Loss of Immune Components During the Processing of Human Milk

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Although milk banks have been in existence for more than 50 years (1), the past decade has seen a resurgence of interest in their functions and systems of operation. In spite of this, there is as yet no consensus as to how breast milk should be processed and stored. Some milk banks prefer to freeze milk (2) to preserve, as far as possible, the immune factors present. Others insist on carefully controlled Holder pasteurization (3), forfeiting some of the immune factors but destroying the majority of potential pathogens (2). In view of the current controversies, we would like to review some of the salient facts as they have been reported so far. Our objective is to scrutinize the relevant data, which will enable us to define the optimal method of processing milk.

THE PATHOGENS

It is uncommon for breast milk to be sterile (4,5). The bacteria present in breast milk include those that can be regarded as harmless commensals, such as coagulase-negative Staphylococcus and Streptococcus viridans, as well as "potential" pathogens (Table 1). These so called "potential" pathogens have, however, only rarely been implicated as causes of neonatal morbidity. There are reports of two nursery outbreaks of Salmonella kottbus infection (7,8), one of Salmonella typhimurium, and one of E. coli, in which human milk was believed to be the source of infection (2,9). In two of these
TABLE 1. The prevalence of bacteria in raw breast milk (%)

<table>
<thead>
<tr>
<th>Presence of bacteria</th>
<th>Method of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water, sterile swab, electric pump (ref. 9)</td>
</tr>
<tr>
<td></td>
<td>Drip milk under supervision (ref. 6)</td>
</tr>
<tr>
<td></td>
<td>Manual (ref. 4)</td>
</tr>
<tr>
<td>Sterile</td>
<td>0</td>
</tr>
<tr>
<td>Commensals</td>
<td>3</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td>65.2</td>
</tr>
<tr>
<td><em>Staphylococcus viridans</em></td>
<td></td>
</tr>
<tr>
<td>Potential pathogens</td>
<td>22</td>
</tr>
<tr>
<td>Coagulase-positive</td>
<td>15</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>12.8</td>
</tr>
<tr>
<td>enterobacteria; group A and B</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td></td>
</tr>
<tr>
<td>Total:</td>
<td>100</td>
</tr>
</tbody>
</table>

outbreaks the milk was heat processed. These observations, therefore, do not resolve the case for or against pasteurization.

It is noted in Table 1 that one group of investigators claimed that 65% of specimens were sterile (6). It is obviously of great importance to establish whether this is the result of personal hygiene and aseptic technique or whether the bacteriological methods employed were less sensitive than those of other investigators. Other studies show that the bacterial flora of breast milk are largely derived from the skin and areola of the breast (5). Additional clinical trials are required within individual units to compare different methods of breast cleansing and milk collecting.

A more elusive threat is that posed by viruses. Herpes viruses including cytomegalovirus (CMV) as well as rubella virus and hepatitis B surface antigen (HBsAg) have been demonstrated in breast milk (10–13). In the case of hepatitis B, there is evidence that the presence of surface antigen in breast milk does not produce a carrier state in the infants of HBsAg carrier mothers (14). Thus, when infants of carrier mothers become infected, infection is not through the breast milk. These observations, however, concern babies fed
on the breast and not babies fed donor milk. Uncertainty also exists about CMV. Some 10 to 17% of women excrete CMV in their milk (15), and there is epidemiological evidence linking viral excretion in milk with infant acquisition of CMV during the first year of life (13). In these infants, virus was excreted in urine and saliva for several years, but no untoward clinical effects were noted. An additional factor to consider is that infants born to CMV-seropositive mothers have natural protection in the form of IgG antibodies. Such protection is absent in the blood of infants born to seronegative mothers. Caution must therefore be exercised in giving milk from CMV-seropositive mothers to seronegative infants. However, to date, there are no convincing descriptions of morbidity in full-term newborns as a result of breast-milk-acquired CMV.

**THE IMMUNE FACTORS**

**Cellular Factors and Specific Antibodies**

The most numerous cells in human milk are the macrophages. Their concentration in breast milk rapidly diminishes after birth and by 6 weeks is 1/100 that at 2 weeks (16). This large and metabolically active phagocyte is capable of synthesizing many of the antibacterial substances present in breast milk, including complement components C$_3$ and C$_4$, lysozyme, and lactoferrin (17). In addition, macrophages seem to exert a helper function on IgA-producing plasma cells *in vitro*; in the presence of macrophages, more IgA is released and for longer periods. The phagocytic function of macrophages and neutrophils has been shown to be directed against *Staphylococcus*, *E. coli, and C. albicans* (18). Oral moniliasis, being easy to detect, might be a useful marker in comparing clinical effects of raw and pasteurized milk.

T and B lymphocytes are present in breast milk in the same relative proportions as in blood. Functionally, however, breast milk lymphocytes respond to mitogens in a manner different from that of blood lymphocytes (19). Moreover, it is believed that particular
clones of lymphocytes accumulate in breast tissue. B lymphocytes in breast milk actively secrete immunoglobulins, mainly IgA, although immunoglobulins G, M, E, and D are also present in small quantities. The total amount of IgA imbibed by the infant remains at the considerable level of 1 g/day throughout lactation. The fate of ingested IgA is unknown. There are several possibilities. In rabbits it has been shown that the neonatal intestine possesses specific receptors for breast milk secretory IgA (20). It remains to be determined whether a similar phenomenon exists in the human infant. Another possibility is that IgA becomes adsorbed onto the mucus overlying the glycocalyx, as is the case in the adult intestine (21). In either case, IgA would be anchored for prolonged periods to the wall of the intestine, where it could serve to prevent bacterial adhesion, promote bacterial agglutination, neutralize viruses, and complex food proteins.

The repertoire of specific antibodies in breast milk is considerable and varies from mother to mother. It is determined to some extent by the bacterial, viral, and food antigens present in the mother's gastrointestinal and respiratory tracts (22,23). Sensitized lymphocytes from intestinal and bronchial-associated lymphoid tissues migrate to breast tissue and there secrete the appropriate antibodies. Antibodies have been described against enteroviruses, rotavirus, herpes simplex, CMV, influenza, arboviruses, rubella, and respiratory syncitial virus (11). One would also like to know whether specific antibodies to hepatitis B virus exist in breast milk of mothers secreting the virus. The presence of such antibodies would be of great theoretical and practical interest and could influence decisions on the best way of processing breast milk.

Cellular immunity, presumably conferred by breast milk T cells, has been studied to only a limited extent. It has been shown that tuberculin-reactive T cells in breast milk can confer this reactivity to the infant's peripheral blood lymphocytes, but only for a brief period (24). How this transfer of cellular immunity from mother's milk to the infant's peripheral blood is achieved is another area for future research.
**Nonspecific Humoral Factors**

Unsaturated lactoferrin is effective against *E. coli* and *C. albicans* (25). Its concentration shows a gradual decline during lactation (26). Lysozyme, which is active against *E. coli* and *Salmonella*, shows a threefold rise. The lactoperoxidase system is active against *Streptococcus, Pseudomonas, E. coli,* and *S. typhimurium*. However, salivary peroxidase activity in the newborn is greater than milk lactoperoxidase, suggesting that the salivary activity represents the more important antibacterial agent (27). All nine components of complement have been detected in breast milk (28). Their function, if any, is a matter of conjecture, as the main intestinal immunoglobulin, IgA, is not complement dependent. Other humoral factors of antibacterial and antiviral potential are chemotactic factors, bifidus factor, lipid-associated staphylococcal resistance factors, monoglycerides with antiviral activity, α1-antitrypsin, and other protease inhibitors (11). In addition, a variety of immunoregulatory substances, including macrophage migratory inhibition factor, IgA-stimulating factors, interferon, and T-cell immunosuppressive substances, have been identified in human colostrum and milk (11).

**Control of Viral Infection**

In addition to specific viral antibodies belonging largely to IgA, various nonspecific factors may help to curb viral infections. Certain free fatty acids and monoglycerides have the capacity to attack the envelopes of some viruses (29). Other nonlipid fractions, which so far have eluded identification, have been shown to be active against vesicular stomatitis virus (30), herpes simplex virus (31), and rotavirus, one of the most common causes of infantile gastroenteritis (30). Free interferon has not been detected in human milk, but milk lymphocytes can be stimulated to produce it. Whether such stimulation does in fact take place is still unknown (32).

**FACTORS ADVERSELY AFFECTING THE ANTIINFECTIVE PROPERTIES OF BREAST MILK**

Some of the antiinfective properties of human milk may be partly or wholly destroyed during processing. Relevant factors include the
type of container used for storing milk, the length of storage, and the effects of cooling to 4°C, of freezing, and of heating.

The Effect of the Container

Goldblum and co-workers (16) have shown that storage of mature milk at 4°C for 24 hr in polypropylene containers caused a significant decrease in lysozyme and lactoferrin, whereas polyethylene containers dramatically reduced the titer of secretory IgA antibodies to *E. coli* somatic antigens without decreasing the concentration of total or secretory IgA. Moreover, there was a significant decrease in the functional capacity of the cellular components irrespective of which container was employed. An additional interesting finding that arose out of these investigations was the fact that colostrum appeared to impart greater stability to its components than did mature milk. None of the cellular or humoral immunological factors investigated was diminished when colostrum was stored at 4°C for 24 hr in any of the containers mentioned.

Effect of Heat

Not surprisingly, milk leukocytes survive best when incubated at near body temperature. Heating milk to 56°C will drastically reduce (33), and Holder pasteurization at 62.5°C for 30 min will completely eliminate (34), all cellular elements in breast milk.

Both pasteurization and lyophilization cause marked reductions of IgG and IgM. Pasteurization at 62.5°C will reduce the concentration of IgA by 33%, and the minute amounts of IgE present in raw breast milk will disappear altogether. Together with the reduction of total IgA, there is a drop in antibodies to *E. coli* (34). Similarly, the amount of lactoferrin is affected by pasteurization, but lysozyme is stable. In view of the many antibacterial and antiviral factors present in breast milk, one cannot draw conclusions from the fact that some factors are destroyed by heating and that others are stable. More meaningful information can be obtained by comparing the total antibacterial activity of raw and pasteurized breast milk. Studies of this type show that the antibacterial activity
may be seriously impaired by pasteurization (35). No similar observations exist on antiviral activity, but as the antiviral factors are more resistant to heat, it is likely that this activity is better preserved on pasteurization.

The Effect of Freezing

Freezing has less effect on the immune components of human milk, particularly if the changes in temperature take place rapidly. Reynolds et al. (36) describe a method of collecting and rapidly freezing breast milk using the breast pump devised by Yamanouchi and Igarashi (37). The advantages of this pump are simplicity and ease of sterilization. From the pump, the milk is transferred to plastic bags designed by the same investigators. These bags are robust and withstand freezing and thawing. If relatively small bags are used, the milk can be frozen rapidly. The authors examined each specimen of milk in the raw state, after storage at −20°C overnight, and after 1 and 4 weeks. Each specimen was examined for bacterial contamination, bacteriostatic activity, cellular and immunological components, fat, and amino acids. The results showed that there was no appreciable rise in bacterial counts on storage, and bacteriostatic activity was preserved for at least 1 week. A surprisingly large number of cells survived freezing and storage.

CONCLUSIONS

The present divergence of opinion on milk processing results largely from the lack of decisive clinical trials comparing pasteurized with raw milk. In these circumstances, our views are based largely on inference from in vitro observations colored by personal bias. In this chapter, we have reviewed some of the extensive literature on the immune properties of breast milk. However, none of the data presented can obviate the need for clinical trials to settle current controversies. One interesting recent clinical study suggested that raw colostrum or breast milk could reduce the prevalence of infections among neonates even when used as a supplement
to formula feeds (6). This important study unfortunately did not examine the use of pasteurized human milk.

In the future, therefore, we must endeavor to answer a number of questions; we would like to define only a few that arise from our study:

1. Can the present methods of collection of breast milk be improved in order to obtain a greater number of sterile specimens?
2. Apart from the four reported outbreaks of infection (Salmonella and E. coli) in which faulty technique was to blame, is there any evidence in properly organized milk banks that bacteria in raw breast milk cause morbidity?
3. Does the presence of HBsAg reflect the existence of HB virus in breast milk? Could this be ascertained by a search for core antigen (HBcAg) or “e” antigen (HBeAg) or the specific DNA polymerase?
4. What is the fate of breast milk IgA in the human intestine?
5. Do ingested intact monocytes and macrophages from breast milk have any role in the infant’s immune regulation?
6. What is the morbidity in two comparable groups of infants treated in the same unit receiving Holder pasteurized milk and rapidly frozen raw milk?

The answers to these questions will not be obtained easily; careful attention to study design and analysis will be vital, but only by objective clinical studies will agreement on the optimal method for milk processing be reached.

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REFERENCES

LOSS OF IMMUNE COMPONENTS