Effect of zinc supplementation on immune and inflammatory responses in pediatric patients with shigellosis[1–3]

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ABSTRACT

Background: Several studies showed benefits of long-term zinc supplementation on the incidence, severity, and duration of diarrhea and on the incidence of respiratory infections. Prolonged zinc supplementation also improves cell-mediated immunity in severely malnourished children.

Objective: We studied the effect of short-term zinc supplementation on intrinsic and specific immune and inflammatory responses in moderately malnourished children with acute shigellosis.

Design: A randomized, double-blind, placebo-controlled trial was conducted in *Shigella*-infected children aged 12–59 mo. Elemental zinc (20 mg) and a multivitamin containing vitamins A and D, thiamine, riboflavin, nicotinamide, and calcium at twice the recommended dietary allowance were given daily for 2 wk to the zinc group ($n = 28$), whereas the multivitamin alone was given to the control group ($n = 28$). Standard antibiotic therapy was given to all patients.

Results: Serum zinc concentrations increased in both groups during convalescence; however, zinc supplementation showed a significant effect. The lymphocyte proliferation response in the zinc group increased relative to that in the control group ($P = 0.002$), but no significant effects were seen on concentrations of cytokines (interleukin 2 and interferon γ) released from mitogen-stimulated mononuclear cells or on concentrations of cytokines (interleukin 2, interferon γ, and interleukin 1β) in feces. Among the antigen [lipopolysaccharide and invasion plasmid-encoded antigen (Ipa)]-specific antibodies, plasma Ipa-specific immunoglobulin G responses at day 30 were significantly higher in the zinc group than in the control group. However, the 2 groups did not differ significantly in the other antigen-specific responses in plasma and stool.

Conclusion: A 14-d course of zinc supplementation during acute shigellosis increases the lymphocyte proliferation response and the Ipa-specific immunoglobulin G response. *Am J Clin Nutr* 2004; 79:444–50.

KEY WORDS Zinc, *Shigella*, specific immunity, cellular immunity

INTRODUCTION

Shigellosis, a bacillary dysentery, is a major cause of childhood mortality and morbidity in developing countries, especially in children aged < 5 y. In developing countries, frequent infection due to *Shigella* may lead to malnutrition, stunting of growth, and even life-threatening complications in children (1, 2). Mortality from shigellosis is highest in severely malnourished children (3). Malnourished children with shigellosis have a lower stimulation index to mitogens, a lower CD4:CD8 ratio, and lower transferrin concentrations than do children who are not malnourished (4).

Zinc deficiency is associated with increased diarrheal and respiratory morbidity (5, 6). Prolonged zinc supplementation in double-blind, placebo-controlled trials reduced the incidence, severity, and duration of acute and persistent diarrhea and dysentery and the incidence of acute lower respiratory infections (7–12). Zinc supplementation has been shown to improve cell-mediated immunity in children (13, 14) although studies on the effects of zinc supplementation on specific immunity in humans are limited (15–19). Zinc is an antioxidant that protects cells from the damaging effects of oxygen radicals generated during immune activation (20, 21). In fact, zinc supplementation of malnourished children with acute or persistent diarrhea reduced intestinal permeability, which reflected enhanced mucosal repair (22). The adverse effects of zinc deficiency on the immune system are likely to increase the susceptibility of children to infectious diarrhea, and chronic or persistent diarrhea may further compromise nutritional status and lead to a zinc-deficient state (6, 10, 23). Therefore, zinc supplementation could conceivably modulate the immune and inflammatory responses to shigellosis in a way that is beneficial to the host. The present study was thus undertaken to investigate the effect of zinc supplementation on innate and specific immune and inflammatory responses in moderately malnourished children with shigellosis.

SUBJECTS AND METHODS

Study subjects

Pediatric patients who had bloody mucoid stools and severe abdominal cramps and attended the Clinical Research Service Center of the International Centre for Diarrhoeal Diseases Re-

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search, Bangladesh (ICDDR,B): Centre for Health and Population Research in Dhaka, Bangladesh, were initially screened for the study. Stool samples were examined microscopically and cultured for enteric bacterial pathogens (24). Children whose stool cultures were positive for *Shigella* organisms were included in the study. Selection of the patients was based on the following criteria: age of 12–59 mo, moderate malnutrition, duration of diarrhea of 0–5 d, and culture-confirmed *Shigella* spp in stool on enrollment. Measurements of weight and length (height) were performed by using the National Center for Health Statistics growth percentile curves. Height-for-age, weight-for-age, and weight-for-height *z* scores were calculated with the use of the PCTL9Z Anthropometry Subroutine (Center for Health Promotion and Education, Centers for Disease Control and Prevention, Atlanta). The exclusion criteria were as follows: measles infection in the past 6 mo; presence of obvious systemic illnesses, such as pneumonia, meningitis, septicemia, leukemoid reaction, hemolytic uremic syndrome, etc; zinc supplementation; severe malnutrition; residence in a location requiring a journey of > 2 h from the Clinical Research Service Center; and refusal of consent. Signed informed consent was obtained from the guardian of each pediatric patient before enrollment according to the guidelines of the Ethical Review Committee of the ICDDR,B.

Study design

The study was designed as a double-blind, placebo-controlled study in which patients were randomly assigned to 2 treatment groups. A block randomization procedure was performed by using a random table to assign an equal number of patients to the following 2 groups: a treatment group, who received zinc acetate with multivitamin syrup, and a control group, who received multivitamin syrup alone (25). Double blinding was done by having identical bottles of syrup labeled with numbers, which were allocated to the children in the 2 groups chronologically according to their serial number within each group. The zinc syrup containing zinc and the multivitamin was masked for taste and flavor and was indistinguishable in consistency, appearance, and taste from the control syrup containing the multivitamin only.

The micronutrient supplements were prepared by Acme Laboratories, Dhaka, Bangladesh. Every 5 mL of the multivitamin syrup contained the following: vitamin A (3000 IU), vitamin D (600 IU), thiamine (1.2 mg), riboflavin (2.0 mg), nicotinamide (6.0 mg), and calcium panthonate (6.0 mg). The zinc preparation contained zinc acetate (20 mg elemental Zn/5 mL) mixed in the multivitamin syrup. The experimental group received the zinc syrup containing the multivitamin (the vitamins were given at twice the recommended dietary allowance) in 2 divided doses daily for 2 wk starting from the day of admission. The control group received the multivitamin (control supplement in which the vitamins were given at twice the recommended dietary allowance) alone. All the pediatric patients received 60 mg pivmecillinum $\cdot$ kg body $wt^{-1} \cdot d^{-1}$ in 4 divided doses for 5 d. In both groups of patients, diarrhea subsided within 3–5 d. During hospitalization all the study children received a standardized diet of 100–125 kcal and 3–4 g protein in mixed food $\cdot$ kg body $wt^{-1} \cdot d^{-1}$. The zinc content of the consumed diet was $\approx$7–8 mg/d. During the stay at the study ward, the children were fed the supplements by the health assistants. When the children were discharged on day 7, their mothers were given the bottles of syrup and were instructed to feed the child the supplement daily at home for the remaining 7 d. On day 14, a health assistant visited the household, measured the amount of syrup taken, and asked the mothers about any problems encountered while feeding the syrups to their children.

Specimen collection

A venous blood sample was obtained from each child at admission (day 1, before supplementation) and 7 and 30 d after admission when the child was brought to the hospital for follow-up sampling. Blood was collected in heparin-coated sterile vials (Vacutainer system; Becton Dickinson, Rutherford, NJ). Peripheral blood mononuclear cells were isolated from heparinized blood with Ficoll-Paque density gradient centrifugation (Pharmacia-Upjohn, Uppsala, Sweden) for 25 min at $939 \times g$ and 25 °C, and plasma was collected as the supernatant fluid from the gradient and stored at -70 °C until analyzed. Blood was also collected in vials that were free of trace elements (Venoject II; Terumo Europe NV, Leuven, Belgium), and serum was separated from cells. In addition, stool samples were collected from the pediatric patients on the day of admission and the follow-up days, extracted as described previously (24), and kept at -70 °C until analyzed.

Zinc status and C-reactive protein

Serum zinc concentrations were measured by using a flame atomic absorption spectrophotometer (BDH) and a zinc standard solution (Spectrosol; BDH). Serum C-reactive protein concentrations were measured by using a fluorescence polarization immunoassay technique (Abbot Laboratories, Abbot Park, IL) on an Abbot TDx analyzer.

Antigen-specific antibodies in plasma and fecal extracts

Enzyme-linked immunosorbent assays were used to test plasma samples for immunoglobulin (Ig) G and IgA antibodies and to test fecal extracts for IgA antibodies (26, 27). The antigens used in the present study were lipopolysaccharide (LPS) from *Shigella flexneri* Y and invasion plasmid-encoded protein antigens (Ipa) from strain Sp-10, an *Escherichia coli* K-12 strain harboring the invasion plasmid and rich in Ipa. Extraction and purification of the antigens were performed as described previously (26). Microtiter plates (Nunc, Roskilde, Denmark) were coated with purified LPS (10 μg/mL in carbonate buffer containing 0.1 mol sodium bicarbonate/L and 5 mmol magnesium chloride/L, pH 9.8) from *S. flexneri* Y (27) or with Ipa (20 μg/mL in phosphate-buffered saline, pH 7.4). Absorption was measured at 450 nm. Antigen-specific responses were expressed as relative titers, which were defined as the absorption multiplied by the dilution factor of the sample (26, 27).

Secretory immunoglobulin A concentrations in fecal extracts

Total secretory IgA concentrations in fecal extracts were measured by using an enzyme-linked immunosorbent assay (26, 28) and pooled human milk having a known IgA concentration of 1 mg/mL as a standard. Microtiter plates (Nunc) were coated with antisecretory component (1:2000 in carbonate buffer, 100 μL/well; Dakopatts AB, Denmark), horseradish peroxidase-conjugated antisecretory component (1:1000; Dako) was used as a conjugate, and the plates were developed by using orthophenylenediamine (Sigma, St Louis) as the substrate.

Measurement of soluble innate mediators in stool and urine

The enzyme activity of myeloperoxidase (MPO; EC 1.11.1.7) was determined in stool extracts by measuring the hydrogen peroxide–dependent oxidation of 3,3',5,5'-tetramethyl-benzidine by using a kinetic spectrophotometer (Beckman DU 640; Beckman Instruments, Fullerton, CA) at 650 nm as described previously (24). The specific activity of MPO was expressed as U/mg total protein in stool extracts. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured in stool samples by using a commercial kit (R&D Systems, Inc, Minneapolis). The assay is based on the SOD-mediated increase in the rate of autooxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzofluorene in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. The rate of change in absorbance at 525 nm per minute was defined as 1 U of enzyme activity. The specific activity was expressed as U/mg total protein. The final products of nitric oxide in vivo are nitrate and nitrite. With the use of a commercial kit (R&D Systems, Inc), concentrations of these metabolites have been used as a quantitative measure of nitric oxide production in urine samples. The creatinine content of urine was measured, and the total concentration of nitrate and nitrite was expressed as a ratio of the creatinine concentration and was referred to as the concentration of nitric oxide metabolites. The cytokines interferon γ (IFN-γ), interleukin 1β (IL-1β), and interleukin 2 (IL-2) were measured in stool extracts by using commercial sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Inc). Concentrations of IL-1β, IL-2, and IFN-γ were expressed as pg/mL stool. The detection limits of the assays were 1 pg/mL for IL-1β, 8 pg/mL for IFN-γ, and 7 pg/mL for IL-2.

Lymphocyte proliferation assay

Peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured with medium alone or stimulated with phytohemagglutinin (2 μL/well; Gibco) in a sterile, 96-well, U-bottomed plate (Nunc) for 3 d in 5% CO_2 and at 37 °C. Cells were pulsed with [^{3}H]thymidine (specific activity of 5 mCi/mmol, 0.5 μCi/well; Amersham Pharmacia Biotech, United Kingdom) 18 h before cell harvest. Labeled cells were collected on glass fiber filter paper (Whatman International Ltd, Maidstone, United Kingdom) by using a cell harvestor (Automesh 2000; Dynatech, Denkendorf, Germany), and [^{3}H]thymidine incorporation into lymphocytes was counted as counts/min with a beta counter (LS 6500; Beckman). For each patient, data are expressed as the mean stimulation index (counts/min of stimulated cells divided by counts/min of unstimulated cells) of triplicates.

Cytokines in cell culture supernatant fluid

Peripheral blood mononuclear cells were cultured as described above with or without PHA (2 μL/well) in a sterile, 96-well, U-bottomed plate (Nunc) for 3 d in 5% CO_2 and at 37 °C. Culture supernatant fluid was collected from unstimulated and PHA-stimulated cells, and the cytokines IFN-γ and IL-2 were measured in the supernatant by using commercial sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Inc). Concentrations of IFN-γ and IL-2 were expressed as pg/mL supernatant fluid.

Statistical analyses

Statistical analyses were performed by using the statistical software SIGMASTAT 2.03 (Jandel Scientific, San Rafael, CA).

TABLE 1
Baseline characteristics of patients supplemented with zinc or placebo[1]

Characteristic	Zinc ($n = 28$)	Placebo ($n = 28$)
Age (mo)	18 (15–28)[2]	24 (18.5–35)
Duration of diarrhea (d)	5 (5–5.7)	4 (3.5–5.5)
Duration of fever[3] (d)	5 (2.5–6)	4 (3–6)
Stools (no./d)	5 (3–8.5)	6.5 (3–13.5)
Blood leukocyte count ($\times 10^3$/mm^3)	12.7 (11.5–15.37)	12 (10.3–16.6)
Blood lymphocyte count (%)	51 (42–60)	47.5 (33–55)
Blood neutrophil count (%)	40 (32–52)	37.5 (33–53)
Weight-for-height *z* score	-1.91 ± 0.75[4]	-1.85 ± 0.99
Weight-for-age *z* score	-2.49 ± 0.91	-2.62 ± 0.78
Height-for-age *z* score	-1.68 ± 1.3	-1.94 ± 0.83

[1] Blood leukocytes, lymphocytes, and neutrophils were counted in a Differential Automatic Hematology Analyzer (Danam Excell, Dallas). There were no significant differences between the 2 groups.
[2] Median; interquartile range in parentheses.
[3] Defined as a temperature > 37.8 °C.
[4] $\bar{x} \pm$ SD.

Data are expressed as geometric means ± SEMs. Two-way analysis of variance (ANOVA) was performed to determine significant interactions between time and treatment, and in the case of a significant interaction, the post hoc Tukey's procedure was performed. For data that were not normally distributed, ANOVA on ranks was applied. For within group (between days) comparisons, one-way ANOVA was performed.

RESULTS

Demographic characteristics of patients

A total of 56 patients with a median age of 23 mo (25–75 percentiles: 15–30 mo) were enrolled in the study. All the patients ($n = 28$ in the zinc group and 28 in the control group) were infected with *S. flexneri* spp. The baseline characteristics of the patients are shown in **Table 1**. There were no significant differences between the zinc group and the control group in any of the baseline characteristics. Microscopic examination of stool specimens on the day of admission showed that 3 patients in the zinc group and 4 in the control group were infected with *Ascaris lumbricoides*. The clinical recovery, growth, and morbidity of the children will be reported elsewhere.

Zinc status and acute phase response

There was no significant difference between the zinc group and the control group in serum zinc concentrations at baseline. In two-way ANOVA, no significant time-by-treatment interaction was observed. Serum zinc concentrations increased in both groups during convalescence. However, both zinc supplementation ($P = 0.01$) and time ($P < 0.001$) showed a significant effect on serum zinc concentrations (**Table 2**). No significant interaction between zinc supplementation and time was observed for serum C-reactive protein concentrations. However, time showed a significant effect ($P = 0.001$) on serum C-reactive protein concentrations.

Innate humoral and inflammatory responses

Concentrations of cytokines (IL-2, IFN-γ, and IL-1β) and innate mediators (MPO, SOD, and urinary nitric oxide metabo-

TABLE 2
Effect of zinc supplementation on zinc status and acute phase response[1]

	Zinc group ($n = 28$)	Control group ($n = 28$)
Zinc (mg/L)		
Day 1	0.59 ± 0.02	0.57 ± 0.02
Day 7	0.73 ± 0.02	0.68 ± 0.01
Day 30	0.79 ± 0.03	0.71 ± 0.02
CRP (mg/dL)		
Day 1	0.94 ± 0.4	0.9 ± 0.3
Day 7	0.56 ± 0.1	0.5 ± 0.08
Day 30	0.5 ± 0.06	0.5 ± 0.07

[1] Geometric $\bar{x}$ ± SEM. CRP, C-reactive protein. In two-way ANOVA, no significant interaction between zinc supplementation and time was observed. However, both zinc supplementation ($P = 0.01$) and time ($P < 0.001$) showed a significant effect on serum zinc concentrations, but only time showed a significant effect ($P = 0.001$) on serum CRP concentrations.

lites) in stool extracts were examined in both groups of patients. No significant differences in the concentrations of cytokines or innate mediators in stool extracts were observed between the 2 groups or within each group over time. Only time showed a significant effect on stool IL-1β concentrations ($P = 0.001$) (**Table 3**).

Cellular immune responses

Peripheral blood mononuclear cells from patients were analyzed for their ability to respond to a mitogen (PHA) by proliferation ([^{3}H]thymidine incorporation) and to produce cytokines (IFN-γ and IL-2) in response to PHA. In two-way ANOVA, no significant interactions between zinc supplementation and time were observed; however, 2 wk of zinc supplementation had a significant effect ($P = 0.002$) on the stimulation index (**Table 4**). No significant differences in the PHA-stimulated secretion of IFN-γ and IL-2 were observed between the 2 groups or within each group over time.

Antigen-specific antibody responses

LPS and Ipa were used as antigens for determination of *Shigella* antigen-specific immune responses in patients. When comparisons were made between the 2 groups by using two-way AVOVA, a significant interaction was identified between time and treatment (zinc supplementation) ($P < 0.003$). Post hoc analysis with Tukey's test showed that the increase in the Ipa-specific IgG response from day 1 to day 30 in the zinc group was significant in comparison with the increase over that time in the control group ($P < 0.001$). In both groups, the Ipa-specific IgG response on day 30 was significantly higher than that on day 1 or day 7 ($P < 0.001$) (**Table 5**). Except for significant effects of time on plasma LPS-specific IgA ($P = 0.012$) and stool s-IgA ($P = 0.026$), no significant differences in antigen-specific antibodies were observed between the 2 groups or within each group over time.

DISCUSSION

The present study showed that a short course of zinc supplementation during a period of acute illness improves both *Shigella* antigen-specific IgG responses and lymphocyte proliferation responses in patients with shigellosis. A decrease in serum zinc concentrations during infection is an acute phase response, but concentrations return to baseline values after clinical recovery (29). However, the significant increase in zinc concentration in the zinc group relative to the increase in the control group suggests that these children probably receive a zinc-deficient diet at home and that the 14-d course of zinc supplementation during the acute phase of the disease made a prominent difference. In line with this, in an earlier study, children who received a 14-d course of zinc supplementation during acute diarrhea had a 25% increase in linear growth from 4 to 9 wk in comparison with the linear growth of the children in the control group (30).

Shigellosis is an acute inflammatory disease that is accompanied by excessive production of proinflammatory cytokines and mediators such as MPO, nitric oxide, and eicosanoids (24, 31, 32). Zinc is known to protect cells from reactive oxygen species–mediated damage during immune activation and to preserve cell membrane integrity (21, 33). A therapeutic effect of zinc in reducing inflammation through reduction of MPO concentrations has been reported in a rat model of Crohn disease (34, 35). Endogenous zinc inhibits LPS- and IL-1β–induced nitric oxide formation and inflammation in the intestine (36). Zinc may also act as a nitric oxide scavenger in in vitro systems (37). However, in the present study, we did not observe a significant effect of zinc on concentrations of cytokines, MPO, nitric oxide metabolites, or SOD.

Zinc supplementation has been shown to improve cell-mediated immunity in young children after prolonged daily supplementation ranging from 3 to 4 mo (13, 14). In terms of a

TABLE 3
Effect of zinc supplementation on innate immune responses in pediatric patients with shigellosis[1]

	Zinc group ($n = 28$)			Control group ($n = 28$)		
Innate mediators in stool extracts	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30
IL-1β (pg/mL)	2562 ± 133	38 ± 22	92 ± 16	2577 ± 871	56 ± 112	68 ± 19
IL-2 (pg/mL)	72 ± 10	79 ± 12	70 ± 12	79 ± 12	78 ± 3	76 ± 2
IFN-γ (pg/mL)	12 ± 2	13 ± 1	13 ± 3	12 ± 2	15 ± 2.3	14 ± 1.3
Myeloperoxidase (U/mg total protein)	3.2 ± 4	0.12 ± 1.8	0.18 ± 0.7	3 ± 3	0.4 ± 1.3	0.65 ± 1
Superoxidase (U/mg total protein)	0.2 ± 0.06	0.34 ± .6	0.15 ± 0.07	0.3 ± 0.2	0.2 ± 0.3	0.2 ± 0.1
Nitrate (μmol/μmol creatinine)	1.2 ± 0.3	1 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.73 ± 0.1	0.72 ± 0.09

[1] Geometric $\bar{x}$ ± SEM. For myeloperoxidase and superoxidase, the rate of change in absorbance at 525 and 655 nm, respectively, per minute was defined as 1 U. One milliliter of liquid stool was equal to ≈0.25 g stool. IL-1β, interleukin 1β; IL-2, interleukin 2; IFN-γ, interferon γ. In two-way ANOVA, no significant differences were observed between the 2 groups or within each group over time. Only time showed a significant effect on stool IL-1β concentrations ($P = 0.001$).

TABLE 4
Effect of zinc supplementation on mitogen-stimulated immune responses in patients with shigellosis[1]

	Zinc group ($n = 25$)	Control group ($n = 28$)
Stimulation index[2]		
Day 1	151 ± 24	112 ± 8
Day 7	176 ± 26	94 ± 19
Day 30	170 ± 42	117 ± 12
Interleukin 2 (ng/mL supernatant fluid)		
Day 1	0.27 ± 0.15	0.4 ± 0.3
Day 7	0.7 ± 0.3	0.5 ± 0.3
Day 30	1.0 ± 0.6	0.8 ± 0.4
Interferon γ (ng/mL supernatant fluid)		
Day 1	12 ± 3.4	15 ± 4
Day 7	28.9 ± 7	28.4 ± 8
Day 30	19 ± 6	26 ± 7

[1] Geometric $\bar{x}$ ± SEM. In two-way ANOVA, no significant interaction between zinc supplementation and time was observed. Only zinc supplementation showed a significant effect ($P = 0.002$) on stimulation index. No significant differences in interleukin 2 or interferon γ were observed between the 2 groups or within each group over time.

[2] Ratio of counts/min of phytohemagglutinin-stimulated cells to counts/min of unstimulated cells.

cutaneous delayed hypersensitivity reaction, the lymphocyte proliferation response to PHA, and the proportion of lymphocyte subsets, the cellular immune status of these children improved with zinc supplementation. In the present study, the 14-d course of zinc supplementation augmented the lymphocyte proliferation response to PHA. However, improvement of the functional aspect of lymphocytes, as indicated by secretion of cytokines in response to PHA, was not evident, although studies using in vitro cell systems have shown that extracellular zinc induces the production of the cytokines IL-1, IL-6, IFN-γ, and tumor necrosis factor α by monocytes (38–41) and of IL-2 and IFN-γ by T cells (41). There may be differences between in vivo and in vitro activation processes.

To determine the effect of zinc supplementation on *Shigella* antigen-specific immune responses, we looked at LPS- and Ipa-specific IgA and IgG antibodies in plasma and stool. A significant increase in the Ipa-specific IgG response from day 1 to day 30 in the zinc group in comparison with the increase in the control group suggests that zinc supplementation may have boosted the mounting of a recall-type memory response in the zinc group. In fact, the mounting of an immune response in children with shigellosis decreases when they are malnourished (4). In addition, compared with adults, pediatric patients with shigellosis have decreased and delayed adaptive immune responses (28), and thus zinc supplementation may have an additive effect in enhancing specific responses. A recent study on oral cholera vaccination of Bangladeshi children (49% had a serum zinc concentration ≤ 60 mg/dL) showed improvement in the immune response after zinc supplementation for 42 d (15). Another study reported a significantly enhanced IgG response to tetanus vaccine in elderly subjects after 1 mo of zinc supplementation (16). Only one study showed a direct correlation between decreased serum zinc concentrations and failure to respond to diphtheria vaccine (18). However, most vaccination studies done on zinc-deficient patients with prolonged zinc supplementation did not show any increase in antibody titer against the vaccine (42–45). Again, zinc supplementation of healthy adults without zinc deficiency significantly increased the serum vibriocidal response to an oral, killed, whole-cell cholera vaccine (46) and the lymphocyte response to mitogens (47), and the beneficial effect of zinc supplementation did not result from a correction of latent zinc deficiency. Animal studies showed that zinc deficiency adversely affects B lymphocyte development in bone marrow, in vitro antibody production, and isotype switching (23, 48–50) and that T-dependent antibody responses are more affected by zinc deficiency than are T-independent antibody responses (20). *Shigella* LPS is a T-independent antigen, whereas because Ipa is a protein, it is a T-dependent antigen. This may explain the effect of zinc supplementation on Ipa-specific responses in plasma.

In conclusion, the results of the present study show that 2 wk of supplementation with 20 mg elemental Zn during acute shigellosis enhances antigen-specific antibody and lymphocyte proliferation responses in the peripheral circulation. The data suggest that instead of prolonged supplementation, a short course of zinc supplementation as an adjunct therapy for common infectious diseases may be equally beneficial in boosting immune

TABLE 5
Effect of zinc supplementation on specific immune responses in patients with shigellosis[1]

Antigen-specific antibody titer	Zinc group ($n = 28$)			Control group ($n = 28$)		
	Day 1	Day 7	Day 30	Day 1	Day 7	Day 30
Plasma						
LPS-IgA	63.3 ± 14	108 ± 16	46 ± 15	61 ± 18	113 ± 20	58 ± 17
LPS-IgG	740 ± 134	997 ± 228	856 ± 280	875 ± 225	1283 ± 272	883 ± 202
Ipa-IgA	14 ± 1.4	12 ± 1.2	9.3 ± 0.8	15 ± 2	13 ± 4	11 ± 1
Ipa-IgG	80 ± 9[a]	117 ± 10[a]	204 ± 34[b]	83 ± 10[a]	97 ± 11[a]	161 ± 18[c]
Stool						
s-IgA	207.6 ± 47	375 ± 102	197 ± 72	184 ± 71	307 ± 69	242 ± 83
LPS-IgA	30.3 ± 4	40.6 ± 4	13.4 ± 5	26 ± 5	43 ± 3	17 ± 4.5
Ipa-IgA	0.57 ± 0.15	0.4 ± 0.12	0.42 ± 0.15	0.53 ± 0.16	0.37 ± 0.12	0.38 ± 0.08

[1] Geometric $\bar{x}$ ± SEM. Except for secretory immunoglobulin (Ig) A (s-IgA), antigen-specific responses are expressed as relative titers, which were defined as the optical density multiplied by the dilution factor of the sample. For s-IgA, values are expressed as μg/mL. LPS, lipopolysaccharide; Ipa, invasion plasmid-encoded antigen. In two-way ANOVA, a significant interaction between zinc supplementation and time was observed for Ipa-specific IgG titers in plasma ($P = 0.003$). Values in the same row with different superscript letters are significantly different, $P < 0.05$ (post hoc Tukey's test). Except for significant main effects of time on plasma LPS-IgA ($P = 0.012$) and stool s-IgA ($P = 0.026$), no other significant main effects or interactions were found.

responses. In underdeveloped countries where micronutrient deficiency is widely prevalent, short-term zinc supplementation may be a useful, practical, and cost-effective therapy from a public health perspective.

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