ROLE OF BREAST-FEEDING IN THE NUTRITIONAL STATUS OF INFANTS

Role of Breast-Feeding for the Nutrition and Immunologic Development of the Infant

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For about 200 million years, mammals have been feeding their babies with their own milk. Humans have been the only species to interrupt the practice by making milk products for artificial feeding. Formulas based on cow's milk became available in many countries, without knowing enough about the consequences of their use.

In the past ten years, new information on human milk has accumulated based on modern scientific research, including biochemistry, nutrition, immunology, endocrinology, and psychophysiology. Human milk is a highly complex and unique secretion quite different from the milk of other species. It contains many constituents which are present in proportions and chemical forms different from those found in milks of other species. We still know very little about many of them, in spite of the hundreds of scientific papers which have been published during the last decade.

NUTRITIONAL COMPONENTS OF BREAST MILK

The composition and output of human milk have been discussed recently in a number of review papers and we shall, therefore, not deal with this aspect of breast-feeding (7,14,33).

IMMUNOLOGIC COMPONENTS OF BREAST MILK

Direct nutritional considerations cannot be viewed in isolation from other related aspects such as host resistance to infections and the consequences of the mother-child relationship. Breast-feeding is much more than the simple supplying of nutrients to the baby. It is a biological interaction of wide importance. Apart from nutritional and metabolic considerations, mammalian milk appears to provide specific protective factors during the time when the
newborns are becoming adjusted to the risks of the extrauterine environment. In man, recent evidence suggests that such factors are not only antiinfective, but also help to protect against the early development of some forms of infant allergy. It has also been shown in animal experiments that breast-feeding influences the development of immunologic capacity.

Although the newborn organism is able to respond to different antigens, the response is quantitatively low and also differs qualitatively from that of the adult organism (28). In some species, including man, this disadvantage is compensated by the transfer of humoral antibodies from mother to the offspring via placenta; in other species (pig, sheep) the transfer of immunoglobulins occurs only through the colostrum and milk. Even though the very life of the human infant does not depend on breast-feeding, the protective effect against infection has been clinically recognized for decades (5).

The protective effect of colostrum and milk is multifactorial, including all known types of immunologic mechanisms, i.e., nonspecific, specific, humoral, and cellular. The antibodies received by the human fetus in utero act largely against certain systemic infections while resistance factors from the breast-milk appear to act mainly within the intestine.

HUMORAL FACTORS

Various protective factors are present in human milk, including immunoglobulins, lysozyme, the bifidus factor, and nutrient-carried proteins which bind vitamin B12, folate, and iron, and limit their availability to intestinal bacteria, especially *Escherichia coli*.

Milk contains large amounts of lactoferrin, an unsaturated iron-binding compound having a strong antibacterial effect, by limiting the availability of iron for bacterial growth. It has been suggested that lactoferrin acts synergistically with IgA, especially against pathogenic strains of *E. coli* and *Candida albicans*. Because lactoferrin competes for iron with enteral organisms, the use of supplementary iron in breast-fed infants may be contraindicated as interfering with the protection afforded by lactoferrin (3).

The *bifidus factor* in human milk, characterized as a nitrogen-containing polysaccharide, facilitates the growth of *Lactobacillus bifidus*, which appears to have the function of checking the growth of undesirable, possibly harmful, organisms such as enteropathogenic *E. coli*, by producing acetic and lactic acid, thereby lowering the pH of the stool (5).

*Lysozyme* (muramidase), the well recognized antiinfective substance in lacrimal secretion, is also found in breast-milk in about 5,000 higher concentration than in cow's milk (12). This enzyme causes the lysis of gram-positive and some gram-negative bacteria by hydrolyzing the linkage between muramic acid and N-acetylglicosamine of the bacterial cell wall.

Recently, evidence has been obtained for the presence of all nine components of complement in human colostrum. They might be activated through
the classic pathway by IgM and IgG antibodies present in low amounts in colostrum and, in addition, by gram-negative bacteria such as *E. coli*. Serum and secretory IgA have been reported to be capable of activating complement, especially when aggregated (2). The high lipase activity in human milk has been shown to be related to its antiviral activity, acting against several alphaviruses, flaviviruses, herpes simplex virus, oncoviruses, and others. The antistaphylococcus factor identified by Gyorgy (9) has been found in the free fatty-acid fraction of milk. The precise mechanism of antiviral activity of lipids is still unknown.

Other substances also occur, which may have protective functions, such as interferon, derived from milk lymphocytes, which may have antiviral properties. Although the IgG, IgM, and IgA classes of immunoglobulins are present in human milk during the whole period of lactation, the highest concentrations, particularly of IgA and IgM classes, are found in the early colostrum. Ig concentrations decline sharply, 5 to 6 days postpartum, as the amount of milk increases and the process of lactation is fully established (21). The levels of IgA remain higher in the colostrum and milk than in the serum. This fact was explained by the finding that IgA is produced locally by cells present in the milk (13) and by experiments in which the selective concentration of serum IgA by mammary glands was proved (11).

The effect of ingested IgA is restricted because it cannot be absorbed from the infant's gut in large amounts. Milk immunoglobulins are absorbed only for a short period after birth (21). Secretory IgA is more resistant to the action of proteolytic enzymes than serum IgA and is found intact in the feces of the infant (17,32). The newborn organism is not able to produce intestinal secretory IgA during the first weeks after birth. Ingestion of secretory IgA in the breast milk leads to high local concentration at potential sites of entry of pathogenic microorganisms and may protect the neonate's gut from invasion of pathogens (5,25). The presence and concentration of milk antibodies depend on the previous and current experience of the mother. Although secretory IgA does not fix complement, it may possibly prevent bacterial adherence to membranes, agglutinate bacteria, and neutralize bacterial toxins (25). The presence of antibodies in milk, mainly directed against antigens occurring in the gut, and the finding of specific cells committed to react with these antigens supports the idea that the mammary gland is a part of the so-called "common mucosal system" in which the migration of cells originating in the gut to mucosal surfaces at distant sites occurs (19,23).

In our own work, we have studied some of the biological activities of immunoglobulins isolated from pig immune colostrum against *E. coli* 055. We have compared the hemagglutinating, hemolytic, and bactericidal activities of these immunoglobulins in pigs, i.e., in the species where colostrum represents the only source of maternal antibodies and is, therefore, of crucial importance. Although the hemagglutinating activity against lipopolysaccharide-sensitized sheep red cells was present in all three immunoglobulins tested, the hemolytic
activity was not present in IgA fraction. Using specific antisera for potentiation, we could also detect anti-LPS activity in IgA immunoglobulin (Fig. 1; refs. 27,30). Using bactericidal reaction as a detection system we did not find any inhibitory effect of IgA antibodies with complement on bacterial growth, and we did not succeed in obtaining any bactericidal effect of the mixture consisting of purified IgA and egg-white lysozyme in the presence of complement (Fig. 2). We could, however, demonstrate the beneficial effect of colostral antibodies present in the intestinal tract of germ-free piglets on artificially induced septicemia (Table 1; ref. 29).

FIG. 1. Hemolytic antibody activity in IgM, IgG, and IgA isolated from the colostrum of pigs immunized with E. coli 055 using sheep red blood cells coated with E. coli 055 lipopolysaccharide. Initial IgM, IgG, and IgA concentration was 0.2%. Facilitation performed by specific antisera against µ, γ, α, and L chains. Hatched bar: direct hemolysis; clear bar: indirect hemolysis.

FIG. 2. Bactericidal antibody activity (against E. coli 055) in IgM, IgG, and IgA isolated from the colostrum of pigs immunized with E. coli 055. Initial IgM, IgG, and IgA concentration was 0.2%. Hatched bar: bactericidal reaction; clear bar: reaction after adding lysozyme.
TABLE 1. Effect of immune colostrum and serum* on survival of germ-free piglets

<table>
<thead>
<tr>
<th>No. of animal</th>
<th>Protein administered</th>
<th>Titer of bactericidal antibodies</th>
<th>Time of death in hr after i.v. contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immune colostrum</td>
<td>$10^{-12}$</td>
<td>Survived</td>
</tr>
<tr>
<td>2</td>
<td>Immune colostrum</td>
<td>$10^{-12}$</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Immune colostrum</td>
<td>$10^{-12}$</td>
<td>Survived</td>
</tr>
<tr>
<td>4</td>
<td>Immune serum</td>
<td>$10^{-15}$</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Immune serum</td>
<td>$10^{-15}$</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>Immune serum</td>
<td>$10^{-15}$</td>
<td>Survived</td>
</tr>
<tr>
<td>7</td>
<td>Immune serum</td>
<td>$10^{-15}$</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>Immune serum</td>
<td>$10^{-15}$</td>
<td>Survived</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>—</td>
<td>8</td>
</tr>
</tbody>
</table>

* About 12.5 ml was administered orally 72 hr after birth.

b Germ-free piglets were intravenously contaminated with a virulent strain of Escherichia coli 055.

Cellular Components

Colostrum and milk contain a large amount of cells which have recently attracted the interest of many workers. Although their fate in the digestive tract of the infant is not fully explained yet, their number, when recalculated in relationship to the amount of milk consumed by the infant, implies that they must play a very important role in the local protection of the intestine. Milk leukocyte counts are in the range of 1 to 3 million/ml. Most cells are of monocytic origin (60–90%) and only about 10% are lymphocytes. The primary cell in human milk appears to be the macrophage. Milk macrophages possess all the typical characteristics such as adherence and spreading on the substrate, high phagocytic activity, alpha-naphtyl esterase positivity, and presence of C3 and Fc receptors. They synthesize lysozyme, complement components, and transferrin. Milk macrophages are more metabolically active than other macrophages, which may be the result of the active phagocytosis of fat particles. The high amount of lactoferrin, components of complement, lysozyme, secretory component, and immunoglobulins of the IgA and IgM classes found in phagocytic cells indicate that they have acquired these proteins by ingestion from the environment. The milk macrophages also release more immunoglobulin than do the lymphocytes, and they may, therefore, serve as a transport vehicle capable of slow, delayed immunoglobulin release (24). Many authors hypothesize that the macrophages are the principal Ig-containing cells in milk and colostrum (6). They can tolerate large variations in the environment and remain active in the milk until digestion, i.e., they may significantly contribute to the defense of the gastrointestinal tract of the infant. Milk lymphocytes are of three main types: T lymphocytes, B lymphocytes, and bearing surface Ig and null cells that cannot be grouped in either the T or B category.
The T lymphocytes respond to antigens that are present in the gut, for example, to K antigens of *E. coli*, but they are unresponsive to others, such as tetanus toxoid or *Candida albicans*. Thus, it seems that the mammary gland is able to select some cells or clones from the repertoire of natural immunologic reactivities by a mechanism similar to that responsible for the local mucosal immunity in the gut (23). Some data suggest that tuberculin-specific cell-mediated immunity can be transferred from the mother to the suckling rat via colostrum and milk. This reactivity can represent an uptake of antigen-sensitized intact cells or mediators released by the maternal T lymphocytes in the intestinal tract of the newborn (10). Clinical studies suggest that a limited number of macromolecules can cross the mucosal barrier even under physiologic conditions in man. In newborns, particularly in premature infants, many of the factors controlling the uptake of intestinal proteins such as secretory IgA, mucoproteins, and adsorbed pancreatic enzymes are undeveloped. This allows for increased access of intestinal antigens to the systemic circulation (31). Many factors in human milk can facilitate the maturation of the intestinal barrier.

Recently, it has been proven in animals that lymphoblasts in gut associated lymphoid tissue come home to the mammary gland and differentiate there into IgA-containing plasmablasts. This phenomenon is limited to lactating recipients and is hormonally induced (15). The ability of lymphocytes originating in the gut and sensitized to intestinal antigens to migrate to the mammary gland can account for the specificity of milk IgA against intestinal microorganisms and the consequent passive protection of the breast-fed infants. Ingestion of antigens during the third trimester of pregnancy can result in the appearance of colostral antibodies against these antigens—this process has been referred to as the enteromammary immune system (1), which forms part of the common mucosal system.

Within a few days after birth, the infant becomes colonized with indigenous bacteria. The early maternal-infant interactions are beneficial to the child not only in terms of emotional adjustment and the initiation of breast-feeding, but also in relation to optimal colonization of intestinal tract and skin. Colonization of the alimentary tract differs in infants fed on cow’s milk or human milk. The bacterial population is predominantly *Lactobacillus bifidus* in the breast-fed, whereas the intestinal flora of babies fed on cow’s milk is made mainly of gram-negative bacteria. The breast-fed infants are well protected, having acquired the mother’s strains and getting breast milk rich in antibodies against those strains. Most outbreaks of epidemic diarrhea in maternity-unit nurseries appeared where neonates were separated from their mothers and bottle-fed. Breast-fed infants either did not contract the disease or, if they did, were able to conquer the infection, whereas, many formula-fed babies died.

Acute necrotizing enterocolitis described in 1974 appears to be related not only to prematurity, but to perinatal hypoxia and to lack of breast milk, even if the etiology seems to be an infection of the intestinal wall damaged by
oxygen lack, by *Klebsiella*, or gram-negative organisms. This relatively common illness in other countries is, however, very rare in Czechoslovakia in spite of the low rate of breast-feeding.

**INFLUENCE OF ARTIFICIAL ORAL COLONIZATION ON THE SYSTEMIC AND LOCAL ANTIBODY RESPONSE IN THE INTESTINE AND BREAST**

In the clinical part of our work, the influence of artificial oral colonization with the nonenteropathogenic *E. coli* strain 083 on the systemic and local humoral antibody response in the intestine and the mammary gland was investigated. Maternal milk cells were isolated and their characteristics and functions studied in naturally colonized and artificially colonized infants.

The substitution of the randomly acquired intestinal flora by a tested nonenteropathogenic *E. coli* strain permits investigation of the immune reactions and keeps the composition of the intestinal flora partly under control (16).

The strain *E. coli* 083 was proved to be nonpathogenic in colostrum-deprived, germ-free, newborn piglets. It has favorable antigenic properties and, in our environment, does not occur as a spontaneous type. According to its biochemical properties, it is a normal classic *E. coli* strain, having no K antigen and not producing enterotoxin, as was confirmed by the WHO reference laboratory in Copenhagen. By using two different electrophoretic methods, it has been proven that the strain *E. coli* 083 does not carry any plasmid. The frequency of transfer of a transferable R plasmid, resistant to antibiotics, from an effective donor to *E. coli* 083 is 100 times lower if compared with that of a standard strain *E. coli* 600.

Twenty-five breast-fed and 25 formula-fed infants were colonized by oral administration of the strain *E. coli* 083. A living suspension was prepared from a 24-hr culture containing $5 \times 10^8$ organisms/ml. One milliliter was given to each child during the first 24 hr after birth and again 3 times a week for 4 successive weeks. Twenty breast-fed and 13 formula-fed infants were followed as controls. Samples of blood and feces were collected from the infants and mothers before colonization and at 2-week intervals after colonization up to 24 weeks of life. Milk samples were taken from the mothers every fortnight during the time of breast-feeding. Smears from the infant's mouth were checked bacteriologically 24 hr after each administration of the suspension. Specific antibodies against *E. coli* 083 in serum, stool filtrates, and milk samples were determined by passive hemagglutination, secretory IgA levels in stool filtrates and milk by radial immunodiffusion.

In another group of 11 mothers of colonized infants and 11 control mothers, milk cells in samples taken between the 8th and 23rd days after colonization were isolated according to the method described by Parmely et al. (22). The presence of cells producing or releasing antibodies was detected by the plaque
technique. Both direct and indirect plaques were detected, the indirect ones after potentiation with anti-IgA and anti-IgA antibodies (30) using four different antigens coupled to sheep red blood cells (SRBC)-standard set E. coli 083, a mixture of E. coli types isolated from stools of mothers whose infants were colonized, from stools of control mothers, and from the stools of the colonized infants.

All infants were kept at the Department for Healthy Infants from birth until the age of six months. Informed consent was obtained from the parents.

The strain of E. coli 083 was detected in stool samples 48 hr after colonization of the infants and predominated over the other spontaneously acquired E. coli strains for several weeks (Fig. 3). The E. coli 083 was also found in all smears taken from the mouth of colonized infants and in some samples of the mother's milk. However, it was never detected in the mother's stool.

Colonization evoked a higher serum antibody response in both colonized groups of infants than in the control groups. The difference was significant from the 2nd to 8th week after colonization. Later, the titers of the two groups did not differ (Fig. 4).

In stool filtrates, higher titers of hemagglutinating copro-antibodies against E. coli 083 were detected in the colonized infants from the 2nd week after colonization. In breast-fed infants, however, the titers decreased after the 4th week, whereas in formula-fed infants the values remained high for 20 weeks if compared with the controls (Fig. 5).

Secretory IgA in stool filtrates measured by immunodiffusion was high in colonized breast-fed and formula-fed infants as well as in breast-fed controls, during the 2nd, 4th, 6th, and 8th week. Later, in breast-fed controls the levels

FIG. 3. Colonization of the intestine with E. coli 083 and other E. coli strains. Bacteriological findings.
FIG. 4. Hemagglutinating antibody against *E. coli* 083 in the infant's serum. There are significant differences (*P* < 0.05) between the 6th and 12th week.

decreased to values found in formula-fed controls, while in both colonized
groups the values still remained significantly high until 20 weeks (Fig. 6).

The level of hemagglutinating antibody against *E. coli* 083 in milk samples
from mothers of the colonized infants was significantly higher during the 2nd,

FIG. 5. Hemagglutinating antibody against *E. coli* 083 in stool filtrates. Significant differences
(*P* < 0.01–0.05) are present at 2, 4, 10, 14, and 20 weeks after colonization.
FIG. 6. Secretory IgA levels in stool filtrates. There are significant differences ($P < 0.01-0.05$) between the 2nd and 12th week after colonization.

4th, and 8th week after colonization than in milk of the control mothers. The values of total IgA in milk were not influenced by colonization (Fig. 7).

The cellular response demonstrated by the number of hemolytic plaques was higher in the mother's milk of colonized infants against both $E.\ coli$ 083
antigens (i.e., the standard and the one isolated from the infant’s stool), than in milk of control mothers, especially after potentiation with the IgA antiserum. No potentiation occurred when IgG antibody was used, nor did plaques develop against noncoated erythrocytes.

The cellular composition of the center of the plaques consisted of clusters of cells microscopically identified mainly as macrophages and noncellular particles coated with fat (Fig. 8). Figure 9 shows a comparison of the number of plaques in breast milk from colonized infants and controls.

The serum and local antibody response induced by oral colonization confirms the results of many authors who demonstrated antibody production after oral vaccination (26). In our study, as in germ-free piglets (28), a living strain was used for colonization which remained in the intestine for a long period of time and represented a permanent antigenic stimulus. The antibody production became detectable sooner than after natural colonization, which was particularly significant in formula-fed infants. In breast-fed infants, the response as far as IgA was concerned was masked by the massive supply from the maternal milk. Antibodies against Enterobacteriaceae do not cross the placenta and the newborn infant is, therefore, not protected against infections caused by those microbes, especially if deprived of breast-feeding. The early induction of antibodies in the intestine might provide local protection against gastrointestinal infections. We used the unusual ability of the strain \textit{E. coli} 083 to persist in the intestine for a long time to demonstrate that it displaces already present pathogens and prevents their spreading into the gut (18).

The ability of the mammary gland to produce specific antibodies was proved by Goldblum et al. (8) in mothers colonized with the same \textit{E. coli} strain 083 as we used in the infants. The precursors of the IgA-producing cells are thought not to be of local origin, but to have emigrated from the intestinal lymphatic tissue after having been sensitized by antigens present in the intestine. In our study, however, the infants were colonized and the absence of \textit{E. coli} 083 in the mother’s stool was repeatedly proved. Even in this model situation, the mammary gland displayed a specific antibody response. Recently, we also

\textbf{FIG. 8.} Photomicrograph of a large lipid-laden macrophage in the center of a hemolytic plaque surrounded by fat particles. \textit{x}1200.
found a higher antibody titer against *E. coli* 083 in the serum of the mothers of colonized infants than against their own *E. coli* isolated from stool samples. We assume, therefore, that the antibody response of the mammary gland is induced by penetration of the antigen, repeatedly found in the infant’s mouth as well as in the milk of the mothers, into the breast macrophages presenting the antigen to lymphocytes. The possible role of subepithelially localized lymphocytes present in the mammary gland and/or migrating cells in the process of specific antibody formation against locally administered antigen cannot be excluded. Campbell et al. (4) have also shown that *Salmonella* organisms introduced into the mouth of the suckling calf lead to raised antibodies against these bacteria in the cow’s milk. They suggested a mechanism of diathetic immunization of the mammary gland which is capable of producing specific antibodies in response to pathogens reaching the breast tissue from the lacteal duct. The antibodies are protecting the gastrointestinal tract and such mechanism can be specific for each mother-infant pair (4).

Ahlstedt et al. demonstrated milk cells actively producing IgA antibodies by indirect plaque formation using anti-IgA and antisecretory component sera (1). However, they did not use morphologic methods confirming the type of the central cell in the plaque. Also, the ratio of 8% of plaque-forming cells to the total amount of cells seems to be high—it would mean that nearly all lymphoid cells are producers of plaques. In the work of Crago et al. (6) dealing with the character of colostral cells, macrophages are described as the main cells containing IgA. Pittard et al. (24) demonstrated macrophages that pinocytose immunoglobulins from the milk and release them gradually.

We may also suggest from our results that the elements present in the center of the hemolytic plaques and analyzed microscopically are macrophages, noncellular globules, and polymorphonuclear leukocytes coated with fat. They are
forming more hemolytic plaques against erythrocytes coated with \textit{E. coli 083} antigen than against \textit{E. coli} antigens prepared from the mother's stool. Mestecky et al. (20) demonstrated hemolytic plaques formed by macrophages and noncellular milk globules against different \textit{E. coli} antigens. Immunoglobulins detected in those elements are thought to originate in the environment. The origin and mode of acquisition of immunoglobulins by these globular elements and phagocytic cells remain unclear.

The fact that milk fat plays a role in the immunologic mechanisms of the milk was demonstrated by Wiman et al. (32). They detected histocompatible antigens in the membranes of the milk fat globules. In our hemolytic plaques, we could always demonstrate the presence of fat around the cellular or noncellular elements present in the center of the plaques.

The possibility of stimulating the mammary gland to increase the antibody production has a direct practical importance since the breast-fed infant swallows the antibodies and cells and they afford the infant a better local protection. Thus, it is not only the breast milk that is important, but it is the process of breast-feeding that provides the immunologic protection and the antigenic stimulation followed by local immune response of the breast as another possible mechanism of protection.

REFERENCES


