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Breast Milk: Role in Neonatal Host Defense

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Immunophysiology
of the Gut

I. INTRODUCTION

The newborn is in a very special situation in many ways when it suddenly has to adapt to the outside world. Not the least surprising is that the neonate can handle the exposure to the many microbes that soon after birth start to colonize mucosal membranes, especially in the oral cavity and in the gastrointestinal tract. This chapter reviews how the mother's milk can help defend the newborn against the threat of early infections resulting from normal and abnormal microbial exposure.

II. QUALITIES AND CAPACITIES OF HUMAN MILK IN HOST DEFENSE

A. Recent Data on Antibodies in Human Milk

The predominant antibody in human milk, secretory immunoglobulin A (SIgA), is produced by lymphocytes in the lactating mammary gland, which form a part of the mucosa-associated lymphoid tissue (Hanson and Brandtzaeg, 1989). The milk SIgA participates together with SIgA from other mucosal sites in the defense of various mucosal membranes. In the breast-fed infant the milk IgA will of course mainly end up on the mucosal membranes of the oral cavity and gastrointestinal tract. SIgA is a major protein component in human colostrum and can amount to several grams per liter. After the first few days of lactation the concentration comes down to around 0.5–1 g/liter but since the milk volume increases in parallel, the total intake per day in a breast-fed baby may amount to around 0.5–1 g of SIgA (Hanson and Brandtzaeg, 1989). This is a very substantial amount of antibodies to obtain every day for a young infant of 3–4 kg. In the early milk there are also IgM and IgG.

Cell transfer experiments in rats suggest that not only SIgA but also the IgM and IgG in milk, at least partly, may be locally produced in the mammary gland by lymphocytes selectively homing to the gland (Dahlgren *et al.*, 1987). Such a local production may occur in humans as well, as suggested by studies of IgG_4 (Keller *et al.*, 1983). The much higher levels of specific IgM and IgG antibodies in milk than in serum from certain IgA-deficient mothers may also result from local production (Hahn-Zoric *et al.*, 1994).

The enteromammaric and bronchomammaric links make lymphocytes home from the gastrointestinal and bronchial tracts to the mammary gland after antigenic exposure (Fishaut *et al.*, 1981; Hanson and Brandtzaeg, 1989). This must be a major explanation for the fact that human milk contains SIgA antibodies of such a remarkable range of specificities

against viruses, bacteria, and parasites. These SIgA antibodies usually remain through lactation. The corresponding antigens represent more different microbes than the mothers can be expected to have been exposed to recently. Since SIgA responses usually are rather short-lived, it is not easy to understand how the milk at one time can carry SIgA antibodies of so many specificities. It may be that the homing of lymphocytes to the mammary glands, which is initiated by the effect of lactogenic hormones on the grandular epithelium, can keep the response polyvalent and consistent by bringing in memory cells from many previous encounters with microbes. This would agree with recent observations of high avidities of SIgA antibodies to microbial antigens in human milk (Roberton *et al.*, 1988). This was seen in the milk from mothers of prematures as well (Sennhauser *et al.*, 1990). Milk SIgA antibody avidities did not increase further in response to parenteral whole-cell cholera vaccination of Pakistani mothers, although the antibody titers increased (Dahlgren *et al.*, 1989).

Cruz and Arévalo (1985) have noticed that milk antibodies, e.g., against rotavirus, can suddenly decrease or vanish for periods. The mechanism is unknown, but has been related to the decreases of milk antibody levels that can occur after oral vaccination with live poliovirus or typhoid bacteria or even after feeding a food protein (Svennerholm *et al.*, 1981; Cruz and Hanson, 1986; Hahn-Zoric *et al.*, 1989).

In some previous studies, but not in others, milk SIgA antibody titers were unaffected by protein undernutrition (Cruz *et al.*, 1982, 1985; Miranda *et al.*, 1983; Cruz and Hanson, 1986). In recent work on the quality of human milk SIgA antibodies to microbes it was noted that the relative affinity, or avidity, was lower in Pakistani than in Swedish women during part of the lactation (Roberton *et al.*, 1988). Similarly, avidities of milk SIgA antibodies in the colostrum, but not in mature milk, were lower in Costa Rican than in Swedish mothers (Hanson *et al.*, 1991). Nutritional deficiencies were not apparent in any of these mothers, but this might still need to be analyzed further to be excluded as the cause of these differences.

In a recent research project in Guatemala, undernourished mothers were given a high- or a low-caloric food supplementation during lactation in a blind randomized fashion. Milk SIgA antibodies to *Escherichia coli* O antigens showed diminishing avidities after 15–20 weeks of supplementation in the low-caloric group. Antibody titers to *E. coli* O antigens did not decrease, but total SIgA in milk also decreased (Cruz *et al.*, 1992; Herías *et al.*, 1993). The avidities of antibodies to tetanus toxoid did not decrease in the milk of the mothers in the low-calorie group, which might be due to the fact that these antibodies were produced by terminally differentiated cells resulting from a vaccine response a long time ago.

The *E. coli* antibodies, in contrast, might be affected by the undernutrition during the ongoing response to continued antigen exposure.

The extent of antigen exposure will of course be an important determinant of milk antibody avidities. Recently Morikawa *et al.* (1991) showed that although Japanese women eat more soy protein than, e.g., Indian women, their milk IgA antibodies to one of the major soybean protein fractions were of significantly lower levels but of higher avidity than those of the Indian mothers.

One factor in nutrition that might be important for the mucosal immune system is vitamin A. In previous and also recent studies in rats and chicken there is evidence that vitamin A deficiency can impair the antibody response (Sirishina *et al.*, 1980; Davies and Fell, 1989). We noted a decrease of the SIgA levels in the bile of rats on a vitamin A-deficient diet. At the same time there was an increase in serum IgA. This might be explained, e.g., by an effect of vitamin A deficiency on the expression of secretory component on the hepatocytes. We also found a SIgA antibody response to an oral cholera vaccine decreased by 90% in the vitamin A-deficient rats compared to pair-fed rats or rats fed *ad libitum* (Wiedermann *et al.*, 1993). The possible effects of vitamin A deficiency on milk antibodies are still unknown.

B. Cells in Human Milk

The lymphocytes, macrophages, and granulocytes found in milk during early lactation are still poorly defined as to their possible role in host defense. In early studies Pitt *et al.* (1977) showed that milk macrophages could prevent necrotizing enterocolitis in a rat model, but this has not been followed up. The milk macrophages, as well as the granulocytes, may well have an important role in defending the mammary gland itself. However, the milk macrophages have receptors for SIgA (Robinson *et al.*, 1991), which might possibly be helpful in enhancing phagocytosis with milk antibodies also in the infant.

The B lymphocytes of milk have been transformed by Epstein-Barr virus and found to mainly produce IgA and IgM antibodies (Hanson *et al.*, 1985). Recent studies of the T lymphocytes in human milk show that many of them carry markers for memory cells (Bertotto *et al.*, 1990, 1991). Such cells could be important for supporting the activities also of memory B cells in the mammary gland discussed earlier. However, there are suppressive effects of human colostrum on T cells (Crago *et al.*, 1981), possibly due to a milk glycoprotein (Mincheva-Nilsson *et al.*, 1990). On the other hand, B-cell activities have rather been stimulated by a component in human milk (Juto, 1985).

C. The Anti-Inflammatory Capacity of Human Milk

A few years ago Goldman *et al.* (1986) stressed that human milk may not use inflammatogenic mechanisms in host defense but rather may be anti-inflammatory. The milk is low in components with the capacity of inducing inflammation, such as IgM and IgG antibodies, complement factors, coagulation components, and kallikrein. In contrast, it is rich in factors that can block inflammatogenic events. One of the major proteins in human milk, SIgA, can prevent microbial contact with mucosal membranes simply by binding the microbes and their products, such as toxins (Table I). This may be one reason why we can demonstrate that SIgA

TABLE I

Anti-inflammatory Capacities of Human Milk

Component	Activity
Secretory IgA antibodies	Prevent IL-6 release from LPS-exposed macrophages and gut epithelium. Prevent microbial attachment to mucosal membranes. Inhibit neutrophil chemotaxis.
Receptor analogues (oligosaccharides and glycocompounds)	Block adherence of microbes and toxins to carbohydrate receptors on epithelial cells.
Lactoferrin	Inhibits complement. Blocks release of IL-6 from LPS-exposed macrophages and gut epithelium. Prevents reactions leading to formation of free radicals.
Lysozyme	Inhibits neutrophil chemotaxis and production of free radicals.
Catalase	Degrades H_2O_2.
Glutathione peroxidase	Prevents lipid peroxidation.
β-Carotene	Lipid anti-oxidant.
Cysteine	Scavenges free radicals.
Ascorbate	Scavenges free radicals. Regenerates reduced form of vitamin E.
Vitamin E, α-tocopherol	Scavenges free radicals. Immunostimulant.
Histaminase	Degrades histamine.
Arylsulfatase	Degrades leukotrienes.
α_1-Antichymotrypsin	Neutralizes inflammatogenic enzymes.
α_1-Antitrypsin	Neutralizes inflammatogenic enzymes.
Prostaglandins E_2, $F_{2\alpha}$	Cytoprotective. Inhibit neutrophil degranulation and lymphocyte activation.
Pregnancy-associated α_2-glycoprotein	Inhibits lymphocyte blastogenesis.
Epithelial growth factors	Strengthen mucosal barriers.

Adapted from Goldman *et al.* (1986, 1989).

isolated from human milk seems to be able to prevent release of the IL-6 that otherwise is obtained from human macrophage and gut epithelial cell lines when they are exposed to lipopolysaccharides (LPS) from gram-negative bacteria. We can register a similar inhibiting capacity of lactoferrin from human milk (Table II). This inhibition was dose dependent. For the human macrophage cell line U-937 a high concentration of lactoferrin, 5500 μg/ml, inhibited the IL-6 release after 24 hr, while for the intestinal epithelial cell line HT-29 a 100-fold lower concentration had the same effect after 2 hr (Table II). The addition of LPS to the cells 15–30 min before the lactoferrin resulted in inhibition of the IL-6 release, beginning within 2 hr for the HT-29 cells and within 4 hr for the U-937 cells. For the U-937 cells this occurred even at a concentration of 1000 μg/ml of lactoferrin.

Furthermore, SIgA may be anti-inflammatory by inhibiting neutrophil chemotaxis and lactoferrin, by inhibiting complement, and when unsaturated by iron also being able to prevent reactions leading to OH formation (Table I). Many components in milk act as antioxidants, which inhibit oxidizing reactions by, e.g., degrading H_2O_2 hr, scavenging free oxygen radicals, inhibiting lipid peroxidation and decreasing the production of H_2O_2 by polymorphonuclear granulocytes. Lysozyme, catalase, glutathione peroxidase, β-carotene, cysteine, ascorbate, and α-tocopherol belong to this category.

Another category of milk components acts as analogues for receptors on epithelial cells for microbes or microbial toxins. Thus human milk contains receptor analogues for the entertoxins from *Vibrio cholerae* and *Escherichia coli* (Holmgren *et al.*, 1981). These analogues may be of ganglioside nature (Kolstø *et al.*, 1983). Human milk also contains receptor

TABLE II

Effect of Lactoferrin (LF) on LPS-Induced Release of IL-6 from an Intestinal (HT 29) and a Macrophage (U 937) Cell Line Exposed to LPS

	% Inhibition	
	HT 29	U 937
LPS(10 μg/ml)	0	0
LPS + LF(5500 μg/ml)[a]	0–30 (2hr)[c]	97 (24 hr)[c]
LPS + LF(50μg/ml)[a]	98 (2 hr)	0–50 (24 hr)
LPS + LF(100μg/ml)[b]	100 (2–4 hr)	86 (4 hr)

[a] LPS + LF added together.
[b] LF added 15–30 min after LPS.
[c] Time of incubation in hours.

analogues for *Haemophilus influenzae* and pneumococci, preventing their attachment to pharyngeal epithelial cells, possibly helping to explain how breast-feeding can prevent otitis media (Andersson *et al.*, 1986).

We assume that in the gut of the breast-fed infant these various milk components act together to keep microorganisms away from the intestinal mucosa and to prevent, for instance, LPS inducing cytokine production. Such cytokines, including IL-1, IL-6, and TNF-α, could induce untoward reactions in the infant, especially in the newborn meeting its first microorganisms, e.g., as gram-negatives in the gut. It is not likely that the capacity of human milk lymphocytes to produce, *in vitro*, interferon (Emödi and Just, 1974) and IL-1 (Söder, 1987) may be functional in the gut. However, TNF-α may also be present in human milk (Mushtaha *et al.*, 1989).

The fact that meconium may also contain analogues to microbial receptors can be another factor supporting the well-being of the neonate. We have noticed that extracts of meconium interact with adhesins of different specificities on *E. coli* strains colonizing newborns (Adlerberth *et al.*, 1991b). For instance, the adhesion of P-fimbriated *E. coli* to intestinal epithelial cells was inhibited by meconium.

III. BREAST-FEEDING OF THE NEONATE IN DIFFERENT POPULATIONS

After the resurrection of breast-feeding during the last decades it is now also the routine in many, but not all, Western hospitals to initiate breast-feeding immediately after delivery. In many communities this constitutes the start of exclusive breast-feeding for a variable period of time.

In several societies exclusive breast-feeding is still rare and a slow start of breast-feeding is rather the rule, if breast-feeding is initiated at all (Hanson *et al.*, 1986). This may have historical reasons. A number of sources, the earliest more than 2000 years old from India, have advocated that the newborn should be given various foods and fluids other than human milk during the first days of life (Fildes, 1986). This is exactly what we found took place in poor populations, as well as in an upper middle class control group in and around Lahore, Pakistan (Hanson *et al.*, 1986; Ashraf *et al.*, 1993). Actually, only some 50% of the newborns in the village group or some 35% of those in a very poor mud hud area had had any human milk at all by 48 hr of age (Fig. 1). In an urban slum and an upper middle class control the figures were somewhat higher. Before that all newborns had been fed honey, clarified butter (ghee), an herb extract, or water. Often a bottle had been used, but they had also been fed by hand or spoon. It is obvious that both what they were fed

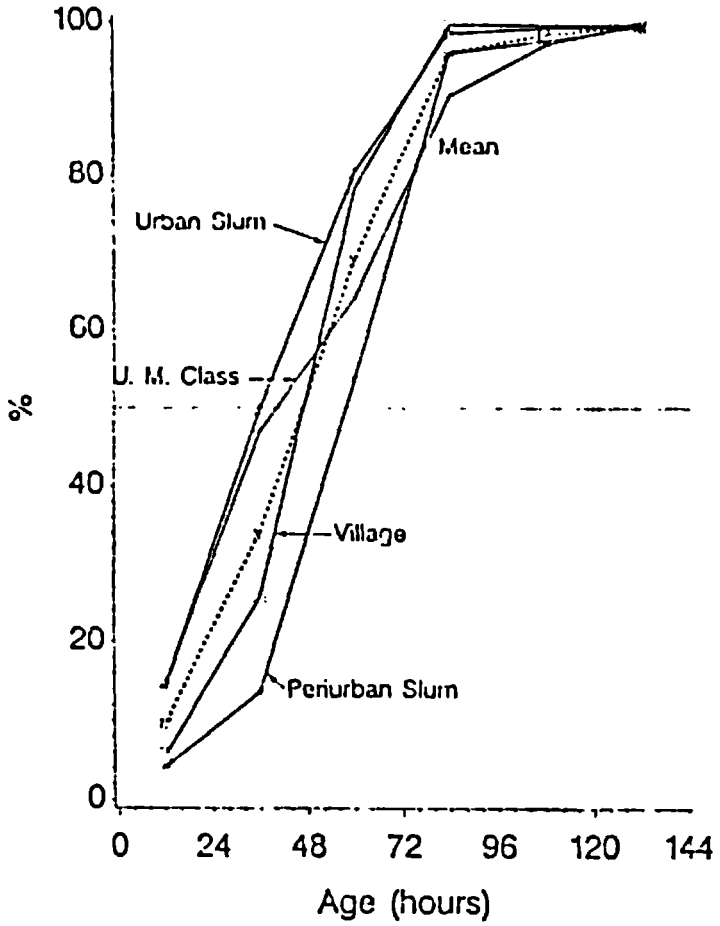

Fig. 1. Age (in hours) at onset of breast-feeding among 1476 neonates in and around Lahore, Pakistan. Three poor groups, from a village, a periurban slum or mud hut area, and an urban slum are compared with an upper middle class group.

and how this was given introduce risks of contamination with potentially pathogenic bacteria. This is of course especially true in poor areas where potable water often is lacking and microbial exposure is high.

Once breast-feeding was initiated most mothers continued with partial breast-feeding (Fig. 2). Initially the mothers were giving extra water to the breast-fed infants (Fig. 3). This was especially striking during the hot season when diarrhea was most frequent and when the protection provided by the maternal milk was most important (Fig. 4) (Jalil *et al.*, 1990; Ashraf *et al.*, 1993). At that time it was also most likely that any foods and fluids other than maternal milk given to the infant were contaminated. The extra water was given under the assumption that the infant would not receive sufficient fluids via only breast-feeding during the hot season. Repeated studies have shown, however, that this is incorrect (Almroth, 1978; Almroth and Bidinger, 1990). Obviously, the thirsty infant in a hot climate sucks more at the breast and more milk is produced, providing sufficient fluid.

Buffalo and cow's milk are the foods most often given to the partially breast-fed Pakistani infants (Fig. 5). The poorer the area, the more often it is diluted, adding to the risk of microbial contamination in addition to

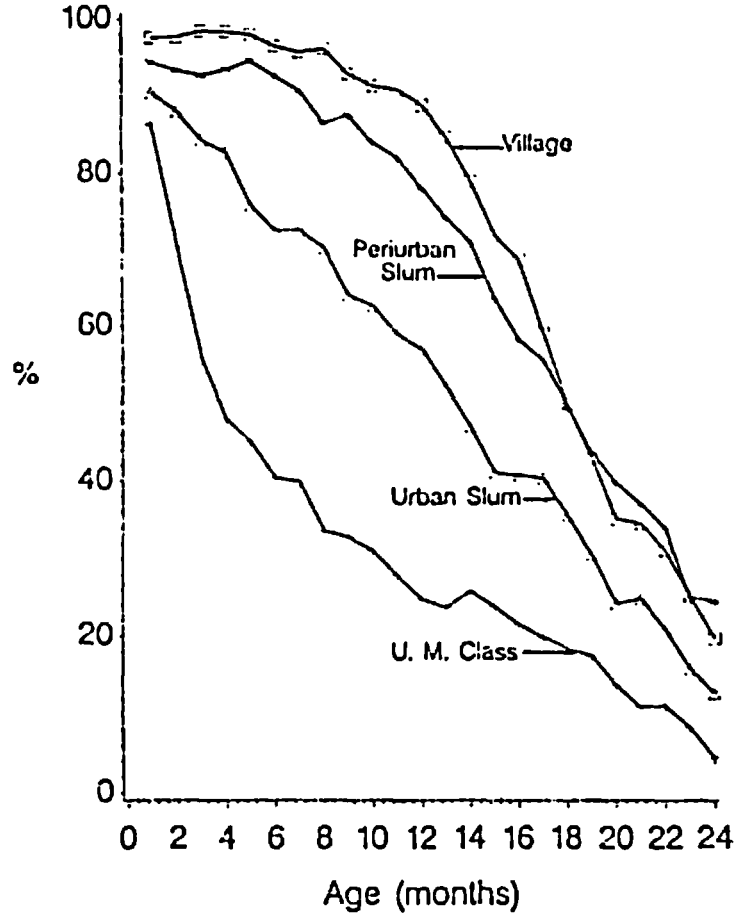

Fig. 2. Exclusive and partial breast-feeding in the four population groups during the first 24 months of life.

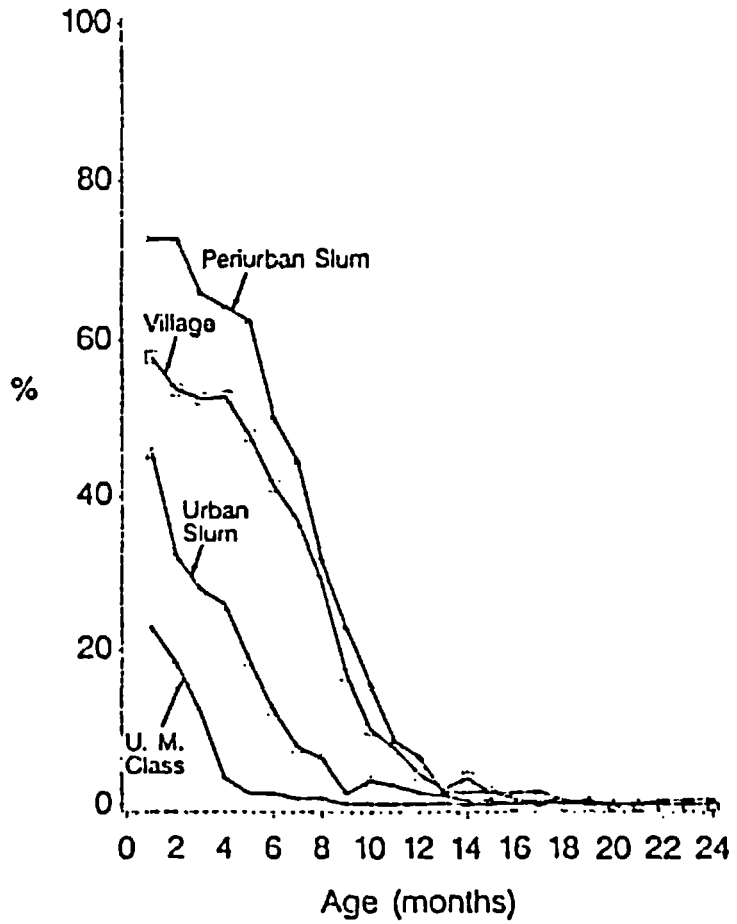

Fig. 3. Infants fed by mother's milk and water.

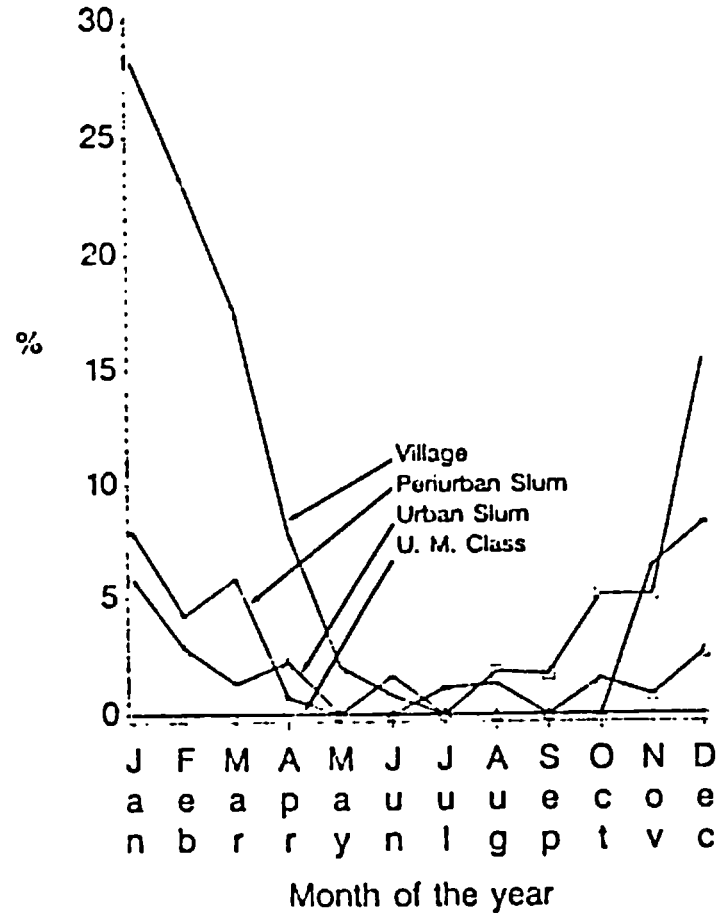

Fig. 4. Exclusive breast-feeding in the four population groups vanishes during the hot season, because the infants are then given extra fluid in the erroneous belief that this is necessary.

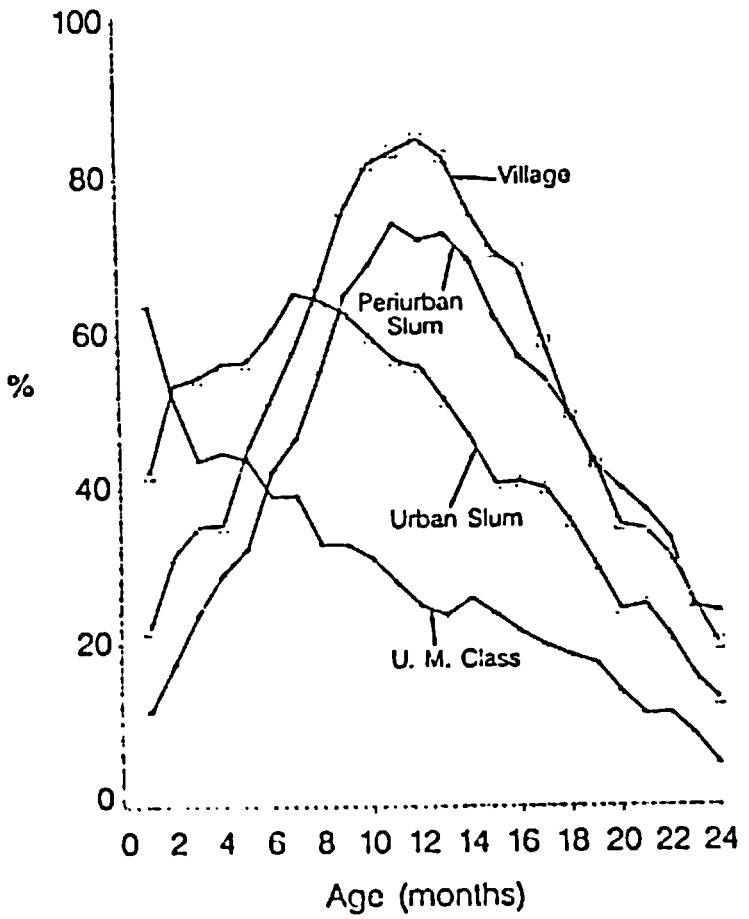

Fig. 5. Breast-feeding plus other foods, mainly buffalo or cow's milk, in the four population groups.

inadequate nutrition (Ashraf *et al.*, 1993). A bottle was used in close to 100% of the breast-fed infants to provide the additional fluids and foods, starting very early in life (Fig. 6). This also adds to the risk of microbial contamination of the foods and fluids given to the infants.

Commercial formulas were rarely used in the three poor areas, but were at 1 month of age given to around 60% of the upper middle class infants (Fig. 7). In this population group encompassing about 2% of the population formulas can presumably be more safely given since the instructions can be understood and the water added is often safe or is used after boiling. The poor population group could presumably not afford the commercial formulas.

Exclusive breast-feeding was rare. Assessing the foods and fluids given to all newborns, 18% were exclusively breast-fed for a period in the village and 10% in the mud hut area. At 1 month of age 9% of the total of 1476 infants followed prospectively were exclusively breast-feeding (Fig. 8). As mentioned above, this mode of breast-feeding was seen only outside the hot season (Ashraf *et al.*, 1993).

It is obvious that the described feeding habits of the neonate and the young child bring considerable risks for exposure to infectious agents by giving various fluids and foods instead of the mother's milk and by using a bottle. In addition, the deprivation of the neonate of the host defense factors of colostrum and the early milk adds to the risk that potential

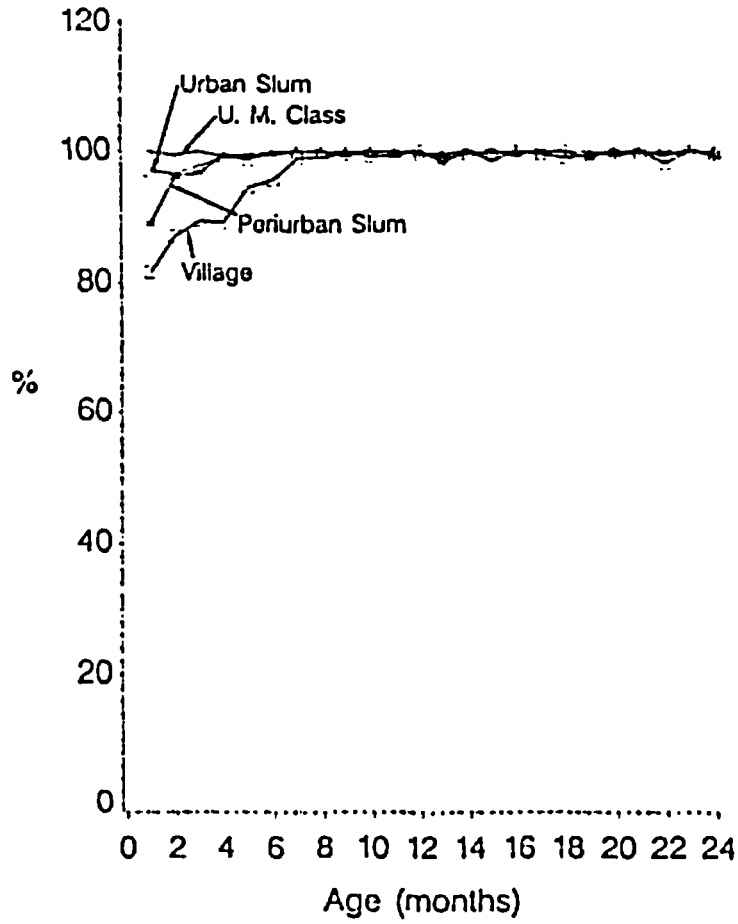

Fig. 6. Use of a bottle for feeding.

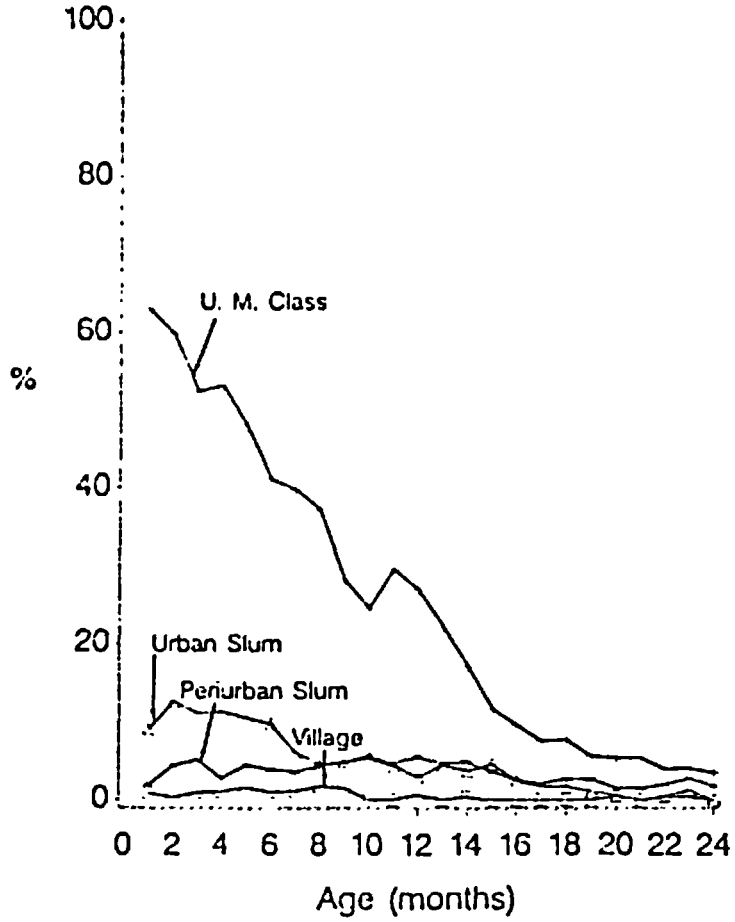

Fig. 7. Use of commercial formulas in the four population groups.

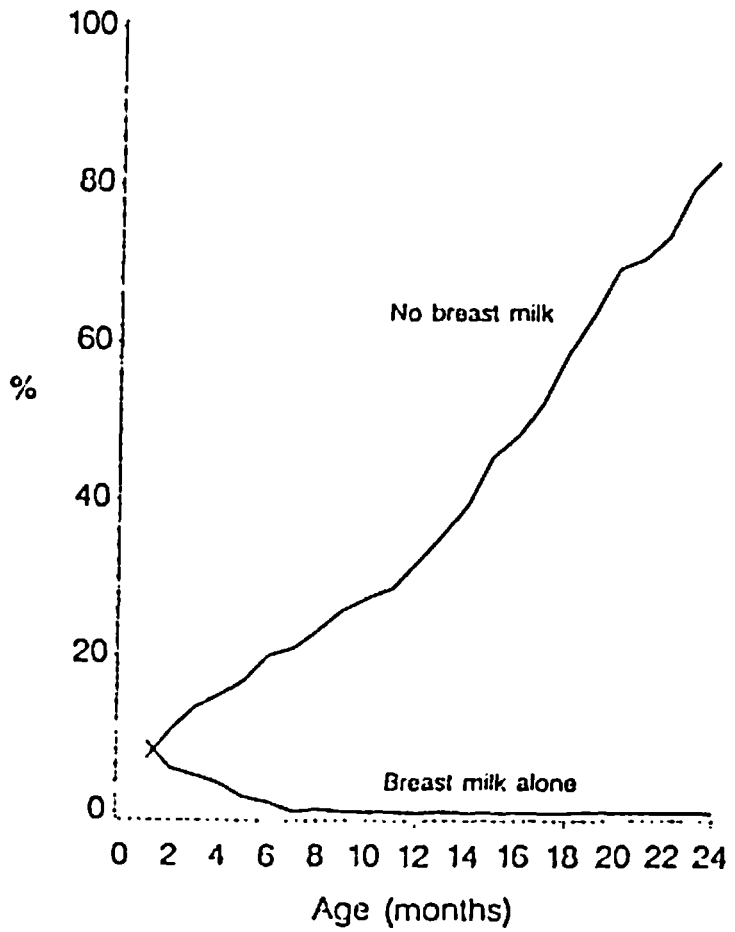

Fig. 8. Exclusive breast-feeding and no breast-feeding in all the 1476 children taken together.

pathogens from these foods and fluids may cause infections. The seriousness of this is illustrated by the fact that the main causes of death in our study area in Pakistan is infections, especially neonatal sepsis and diarrhea (Khan *et al.*, 1993); 83% of these deaths occur within the first 3 months of life.

It might be said that the most common immunodeficiency in the young infant may be the lack of SIgA and other human milk defense factors in the non-breast-fed infant.

IV. THE EFFECTS OF BREAST-FEEDING ON THE NEONATE

A. Effects on the Intestinal Colonization and the Stool Flora of the Neonate

In the Pakistani study the heavy microbial exposure of the newborns resulted in gut colonization with gram-negative aerobes, in many during the first day of life (Adlerberth *et al.*, 1991a). Actually, in the hospital the vaginally delivered and those delivered by cesarean section were equally early colonized. Compared with Swedish newborns the Pakistani ones were colonized much earlier with gram-negatives. Whereas the Swedish had one or a few serotypes of *E. coli* or *Klebsiella* to dominate the aerobic gut flora, the Pakistani newborns had a much more variable stool flora with many different bacterial genera present. Although few Pakistani infants were exclusively breast-fed, after the initial prelacteal feeds it was possible to show that significantly fewer of them had gram-negatives such as *Proteus*, *Citrobacter*, and *Klebsiella* in the stool flora than those not breast-fed (Adlerberth *et al.*, 1991a).

Human milk can obviously influence the flora present in the stool. Colonizing infants during the first week of life with a harmless *E. coli* 083 it was found that breast-feeding would enhance the colonization of the type 1 fimbriated form of *E. coli* 083 (Lodinová-Zádniková *et al.*, 1991). These *E. coli* 083 bacteria from the breast-fed infants also adhered better to the colon epithelial cell line HT29 than those from the non-breast-fed. This adherence occurs via type 1 fimbriae. It may be that the type I fimbriation makes these *E. coli* less virulent, since such fimbriae cause bacteria to be quickly phagocytized via their binding to mannose residues on granulocytes (Söderström and Öhman, 1985). Via type 1 fimbriae bacteria also bind to the carbohydrate moiety of SIgA antibodies (Wold *et al.*, 1990). Since macrophages can carry Fc receptors for SIgA antibodies, such bound bacteria may be quickly killed by macrophages.

Another sign of the effect of human milk on intestinal bacteria comes from studies of the sensitivity of *E. coli* to bactericidal antibodies. It was

found that the *E. coli* from the stool of breast-fed infants were clearly more sensitive than those from formula-fed infants (Gothefors *et al.*, 1975). Bacteria that are more sensitive to bactericidal antibodies are generally regarded to be of lower virulence. It is not known how milk components can affect the *E. coli* in such a manner, but similar changes have been noted for *E. coli* carried for a long time in the urinary tracts of humans or rats (Lindberg *et al.*, 1975; Mattsby-Baltzer *et al.*, 1982).

B. Breast-Feeding Protects Against Neonatal Sepsis

It is likely that the microorganisms that cause neonatal sepsis and/or meningitis often may originate from the intestinal flora. The disturbed intestinal flora we have noted in the Pakistani newborns with a delayed onset of breast-feeding may put them at risk. As already mentioned, neonatal sepsis and also early diarrhea are the two most common causes of morbidity and mortality in this population group.

In a study of Winberg and Wessner (1971) it was shown that breastfeeding may protect against neonatal sepsis. Narayanan *et al.* (1980, 1981, 1982) showed in a series of papers that feeding with banked human milk in prematures decreased their high risk of developing neonatal infections.

Recently we investigated the mode of feeding among 42 cases of neonatal sepsis from a hospital in Lahore, Pakistan, compared with that of 270 controls matched as to birth date and socioeconomic conditions (Ashraf *et al.*, 1991). A number of confounding factors could be excluded, and the only factor found to be related to the risk of attracting the infection was the mode of feeding (Table III). So many more of the cases than the controls had been fed formula or animal milk that the odds ratio to get the infection in the artificially fed compared to the breast-fed was as high as 18. Since exclusive breast-feeding only occurred in one infant in the study, this meant that partial breastfeeding could provide this degree of protection. Furthermore, this occurred in infants who had been given various foods and fluids other than mother's milk before breast-feeding started. Thus these neonates should have attracted the "risk" flora in the gut already alluded to (Adlerberth *et al.*, 1991a). Still, even partial breastfeeding could prevent disease so efficiently.

C. Breast-Feeding Protects Against Early Gastroenteritis and Necrotizing Enterocolitis

Numerous studies have shown that breast-feeding can protect against diarrheal diseases as reviewed by Feachem and Koblinsky (1984) and Cunningham *et al.* (1991). However, the methodological problems of

TABLE III

Comparison of Variables Between Cases of Neonatal Septicemia (n = 42) and Controls (n = 270)

Variables	Significance
Modes of feeding (partial breast-feeding vs. animal milk or formula)	$p < 0.001$
Age	NS[a]
Sex	NS
Median birth order (1–10)	NS
Place of delivery	
Hospital	NS
Home	NS
Birth attendant	
Doctor	NS
Qualified midwife	NS
Untrained midwife	NS
Mode of delivery	
Spontaneous	NS
Forceps	NS
Cesarean	NS
Time between rupture of membranes and delivery	
0–24 hr	NS
>24 hr	NS
Instruments used for delivery	
Sterilized	NS
Unsterilized	NS
Tying of cord	
Clamp	NS
Sterilized thread	NS
Unsterilized thread	NS
Care of cord	
Spirit/gentian violet	NS
Antibiotics	NS
Oil or butter	NS
Ash	NS
Nothing	NS

From Ashraf *et al.* (1991).

[a] Not significant.

these studies are many because the confounding factors are multiple (Jalil *et al.*, 1990; Victora, 1990). In our study in Lahore, Pakistan, of 1476 prospectively followed children, significant protection against diarrhea by breast-feeding was seen during the first 24 months of life in the village and mud hut area and for 9 months in the city slum and for 6 months in the upper middle class control group (Hanson *et al.*, 1991; Jalil *et al.*, 1993). The protection was seen especially during the hot season when

diarrhea is more frequent. This occurred in spite of the fact that the mothers erroneously believe that they need to give their infants extra water, as mentioned earlier. The efficacy of the protection during the first weeks of life in the poor groups was as high as 60–80% and in the upper middle class group around 40% (Jalil *et al.*, 1993). Again it is a surprise that partial breast-feeding, which is the predominant mode of feeding, can protect so well.

Previous studies have shown that protection in breastfed infants against cholera, enterotoxigenic *E. coli*, and *Campylobacter* relates to the content in the mother's milk of SIgA antibodies against these pathogens (Glass *et al.*, 1983; Cruz *et al.*, 1988; Ruiz-Palacios *et al.*, 1990). It is quite likely that other milk components could be important as well, but this has not been demonstrated.

In a recent study Lucas and Cole (1990) found that breast-feeding protected against necrotizing enterocolitis. They figured that breast-feeding could prevent as many as 500 cases a year in the United Kingdom, of which about 100 would otherwise die.

It has been difficult to prove that breast-feeding decreases morbidity in lower-respiratory-tract infections, but evidence of this has been presented (Wright *et al.*, 1989). A careful epidemiological study in Brazil shows significantly fewer cases of death from pneumonia among breast-fed compared to non-breast-fed infants (Victora *et al.*, 1987; Victora, 1990).

D. Breast-feeding May Enhance Vaccine Responses in the Infant

The SIgA and IgM antibodies against *E. coli* and poliovirus were found in Swedish newborns (Mellander *et al.*, 1986). This was surprising since such antibodies are not known to pass from the mother to the fetus. That these antibodies really had been produced by the fetus was shown by the fact that they also occurred in newborns of mothers lacking IgA and/or IgM because they had hypogammaglobulinemia or IgA deficiency (Hahn-Zoric *et al.*, 1992). Since vaccine or wild poliovirus strains do not exist in Sweden after exclusive use of inactivated poliovirus vaccine, and no cross-reactions giving such a response were known, the possibility was considered that the immune system of the fetus could have been stimulated by IgG anti-idiotypic antibodies from the mother. Such antibodies were in fact found in the commercial immunoglobulin given to the mothers with hypogammaglobulinemia and in the cord sera of the neonates (Hahn-Zoric *et al.*, 1992, 1993).

Anti-idiotypic antibodies to poliovirus were also identified in human milk (Hahn-Zoric *et al.*, 1993). It was considered possible that the

presence of such antibodies in the milk could be one explanation why breast-fed infants responded better to parenteral tetanus, diphtheria, and peroral poliovirus vaccines than formula-fed infants (Hahn-Zoric *et al.*, 1990). The saliva SIgA and the stool IgM responses to parenteral tetanus and diphtheria toxoids and oral poliovirus vaccines were higher among the breast-fed than the formula-fed infants after the first vaccine doses. At 21–40 months of age the serum IgG to diphtheria toxoid and poliovirus neutralizing activity was also significantly higher in the breast-fed. These findings are in agreement with Pabst *et al.* (1989; Pabst and Spady, 1990) who showed an increased T-lymphocyte response after BCG vaccination and an increased serum antibody response to a conjugate vaccine in breast-fed compared to non-breast-fed infants.

V. CONCLUSIONS

Human milk is rich in host defense factors, although so far only SIgA antibodies have been shown to be protective per se. Defense via human milk may largely be anti-inflammatory. Thus, milk lactoferrin seems to be able to prevent IL-6 release after exposure of human macrophages and gut epithelial cells to LPS.

The start of breast-feeding is traditionally delayed during the first days of life in some, possible many, societies in the developing world (e.g., in Pakistan). Thereafter partial breast-feeding is most common with the addition of animal milk and extra water using a bottle, especially during the hot season when there is increased incidence of diarrhea.

Such partial breast-feeding protects strongly against neonatal sepsis and early diarrheal diseases. Breast-feeding also protects against necrotizing enterocolitis. Mortality in lower respiratory tract infections is decreased as well by breast-feeding.

Preliminary data suggest that breast-feeding may also enhance vaccine responses in the offspring, possibly via the anti-idiotypic antibodies present in human milk.

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