Vitamin A Modifies the Intestinal Chemokine and Cytokine Responses to Norovirus Infection in Mexican Children\textsuperscript{1,2}

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Abstract

Vitamin A supplementation is associated with divergent clinical norovirus (NoV) outcomes in Mexican children. Fecal cytokine concentrations following NoV genogroup infections among 127 Mexican children 5–15 mo old enrolled in a randomized, double-blind, placebo-controlled, vitamin A supplementation trial were determined to clarify the role the gut immune response plays in these associations. Stools collected from supplemented children (20,000 IU retinol (3.3 IU = 1 μg retinol) for children < 12 mo of age; 45,000 IU for children ≥ 12 mo) or children in the placebo group were screened for NoV genogroups I (GI) and II (GII). Monocyte chemoattractant protein-1 (MCP-1), TNF\textalpha, IL-5, IL-6, IL-8, IL-4, IFN\gamma, and IL-10 fecal concentrations were also determined. Differences in cytokine levels between the 2 groups following GI and GII infections were determined using ordered logistic regression models. MCP-1 and IL-8 levels were greater among GI- and GII-infected children, respectively, compared with uninfected children, whereas IL-5 levels were greater following both genogroup infections. MCP-1, IL-8, and IL-6 fecal levels were reduced among supplemented children with GII-associated diarrhea compared with the placebo group. Vitamin A–supplemented, GII-infected children had reduced MCP-1 and TNF\textalpha levels compared with GII-infected children in the placebo group (P\textsuperscript{interaction} = 0.02 and 0.03, respectively). Supplemented children with GI-associated diarrhea had higher TNF\textalpha and IL-4 levels compared with children in the placebo group with diarrhea (P\textsuperscript{interaction} = 0.02 and 0.02, respectively). The divergent effects of supplementation on NoV outcomes may result from the different effects vitamin A has on the genogroup-specific immune responses. J. Nutr. 141: 957–963, 2011.

Introduction

Vitamin A supplementation has been promoted as an important cost-effective intervention for improving childhood survival in developing countries after randomized clinical trials reported that supplementation reduces overall infant mortality, diarrhea-associated infant mortality, and the severity of diarrheal disease episodes (1–3). A number of unanswered questions still remain regarding the efficacy of vitamin A, because no clear effect of supplementation has been found on diarrheal disease morbidity in these trials (3–7). This lack of effect may relate to the fact that diarrheal disease is caused by a wide variety of bacterial, viral, and parasitic enteric pathogens. Each of these pathogens may induce a unique immune response that would influence disease pathogenesis and recovery. Vitamin A may partly determine the appropriateness of generated responses, because it differentially regulates the innate and adaptive immune responses (8–11). The simultaneous misclassification of diarrheal disease as an outcome and the failure to consider the pathogen-specific effect of vitamin A may bias findings of associations between vitamin A supplementation and diarrheal disease and therefore be responsible for previously reported inconsistent effects.

Norovirus (NoV)\textsuperscript{10}, which belongs to the Caliciviridae family, is now recognized as the leading cause of epidemic and endemic nonbacterial gastroenteritis. In industrialized countries, NoV may be responsible for 68–93% of nonbacterial gastroenteritis outbreaks (12–14). More importantly, studies in develop-

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\textsuperscript{10} Abbreviations used: GI, genogroup 1; GII, genogroup 2; MCP-1, monocyte chemoattractant protein-1; NoV, norovirus; Th, T helper.
oping countries have shown that NoV is a major cause of acute diarrhea in children (14--17). NoV can be divided into 5 genogroups (GI--GV), of which GI and GII cause the majority of infections in humans (18). Each genogroup is further divided into genotypes or genetic clusters (19). The T cell component of the immune response after challenge with NoV is weighted more toward a T helper type 1 (Th1) response, with increases in IFNγ and IL-2 production (20).

We have addressed the efficacy of vitamin A supplementation on NoV infections as part of a larger randomized and placebo-controlled, double-blind trial concerned with the pathogen-specific effects of supplementation in children from marginalized peri-urban communities of Mexico City, Mexico. We previously reported that vitamin A supplementation has divergent effects on overall and genogroup-specific NoV outcomes (21). We now want to determine whether the effect that vitamin A supplementation has on the NoV mucosal immune response may be the mechanism underlying and responsible for these divergent effects. We hypothesized, given the immune regulatory effects of vitamin A, that the reduced prevalence of specific NoV genogroups and increased resolution of NoV infection are associated with the upregulation of a humoral response. Accordingly, we report here the effect of vitamin A on the innate and adaptive cytokine responses following NoV infection and onset of diarrhea among the study children.

Materials and Methods

Study population. A census was carried out of all children < 2 y of age living within 9 neighborhoods (colonias) that are part of the community of La Magdalena Atlípcac, located along the eastern perimeter of Mexico City. Mothers of all children from 5 to 15 mo of age were invited to participate in the study, as described elsewhere (18,19). Children were excluded if they had diseases that caused immunosuppression or any congenital or acquired alteration of the digestive tract (such as chronic diarrhea) that could alter the absorption of micronutrients. Children who were taking vitamin supplements were also excluded. For the overall study, 200 children living in this community were identified and enrolled over a 10-mo period beginning in January 1998 after their parents consented to their participation.

Study design. On enrollment, each child was randomly assigned to receive vitamin A or a placebo by using a randomized sequence generated by an epidemiologist at the National Institute of Public Health, Mexico. Children < 12 mo of age in the vitamin A group were administered a solution containing 20,000 IU of retinol (3.3 IU = 1 μg retinol) at baseline and every 2 mo thereafter, and children ≥ 12 mo of age received a solution containing 45,000 IU of retinol. Testing and preparation of the placebo and vitamin A solutions were done by personnel at the National Institute of Nutrition to ensure similarity in taste, viscosity, and color to ensure that the field personnel and principal investigator were unaware of the regimens. The field team was in charge of administering the supplements and placebos to children from identical opaque plastic droplet bottles.

During the baseline visit, information was collected regarding the number of household members; their ages, education level, and income; household water sources and sanitation facilities; and household possessions. Information was also collected during the first visit to determine the child’s feeding and weaning patterns as well as occurrences of morbidity during the previous 2 wk. A previously validated questionnaire was used in the collection of these data by project personnel who had received training in its application.

Recruited children were then followed-up prospectively for up to 15 mo during which time households were visited twice a week. At each visit, mothers or caretakers of children were interviewed to determine the presence of the following symptoms: diarrhea, the number and consistency of evacuations, the presence of blood and mucus in stool, fever, cough, and respiratory difficulty. A stool sample was collected twice a month among healthy children, and up to 3 stool samples were collected during a week beginning with a diarrheal episode. No initial or final blood samples were taken from the study children, because parents did not consent to blood being drawn. Project supervisors accompanied project personnel for ~5% of all household visits to ensure quality of data collection. Children were referred to the study physician for diagnosis and treatment when fieldworkers or caregivers were concerned about the child’s health status.

Identification of NoV in stools. The analysis of the effect of vitamin A supplementation on NoV infection was restricted to children monitored during the summer months (June through August). A previous study by Ko et al. (22) suggested that NoV infections are an important cause of traveler’s diarrhea during the summer and so may also be an important cause of pediatric diarrheal episodes that peak during this season. Fresh stool samples collected from children during these months were placed in sealed test tubes on ice and then frozen within 4 h of their collection at ≤20°C.

NoV was detected by RT-PCR (22). Accordingly, viral RNA was extracted from 140 mL of a 10% stool suspension by a spin column technique in accordance with the instructions in the QIAamp Viral RNA Mini Kit handbook (QIAGEN). Viral RNA was assayed in a generic RT-PCR with 2 sets of primers, G1SKF/G1SKR and G2SKF/G2SKR, to detect the capsid region of NoV GI and GII, respectively (23). Viral RNA was reverse transcribed for 60 min at 42°C, followed by 15 min at 95°C to activate the Taq polymerase. Thermocycling conditions for PCR consisted of 40 cycles of 1 min at 94°C, 1 min at 40°C, and 1 min at 72°C, with a final extension for 10 min at 72°C. Amplified products were analyzed on ethidium bromide-stained 2% agarose gels. To prevent any possible cross-contamination, standard operating procedures were strictly adhered to for RT-PCR, as described elsewhere (17). All RT-PCR products positive for NoV were purified by use of the QiAquick PCR Purification kit (Qiagen) in accordance with the manufacturer’s protocol and were then sequenced on an ABI 3730XL sequencer by a commercial company (SeqWright). Stool samples were also screened for enteropathogenic Escherichia coli, enterotoxigenic E. coli, Shiga toxin-producing E. coli, enteroinvasive E. coli, and Giardia lamblia (24).

Determination of fecal cytokine concentrations. Fresh stools collected from children during the summer months were placed in sealed test tubes in ice and then frozen within 4 h after their collection at ~28°C. Samples were extracted by homogenization and centrifugation (15 min, 10,000 × g) at 48°C with PBS-containing protease inhibitors [benzenesulfonyl, pepstatinA, leupeptin, and aprotinin (1:10 wt:vol); Sigma]. The supernatants were collected, frozen, and stored at ~70°C until assayed for the chemokines monocyte chemoattractant protein-1 (MCP-1) and IL-8, the proinflammatory cytokines IL-6 and TNFα, the Th1 cytokine IFNγ, the Th2 cytokines IL-4 and IL-5, and the T regulatory cytokine IL-10 by an ELISA using paired ELISA-specific capture and biotinylated detecting antibody (Pierce-Endogen and R&D Systems). Peroxidases conjugated to streptavidin (Pierce-Endogen) were used to detect the capture of antibody; peroxidase activity was measured using ABTS substrate and read at a wavelength of 405 nm. Recombinant cytokines were used to generate a standard curve, and levels of these cytokines from the stool extracts were determined using the standard curve in 96-well plates according to the manufacturer’s protocol. Cytokine concentrations (ng/L) were normalized to stool protein concentration (g/L). The detection limit for these cytokine assays was 10 ng/L.

Study outcomes. The endpoints for this analysis were the levels of the chemokines and cytokines in children in the vitamin A and placebo groups following infections by GI and GII and following the onset of diarrheal episodes associated with these infections.

Data analysis. Stools collected from children followed during the summer months were used in this analysis. Data were entered in Visual Fox Pro 6.0 (Microsoft), verified, and checked for range and consistency. Because an important proportion of samples had no detectable levels of...
cytokines, conventional analytic techniques could not be used. Accordingly, we used ordered logistic regression analysis, which models the probability distributions of cytokine values categorized into 3 levels ordered from lowest to highest: undetectable, less than median of positives, and greater than median of positives. The inclusion of the vitamin A variable in the model tests the hypothesis that the probability distributions of categorized cytokine values in the supplemented group will be greater than distributions in the placebo group expressed as an OR.

The overall analyses of the modifying effect of vitamin A on the kinetics of the immune response have been carried out in 3 broad stages. We previously modeled the probability that categorical levels of MCP-1, IL-6, IL-8, TNFα, IFNγ, IL-4, IL-5, and IL-10 among children randomized to vitamin A were different from levels among children in the placebo group (25). For the present analysis, non-randomized comparisons of categorical levels of fecal chemokines and cytokines were first conducted on children stratified by the presence or absence of GI and GII infections and by the presence or absence of diarrheal symptoms associated with GI or GII infections. A GI or GII infection was defined as any period encompassing 1 or more stools that are positive for NoV1 or NoVII. Stools collected in the subsequent 3 wk following the positive stool were defined as those during which the immune responses to these infections were generated and so were used in these analyses. A diarrheal episode was defined as the mother reporting symptoms in the child and confirmed by the passage of ≥3 liquid stools in 1 d. A period of ≥3 symptom-free days was used to define the end of an episode.

Comparisons of chemokine and cytokine levels among children in the vitamin A and placebo groups were then carried out stratified by the presence or absence of infections by the GI or GII genogroups and by the presence or absence of diarrheal symptoms. An interaction term was included in these stratified analyses to test whether the effect of vitamin A was significantly different between strata. Significance was set at $P < 0.05$ and $< 0.1$ for interactions. Data were analyzed using the OLOGIT procedure in STATA (version 10) software.

The sample size for the overall study was calculated assuming that the study population had a diarrheal disease rate of 3 episodes/child each year and that the vitamin A supplement would reduce the incidence rate of diarrhea by ~20%. A sample size of 100/group was required to detect a 20% difference between the control and treatment group with a power of 80%, a 95% significance level, and an expected loss to follow-up of 20%. This calculation allowed for repeated measurements of the outcome and a correlation between measurements at different time points of 0.7 (26).

The study was approved by the ethical review committees from the National Centre for the Health of Infants and Adolescents of Mexico and the Harvard School of Public Health.

**Results**

Fecal cytokines and NoV infection were studied in a subsample of 127 children during the rainy summer months of June, July, and August. An additional 61 children were not enrolled until the drier fall season and thus were not included in the analysis. As previously reported, there were no differences in the distribution of socio-demographic characteristics of study children and households between children administered vitamin A and those in the placebo group (21). There was no access to piped water in 42–45% of households and 65% had no indoor toilets. Twenty-five to 30% of the children were stunted.

A total of 374 stool samples were collected from these 127 children, 193 samples from 70 children administered the placebo, and 181 samples from 57 children administered the vitamin A supplement. NoV was isolated from 114 (30.5%) of 374 stool samples collected during these summer months. GI and GII were found in 62 (54.4%) and 52 (45.6%) of the 114 positive samples, respectively. Approximately 11% of the study children during the summer months had sequential infections by different GI and GII strains. Thirty-seven (33.0%) of the overall 114 NoV infections 22 (35.5%) of 62 GI infections and 12 (23.1%) or 52 GII infections were associated with diarrheal episodes.

The proportion of samples that had detectable cytokine levels in stool ranged from 42% for IL-6 to 53% for IL-4. Using ordered logistic regression models, children with GI and GII infections had an increased probability of having higher levels of the chemokines MCP-1 and IL-8 in their stool compared with children without infections (Table 1). Children infected with either GI or GII had increased probabilities of higher fecal IL-5 levels compared with children without infections.

In contrast, children with GI-associated diarrhea had reduced odds of higher fecal levels of the chemokines MCP-1 and IL-8 compared with children without GI-associated diarrhea (Table 2). Children with GI-associated diarrhea had

### Table 1: Fecal chemokine and cytokine levels in Mexican children with NoV GI and GII infections and in uninfected children

<table>
<thead>
<tr>
<th>Chemokine or cytokine</th>
<th>Stools collected,(^1) n/children, n</th>
<th>Not detectable,(^2) %</th>
<th>Concentration,(^3) pg/mg protein</th>
<th>OR (95% CI)(^4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCP-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No NoV GI infection</td>
<td>241/108</td>
<td>38.5</td>
<td>198 ± 582</td>
<td>2.12 (1.05–4.30)</td>
<td>0.03</td>
</tr>
<tr>
<td>NoV GI infection</td>
<td>88/43</td>
<td>35.2</td>
<td>243 ± 978</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No NoV GII infection</td>
<td>290/109</td>
<td>45.9</td>
<td>74 ± 207</td>
<td>2.25 (1.00–5.07)</td>
<td>0.04</td>
</tr>
<tr>
<td>NoV GII infection</td>
<td>69/33</td>
<td>44.5</td>
<td>203 ± 875</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL-5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No NoV GI infection</td>
<td>263/108</td>
<td>78.7</td>
<td>70.2 ± 211</td>
<td>2.85 (1.16–7.00)</td>
<td>0.02</td>
</tr>
<tr>
<td>NoV GI infection</td>
<td>95/43</td>
<td>65.3</td>
<td>133 ± 310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No NoV GII infection</td>
<td>291/109</td>
<td>76.6</td>
<td>62.6 ± 263</td>
<td>2.32 (1.07–5.00)</td>
<td>0.03</td>
</tr>
<tr>
<td>NoV GII infection</td>
<td>67/33</td>
<td>68.7</td>
<td>73.9 ± 277</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Number of stools screened for chemokines and cytokines and number of children from which they were collected. Stools were collected during the 3-wk period following NoV infection.

\(^2\) Percentage of stools that had no detectable chemokine or cytokine concentrations.

\(^3\) Values are median ± IQR.

\(^4\) OR calculated using ordered logistic models represents the odds that chemokine or cytokine (categorized into 3 levels: nondetectable, less than median, greater than median) will have different values among children with NoV infections. Treatment arm and presence or absence of diarrheal episode included in model along with age of child.

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TABLE 2  Fecal chemokine and cytokine levels in Mexican children with NoV GII-associated diarrhea and in children without diarrhea

<table>
<thead>
<tr>
<th>Chemokine or cytokine</th>
<th>Stools collected, n/children</th>
<th>Not detectable, %</th>
<th>Concentration, pg/mg protein</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No GII-associated diarrhea</td>
<td>302/114</td>
<td>35.4</td>
<td>303 ± 800</td>
<td>0.27 (0.07–1.02)</td>
<td>0.05</td>
</tr>
<tr>
<td>GII-associated diarrhea</td>
<td>30/11</td>
<td>36.7</td>
<td>265 ± 924</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No GII-associated diarrhea</td>
<td>305/114</td>
<td>60.3</td>
<td>73.6 ± 207</td>
<td>0.18 (0.03–1.00)</td>
<td>0.05</td>
</tr>
<tr>
<td>GII-associated diarrhea</td>
<td>31/11</td>
<td>74.2</td>
<td>58.9 ± 588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No GII-associated diarrhea</td>
<td>328/114</td>
<td>44.5</td>
<td>92.8 ± 266</td>
<td>0.24 (0.07–0.84)</td>
<td>0.02</td>
</tr>
<tr>
<td>GII-associated diarrhea</td>
<td>35/11</td>
<td>60.0</td>
<td>69.1 ± 588</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Number of stools screened for chemokines and cytokines and number of children from which they were collected. Stools were collected during the 3-wk period following GII-associated diarrhea.
2 Percentage of stools that had no detectable chemokine or cytokine concentrations.
3 Values are median ± IQR.
4 OR calculated using ordered logistic models represents the odds that chemokine or cytokine (categorized into 3 levels: nondetectable, less than median, greater than median) will have different values among children with GII-associated diarrhea. Treatment arm and age of child included in model.

similar decreases in the odds of having higher levels of the inflammatory cytokine IL-6 in stool compared with children without GII diarrhea. No significant differences were found in chemokine or cytokine levels in stool between children with and without GII-associated diarrheal episodes.

The probability of having higher MCP-1 levels in stool was reduced among GII-infected children supplemented with vitamin A compared with uninfected children (Table 3). These reductions differed significantly from the impact vitamin A had on this probability among supplemented children not infected with GII (P-interaction = 0.02). The odds for greater fecal TNFα levels among vitamin A-supplemented, GII-infected children were not significantly different from that of infected children in the placebo group. This nonsignificant (P = 0.13) reduction in odds among the supplemented, infected children, however, was significantly different from the increased probability of higher TNFα levels among supplemented children who were not infected (P-interaction = 0.03). Vitamin A supplementation was not associated with significant differences in fecal cytokine levels following GII infections.

The probability of higher fecal TNFα concentrations increased among vitamin A-supplemented children with GII-associated diarrhea compared with children in the placebo group (P = 0.02) (Table 4). These probabilities differed from the associations among supplemented children who did not have NoV diarrhea (P-interaction = 0.02). Vitamin A-supplemented children with GII-associated diarrhea tended to have higher fecal IL-4 levels compared with children in the placebo group (P = 0.02) (Table 4). In contrast, supplementation was associated with nonsignificant reductions in the probability of higher IL-4 concentrations among children who did not have GII-associated diarrhea (P = 0.09). The differences between probabilities of increased IL-4 levels were significantly different between children with and without GII-associated diarrhea (P-interaction = 0.02). Fecal cytokine levels did not differ between vitamin A-supplemented children and children in the placebo group following GII-associated diarrhea.

Discussion

This study provides unique information about the intestinal cytokine responses following GI and GII infections among young children enrolled in a community-based, randomized clinical trial and how these responses may be modified by vitamin A. Increased fecal levels of the chemokines MCP-1 and IL-8 were found following GI and GII infections, respectively, whereas increased levels of IL-5 were found following infections by both genogroups. In contrast, children had reduced levels of the inflammatory cytokine IL-6 and reduced chemokine levels following GII-associated diarrhea. Vitamin A-supplemented

TABLE 3  Effect of vitamin A supplementation on fecal chemokine and cytokine levels in Mexican children following NoV GII infections

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Innate immune response</th>
<th>Vitamin A</th>
<th>Placebo</th>
<th>OR (95% CI)</th>
<th>P-value</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>No GII infection</td>
<td>122/46</td>
<td>143/63</td>
<td>0.85 (0.53–1.34)</td>
<td>0.48</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>GII infection</td>
<td>25/12</td>
<td>39/21</td>
<td>0.11 (0.02–0.51)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>No GII infection</td>
<td>118/46</td>
<td>150/63</td>
<td>1.40 (0.89–2.2)</td>
<td>0.14</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>GII infection</td>
<td>21/12</td>
<td>38/21</td>
<td>0.32 (0.07–1.41)</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

1 The OR calculated using ordered logistic models represents odds that a cytokine (categorized into 3 levels: undetectable, less than median, greater than median) will have a higher value among vitamin A-supplemented children.
2 P-interaction indicate whether the effect of vitamin A was significantly different between strata.
3 Stools were collected during the 3-wk period following NoV infection.
children with GII infections had reduced fecal MCP-1 and TNFα responses but greater TNFα and IL-4 responses following GI-associated diarrhea. These findings suggest that the effect of vitamin A supplementation on the mucosal immune response following NoV infections is dependent on the genogroup infecting the child and the resulting pathological processes induced by each.

Previous studies have reported a clear Th1 cytokine response following NoV infections or NoV challenge. Tacket et al. (27), e.g., reported that peripheral blood mononuclear cells from individuals challenged with Norwalk GI virus-like particles had increased secretion of IFNγ and IL-2. Significant increases in IFNγ and IL-2 have also been found in serum among volunteers challenged with Snow Mountain Virus, which is a GII reference strain, or in fecal samples from travelers with NoV gastroenteritis (20,28). However, Lindesmith et al. (20) reported no significant increase of IFNγ secretion by peripheral blood mononuclear cells from Snow Mountain Virus-challenged individuals when restimulated with a GI Norwalk strain but did find a significant increase in the secretion of IL-5. The similar cytokine response patterns found in our study may have resulted from sequential infections by different GI and GII strains, because ~11% of the study children had such multiple exposures.

Previous studies have not determined MCP-1 and IL-8 responses following GI and GII infections, which may be compared with our findings. Chachu et al. (29) recently reported that effective immunity against NoV requires a broad immune response involving CD4+ T cells, CD8+ T cells, and B cells. MCP-1 and IL-8 may be part of this broader immune response. MCP-1 is principally involved in the attraction of CD4+ and CD8+ lymphocytes as well as monocytes, inducing the latter t release arachidonic acid and cytokines (30–33). It also induces the maturation of dendritic cells that are involved in the activation of these T-cell populations (34,35). IL-8, a chemokine produced by macrophages and other cell types such as epithelial cells that induces chemotaxis of neutrophil granulocytes and is involved in local inflammation, may also be part of this broader immune response to NoV (36).

We have found that fecal chemokine and inflammatory cytokine levels are reduced in the 3-wk period following a GII-associated diarrheal episode. These findings are partly consistent with reports that IL-6 responses in the gut are low following NoV infections (20,37). However, they are not consistent with findings that IFNγ and IL-12 levels, reflecting a strong Th1 response, are elevated in travelers with NoV diarrhea and among pigs with diarrhea induced by the human NoV GII.4 strain (28,37). Further community studies are needed to address the intestinal response of these chemokines and cytokines following NoV infections in a setting where children have sequential infections by different genotypic NoV strains or other, different types of diarrheal pathogens.

There are no comparable studies to our knowledge that have determined the effect of vitamin A on the gut or systemic cytokine responses following NoV infections among children or in animal models. Bai et al. (38) has reported that lamina propria mononuclear cells from retinoic acid-treated mice produced lower levels of TNFα similar to the reduced levels of this cytokine we found following GII infections. We previously reported that vitamin A-supplemented children had reduced overall MCP-1 levels in these same samples compared with MCP-1 levels among children in the placebo group (39). The reduced probability of higher levels of MCP-1 among supplemented children following GII infections reported here may possibly explain the overall effect. These findings suggest that vitamin A may be reducing the inflammatory response in the gut of children following infections by the GII genotype.

These responses contrast with the higher fecal levels of TNFα as well as higher IL-4 levels found among vitamin A-supplemented children following GI diarrhea. An upregulated Th2 response with increased production of IL-4 has been reported among retinoic-acid-treated mice (8,40,41). We previously reported higher IL-4 fecal levels among supplemented children with diarrhea during this period similar in magnitude to the increase found following GI diarrhea (23). As a result, it is most likely that the increased probability of higher levels of IL-4 among supplemented children following GI diarrhea infections reported here may possibly explain the overall effect.

The association of vitamin A supplementation with quite different cytokine responses following GI or GII infections and diarrhea may be due to the qualitatively different responses that are generated by each of these genogroups. There is evidence that IgG and cytokine responses to one GII strain cross-react with other GII strains but not to GI strains (42–44). The qualitatively distinct immune responses to each strain may be a result of distinct abilities of these different strains to bind to the histoblood group antigens. The generation of these NoV strain-specific responses may then be differentially regulated by vitamin A and so underlie the genogroup-specific effect of vitamin A supplementation on the cytokine response found in our study.

There are several limitations of the study that need to be addressed. One limitation is that we have no indicators of the...
children’s vitamin A status, because no blood samples were collected from children. Differences in the initial status of study children could determine the effectiveness of supplementation. A probabilistic national survey conducted in Mexico has shown that overt and subclinical vitamin A deficiency among children living in Mexico City is minimal but that low levels of serum retinol are prevalent (49). A second limitation may have been that the study was not sufficiently powered to address the genogroup-specific effect of vitamin A supplementation. The distinct NoV genotype effects associated with vitamin A supplementation suggests that this may not be such an important issue. It is also important to acknowledge that these associations may not represent the development of a protective immune response in the gut. They may reflect the development of an inadequate response that can lead to prolonged or more severe infection.

We have found that the fecal cytokine response patterns following NoV infections and the effect vitamin A has on these responses varies by specific NoV genogroup outcomes. These results further confirm that the regulatory effect of vitamin A on the gut mucosal immune response is conditioned by whether a child is infected by a pathogen, the type of pathogen infecting the child, and the onset of pathology. This complexity may explain the inconsistent effects of vitamin A on different health outcomes reported in previous studies (5,7,45,46). These findings are suggestive at this point; thus, further work is required to confirm that vitamin A has this differential effect on the immune response. Such confirmation could then lead to the design of vitamin A supplementation programs that target groups of children who would benefit most from such supplementation using household and community markers of infection.

Acknowledgments
We thank Matilde Juarez of the Clinical Microbiological Laboratory, Hospital La Perla, Secretary of Health, Cda Netzhahuaycoytli, for her assistance in the laboratory. K.Z.L., J.L.R., and J.L.R. were involved in the design of the project, procurement of funds, and project supervision; C.G. and G.P.K. were involved in the isolation and characterization of NoV in stools; N.N. determined fecal cytokine concentrations; K.Z.L. and A.A.M. were involved in the data analysis; K.Z.L. was responsible for the preparation of the article with input from J.L.S., J.L.R., C.G., H.L.D., and G.P.K. All authors read and approved the final manuscript.

Literature Cited


