquid twice daily. Five extra sachets were provided to enable repeat dosing if vomiting occurred within 15 min of administration. Probiotic and placebo sachets were provided in-kind by Lallemand Health Solutions, which performed quantitative bacterial culture of the investigational product on the completion of trial batch administration to confirm the quantity and shelf-life stability of the investigational product.

Research assistants blinded to patient allocation collected demographic and clinical data and completed trial interventions in the ED. Caregivers completed electronic or telephone follow-up surveys every 24 h until both vomiting and diarrhea had ceased for 24 h. All microbiological testing and sIgA quantification were performed blinded to clinical data and treatment allocation.

Sample size calculation

At the time of study planning, no prior study had assessed changes in sIgA stool concentrations after probiotic administration to children with AGE. The most relevant study to date evaluated sIgA in infants administered a milk formula containing a prebiotic (35). The authors of that study reported a between-group difference in sIgA concentrations (i.e., prebiotic compared with placebo) of ∼300 μg/g of stool (27). Therefore, we sought to detect a minimal difference in stool sIgA concentrations in the probiotic group relative to the placebo group of 300 μg/g with an SD of 500 μg/g. Sample size calculations, employing a power of 80% and a 2-sided type I error of 0.05, yielded a minimal sample size of 50 subjects who provided all 3 stool specimens in each group.

Enteropathogen identification

See **Supplemental Methods** for a detailed description.

Secretory IgA quantification

Quantification was performed at the Hospital for Sick Children, Toronto, Ontario, Canada. Prior to dilution, stool samples were weighed with extraction buffer and dilution factors calculated for each sample. A lower dilution factor was used for samples with input weights below the manufacturer's suggested limit to ensure data points would register in the linear range on a standard curve. sIgA stool concentrations were determined using the sIgA ELISA kit (36) (Eagle Biosiences). A 4-parameter logistic standard curve was generated, with samples run in duplicate. Samples were retested if the CV was >10% or absorbance reads at 450 nm against 620 nm were outside the dynamic range of standards provided. The lower limit of detection was 3.1 ng/mL.

Statistical analysis

All analyses were specified *a priori* (22). Per-protocol analysis was implemented for all patients who submitted specimens on days 0, 5, and 28. For the primary outcome, the change in stool sIgA concentrations (day 0 to day 5) was compared between groups using the Mann–Whitney *U* test. Effect size estimates for each comparison were calculated as described by Fritz et al. (37) and denoted as Cohen's *d*. Within treatment group day 0 and day 5 comparisons were assessed by Wilcoxon's signed-rank sum test. Factors such as age, sex, frequency of vomiting and diarrhea in the 24-h period before enrollment, recent antibiotic use, breastfeeding status, and the presence of enteropathogens (i.e., virus, bacteria, and codetection) may affect baseline sIgA concentrations. Therefore, secondary analysis of the primary outcome employed generalized linear regression mixed modeling using γ distribution and log link with random effect intercept for subjects adjusted for these *a priori* identified covariates. We also included the variable "day of stool collection," measured on a continuous scale, to evaluate the effect of time on sIgA concentrations across the 28-d study period, independent of probiotic treatment, as well as through the inclusion of an interaction term between day of stool collection and treatment assignment. Interaction between treatment assignment and time of stool collection (i.e., day 0 or day 5) was tested in the model.

The secondary outcome of change in stool sIgA concentrations between day 5 and day 28 was analyzed as described for the primary outcome. The association between day 0 stool sIgA concentrations and baseline MVS score was analyzed using a generalized linear model with a log-link function and γ distribution adjusted for age, sex, treatment assignment, recent antibiotic use, breastfeeding status, and enteropathogen status. The same analytic approach was employed to correlate the day 5

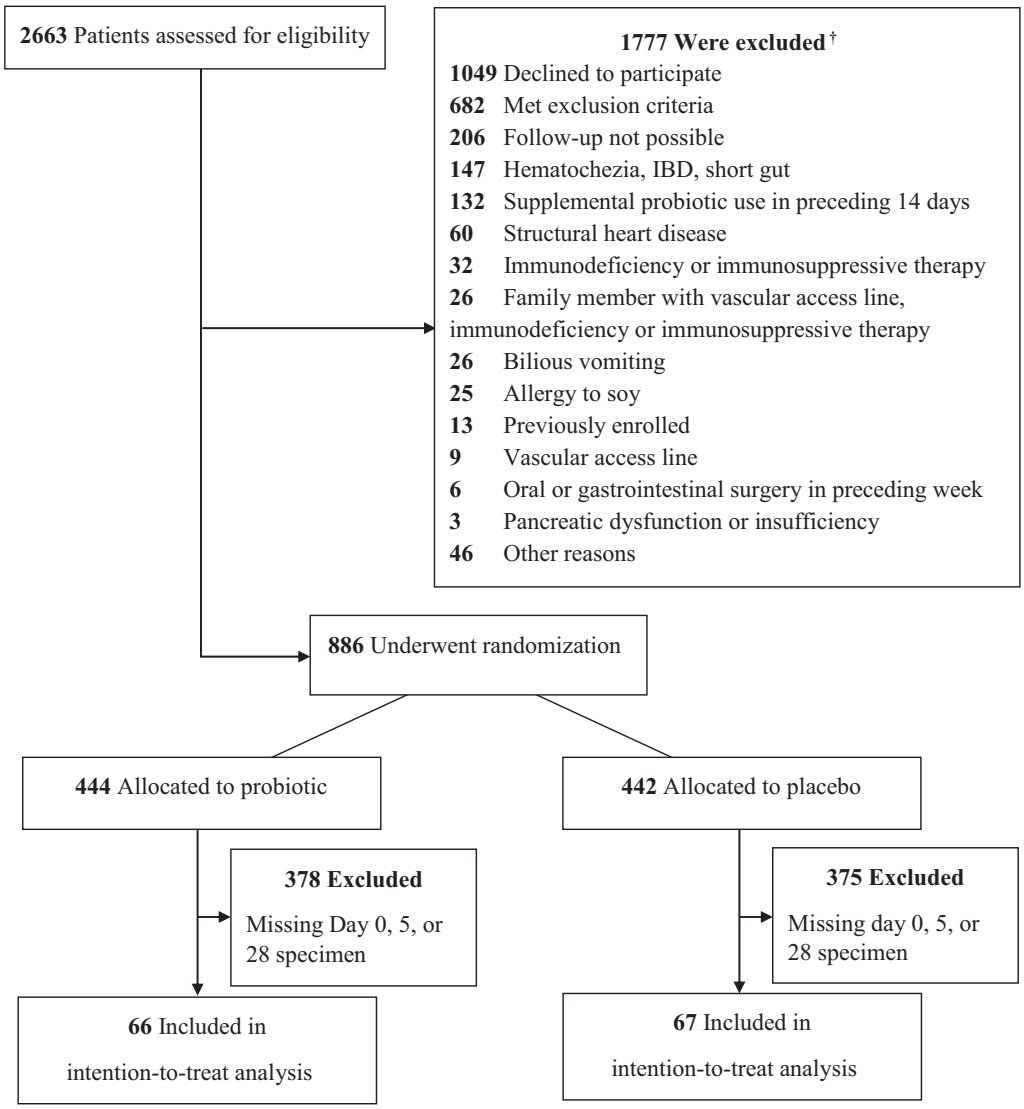

FIGURE 1 Enrollment, randomization, and outcome assessments. IBD, inflammatory bowel diseases. †Patients may have met more than 1 criterion.

and day 28 stool sIgA concentrations with the postrandomization, follow-up MVS score.

Between treatment group sIgA differences were compared at each time point based on enteropathogens identified at group (i.e., virus and bacteria) and individual virus (i.e., norovirus, rotavirus, and adenovirus) concentrations using the Mann–Whitney *U* test. The independent effects of enteropathogen status on stool sIgA concentrations at baseline, day 5, and day 28 were assessed, respectively, using a generalized linear model with a log-link function and γ distribution to fit skewed stool sIgA data, including the covariates described for the primary outcome. To further evaluate the effect of individual enteropathogens and treatment status on sIgA concentrations, changes from day 0 to day 5 and from day 5 to day 28 were evaluated as described for the primary outcome for participants who were virus positive, bacteria positive, virus–bacteria codetected, adenovirus positive, norovirus positive, and rotavirus positive, respectively. Regression analyses were performed when the minimum number of participants was ≥15.

Analyses were conducted using R version 3.5.6 (R Foundation for Statistical Computing) using packages lme4 and lmne for mixed-model γ regression with log link. Additional descriptive statistics and graphing were performed using SPSS version 24.0.0.1 (IBM) and GraphPad Prism (version 8.0.1). We did not use multiple imputations in the calculations because only 1 participant had incomplete clinical data (38). We calculated 2-tailed *P* values and set the significance level α at 0.05. To account for the testing of numerous secondary outcomes ($n = 27$), a Bonferroni correction was applied, and consequently the *P* value of significance for secondary outcomes was set at 0.002 (39).

Results

Participants

Between November 5, 2013, and April 7, 2017, 886 children were enrolled, of whom 172, 182, and 107 submitted day 0, day 5, and day 28 stool specimens, respectively (**Figure 1**). A total

TABLE 1 Clinical characteristics by treatment group[1]

	L. rhamnosus/helveticus ($n = 66$)	Placebo ($n = 67$)
Clinical features		
Age, mo	14.0 (10.0, 23.3)	12.0 (9.0, 21.0)
Male sex, *n* (%)	36 (54.5)	39 (58.2)
Weight, kg	10.5 (9.1, 13.0)	10.3 (8.8, 11.8)
Exclusively breastfed, *n* (%)	3 (4.5)	5 (7.5)
Received antibiotics in previous 14 d, *n* (%)	7 (10.6)	11 (16.4)
Received rotavirus vaccine, *n* (%)	43 (65.2)	32 (47.8)
Duration of illness,[2] h	40.4 (25.6, 61.3)	49.9 (34.8, 61.0)
Index emergency department visit Modified Vesikari Scale score[3]	11.0 (9.8, 13.3)	11.0 (10.0, 13.0)
History of vomiting, *n* (%)	52 (78.8)	56 (83.6)
No. of vomiting episodes in preceding 24 h[4]	4 (1, 5)	4 (3, 8)
No. of diarrhea episodes in preceding 24 h	5 (4, 9)	5 (4, 9)
History of fever,[5] *n* (%)	31 (47.0)	28 (41.8)
Clinical Dehydration Scale score[6]	1 (0, 2)	1 (0, 2)
Received ondansetron at index emergency department visit, *n* (%)	16 (24.2)	20 (29.9)
Received antibiotics at index emergency department visit or recommended at discharge, *n* (%)	2 (3.0)	1 (1.5)
Received intravenous rehydration at index emergency department visit, *n* (%)	7 (10.6)	5 (7.5)
Admitted to hospital at index visit, *n* (%)	0 (0)	2 (3.0)
Pathogens detected, *n* (%)		
Bacteria	6 (9)	10 (15)
Virus	36 (54.5)	27 (40)
Co-detection (positive both bacteria and virus)	4 (6)	7 (10)
Pathogen positive (bacteria or virus)	46 (69.7)	44 (65.7)
Pathogen negative (no bacteria or virus)	20 (30.3)	23 (34.3)
Rotavirus	11 (16.6)	6 (8.9)
Norovirus	17 (25.8)	23 (34.3)
Adenovirus	14 (21.2)	7 (10.4)

[1] Values are medians (IQRs) unless stated otherwise.
[2] This variable was defined according to the duration of vomiting or the duration of diarrhea before enrollment, whichever was greater.
[3] Scores on the Modified Vesikari Scale range from 0 to 20, with higher scores indicating greater disease severity.
[4] Only children with a history of vomiting were included.
[5] Fever was defined as a documented adjusted rectal temperature of at least 38.0°C whether at home or in the emergency department at the time of enrollment.
[6] Scores on the Clinical Dehydration Scale range from 0 to 8, with higher scores indicating more severe dehydration (40, 41).

of 133 children ($n = 66$ probiotic, $n = 67$ placebo) provided all 3 stool specimens. Study groups did not show a meaningful difference in any baseline characteristics (**Table 1**). Respective median stool sIgA concentrations on days 0, 5, and 28 did not differ between groups at any of the time points (**Table 2**).

Primary outcome

There were no significant changes in stool sIgA concentrations between day 0 and day 5 within *L. rhamnosus/helveticus*- or placebo-treated groups (**Figure 2**). In addition, stool sIgA concentrations did not differ between the *L. rhamnosus/helveticus* and the placebo groups (Table 2). In the regression model, we found no effects of treatment allocation ($P = 0.26$) or interaction between treatment and specimen collection day ($P = 0.95$). No covariates were associated with the stool sIgA concentrations between day 0 and day 5 (**Supplemental Table 2**).

Secondary outcomes

Although there were significant differences between day 5 and day 28 median stool sIgA concentrations within both the *L. rhamnosus/helveticus* and the placebo treatment groups ($P < 0.001$; Figure 2), no significant differences between treatment groups were found (Table 2). In the regression model, evaluating the sIgA concentration measured on days 5 and 28, we found no effects of treatment allocation [β: −0.24 (−0.72, 0.24); $P = 0.32$] or interaction between treatment and specimen collection day [β: 0.004 (−0.02, 0.02); $P = 0.65$]. The only covariate statistically associated with a decrease in stool sIgA concentrations between day 5 and day 28 was a longer time interval between stool collection days [β: −0.03 (−0.04, −0.02); $P < 0.001$], indicative of the expected decrease in sIgA concentration following recovery from AGE (**Supplemental Table 3**).

No relation between baseline stool sIgA concentrations and baseline MVS score (i.e., symptom severity prior to and up to the time of stool specimen submission) was found. With mild disease (MVS score <9) as the reference group, moderate and severe disease were not associated with stool sIgA concentrations: β: 0.51 (−0.18, 1.20) ($P = 0.15$) and β: 0.48 (−0.14, 1.10) ($P = 0.13$), respectively (**Supplemental Table 4**).

In the adjusted model evaluating the association between day 5 stool sIgA values and the postrandomization MVS score, we found no relation between moderate [β: −0.10 (−0.76, 0.56); $P = 0.77$] or severe [β: −0.34 (−0.99, 0.30); $P = 0.30$] disease

TABLE 2 Stool sIgA concentrations, change in stool sIgA concentrations, and clinical disease severity scores across study time points[1]

	L. rhamnosus/helveticus ($n = 66$)	Placebo ($n = 67$)	Difference in median	95% CI of the difference	*P* value[2]	Effect size[3]	Test statistic[4]
Day 0, stool sIgA	1999 (768, 4071)	2198 (702, 5278)	199	−337, 1101	0.27	0.17	1998
Day 5, stool sIgA	2505 (1111, 5310)	3207 (982, 7080)	701	−434, 1551	0.19	0.16	2005
Day 28, stool sIgA	1377 (697, 2248)	1779 (660, 3977)	402	−200, 781.2	0.27	0.19	1967
Change day 0 to day 5, sIgA	500 (−1135, 2362)	362 (−1122, 4256)	−139	−989, 1459	0.77	0.08	2115
Change day 5 to day 28, sIgA	− 1035 (−3130, 499)	− 1260 (−4437, 843)	−225	−1313, 892.3	0.70	0.07	2125
Change day 0 to day 28, sIgA	− 461 (−2001, 723)	− 204 (−3798, 1548)	257	−909, 1205	0.76	0.05	2144
Modified Vesikari Scale score, illness onset to day 0	11 (9, 13)	11 (10, 14)	0.39	−0.5, 1.3	0.39	0.13	0.853
Modified Vesikari Scale Score, illness day 0 to end of illness	5 (2, 8)	5 (3, 8)	0.47	−0.8, 1.7	0.45	0.13	0.743

[1]Values are medians (IQRs) unless stated otherwise. sIgA concentrations in micrograms per gram. sIgA, secretory immunoglobulin A.
[2]*P* values reflect results of Mann–Whitney *U* test except for analyses comparing Modified Vesikari Scale scores, which employed the Welch's *t* test.
[3]Effect size reflects the Cohen's *d* effect size estimates, where $d = 0.2$, $d = 0.5$, and $d = 0.8$ refer to small, medium, and large effect sizes, respectively.
[4]Test statistic reflects the Mann–Whitney *U* test statistic except for analysis comparing Modified Vesikari Scale scores, which reflects the Welch's *t* statistic.

and day 5 stool sIgA concentrations (**Supplemental Table 5**). The results were similar with respect to day 28 stool sIgA values, with the exception of a positive but not statistically significant association between day 28 stool sIgA values and the detection of viral pathogens in the stool—that is, higher day 28 values for this group of children [$\beta = 0.43$ (0.05, 0.81); $P = 0.03$] (**Supplemental Table 6**).

Overall, 67.7% (90/133) of the participants were pathogen positive, with viral pathogens detected most frequently (47.4%; 63/133) (Table 1). There were no differences in baseline, day 5, or day 28 stool sIgA concentrations between treatment groups regardless of pathogens identified (**Table 3**). Similarly, no differences were noted in the change in stool sIgA between treatment groups, when analyzed by pathogen status, between day 0 and day 5, day 0 and day 28, or day 5 and day 28.

Within the groups of identified individual viral agents, differences between probiotic and placebo groups in stool sIgA concentrations (absolute values or changes over time) were statistically significant after correction for multiple secondary outcomes (**Figure 3**, **Supplemental Table 7**). Adjusted models evaluating pathogen-level changes in stool sIgA over time also found no significant treatment group effects (**Supplemental Tables 8–11**).

Discussion

In this study, we quantified the effect of *L. rhamnosus/helveticus* administration on stool sIgA concentrations in preschool-aged children with AGE. Neither the *L. rhamnosus/helveticus* group nor the placebo treatment group had

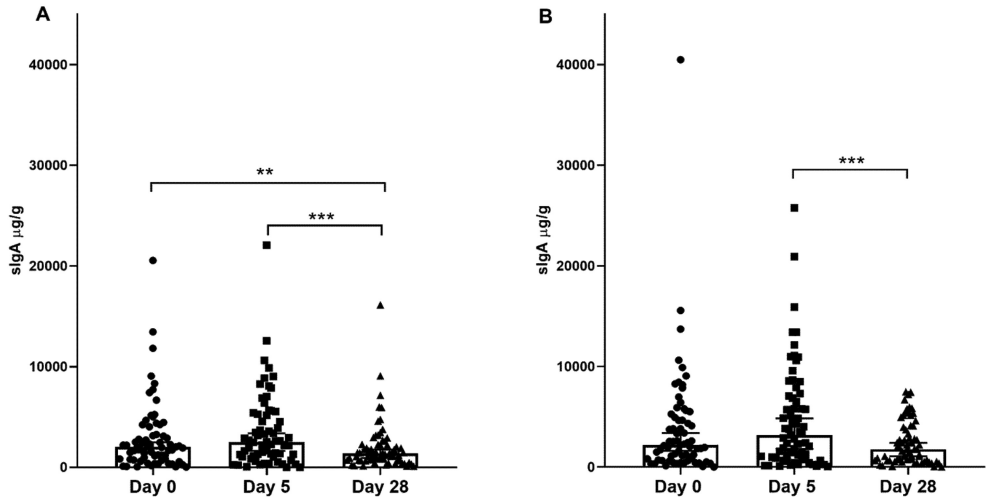

FIGURE 2 Stool sIgA concentrations at baseline and 5 and 28 d postrandomization treatment for both the probiotic (A) ($n = 66$) and placebo (B) ($n = 67$) treated groups. An outlier analysis was performed, and outliers were removed; however, because this did not alter the findings, the outliers were retained. The median difference for the day 5 to day 28 change in the probiotic group was 1035 μg/g (95% CI of the difference: 85, 1951). The median difference for the day 0 to day 28 change in the probiotic group was −461 μg/g (95% CI of the difference: −1281, 98). The median difference for the day 5 to day 28 change in the placebo group was 1260 μg/g (95% CI of the difference: 60, 2077). Significance denoted as $^{*}P = 0.01$, $^{**}P = 0.0005$, and $^{***}P = 0.0006$. sIgA, secretory immunoglobulin A.

TABLE 3 Stool sIgA concentrations across study time points and enteropathogen status[1]

	L. rhamnosus/helveticus	Placebo	Difference in median	95% CI of the difference	*P* value[2]	Effect size[3]	Test statistic[4]
Bacteria positive ($n = 16$)							
Day 0, stool sIgA	2257 (1478, 2869)	2574 (1034, 8202)	316	−1295, 5682	0.63	0.27	25
Day 5, stool sIgA	1740 (1044, 5131)	1110 (260, 3137)	−630	−3980, 1873	0.42	0.44	22
Day 28, stool sIgA	1154 (580, 3140)	2000 (1210, 4703)	846	−959, 3521	0.21	0.44	18
Change day 0 to day 5, sIgA	−330 (−693, 2263)	−191 (−4389, 772)	138	−5944, 1020	0.56	0.69	24
Change day 5 to day 28, sIgA	−1225 (−4232, 2592)	891 (−735, 2551)	2115	−3716, 5778	0.36	0.33	21
Change day 0 to day 28, sIgA	−1554 (−1787, 1899)	227 (−4404, 1227)	1781	−4950, 2751	0.95	0.50	29
Virus positive ($n = 63$)							
Day 0, stool sIgA	1702 (572, 3995)	1850 (521, 4347)	149	−802, 1120	0.74	0.08	462
Day 5, stool sIgA	3232 (1475, 5662)	5701 (1225, 8565)	2469	−756, 3427	0.29	0.27	409
Day 28, stool sIgA	1417 (822, 2518)	2434 (756, 5146)	1017	−264, 1638	0.19	0.33	392
Change day 0 to day 5, sIgA	1260 (−453, 3740)	2136 (−960, 6671)	876	−1299, 3628	0.36	0.23	420
Change day 5 to day 28, sIgA	−1199 (−3607, 45)	−2171 (−5209, 164)	−972	−2236, 1001	0.49	0.18	436
Change day 0 to day 28, sIgA	−207 (−1524, 791)	180 (−2144, 2550)	387	−737, 2324	0.28	0.28	408
Codetection ($n = 11$)							
Day 0, stool sIgA	672 (92, 3323)	1235 (144, 3817)	563	−2783, 3710	0.78	0.23	12
Day 5, stool sIgA	2355 (552, 2799)	3522 (2806, 10,934)	1166	−81, 10,865	0.07	1.39	4
Day 28, stool sIgA	790 (424, 5599)	1779 (392, 2709)	989	−5380, 2363	0.92	0.11	13
Change day 0 to day 5, sIgA	600 (−873, 1893)	2113.3 (186, 7117)	1513	−1111, 9272	0.16	1.02	6
Change day 5 to day 28, sIgA	−591 (−1855, 3553)	−2415 (−10,482, 18)	−1824	−10,817, 1986	0.16	1.02	6
Change day 0 to day 28, sIgA	406 (−2265, 4586)	204 (−434, 2565)	−202	−5719, 3303	0.65	0.35	11
Any pathogen positive ($n = 90$)							
Day 0, stool sIgA	1702 (598, 3171)	1905 (541, 4288)	204	−524, 1004	0.59	0.12	944
Day 5, stool sIgA	2783 (1407, 5556)	3685 (967, 8354)	902	−830, 2210	0.49	0.15	926
Day 28, stool sIgA	1405 (741, 2355)	2084 (799, 4387)	680	−120, 1268	0.11	0.34	815
Change day 0 to day 5, sIgA	1042 (−586, 2631)	1581 (−967, 5296)	539	−1080, 2195	0.55	0.13	937
Change day 5 to day 28, sIgA	−1205 (−3557, 205)	−1769 (−4704, 903)	−564	−1643, 1179	0.81	0.05	982
Change day 0 to day 28, sIgA	−207 (−1648, 835)	192 (−2028, 2011)	399	−644, 1680	0.36	0.20	898
Pathogen negative ($n = 43$)							
Day 0, stool sIgA	2306 (1612, 4931)	3394 (1331, 5910)	1088	−884, 2701	0.41	0.26	195
Day 5, stool sIgA	1268 (335, 3633)	2076 (982, 5750)	808	−598, 2061	0.28	0.34	185
Day 28, stool sIgA	1188 (482, 1875)	785 (521, 3977)	−402	−702, 642.7	0.97	0.02	228
Change day 0 to day 5, sIgA	−257 (−1672, 765)	27 (−2563, 602)	284	−1571, 1785	0.70	0.12	214
Change day 5 to day 28, sIgA	−16 (−2529, 742)	−954 (−3639, 674)	−938	−2031, 1662	0.67	0.13	212
Change day 0 to day 28, sIgA	−1117 (−3346, −241)	−1748 (−4907, 23)	−630	−2672, 1190	0.52	0.20	203

[1] Values are medians (IQRs) unless stated otherwise. sIgA concentrations in micrograms per gram. sIgA, secretory immunoglobulin A.
[2] *P* value reflects results of the Mann–Whitney *U* test. Statistical significance set at $P < 0.002$ after correction for multiplicity ($n = 27$).
[3] Effect size reflects the Cohen's *d* effect size estimates, where $d = 0.2$, $d = 0.5$, and $d = 0.8$ refer to small, medium, and large effect sizes, respectively.
[4] Test statistic reflects the Mann–Whitney *U* test statistic.

detectable increases in stool sIgA concentrations over the 5 d following the ED visit. After controlling for repeated measures and trial site, there was no effect of treatment on stool sIgA concentrations. Although we found significant reductions in stool sIgA concentrations between day 5 and day 28, this was not related to treatment allocation. In addition, we found no relation between disease severity and stool sIgA concentrations.

Only 1 prior study has evaluated stool sIgA in children with AGE; it reported a positive effect on fecal sIgA from the administration of 4×10^8 CFU/d of *Lactobacillus casei* for 7 d (21). Although it is unclear why our results differed, strain and disease specificity are important characteristics of probiotics (42), and thus strain differences may account for the differential findings. Study rigor may play a role because although the previous study mentioned placebo, the contents were not described, and blinding was not reported. Moreover, the between-group differences, which are reported as $P < 0.05$, appear to be minimal, do not adjust for baseline values, and there

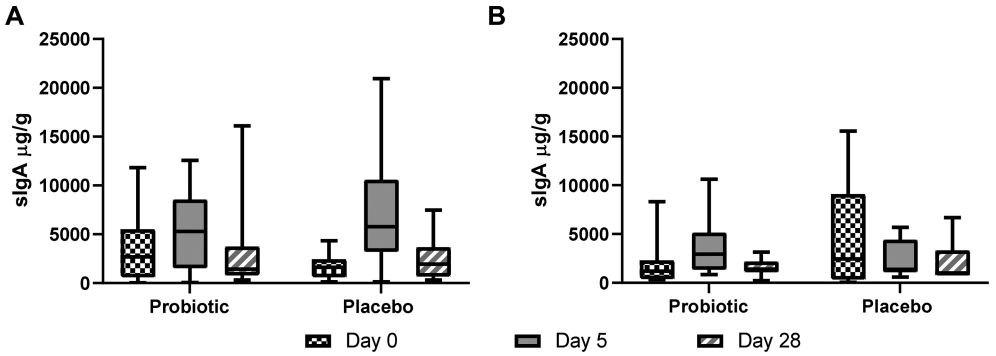

FIGURE 3 Stool sIgA concentrations at baseline and 5 and 28 d postrandomization treatment for both norovirus- (A) and rotavirus- (B) infected children. Norovirus-infected children: $n = 40$; probiotic = 17, placebo = 23. Rotavirus-infected children: $n = 17$; probiotic = 11, placebo = 6. No significant differences were found after correction for multiple comparison. sIgA, secretory immunoglobulin A.

do not appear to be adjustments made for multiplicity (21). Many of the studies that report increased fecal sIgA concentrations in otherwise healthy infants provide the intervention for a much longer period of time (17, 43).

It is presumed that sIgA is a critical factor in protecting mucosal surfaces against viral infections. Human studies have correlated virus-specific sIgA increases with the cessation of stool virus excretion or protection against infection and disease (44, 45). However, it has been difficult to discern the importance of the sIgA response to gastrointestinal viruses because there is no overt clinical profile in IgA-deficient humans. In addition, few animal models of enteropathogenic gastrointestinal viral infections realistically replicate human infection.

Although we found similar changes over time in sIgA across treatment arms overall, among the subgroup of norovirus-infected children, a clinically but not statistically significant difference [day 0 to day 5: an increase of 1181 μg/g (IQR: −554, 2308) compared with 4544 μg/g (1725, 7900); $P = 0.008$; Cohen's $d = 0.91$; and day 0 to day 28: a median change of −721 μg/g (IQR: −2928, 456) compared with a median increase of 544 μg/g (−1060, 2565); $P = 0.04$; Cohen's $d = 0.67$] was identified. This reflects either a greater sIgA increase among placebo-treated children or a suppression of the sIgA response in children treated with probiotics. Although animal models provide limited insight into human norovirus infection and sIgA response, gnotobiotic pig anti-norovirus-specific IgA is detected as early as 6 d following virus exposure, and diarrhea severity in this species is moderately correlated with convalescent-phase intestinal sIgA antibody titers (46). Epidemiological data from human studies suggest a link between sIgA concentrations induced by norovirus infection and nonreplicating vaccine administration (47, 48).

Our findings should be considered in the context of emerging evidence that gastrointestinal tract colonization by orally administered probiotics and fecal sIgA concentrations are likely person-specific. This reasoning stems from research that has demonstrated person-, region-, and strain-specific mucosal colonization patterns, hallmarked by predictive baseline host and microbiome features (49). As a result, probiotics may induce individualized impacts on mucosal community structures; therefore, approaches to probiotic therapy may benefit from personalized probiotic therapy. Thus, the lack of sIgA induction detected in our study might reflect insufficient colonization by probiotics across the patient population.

Our study has several limitations. It is possible that stool specimen collection dates did not correspond to the peak timing of *L. rhamnosus/helveticus*-induced sIgA production. However, in mouse models, pathogen-specific IgA responses are seen as early as 3 d following viral inoculation (50). Despite conducting this study at 6 sites, we obtained repeat stool specimens from only a relatively modest proportion of the PROGUT study cohort. This occurred due to the outpatient nature of the study and the self-limited disease process that made stool retrieval a challenge. Although those who provided all stool specimens were less likely to be infected by rotavirus, they did not differ in any other baseline characteristics from those who did not provide all specimens (**Supplemental Table 12**). We also did not collect serum nor attempt to correlate fecal sIgA concentrations with IgA concentrations in serum. However, note that individuals with low concentrations of serum IgA likely have sufficient secretory IgA at their mucosal surfaces to remain asymptomatic (51). Also note that other mechanisms of probiotic action were not evaluated, including the impact on the luminal and mucosal microbiomes (52) or via altering fecal metabolites (53). We had intended to conduct bacteria-specific analyses as well; however, given the small numbers of individual pathogens, we are unable to report these findings.

In conclusion, among children with AGE presenting to an ED, *L. rhamnosus/helveticus* treatment did not increase stool sIgA concentrations relative to placebo. The lack of effect identified in this evaluation of the intestine's immune system is consistent with the lack of clinical efficacy reported in the recent PROGUT clinical trial.

The authors' responsibilities were as follows—SBF and PMS: conceived the study and co-wrote the study protocol; JX, SW-U, LC, X-LP, BL, SS, YF, SG, KJF, NP, KH, and DS: designed the study; RH, KJ-H, and PMS: provided major input into the concept and analysis of the study; SBF, JX, SW-U, LC, X-LP, BL, SS, YF, SG, KJF, NP, KH, DS, and PMS: drafted the manuscript and revised the manuscript for intellectual content; RH and KJ-H: provided major input into drafting and revision of the manuscript; and all authors: read and approved the final manuscript. SBF reports receiving research support from Lallemand Health Solutions and serves as a consultant for Takeda Pharmaceutical Company, Eligo Bioscience, and RedHill Biopharma. PMS receives research support from Lallemand Health Solutions and honoraria from Abbott Nutrition, Mead Johnson Nutritionals, and Nestle Nutrition related to the use of probiotics in humans. All other authors report no conflicts of interest.

Data Availability

The full study protocol, statistical analysis plan, informed consent forms, deidentified individual participant data, and a data dictionary defining each field in the set are available upon request from the corresponding author following the provision of ethics approval, with a signed data access agreement.

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