HUMAN LACTATION 2

MATERNAL AND ENVIRONMENTAL FACTORS

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EFFECTS OF ETHNICITY ON IMMUNOLOGIC COMPONENTS IN HUMAN MILK

José Ramiro Crúz, Carolina Arevalo and Lars Å. Hanson

Chief, Nutrition Infection and Immunology Program INCAP	Scientist, Nutrition Infection and Immunology Program INCAP	Chief, Department of Clinical Immunology University of Gothenburg

Human milk contains various defense components, such as bifidus and antistaphylococcal factors, lysozyme, lactoferrin, receptor analogues, and lipids, which have been shown to inhibit microbial adhesion to host cells, function, proliferation and/or expression in vitro.[1-5] The biologic significance of these substances in the gastrointestinal tract of the breast-fed infant needs further elucidation. In addition, human colostrum and milk contain large numbers of cells involved in immune reactions, such as macrophages, neutrophiles, B- and T- lymphocytes, and immunoglobulins G, M, and A.[6-9] The secretory immunoglobulin A (SIgA), is formed by two IgA monomers, a joining (J) chain, and a secretory component (SC); this particular arrangement makes the molecule of SIgA resistant to enzymatic degradation and, therefore, functional in the gastrointestinal tract.[10]

Glass and coworkers[11] have shown that high levels of milk specific SIgA antibodies are associated with protection against cholera in the breast-fed child, even in the presence of infection. Furthermore, feeding breast milk with SIgA antirotavirus antibodies to severely immunodeficient children suffering from rotavirus-associated gastroenteritis stops viral shedding and symptomatology.[12] This information, coupled with that obtained experimentally, suggests that SIgA may be the most important single protective factor in human colostrum and milk. It is appropriate, therefore, to examine the concentration of SIgA and the content of specific antibodies of the SIgA class in colostrum and milk of women with different ethnic characteristics in order to estimate their protective capacity.

Several authors have approached the question by examining the content of total SIgA of colostrum and milk. Although the results may vary because of different methodologies employed, it is clear that human colostrum contains between 1.7 and 5.6 g of SIgA/l; the mean values fluctuate around 2.0 g (Table 1).[13-18] This figure seems to be independent from the nutritional status of the lactating woman, although one study[15] reported that deficient nutritional status resulted in the production of colostrum which was deficient in immune components. This detrimental effect of undernutrition was not seen in specimens collected two weeks postpartum. The concentration of SIgA is lower in mature milk than in colostrum. The concentrations reported in studies done in different locations

vary from about 0.5 to 1.6 g/l one month after delivery (Table 1). These values persist for as long as one year, with no clear differences among groups of various ethnic backgrounds.

Table 1. SIgA Concentration (g/l) in Milk Samples from Women from Different Locations

Location	Time Postpartum						Reference
	2-3d	1m	3m	6m	9m	12m	
Texas, USA	2.0	1.0	0.5	0.5	0.8	1.0	13
The Gambia[a]			0.5	0.4	0.3	0.3	14
Cambridge, England			0.4	0.3			14
Colombia (WN)[b]	2.6		0.5	0.6			15
Colombia (UN)[c]	2.0		0.5	0.5			15
India (WN)	3.4		1.2				16
India (UN)	3.7		1.2				16
India	3.4		1.0	0.8	1.0	1.1	16
Ethiopia	1.7	0.4					17
Ethiopia	5.6	0.6					17
Sweden	2.5	0.7					17
Guatemala, rural		0.6	0.4	0.4	0.4	0.6	17
Guatemala, urban		0.8	0.6				17
Guatemala, urban		1.0	0.6				17
Guatemala, rural	3.0	1.6			1.5		18

[a] Estimated from the published curve.
[b] WN: well nourished.
[c] UN: undernourished.

Some authors have analyzed the amount of SIgA that a mother transfers to her infant in a day, [14, 17, 19] by determining SIgA concentration in colostrum and milk and by estimating the volume of milk that a breast-fed child ingests in a 24-h period. There may be some methodologic differences, but despite them, SIgA daily intake at one month of age is 0.2 to 0.6 g (Table 2). The amount of SIgA ingested tends to be lower as lactation progresses, probably due to a decrease in the volume of milk. An important consideration is the quantity of SIgA that a child ingests in relation to his body weight. Recently, Butte and colleagues [19] have shown that, during the first month of life, an infant may receive around 130 mg/kg/day of SIgA. There is a gradual decrease in the amount, to approximately 80 mg/kg/day four months postpartum. We have calculated the values for mother-infant pairs from Guatemala and found an identical pattern (Table 3). Moreover, the mean figures and the standard deviation for the urban, privileged group one month postpartum are almost identical to those observed among women from Texas. Goldman and coleagues (20) have shown that during weaning there is a decrease in the amount of milk that children ingest. There is, however, a concomitant increase in milk SIgA concentration. In our series of individuals, the volume of milk ingested correlated with the child's weight at the time of sampling; this variable also correlated with birth weight.

Table 2. Daily SIgA Intake (g) by Breast-fed Children from Different Locations

Location	Time Postpartum					Reference
	1m	3m	6m	9m	12m	
Tha Gambia[a,b]		0.3	0.2	0.2	0.2	14
Cambridge, England[b]		0.3	0.2			14
Texas, USA[b]	0.6	0.5				19
Sweden	0.5	0.4	0.3	0.3		17
Guatemala, rural	0.3	0.3	0.3	0.2	0.3	17
Guatemala, urban (poor)	0.4	0.3				17
Guatemala, urban (privileged)	0.5	0.3				17

[a] Original data given as mg/12h.
[b] Estimated from the published curve.

Table 3. Daily Milk SIga Intake (mg) per Kg of Body Weight by Children from Different Locations

Location		Time Postpartum						Reference
		1m	2m	3m	4m	9m	15-19m	
Texas, USA[a]	$\bar{x}$	132	103	82	78			19
	S.D.	60	45	38	35			
Guatemala, rural	$\bar{x}$	96		49	45	38	48	
	S.D.	28		18	26	16	23	
Guatemala, urban (poor)	$\bar{x}$	75		56	48			
	S.D.	32		28	13			
Guatemala, urban (privileged)	$\bar{x}$	122	74	55				
	S.D.	62	23	16				

[a] Estimated from the published curve.

In summary, the analysis of SIgA concentration in milk specimens collected from individuals of different ethnic groups indicates that ethnicity is not a determinant of the quantity of SIgA that women transfer via colostrum and milk to their infants.

Studies on specific antibodies in human milk are more difficult to interpret, since their presence in milk depends on maternal exposure to the antigens. Several investigators have determined milk SIgA antibodies specifically directed against Escherichia coli. Carlsson, et al. [21] have shown that women from Pakistan and Sweden have antibodies against somatic antigens of some serogroups of E. coli in similar concentrations. Another study (17) has provided information indicating that women from different

ethnic groups have different levels of anti-E. coli antibodies. Milk specimens of women from a high socioeconomic group in Guatemala City have similar content of antibody to that of Swedish women, while women from lower socioeconomic urban and rural areas of Guatemala have similar titers between them, but lower than those seen in the other two population groups (Table 4).

Table 4. Anti-E. coli IgA Antibodies (% of Reference) in Milk Samples from Different Countries

Population Group		Time Postpartum					
		3d	5d	1m	3m	6m	9m
Ethiopian,							
underprivileged	$\bar{x}$	47	28	15			
	S.D.	30.9	26.5	13.3			
privileged	$\bar{x}$	121	47	34			
	S.D.	74.5	11.1	14.3			
Swedish	$\bar{x}$			48	50	72	92
	S.D.			46.3	28.8	56.9	62.0
Guatemalan,							
rural	$\bar{x}$			14	13	22	12
	S.D.			7.8	13.9	19.7	6.21
urban poor	$\bar{x}$			17	25		
	S.D.			12.5	13.9		
urban privileged	$\bar{x}$			37	55		
	S.D.			16.2	34.0		

Source: 17.

A similar observation has been made with regard to anti-Salmonella and anti-Shigella antibodies in the same population groups. [22] Obviously the variable of exposure and its timing cannot be controlled in these epidemiologic studies. Nevertheless, Holmgren and collaborators [23] have shown, with pathogens that have a more defined geographic distribution such as Vibrio cholerae and ETEC, that women who live in endemic areas have antibodies against cholera and coli entero toxin in their milk, while those women who have never traveled to those areas do not show antibody activity.

These facts have made it necessary to study the immune response of lactating women to a particular antigen, given under controlled conditions. We have studied the rate, magnitude and duration of the milk immune response of women from rural Guatemala in comparison with women from the capital city [24] after administrating a food protein antigen. There was no difference in the rate of lactoconversion (appearance of specific IgA antibodies in milk) between the two groups of women. The magnitude of the response and the duration of antibodies in milk were similar among the women in the two groups. Furthermore, when the women were classified according to their nutritional status, based on weight and height,

there was no difference in the response of the well-nourished women when compared to women with deficit of weight-for-height. These results indicate that the milk SIgA response is not affected by either ethnicity or nutritional status.

Table 5. Correlation between SIgA Concentration and Specific Antibody Levels in Human Milk[a]

Subject No.	Observed correlation (r_s) value			Critical r_s value
	E. coli LT	*Shigella*	Rotavirus	(p = 0.975)
1	0.500	0.308	0.410	0.50
2	0.530	0.406	0.151	0.58
3	0.033	0.424	0.270	0.52
4	0.494	0.510	0.470	0.52
5	*0.810*	0.000	0.220	0.55
6	0.387	*0.620*	0.230	0.52
7	0.004	0.415		0.55
			0.058	0.61[b]
8	0.056	0.055	*0.699*	0.55
9	0.399	*0.580*	*0.587*	0.50
10	0.145	0.160	0.080	0.52

[a] Spearman correlation test.
[b] For RTV only.
When observed $r_s \leq$ critical r_s value IgA antibody levels are independent of total SIgA. Dependent levels are underlined.
Source: 26.

One factor that seems to be important in the persistence of specific antibodies in milk is the presence of antigen in the intestine of the lactating woman. Hanson and colleagues [25] have shown that a single dose of live, attenuated oral polio vaccine can induce a dramatic fall in the levels of preexisting milk anti-poliovirus antibodies. We have shown that specific antibodies tended to disappear in milk specimens from lactopositive women who were given a food protein antigen orally. Prospective studies in a group of rural women from Guatemala [26] have shown that levels of specific SIgA antibodies may fluctuate as lactation progresses. Fig. 1 summarizes the findings in one representative individual. This finding does not seem to be associated with ethnic factors, since Goldman, et al., [13] and Cukor and coworkers [27] have made similar observations in women from two different communities in the United States. With the purpose of comparing these findings with those of Guatemalan women from urban settings, we analyzed milk specimens collected serially for the presence of anti-*E. coli* labile toxin (EcLT), anti-*Shigella* B6 and anti-rotaviruses antibodies. We were unable to demonstrate the same fluctuations as those observed in rural women (Fig. 2). Furthermore, when the relationships of the changes in specific antibodies found among rural women were analyzed in each of the subjects, it was clear that the fluctuations are independent from the titers of other specific antibodies as well as of the concentrations of total SIgA (Table 5).

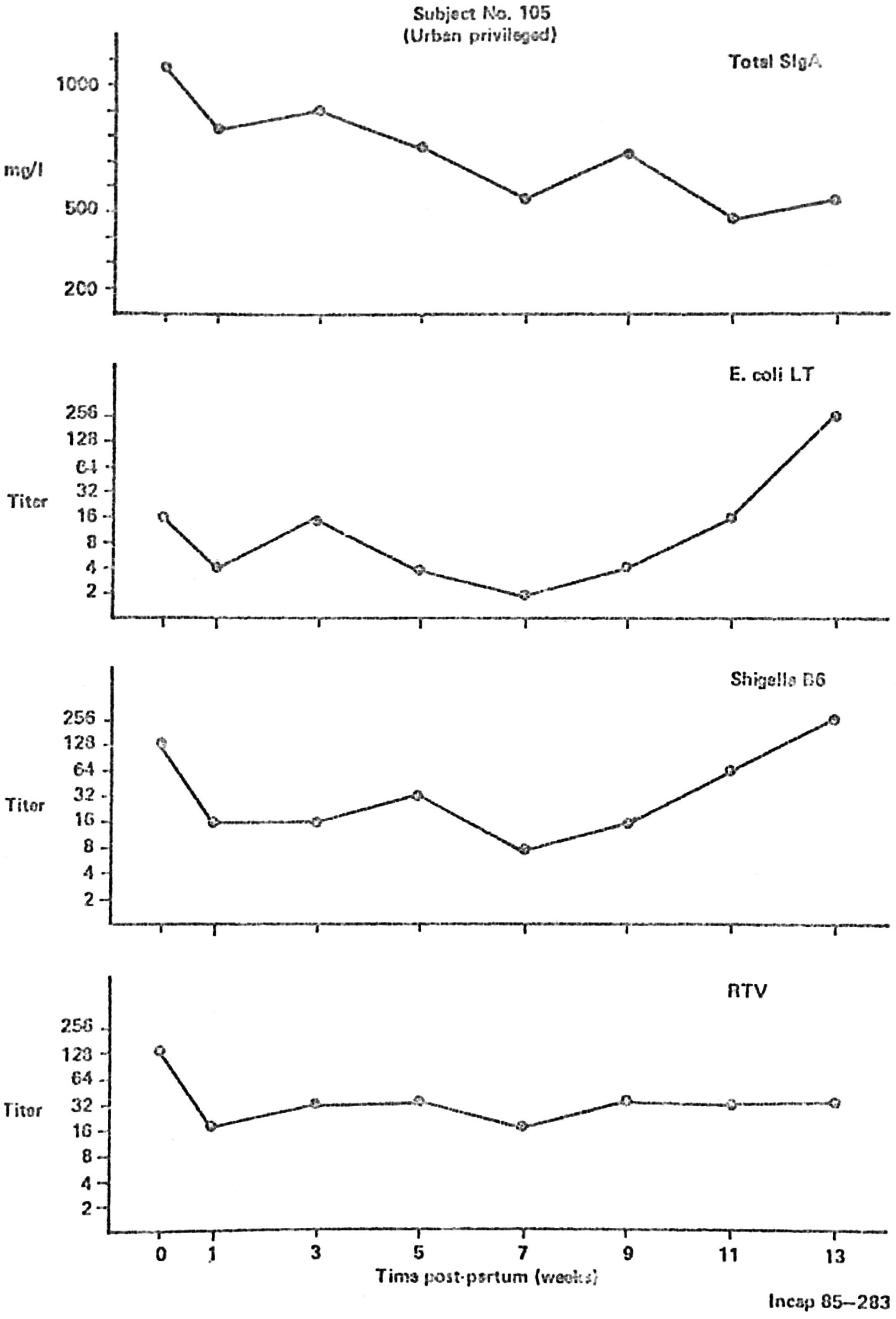

Fig. 1. Secretory IgA concentration (mg/l) and levels of specific anti-E. coli LT, Shigella B6 somatic and rotavirus antibodies in milk specimens of one representative rural woman.

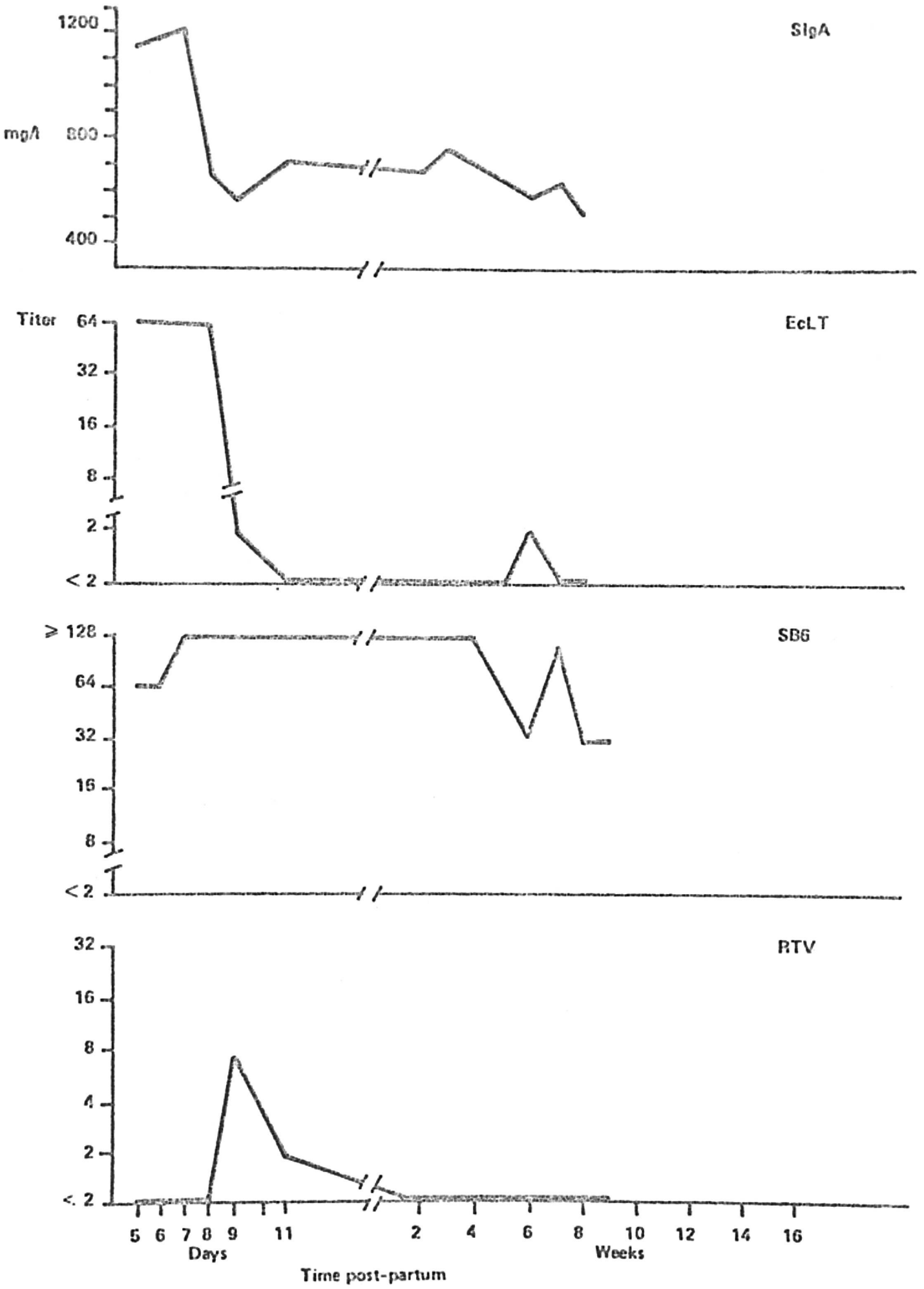

Fig. 2. Secretory IgA concentration (mg/l) and levels of specific anti-E. coli LT, Shigella B6 somatic and rotavirus antibodies in milk specimens of one representative urban woman.

These findings would explain why some authors have not demonstrated milk specific antibodies against pathogens, which are known to infect practically the totality of the population during the early stages of life, such as rotavirus.[28] Table 6 summarizes some of the findings, in terms of proportions of lactopositivity against rotaviruses in different population groups. Colostrum specimens tend to have high levels of specific anti-rotavirus antibodies; moreover, almost all samples tested (86-100%) contained measurable antibody activity. Samples of mature milk from the same locations not only have lower levels of antibody, but also the percent of those specimens with antibodies is not as high (36-94%) when compared to colostrum.[18, 27, 29-32]

Table 6. Presence of SIgA Anti-rotavirus Antibodies in Colostrum and Milk Specimens from Different Locations

Material	Location	Anti-rotavirus antibodies		Reference
		Number tested	Number (%) positive	
Colostrum	Guatemala	10	9(90)	29
	"	18	18(100)	18
	"	12	12(100)	30
	Costa Rica	21	18(86)	18
	"	21	19(90)	18
	"	32	32(100)	29
	"	43	43(100)	30
	Virginia, USA	12	11(92)	18
	Washington, DC, USA	12	12(100)	28
	Boston, USA	15	12(80)	27
	Norway	5	5(100)	32
Milk	Guatemala	25	21(84)	18
	"	25	22(88)	18
	"	95	89(94)	29
	"	38	28(74)	30
	Boston, USA	105	38(36)	27
	Bangladesh	39	35(90)	18
	"	39	34(87)	18

The practical implications of these findings are important. Ethnicity and nutritional status do not seem to have a marked effect on the quantity of milk SIgA and on the levels of specific antibodies in milk. Infections and intestinal colonization in the lactating women, however, would be more important in the limitation of the enteromammaric traffic of IgA-committed lymphocytes and, therefore, in the appearance and/or persistence of specific antibodies in human milk. In this regard, infections in the mother, be they symptomatic or asymptomatic, may increase the likelihood of an infection in the breast-fed child resulting in diarrhea.

Other protective factors, including complement, also have been analyzed in breast milk. Various methodologies have been employed for measurement of complement in milk, but they do not seem to play an important role in the differences reported from different population groups. In one report from Colombia, [15] high levels (133-184 mg/dl of C3, and 23-31 mg/dl of C4) of different components of the complement system were detected, the values representing 50-75% of normal adult serum concentrations. Another study [33] from Japan has shown that colostrum has a lytic capacity of about 7% of normal serum. Women from other population groups do not have those high levels of complement components and, in fact, may show none. Only 18% of Brazilian women showed C1q and C3. [34] Mata and Wyatt [35] studied women from rural Guatemala and obtained similar results to those observed by us more recently (Table 7). The finding that the presence and concentration of C3 and C4 do not persist following delivery is of importance; specimens obtained serially from various women do not follow a particular pattern. The factors that induce the appearance of components of the complement system in milk still need further elucidation.

Table 7. C4 and C3c Concentration in Samples of Colostrum and Milk[a]

Age (years)	Colostrum C4 (mg/dl)	Colostrum C3c (mg/dl)	Milk C4 (mg/dl)	Milk C3c (mg/dl)
26 ± 5	4.4 ± 0.05	4.9 ± 2.3	4.6 ± 3.5	5.6 ± 3.0
	6/22[b]	8/22	2/22	3/22

[a] Mean ± S.D.
[b] Positive/tested

Since human milk is known to contain maturation factors, with the collaboration of Dr. Allan Goldstein (George Washington University) the content of thymosin in specimens obtained from rural and urban women from Guatemala was determined. The results show that these samples contain thymosin, although there are variations that seem to be associated with the time after delivery (Table 8) The presence of thymosin in milk may be important in the maturation of the gut immune response, both in terms of cell-mediated immunity, which is known to be functional in the intestine, and in terms of the SIgA response, which is known to be T-cell dependent. [37]

Table 8. Thymosin α_1 Levels in Mature Human Milk (>15 Days) from Urban and Rural Mothers in Guatemala

		Months Postpartum 1	2-3	6	9	15-18
Rural	-Not detectable	1	2	2	5	4
	-Trace	1		3	1	1
	-Quantifiable					
Urban	-Not detectable		6			
	-Trace	2	4			
	-Quantifiable	5[a]	3[b]			

[a] range 370-690 pg/ml [b] range 350-730 pg/ml

In summary, it is apparent that ethnicity and nutritional status do not seem to be important determinants of the quantity of immune factors in human milk. A more important variable is the level of intestinal exposure to antigens by the lactating woman, since exposure may determine the appearance, level and persistence of SIgA antibodies in milk.

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