Human Milk Vacuolating Cytotoxin A Immunoglobulin A Antibodies Modify Helicobacter pylori Infection in Gambian Children

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We collected data, including the weights, urea breath test results, and presence of maternal milk cytotoxin-associated gene–specific and vacuolating cytotoxin A–specific immunoglobulin A monthly from 48 mothers and infants (to 44 weeks of age) in The Gambia. In all, 11 children (23%) had negative urea breath test results, and 37 (77%) had positive results. Weight loss associated with Helicobacter pylori colonization was restricted to children whose mothers did not produce anti–vacuolating cytotoxin A antibodies in their milk (P = .028, by t test).

Throughout infancy, children from developing countries are challenged by a range of intestinal pathogens. Initial protection from the harmful effects of these is offered through nonimmune defenses and transferred maternal factors. We have shown that maternal milk IgA antibodies directed against bacterial urease delays Helicobacter pylori colonization among infants in The Gambia [1], a population among whom early colonization is associated with enteropathy and stunted growth [2]. Whether H. pylori causes this enteropathy is unknown; a transient gastric hypochlorhydria induced by H. pylori may predispose infants to recurrent enteric infections [3], but other mechanisms may be involved.

Among H. pylori products associated with virulence, 2 in particular have excited considerable research. These are the vacuolating cytotoxin A (VacA) and the product of the cytotoxin-associated gene (CagA). Both could induce epithelial damage and thus initiate the development of enteropathy in Gambian infants. Type 1 signal sequence (s1m-2 alleles) of VacA have been demonstrated to have enterotoxigenic properties in vitro and in animal models [4–6]. CagA mediates epithelial damage by acting as a type IV secretion system and mediating phosphorylation of a wide number of intracellular signaling proteins via an Src phosphatase [7–9]. We sought evidence that maternal milk antibodies directed against CagA and VacA in Gambian mothers could influence the growth of children exposed to H. pylori.

Methods. We used samples collected in a previously reported cohort study [1], which took place at the Medical Research Council Research Station at Keneba, The Gambia, in a village of rural subsistence farmers. All infants born during a 15-month period and their mothers were recruited, subject to informed parental consent obtained by local field workers. Study protocols were approved by the Joint Medical Research Council and Gambian Government Ethical Committee, as well as by a meeting of village elders. Eighty-one infants were born from August 1993 to October 1994; of these, 65 were included in the study. All mothers breast-fed their infants throughout the study. Each month, mothers collected a 5-mL sample of milk from each breast by manual expression before feeding their children. In addition, 0.5-mL capillary blood samples were obtained from the mothers 1 time. From the age of 12 weeks, 13C-urea breath tests were performed on the children at 4-week intervals, according to a previously described and validated protocol [10]. The weight of each infant was recorded at the time each breath test was performed (3 separate measurements were obtained to check reproducibility on regularly calibrated electronic scales [Seca]). These data were expressed as weight-for-age z scores, compared with the National Centre for Health Statistics standards.

Milk samples were frozen within 1 h of collection, stored at −20°C, and transported in a frozen state to the United Kingdom for analysis. Prior to performance of the assay, the aqueous phase was separated from cellular debris and the fatty layer, then absorbed at 4°C overnight against monoclonal antibodies to Lewis b antigen (Alpha Laboratories) and crude Campylobacter jejuni antigen. Absorbed milks were used in the assays at a final concentration of 1:200. Recombinant CagA and VacA antibodies, which have been characterized and described else-

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where [11, 12], were coated onto assay plates in carbonatebicarbonate buffer at a concentration of 5 μg of protein per mL. Assaying of specific milk IgA and specific serum IgG levels in mothers were performed, as described elsewhere [1, 13]. Results were expressed as optical density ratios, allowing comparison of continuous variables, or where appropriate, conversion to binary values, as described below.

Simple regression was used to compare continuous variables. Unpaired t tests were used to assess differences in mean values between normally distributed groups of results, and χ² tests were used to assess relationships between categorical variables. All analyses were performed using SPSS software, version 11.0 (SPSS).

Results. Only limited volumes of milk samples remained after previous analyses, which had demonstrated that, although levels of specific milk antibodies do vary throughout lactation, changes in individual mother’s antibody levels during the first 12 weeks of lactation were small [1, 14]. We, therefore, pooled milk samples of individual mothers from weeks 4–12, producing sufficient volumes of samples to include 48 (74%) of 65 children in this study. Of these, 11 (23%) had sequential negative urea breath test results from 12 to 48 weeks. We classified these infants as not having been exposed to H. pylori; the remaining 37 (77%) had ≥1 positive breath test result and were classified as having been exposed to H. pylori. Further details of urea breath test results in this cohort of children are described elsewhere [10, 15].

All 48 mothers were H. pylori IgG seropositive. Maternal milks produced a range of IgA reactivities against CagA in an essentially normal distribution with a mean (± SD) optical density ratio of 0.39 ± 0.11 (figure 1). There were no differences in maternal milk CagA antibody levels between the 11 children not exposed to H. pylori (mean optical density ratio, 0.39 ± 0.11) and the remaining 37 (mean optical density ratio, 0.38 ± 0.03).

Maternal milk anti-VacA antibodies, however, were distributed in 2 clusters (figure 1). One cluster (23 infants) was tightly distributed around the blank optical density ratio of 0.3, and a second cluster (25 infants) exhibited a range of reactivities, approximately corresponding to a positively skewed normal distribution. On the basis of this apparent bimodal distribution, we classified these 2 clusters as VacA antibody negative and VacA antibody positive, respectively. Only 1 (9%) of the 11 children not exposed to H. pylori came from a mother whose milk was VacA antibody positive (P = .005, by χ² test).

At 44 weeks of age, girls’ weights ranged from 5.49 kg to 10.13 kg (mean weight-for-age, 9.0 kg; z score = 0.95 kg). Boys’ weights ranged from 5.77 kg to 9.66 kg (mean weight-for-age, 9.6 kg; z score = 1.0 kg). The level of CagA antibodies in maternal milk was not related to the change in infant weight-for-age z score between 4 and 44 weeks of age (R² = 0.14; P = .365). There was, however, an association between increased infant weight gain and the presence of maternal milk VacA antibodies (P = .028, by t test; figure 2). Weight loss associated with early H. pylori colonization in this study (amounting to >0.5 z scores of difference in mean values, compared with noncolonized peers; ~0.5 kg at this age) was restricted to children whose mothers did not produce anti-VacA antibodies in their milk (figure 2).

Discussion. VacA-specific (but not CagA-specific) IgA antibodies in maternal milk significantly reduced the often-observed reduction in infant growth in Gambian children colonized with H. pylori. Children exposed to H. pylori by mothers without anti-VacA antibodies in their milk showed a decrease in weight gain between 4 and 44 weeks of age, compared with their peers, corresponding to a clinically relevant increased risk of morbidity and mortality from all causes among this population. These data extend our previous findings on the clinical effects of specific human milk antibodies on H. pylori infection among infants. We have already demonstrated that specific antiurease milk IgA delays H. pylori colonization among infants [1]; there is no evidence that either VacA-specific or CagA-specific antibodies in human milk do likewise. Indeed, we predominantly detected anti-VacA IgA in the mothers of children exposed to H. pylori. Although it is possible that these antibodies predispose infants to colonization, it is more likely that the mothers experience an increase in specific antibody after their infants became colonized with H. pylori, as was also observed with milk antibodies against recombinant urease [1].
This implies that primed mothers undergo increased enteral antigen exposure following infant colonization, potentially from the newly colonized infant, with entero-mammary circulation leading to increased excretion of specific milk antibodies.

We have not detected any clinical effects of anti–CagA IgA in human milk, but anti–VacA IgA may ameliorate the growth stunting associated with early H. pylori colonization of infants in several Gambian studies [2, 3]. It is, therefore, possible that VacA plays a role in the development of the enteropathy characteristically associated with this growth failure. Although VacA is produced by H. pylori in the stomach, it may exert an effect upon more distal regions of the gut. In vitro studies suggest that VacA needs to be activated within an acidic pH, and transfer of toxin to the host epithelium requires contact-dependent mechanisms [4]. In both gastric and small bowel epithelia, VacA produces acidic ballooning of the endoplasmic reticulum and permeabilization of cell membrane in a manner independent of the cytopathic effects observed [5]. Dogs, however, have developed secretory diarrhea when given large enteral doses of VacA [16]. Extreme purgative diarrhea has been shown to increase shedding of infectious H. pylori [17], and it is possible that the development of enteropathy with secretory diarrhea may increase fecal and/or oral transmission of the bacterium among infants in this population.

Our data suggests that VacA may play an important role in disease expression—and possibly facilitate transmission—during colonization before infants develop their own specific anti-VacA antibodies. Throughout this vulnerable period, passive immunity to H. pylori may be particularly advantageous to infants. It is, therefore, likely that the ability of Gambian mothers to produce specific milk antibodies directed against key bacterial virulence determinants, such as VacA, is of major importance in enabling their babies to gain maximum benefit from feeding during the first few months of life.

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References


Figure 2. Change in weight-for-age z-score between 4 and 44 weeks of age among children exposed to Helicobacter pylori whose mothers had negative vacuolating cytotoxin A (VacA) antibody test results, children exposed to H. pylori whose mothers had positive VacA antibody test results, and children who were not exposed to H. pylori. UBT, urea breath test.