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Studies of Human Milk

II. *Concentration of Antibodies against Salmonella and Shigella in Milk of Women from Different Populations and the Daily Intake by their Breast-fed Infants*

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ABSTRACT. Cruz, J. R., Carlsson B. V. M., Hofvander Y., Holme D. T. and Hanson, L. Å. (Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, Department of Clinical Immunology, University of Göteborg, the Institute of Nutrition, University of Uppsala, Uppsala and the Department of Bacteriology, Karolinska Institute, Stockholm, Sweden). Studies of human milk, II. Concentration of antibodies against Salmonella and Shigella in milk of women from different populations and the daily intake by their breast-fed infants. Acta Paediatr Scand 74: 338, 1985.

The concentration in human milk of IgA antibodies against six Salmonella and two Shigella groups was determined in specimens obtained from Swedish and Guatemalan nursing mothers of three different socioeconomic levels. The daily intakes of milk antibodies by their children were also estimated. The results show that the concentrations of specific IgA antibodies in milk vary among the different population groups. There is, however, no difference in daily intake of specific IgA by the children. *Key words: Human milk, secretory IgA, IgA specific antibodies, Salmonella, Shigella.*

Epidemiological studies have shown that breast-feeding can protect the breast-fed infant from diarrheal disease (1, 2). This protective effect may be due in part to the antiinfective factors present in human milk, such as macrophages, lymphocytes, lactoferrin, lysozyme, and specific antibodies (3–5). Recently, Glass et al. (6) have shown that the levels of specific secretory IgA (SIgA) antibodies in milk are important in the prevention of *V. cholera*-induced disease, although their presence in milk does not reduce the likelihood of infection by the microorganism. In developing areas of the world, where malnutrition is prevalent and a very high proportion of mothers breast-feed their children for prolonged periods of time, infantile diarrheal disease continues to be a major health problem (7, 8). The combination of these observations has prompted some investigators to suggest that mothers from less developed areas have an impaired capacity to produce milk SIgA. The data reported on the concentration of antiinfective factors in milk samples obtained from underprivileged women are, however, inconclusive (9–11). The levels of specific SIgA antibodies in milk may be influenced by antigenic exposure, by the capacity of the mother to respond adequately to that stimulus, or by the volume of breast milk produced. With the purpose of comparing the milk content and the daily intake by children of specific SIgA milk antibodies against common enteropathogens, subjects from different ecosystems were studied.

MATERIAL AND METHODS

Specimens. Milk samples were collected from Guatemalan rural (Santa María Cauqué), urban poor, urban privileged (Guatemala City), and from Swedish mothers by means of a manual breast pump one

month after delivery. The characteristics of the women and the methodology to collect and process the samples, as well as that for estimation of breast milk intake by the children have been reported (10).

Quantitation of SIgA. A modification of the enzyme-linked immunosorbent assay (ELISA) was employed to specifically quantitate SIgA (12).

Antibody determinations. A modified direct ELISA was used for determination of antibodies against the somatic antigens of *S. paratyphi* (Group A), *S. typhimurium* (Group B), *S. thompson* (Group C_1), *S. newport* (Group C_2), *S. enteritidis* (Group D_1), *S. anatum* (Group E), *Sh. flexneri 6* (Group B_6), and *Sh. sonnei* (Group D). Plastic tubes were coated with optimal concentrations (2.5–10.0 μg/ml) of the isolated lipopolysaccharides (LPS). The antibody levels were determined using rabbit antihuman colostral IgA antiserum (Dakopatts AS, Copenhagen, Denmark) conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, USA). The milk IgA antibody levels are expressed in percent of a reference serum. The reference serum for *Salmonella* antibodies (kindly provided by Dr B. Kaijser, Department of Clinical Bacteriology, Göteborg, Sweden), was obtained from a patient after a systemic *Salmonella* B infection and showed an agglutination titer of 1/640 against *Salmonella* B. The reference serum for *Shigella* antibodies was obtained from a patient after vaccination with *Sh. flexneri* vaccine. The value used as reference to calculate the antibody levels in milk samples was obtained by reacting the reference sera with their homologous antigen. The daily intake of antibodies was calculated by adjusting their concentration to 24 h milk volumes, as described (10). The data were analyzed using the Kruskal-Wallis test (13).

RESULTS

The levels of specific antibodies in milk, expressed as percent of the reference standard, are presented in Table 1. The content of antibodies against *Salmonella* A was similar in samples from all four groups of mothers; the Swedish specimens, however, had significantly lower levels of all the other *salmonella* antibodies than those of the three Guatemalan groups. These differences were accounted for mainly by the mothers in the high socioeconomic Guatemalan group, who showed the highest content of all antibodies determined. The rural mothers had lower anti-*Shigella* D antibody levels than the privileged Guatemalan subjects.

Table 1. *IgA antibodies against* Salmonella, *and* Shigella *(in % of reference) in milk samples of Guatemalan and Swedish mothers, one month after delivery*

Population group	SIgA (g/l)	Antibodies against							
		Salm A	Salm B	Salm C_1	Salm C_2	Salm D_1	Salm E	Sh B_6	Sh D
Guatemalan									
Rural, $n=10$	0.60[a] (0.41–1.09)[c]	8.3 (5–11)	9.5 (6–19)	11.2 (6–21)	8.5 (4–16)	7.8 (4–11)	14.5 (9–36)	80.4 (27–250)	13.8[b] (8–30)
Urban poor, $n=10$	0.65 (0.21–1.88)	8.6 (5–25)	8.3 (4–42)	15.1 (6–75)	9.9 (5–44)	8.0 (5–31)	16.2 (7–50)	99.3 (41–279)	24.8 (7–322)
Urban privileged, $n=10$	0.88 (0.31–2.64)	11.0 (4–24)	10.4 (4–24)	23.7 (10–59)	10.1 (6–27)	11.0 (6–28)	24.5 (8–55)	105.4 (52–206)	42.7 (15–217)
Swedish, $n=15$	0.78 (0.34–1.65)	5.8 (1–35)	3.8[d] (1–15)	7.7[d] (1–57)	4.4[d] (1–22)	5.2[d] (2–34)	6.6[d] (3–46)	31.7[d] (5–97)	24.2 (0–66)

[a] Geometric mean.
[b] Lower than the urban privileged.
[c] Range.
[d] Lower than the other three groups.

When the antibody concentrations were adjusted for volumes of milk ingested by the children in 24 h, the differences were no longer observed, except for IgA anti-*Shigella* B_6 levels, which were lowest among the Swedish mothers when compared to the three groups of Guatemalan subjects (Table 2).

DISCUSSION

The results of the present investigation show that the milk concentrations of IgA antibodies against enteropathogens vary among different population groups. Nevertheless, and more important, the data indicate that the children of the four groups studied receive similar daily amounts of specific IgA antibodies, except for those directed against *Shigella* B_6, which were shown to be lowest in the Swedish population. These and other observations presented in the literature (9, 10), strongly suggest that the occurrence of diarrheal disease among breast-fed infants of developing areas of the world is not due to an impairment in milk IgA response that could be directly associated with nutritional status of the mothers. This suggests that environmental factors might be more important in determining the differences in the rates of diarrheal disease morbidity seen among the various groups of children, especially since extra food and fluid is consistently given to breastfed infants in developing countries. One such factor is the degree of microbial contamination: children living under poor hygienic conditions may constantly be exposed to and ingest large numbers of enteropathogens. These high doses of microorganisms, once in the intestine of the breast-fed infant, may overcome the binding capacity of the specific SIgA antibodies present in the mother's milk and, therefore, induce disease. This is supported by observations published by Glass et al. (6), who showed that human milk with no or low antibody titers against *V. cholera* was not capable of preventing disease in the breast-fed child, while those children who received milk with high levels of antibodies were protected against disease although they were infected by the bacterium. Mata and colleagues (14) reported that breast-fed children living in Santa María Cauqué may shed *Shigella* in their feces in the absence of illness.

Table 2. *Daily intake of milk anti-*Salmonella, *and* Shigella *IgA antibodies (% of reference) by Guatemalan and Swedish infants, one month after delivery*

Population group	SIgA (g/24 h)	Antibodies against							
		Salm A	Salm B	Salm C_1	Salm C_2	Salm D_1	Salm E	Sh B_6	Sh D
Guatemalan									
Rural,	0.30[a] (0.16–0.53)[b]	4.6 (2–7)	4.8 (3–8)	5.9 (3–12)	4.6 (2–9)	4.2 (2–7)	7.5 (4–18)	41.3 (13–146)	7.1 (3–17)
Urban poor,	0.28 (0.03–0.69)	3.8 (2–9)	3.5 (2–7)	6.3 (2–47)	4.1 (2–7)	3.4 (1–5)	7.0 (4–19)	42.0 (12–124)	13.2 (4–49)
Urban privileged,	0.40 (0.17–0.81)	5.0 (1–17)	4.7 (2–22)	11.4 (2–43)	4.6 (2–19)	5.0 (1–20)	9.5 (2–39)	53.6 (6–123)	20.2 (2–108)
Swedish,	0.47 (0.23–0.89)	3.9 (1–26)	2.5 (1–11)	4.8 (1–44)	2.7 (1–10)	3.3 (1–25)	4.2 (1–34)	19.7[c] (2–72)	15.6 (0–49)

[a] Geometric mean.
[b] Range.
[c] Lower than the other three groups.

All these observations strongly suggest that, in spite of the high rates of diarrhea morbidity among infants of rural areas of the developing countries, breast milk of the mothers from these areas may be important, not only in reducing the incidence of diarrheal episodes, but also in decreasing their severity. This latter effect of breast-feeding has been suggested by Frank and colleagues (15) for respiratory infections as well. Controlled studies aimed at determining the degree of protection by breast-feeding against diarrhea, not only in terms of incidence rates but also in regard to severity of the episodes among underprivileged populations are needed.

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