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ASSOCIATION OF HUMAN MILK SIgA ANTIBODIES WITH MATERNAL INTESTINAL EXPOSURE TO MICROBIAL ANTIGENS

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INTRODUCTION

Human colostrum S-IgA and mature breast milk contain large quantities of secretory IgA (S-IgA)[1-3]. In general, the concentration of milk S-IgA is higher in the early postpartum period; than in the later stages of lactation[3-5]. The content of S-IgA is the result of the additive presence of a great variety of specific S-IgA antibodies that are formed locally in the mammary gland by lymphocytes that had been primed at the intestinal level by microbial and food antigens, and that migrated to the mammary gland under the influence of hormones[6-8]. In such a way, antibodies directed against *Shigella, Salmonella* and *Escherichia coli* somatic antigens, *Vibrio cholera* and *E. coli* toxins, rota- and polioviruses, and cow milk, black beans and soy beans proteins, have been detected in human colostrum and milk samples obtained in different ecosystems[1,9-14]. The ingestion of human milk containing high levels of specific antibodies has been shown to be associated with protection against diarrheal illnesses[15,16] and food allergies[17] in the breast-fed infants. Nevertheless, prospective studies have demonstrated that the presence and levels of specific S-IgA antibodies in milk do not remain constant during lactation[18,19], suggesting that its protective capacity also varies over time.

Based on observations that oral ingestion of antigens induces a temporary decrease in pre-existing, specific homologous S-IgA milk antibodies[20,21], we have suggested that intestinal infections in lactating women may induce a transient diminution in the concentration of S-IgA antibodies in milk. To explore this hypothesis, we have conducted a prospective longitudinal study among mother-infant pairs in a rural community in Guatemala. Here we present a summary of our initial findings.

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MATERIALS AND METHODS

Population

The mothers were recruited in Santa María de Jesús, a rural community 50 km from Guatemala City[22]. This traditional society is mainly formed by Maya-Cackchiquel natives among whom breastfeeding is almost universally practiced. For the purpose of this report, 15 mothers were studied. Their ages ranged from 19 to 39 years (mean = 28.3; SD = 6.1 years) at the time of delivery. They were enrolled in the program 5-7 days post-partum and we visited their homes every week. Fecal and milk samples were collected routinely every two to three weeks and, in addition, whenever an episode of diarrhea was detected among the women or their children.

Laboratory Procedures

Fecal specimens were collected in paper cans and processed as described[23]. Detection of *Shigella* spp., *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, *Plesiomonas shigelloides* and enteropathogenic, enterotoxigenic and adherent *E. coli*, as well as rotaviruses, *Giardia lamblia* and *Cryptosporidium* was done following standardized procedures detailed elsewhere[23]. To detect specific IgA antibodies in milk, *Shigella* spp. lipopolysaccharides were prepared by the method described by Cáceres *et al.*[24].from the homologous bacterial strains, and *Giardia* antigen was obtained from cultured trophozoites of the Portland strain[25]. All antibodies were detected by means of the enzyme-linked immunosorbent assay, as described[19], using alkaline phosphatase-labeled anti-human IgA antiserum (Tago, Inc., Burlingame, CA, USA).

RESULTS

Intestinal Infections in the Women

Five women were shown to shed *Giardia lamblia* cysts during the observation period. In three of them, the excretion of *Giardia* cysts was short-lived (1-2 weeks), one subject had positive samples taken 6 weeks apart, and one individual shed *Giardia* for at least 4 months. None of these infections were associated with diarrhea. *Shigellae* were found in 6 of the 13 women in which the *Shigellae* studies were complete (fecal culture and anti-*Shigella* antibodies). Two women had two different infections by *Shigellae*. In total, we isolated *Shigella flexneri* 2 from three cases, *Shigellae flexneri* 6 from four cases, *Shigella boydii* 4 from one and *Shigella boydii* 10 from one woman. Only one infection due to *Shigella flexneri* 2 resulted in diarrhea.

Specific IgA Antibodies in Milk

Anti-*Giardia* antibodies were detected in 10 (66.7%) of the 15 women. The antibody levels varied between 1:2 and 1:64. The women who were lacto-negative at the beginning remained negative for the duration of the follow-up. Of those who had antibodies in the milk samples taken at the start of the study, one had an increase ($\geq$ 4-fold difference), eight had a decrease ($\geq$ 4-fold difference or from positive to negative) and in one case the levels remained unchanged. In regard to anti-*Shigella* antibodies, only three (23%) women were lacto-negative. Among these three cases, we documented an increase in antibody titers in one instance; the levels did not change in the two remaining ones. Among the lacto-positive cases, in which the titers varied between 1:8

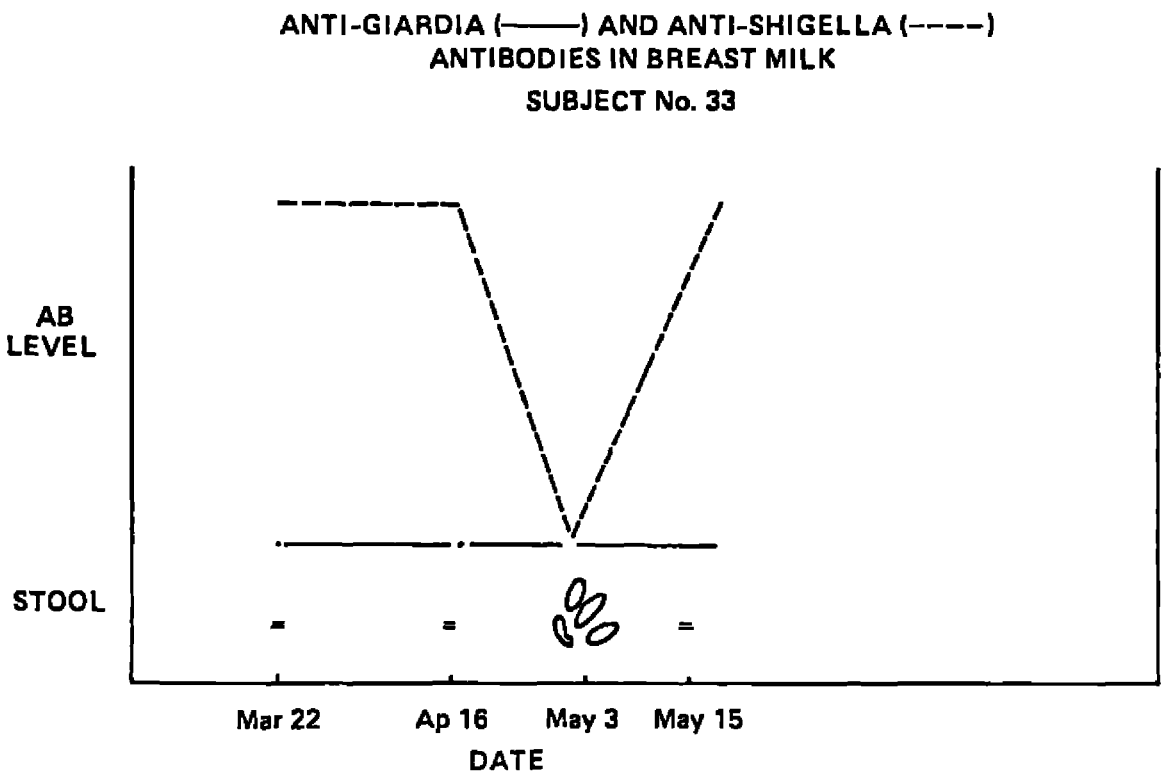

Figure 1. Association of milk antibody levels and detection of *Shigella* spp. in stool samples[a] from a woman.

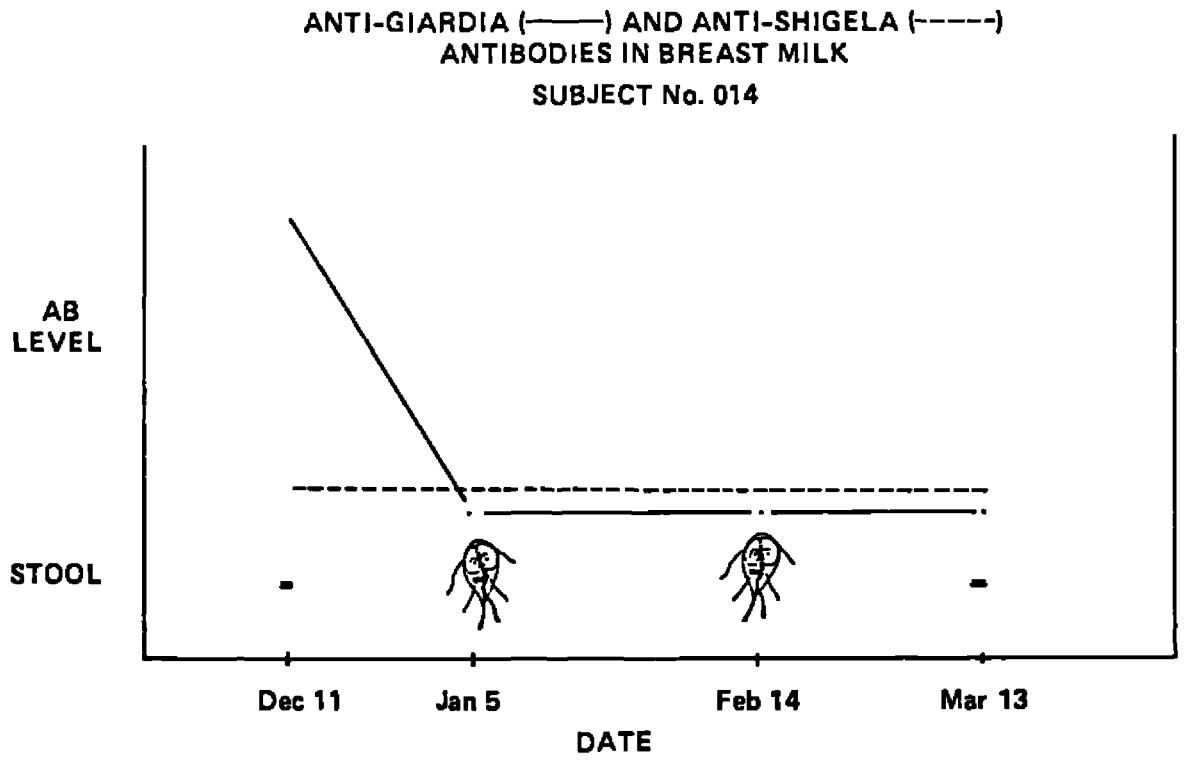

Figure 2. Association of milk antibody levels and detection of *Giardia* cysts in stool samples from a woman. Milk and stool samples were collected concomitantly on the dates shown in the horizontal axis.

and 1:256, we observed a fall in antibody levels in 7 instances (6 individuals); there were 4 women whose titers remained constant.

Association of Changes in Milk IgA Antibody Levels with Intestinal Infections

Five of the eight documented decreases in anti-*Giardia* IgA antibodies followed detection of *Giardia* cysts in the fecal samples obtained from the women. Fig. 1 depicts one case. Three falls and one increase in anti-*Giardia* antibodies were not associated with documented excretion of *Giardia* parasites. Likewise, five of the decreases in anti-*Shigella* antibodies followed infections by *Shigella* spp. (Fig. 2); lacto-conversion was seen after excretion of *Shigella*. In two cases in which infection was not documented, antibody falls were observed. In total, among 11 proven infections, a fall in antibody levels was seen in 10 cases (91%); only 5 (31%) cases of decreased titers were seen among non-infected individuals (p = 0.0047, Fisher exact test, Table 1).

Table 1. Changes in Anti-*Giardia* and Anti-*Shigella* IgA Milk Antibody Levels in Relation to Intestinal Infections in the Mother

		Decrease in antibody levels[a]		
		Present	Absent	Total
Intestinal infection documented	Yes	10[b]	1	11
	No	5	11	16
	Total	15	12	27

[a]number of individuals
[b]odds-ratio: 22; 95% CI: 1.89 - 1038.72 p=0.004 (Fisher exact test).

DISCUSSION

IgA-committed lymphocytes that are primed in the intestine migrate to the mammary gland, where they produce IgA dimers that, after being coupled with secretory component, are secreted into milk[6-8]. The ingestion by the breast-fed infant of such antibodies in high titers has been proven to be associated with protection against diarrheal disease caused by *V. cholera* and enterotoxigenic *E. coli*[15,16] and against cow milk-induced allergy[17]. These studies clearly underscore the importance of the presence and high levels of specific antibodies in human milk for breastfeeding to be protective against intestinal pathologies.

On the other hand, diarrheal illnesses are very common among children of rural areas of the developing world, where breastfeeding is practiced almost universally for prolonged periods[26,27]. These observations prompted some authors to put forward the hypothesis that underprivileged women, who commonly show nutritional deficits, have an impairment in their capacity to produce milk in adequate quantity and/or immunological quality[28]. Several studies have not been able to document deficiencies either in the volume of milk produced by underprivileged mothers or in its content of S-IgA[5,29,30]. Nevertheless, prospective longitudinal observations both in developed and developing communities have shown that the levels of

specific antibodies in milk fluctuate over time, independently of the total concentration of S-IgA and of other specific antibodies[18,19]. These changes in antibody levels do not follow specific patterns and seem to be of greater magnitude and more common among women who live in highly contaminated areas than among women of urban, more hygienic, environments. These findings, coupled with the fact that oral immunization with either live poliovirus[20] or with a vegetable protein extract[21] results in the temporary decrease of pre-existent antibodies, motivated us to suggest that intestinal infections (be they asymptomatic or symptomatic) in the lactating woman induce a drop in the content of homologous milk IgA antibodies.

The results presented here support our hypothesis. Documented infections by *Giardia lamblia* and different *Shigella* spp. in lacto-positive women resulted in decreases in antibody levels in 91% of the cases. The magnitude and duration of the observed changes varied widely among the individuals, suggesting that factors such as duration of the infections, infectious dose of the organisms, and even type of microbial agent, may play a role in determining the behavior of milk antibodies after a given infection. It is necessary to note that decreases in the levels of anti-*Giardia* and anti-*Shigella* antibodies were also observed in the absence of documented fecal excretion of these microorganisms. It is possible that our laboratory methodology to detect *Giardia* and *Shigellae* in fecal material is not 100% sensitive, especially when examining samples collected from healthy individuals. It is also very likely that other factors, different from infectious processes, influence the behavior and/or transit of IgA-commited lymphocytes that under normal conditions are programmed to migrate to the lactating mammary gland tissue.

We consider it of importance to extend these studies to a larger number of individuals and to a greater variety of diarrheagenic agents such as rotavirus, toxigenic *E. coli* and *Campylobacter*, that are among the most common pathogens associated with gastroenteritis in our populations. Of greater importance, however, is to explore the implications for the health of the breast-fed infant of these changes in milk antibody content. With the present knowledge, however, it is valid to recommend that for the protective role of breastfeeding to be expressed in its maximum capacity, efforts must be made to reduce the risk of intestinal infections in the lactating mother.

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CONCLUSIONS

Intestinal infections by *Giardia lamblia* and *Shigella* spp. in the lactating woman induce a temporary decrease in S-IgA specific homologous antibodies

found in breast milk. Other non-infectious factors may also be associated with changes in S-IgA antibody concentrations in human milk.

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