A joint meeting of the Nutrition Society and the British Society for Immunology was held at the Royal College of Physicians, London on 9 February 2000

Symposium on 'Nutrition and immunity'

Role of breast-feeding in managing malnutrition and infectious disease

Suzanne M. Filteau
Centre for International Child Health, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

Breast-feeding policy tends to be an emotive issue. International agencies recommend exclusive breast-feeding for 4–6 months followed by continued partial breast-feeding into the second year of life in order to promote infant and child health and minimize the damage caused by the malnutrition–infection cycle. To what extent are these recommendations supported by the experimental evidence? Are they a simplification for emotional reasons or public health purposes? Breast-feeding is believed to benefit infants because breast milk contains the ideal mix of nutrients for infants, because it contains factors which promote development of the infant's gut and immune system and which prevent pathogen invasion, and because exclusive breast-feeding prevents intake of pathogens in food or water. However, some apparently contradictory evidence exists. First, in environments which are not highly contaminated breast-fed infants tend to growth falter relative to those fed formula. Second, in such environments partial breast-feeding is not associated with significantly increased gut damage relative to exclusive breast-feeding, suggesting that active promotion of gut development by breast-feeding is more important than simple avoidance of pathogens from other foods. Third, many immune factors in breast milk are probably present primarily to protect the mother, not the infant. Finally, breast milk itself may contain bacteria or viruses. This problem has come to the fore with the human immunodeficiency virus epidemic, since it is clear breast-feeding is an important mode of mother-to-child transmission. The present review will examine these challenges to the basis of the international infant feeding recommendations and will suggest that the science does actually support the policy.

Breast-feeding: Infants: Maternal health: Immunity: Human immunodeficiency virus

Breast-feeding is a key component of infant and child health programmes. The benefits in terms of preventing the malnutrition–infection cycle are especially great in less-developed countries (Brown *et al*. 1989), but infections are also less prevalent in breast-fed infants in industrialized countries (Howie *et al*. 1990; Wright *et al*. 1998). There is little need to explain to this audience the scientific bases for the international policy that all infants should be exclusively breast-fed, i.e. not given any other foods or liquids including water, for the first 4–6 months of life and partially breast-fed into the second year (World Health Organization, 1993). The information supporting these recommendations is well reviewed in WHO publications concerning breast-feeding (World Health Organization, 1993) and complementary feeding of infants (Brown *et al*. 1998). Breast-feeding is considered to decrease the incidence and severity of infection and malnutrition by three main mechanisms: (1) it is nutritionally ideal for infants, whereas weaning foods in poor communities are often of low energy density or lacking in micronutrients; (2) weaning foods may be contaminated and cause diarrhoea and gut damage; (3) breast milk contains factors which both passively prevent infection and actively promote the infant's own immune function. The present review will consider recent evidence undermining these mechanisms and possibly challenging the recommendations. I hope to convince the audience that, in spite of apparently conflicting evidence, the infant feeding recommendations are truly based on science and are not being promoted merely for philosophical or emotional reasons.

Abbreviations: HIV, human immunodeficiency virus; IL, interleukin; SIgA, secretory immunoglobulin A.

Corresponding author: Dr Suzanne Filteau, fax +44 (0)20 7404 2062, email sfilteau@ich.ucl.ac.uk

Breast milk as the best source of nutrition for infants

Breast milk is considered the ideal food for infants for about the first 6 months of life, after which growth and status of certain micronutrients, particularly Fe, are improved by adding appropriate complementary foods to continued partial breast-feeding (Brown *et al*. 1998). Those agencies asserting the nutritional superiority of breast-feeding have recently had to deal with the unfortunate experimental evidence that both parts of the international recommendations, for early exclusive breast-feeding and prolonged partial breast-feeding, are associated with growth faltering relative to relevant controls. Breast-fed infants grow at least as well as the largely non-breast-fed cohort of the widely-used National Centre for Health Statistics/WHO standards during the period of exclusive breast-feeding (World Health Organization Working Group on Infant Growth, 1994). However, in areas where ambient infection is low, these infants growth falter compared with the standards in the second half of infancy when partially breast-fed. Children breast-fed for prolonged periods, defined variously as more than about 12–18 months, have often been shown to have poorer anthropometric indices than children given breast milk early but weaned by the time of measurement (Victora *et al*. 1984; Brakohiapa *et al*. 1988; Briend & Bari, 1989; Nube & Asenso-Okyere, 1996). The results are well acknowledged to be confounded by factors such as: (1) socio-economic status, which might result in the poorest who are least able to afford quality complementary foods continuing to breast-feed longer; (2) reverse causality, such that a mother might want to continue breast-feeding a child which was growth faltering or ill and unwilling to eat sufficient complementary foods; (3) early death of non-breast-fed children in poor households (Briend & Bari, 1989), so that they do not contribute to anthropometric data later. However, when such confounders are accounted for statistically, children breast-fed for prolonged periods still have significantly poorer growth (Victora *et al*. 1984; Nube & Asenso-Okyere, 1996).

To deal with these potential arguments against international feeding recommendations, WHO is developing new growth standards based on growth of infants fed according to the recommendations. While such action may seem to involve circular logic and a manipulation of growth curves to fit a particular philosophy of infant feeding, it is worth remembering that the aim of maximal and rapid growth comes largely from agriculture, and what is optimum for an animal raised for meat is not necessarily so for a human infant. The importance of maximal growth is especially debatable among populations prone to obesity. The immunological (Goldman, 1993) and possible cognitive (Anderson *et al*. 1999) benefits of breast-feeding are more important for human infants. Finally, it should be remembered that the growth differential favouring non-breast-fed infants may be erased in areas where the lack of safe nutritionally adequate complementary or alternative foods results in severe growth faltering or death of non-breast-fed infants. Although breast-fed Bangladeshi children, 12–35 months old, had slightly lower weight-for-age than completely-weaned children, the former had much lower risk of dying and breast-feeding was most protective for the most malnourished children (Briend & Bari, 1989).

Complementary or alternative foods and gut integrity

The inevitable need to wean means that in communities without access to clean water, the prevention of the malnutrition–infection cycle by exclusive breast-feeding eventually will end. The weaning period is the time of greatest risk of infection and stunting. Do weaning foods themselves cause this situation or is it some other aspect of reducing the proportion of dietary intake from breast milk? Especially in resource-poor countries weaning foods may be nutritionally inadequate in terms of low energy density and high bulk, and deficiencies in many micronutrients (Brown *et al*. 1998). Furthermore, consumption of other foods is more affected by illness-induced anorexia than is consumption of breast milk (Brown & Perez, 1992). Breast-feeding during acute diarrhoea reduced the amount of oral rehydration fluid required and improved recovery in Burmese infants (Khin-Maung-U *et al*. 1985). Breast-milk consumption during acute diarrhoea reduced the risk of developing persistent diarrhoea, although with the small number of patients the difference was not significant (Sazawal *et al*. 1992). Persistent diarrhoea carries a high risk of mortality, so its prevention is of great clinical importance. Thus, continued partial breast-feeding can make up for the nutritional shortcomings of many complementary foods during periods of both health and illness.

Weaning foods may be a source of pathogens and cause diarrhoea. Recent work has shown that breast milk may overcome the risk of gut damage from weaning foods. Numerous nutrients, e.g. nucleotides (Uauy *et al*. 1990) and vitamin A (Warden *et al*. 1997), and growth factors (Donovan & Odle, 1994) present in milk promote gut integrity and repair of tissue damaged by infection so that minor inflammation does not progress to major pathology. In a large cross-sectional study breast-fed Guatemalan infants had lower intestinal permeability, as measured by dual-sugar absorbance tests, than did non-breast-fed infants (Goto *et al*. 1999). Such permeability changes are probably of physiological importance, since they have been shown in Gambian infants to be correlated with lactose maldigestion (Northrop-Clewes *et al*. 1997) and growth faltering (Lunn *et al*. 1991). Although cows' milk or other foods may damage the gut mucosa, it is also possible that the permeability differences are due to positive actions of breast milk for promoting gut integrity. In Guatemala permeability was negatively correlated with the age of cessation of breast-feeding but not with the amount of time since cessation, which suggested the positive effects of breast milk were paramount (Goto *et al*. 1999). We have recently conducted a longitudinal study of gut function of infants of human immunodeficiency virus (HIV)-infected South African women in an area where most of the population had access to piped water from the city supply. Infants given no breast milk had increased intestinal permeability, but permeability did not differ between exclusively and partially breast-fed infants (NC Rollins, SM Filteau, KE Uebel, A Coutsoudis and A Tomkins, unpublished results). This study

provides further evidence that in areas where water supplies and sanitation are not too bad, complementary foods are not themselves the major problem, and it is the active role of breast milk which is primary for maintaining gut integrity. Thus, although we may have overestimated the damage caused by weaning foods, we may have underestimated the gut protective effects of breast milk.

Immunological factors in breast milk

Breast milk contains a wide range of immunologically active factors which both passively protect the infant against infection and actively stimulate development of its immune system. Older research has focused mainly on passive protection mediated by defined molecules such as secretory immunoglobulin A (sIgA), lactoferrin, lysozyme and oligosaccharides. Milk also passively protects by virtue of its wide range of anti-inflammatory factors which serve to minimize gut damage. This work has been reviewed elsewhere (Goldman, 1993; Filteau & Tomkins, 1994; Grazioso & Buescher, 1996; Newberg, 1997; Filteau, 2000) and will not be further discussed here.

Additional less-immunologically-specific passive protection is provided by milk fat globules. This area has been little investigated, but is probably a key part of the mechanism whereby breast milk protects infants from infection. Fat globules can bind potential pathogens and prevent their attachment to infant cells, a first step in infection (Peterson *et al*. 1998). Fat globules may sequester and thus protect specific milk immune factors during passage through the infant stomach (Garofalo *et al*. 1995; Filteau *et al*. 1999*a*; Schroten *et al*. 1999). Once hydrolysed from triacylglycerols, milk fatty acids can damage bacteria by disrupting their cell membranes (Hamosh, 1998).

Recent research has investigated cytokines in milk, in part because they may be responsible for some of the active immune stimulation, rather than passive protection, of the infant. Some immunological differences between breast-fed and formula-fed infants which are not easily explained by passive protection or avoidance of inflammation are shown in Table 1. For investigation of which milk components are key immunostimulants it is not sufficient, as most of us have usually done, to simply demonstrate the presence of a particular factor in milk. It is also necessary to consider whether the amounts in milk are adequate for biological activity, whether there co-exist factors in milk which inhibit the function of the postulated immunoenhancer (especially important for cytokines) and whether the factor is able to survive passage through the infant stomach to the intestine for either absorption or interaction with gut leucocytes or epithelial cells.

Table 1. Evidence for active promotion of immune system development by breast-feeding

Difference in breast-fed relative to formula-fed	Reference
Increased urinary IgA	Prentice (1987), Goldblum *et al.* (1989)
Lymphocyte subpopulation profile	Hawkes *et al.* (1999)
Larger thymus size	Hasselbalch *et al.* (1996, 1999)
Protection from *Hemophilus influenzae* even after complete weaning	Silfverdal *et al.* (1997)
Increased antibody response to *Hemophilus influenzae* B vaccine	Pabst & Spady (1990)
Increased antibody response to oral polio vaccine	Hahn-Zoric *et al.* (1990), Pickering *et al.* (1998)

IgA, immunoglobulin A.

The extent to which cytokines survive passage through the infant stomach is largely unknown, but recent work has suggested that some hormones and cytokines may be sequestered and protected until they reach the intestine. Erythropoietin (Kling *et al*. 1998) and granulocyte colony-stimulating factor (Calhoun *et al*. 1999) are stable in milk when subjected *in vitro* to infant gastric secretions and low pH typical of the infant stomach. Similar studies with other cytokines would be useful. Milk macrophages contain sIgA which they cannot themselves synthesize (Crago *et al*. 1979), and may possibly be serving as carriers of sIgA past the stomach. Although this possibility has not been investigated, cytokines may be similarly transported in milk and released by lysis of the macrophages in the gut. Measurable concentrations of both interleukin (IL)-10 (Garofalo *et al*. 1995) and transforming growth factor-β (Filteau *et al.* 1999*a*) increased in milk after treatment of the samples with bile salts, suggesting these cytokines may be sequestered in the lipid globule and released in the infant intestine. Transforming growth factor-β is the cytokine for which perhaps the best evidence exists for its survival and indeed, activation, during passage through the infant gut (Ishizaka *et al.* 1994; Letterio *et al.* 1994). Transforming growth factor-β may promote immunoglobulin A synthesis (Defrance *et al.* 1992) and oral tolerance (Lundin *et al.* 1999), and decrease inflammation and promote healing of intestinal cells damaged by cytokines or infection (Dignass & Podolsky, 1993; Planchon *et al.* 1994).

Much work on milk cytokines has been done by dairy researchers who have little interest in calf health and are concerned about the cow. Since mastitis is a major problem in the dairy industry, resulting in substantially decreased milk production, controlled studies of the cytokine mediators of mastitis have been conducted. Comparison of mastitis severity with cytokine concentrations and of time-courses of cytokine production with key features of the disease (mammary epithelial permeability and neutrophil recruitment) suggest important roles for IL-1, IL-6, IL-8 and tumour necrosis factor-α (Shuster *et al.* 1993, 1995, 1996; Waller *et al.* 1997; Barber & Yang, 1998). The work also supports previous suggestions from the human lactation literature that the main role of milk neutrophils is in maternal protection, and that once secreted neutrophils are 'used' and capable of only minimal further activation and function (Buescher & McIlheran, 1988, 1993; Keeney *et al.* 1993). Finally, even classical immune factors protecting infants may also serve to protect the mammary gland. Gambian women with mastitis in one breast had lower levels of sIgA, complement component C3, and lactoferrin in milk from their unaffected breast than was found in milk samples from women in the community without mastitis, suggesting that low levels of these immune factors may have predisposed these women to mastitis (Prentice *et al.* 1985).

https://doi.org/10.1017/S002966510000080X Published online by Cambridge University Press

Thus, possibly many immune factors do not need to get through the infant gut intact since they have already done their job in the mother.

The importance of women's health

The studies on mastitis remind us of the importance of considering women's health when trying to understand the benefits of breast-feeding. Most work on the subject has investigated the effects of maternal undernutrition on milk volume and energy (Rasmussen, 1992; Perez-Escamilla *et al.* 1995; Gonzalez-Cossio *et al.* 1998) and immune factors (Miranda *et al.* 1983; Chang, 1990; Herias *et al.* 1993). The work on immune factors is confounded by the fact that undernourished women often live in areas of high microbial contamination, which tends to increase the concentration of some milk immune factors (Lonnerdal *et al*. 1976; Prentice *et al*. 1983) and which could obscure effects of maternal undernutrition. For example, a randomized controlled trial of vitamin A supplementation of deficient Bangladeshi women showed no effects on milk concentrations of sIgA, lactoferrin, lysozyme and IL-8, at least partly because these concentrations were normal, even in the placebo-treated women (Filteau *et al*. 1999*b*).

There have been relatively few studies of the effect of infection on milk quantity and quality. Peruvian women with established lactation who suffered an acute febrile infection exhibited no changes in milk volume or concentrations of protein, casein, lactoferrin or trace metals despite the expected acute-phase changes in serum proteins and minerals (Zavaleta *et al*. 1995). Maternal fever and clinical symptoms of infection in the first 48 h post partum also did not adversely affect initiation of lactation (as indicated by full lactation by day 14 post partum) or protein or mineral contents in colostrum and early milk, although lactoferrin concentration was decreased (Lonnerdal *et al*. 1996). This study did not investigate milk volume, whereas other work from this group has shown that a very stressful labour and delivery can result in delayed initiation of lactation and decreased milk production on day 5 post partum (Chen *et al*. 1998).

Mastitis, i.e. localized mammary infection or inflammation, appears to have greater effects on lactational performance than does maternal systemic infection. Subclinical mastitis, although long recognized as decreasing milk production in cows (Peaker, 1974; Shuster *et al*. 1995), has only recently been investigated in human subjects and appears to be very common (Filteau *et al*. 1999*a*,*b*; Willumsen *et al*. 2000). Subclinical mastitis lacks symptoms but otherwise shares many features with clinical mastitis, i.e. both are often unilateral and are associated with raised milk Na, pH and inflammatory cytokines (Willumsen *et al*. 2000). We use raised milk Na:K, which is easy and cheap to measure in spot milk samples, to diagnose subclinical mastitis. The opening of paracellular pathways between mammary epithelial cells during this local inflammation results in elevated concentrations of most immune factors which have been measured, i.e. immunoglobulins, lactoferrin, lL-8 and transforming growth factor-β (Prentice *et al*. 1985; Filteau *et al*. 1999*a*,*b*; Semba *et al*. 1999*b*). Lysozyme was not increased concurrently with subclinical mastitis (Filteau *et al*. 1999*b*; Semba *et al*. 1999*b*), and was increased in clinical mastitis only 1 week after diagnosis (Prentice *et al*. 1985).

Subclinical mastitis has been associated with poor weight gain of Bangladeshi (Filteau *et al*. 1999*b*) and American (Morton, 1994) infants. Milk stasis may be the cause of the inflammation, but may also become a consequence. The raised Na imparts a salty taste which the infant may refuse (Connor, 1979). An infant refusing the breast may encourage the mother to give additional foods, thus further decreasing milk volume, and resulting in further stasis and possibly cessation of lactation. Other likely causes of subclinical mastitis are maternal systemic infection or deficiencies of antioxidant micronutrients (Filteau *et al*. 1999*a*). The problem of local clinical or subclinical mastitis can usually be treated by encouraging women to continue breast-feeding or to express milk from the affected breast, although in serious cases antibiotics or surgery may be required (Thomsen *et al*. 1984). Similarly, intensive lactation counselling before subclinical mastitis had a chance to occur reduced the prevalence of the condition among Bangladeshi women (M Flores & SM Filteau, unpublished results).

In addition to milk quality and quantity, another potential problem with breast-feeding by mothers with local or systemic infections is the transmission of the infection to the infant. Bacteria can frequently be cultured from breast milk, especially since milk is rarely expressed under sterile conditions and may contain skin bacteria (Thomsen *et al*. 1984). Nevertheless, even among women who were themselves infected in the early post-partum period, most milk bacteria were non-pathogenic (Narayanan *et al*. 1984). In this study, an unusual trial of infections in high-risk Indian infants randomized to receive raw or pasteurized breast milk, with or without additional formula, the infants receiving raw human milk suffered fewer infections than infants receiving pasteurized milk (Narayanan *et al*. 1984). Infants receiving formula in addition to raw or pasteurized milk had more infections than the respective groups receiving breast milk alone. The results indicate that immune factors in milk, some of which are heat labile and destroyed by pasteurization, outweigh the extra risk to an infant of infection caused by receiving pathogen-contaminated breast milk. Thus, maternal bacterial infection, whether systemic or in the mammary gland, should not preclude breast-feeding.

Human immunodeficiency virus

Maternal viral infections may be a different case. There is evidence that several viruses, including HIV (Leroy *et al*. 1998; Miotti *et al*. 1999), hepatitis C (Kumar & Shahul, 1998) and cytomegalovirus (Vochem *et al*. 1998), can be transmitted by breast-feeding, with possible detriment to the infant. The HIV epidemic in Africa, where breast-feeding is crucial to child survival, has intensified research in this area. Prolonged breast-feeding may be advisable for most mothers, but among HIV-infected mothers prolonged breast-feeding increases the risk of breast-milk transmission of HIV to the infant, particularly if the mother first becomes HIV-infected while lactating (Dunn *et al*. 1992). Even if the

https://doi.org/10.1017/S002966510000080X Published online by Cambridge University Press

infant itself escapes HIV infection, it may grow up in a family where the adults are too ill and stressed to be maximally economically productive or involved in child care, or where family resources are depleted by medical expenses. Thus, although breast-feeding is an acknowledged risk factor for mother-to-child HIV transmission, these high-risk infants may particularly need the health benefits of breast-feeding. It is difficult and expensive to determine at birth which infants are already infected and which are still at risk of infection from breast-feeding, so infant feeding advice directed towards HIV-infected women needs to maximize the health benefits to all their infants. The advice must not undermine support for the international policy for the uninfected women in the area, a difficult problem since most women do not know their HIV-infection status.

Although HIV-infected women who have uninterrupted access to safe breast-milk substitutes should probably avoid breast-feeding, this problem leaves many women and health workers in Africa with a serious dilemma. Recent evidence suggests that advocacy of exclusive breast-feeding to these women may be possible as well as being appropriate for women of negative or unknown HIV status. Mother-to-child HIV transmission by 3 months of age was not different between South African infants given no breast milk and infants exclusively breast-fed, whereas infants receiving breast milk and other foods were more often infected (Coutsoudis *et al*. 1999). Recently we have been investigating mechanisms for this observation in the same cohort (JF Willumsen, SM Filteau, A Coutsoudis, ML Newell, AM Tomkins, unpublished results).

Subclinical mastitis has been associated with increased milk viral load among HIV-infected South African (Willumsen *et al*. 2000) and Malawian (Semba *et al*. 1999*a*) women, and with higher mother-to-child HIV transmission (Semba *et al*. 1999*a*). These findings further the work on clinical mastitis or other overt breast pathology, such as cracked nipples, which increase the risk of infant HIV infection (Nicoll *et al*. 1995). It is likely that during clinical or subclinical mastitis the virus enters the milk either with leucocytes recruited into the milk under the influence of inflammatory cytokines, or non-specifically through the leaky mammary epithelium. In a cross-sectional study of South African women not screened for HIV, mixing breast milk with formula was associated with higher milk Na:K than was exclusive breast-feeding. A similar, but non-significant, trend was seen among the HIV-infected cohort (JF Willumsen, SM Filteau, A Coutsoudis, ML Newell and AM Tomkins, unpublished results).

Another possible mechanism whereby adding other foods to breast milk may have increased HIV transmission in the South African cohort is by increasing infant gut permeability. However, gut permeability was not increased in mixed-fed infants (NC Rollins, SM Filteau, KE Uebel, A Coutsoudis and A Tomkins, unpublished results). It is possible that HIV transmission rate would be even higher in mixed-fed infants in an area without a piped water supply and where addition of other foods may cause greater damage to the infant gut.

Another mechanism which is at present largely speculative is that the amount of milk protective factors, e.g. sIgA or immunoglobulin M against HIV (Van de Perre, 1995), lactoferrin (Harmsen *et al*. 1995), or gp120-binding glycosaminoglycan (Newberg *et al*. 1995) was sufficient in exclusively breast-feeding infants, but not in those receiving other foods and probably less breast milk, to prevent free HIV from infecting the infant. This finding would be analogous to raw human milk being more protective than pasteurized milk, and milk alone being more protective than milk supplemented with formula (Narayanan *et al*. 1984).

In the South African trial the feeding groups were not chosen randomly (Willumsen *et al*. 2000) and it is probably unwise to speculate as far as I have on the mechanisms and the public health importance of the protective effect of exclusive breast-feeding for HIV transmission. The key question for an individual woman and her health care worker is whether lactation support, especially of women experiencing problems, can reduce her milk viral load. The key public health question is whether widespread advocacy and uptake of exclusive breast-feeding can reduce mother-to-child HIV transmission in Africa. Until further research is conducted, this situation remains one where advocacy of exclusive breast-feeding is probably based more on philosophy than science.

In conclusion, I suggest that we can, as scientists, support the international infant feeding policy as long as we broaden our support for women. Our support needs to go beyond lactation counselling and include reducing undernutrition, improving status of antioxidant micronutrients, and providing widespread advice and support to decrease the number of women becoming HIV-infected.

Acknowledgements

The author's work presented was funded by the Department for International Development, UK, the Child Health Research Appeals Trust, Institute of Child Health, and UNICEF, South Africa.

References

Anderson JW, Johnstone BM & Remley DT (1999) Breast-feeding and cognitive development: a meta-analysis. *American Journal of Clinical Nutrition* **70**, 525–535.

Barber MR & Yang TJ (1998) Chemotactic activities in nonmastitic and mastitic mammary secretions: presence of interleukin-8 in mastitic but not nonmastitic secretions. *Clinical and Diagnostic Laboratory Immunology* **5**, 82–86.

Brakohiapa LA, Bille A, Quansah E, Kishi K, Yartey J, Harrison E, Armar MA & Yamamoto S (1988) Does prolonged breastfeeding adversely affect a child's nutritional status? *Lancet* **ii**, 416–418.

Briend A & Bari A (1989) Breastfeeding improves survival, but not nutritional status, of 12–35 month old children in rural Bangladesh. *European Journal of Clinical Nutrition* **43**, 603–608.

Brown K, Dewey K & Allen L (1998) *Complementary Feeding of Young Children in Developing Countries*. Geneva: WHO.

Brown KH, Black RE, de Romana GL & de Kanashiro HC (1989) Infant-feeding practices and their relationship with diarrheal and other diseases in Huascar (Lima), Peru. *Pediatrics* **83**, 31–40.

Brown KH & Perez F (1992) Determinants of dietary intake during childhood diarrhea and implications for appropriate nutritional therapy. *Acta Paediatrica* Suppl. 381, 127–132.

Buescher ES & McIlheran SM (1988) Antioxidant properties of human colostrum. *Pediatric Research* **24**, 14–19.

Buescher ES & McIlheran SM (1993) Polymorphonuclear leukocytes and human colostrum: effects of in vivo and in vitro exposure. *Journal of Pediatric Gastroenterology and Nutrition* **17**, 424–433.

Calhoun DA, Lunoe M, Du Y, Staba SL & Christensen RD (1999) Concentrations of granulocyte colony-stimulating factor in human milk after *in vitro* simulations of digestion. *Pediatric Research* **46**, 767–771.

Chang S-J (1990) Antimicrobial proteins of maternal and cord sera and human milk in relation to maternal nutritional status. *American Journal of Clinical Nutrition* **51**, 183–187.

Chen DC, Nommsen-Rivers L, Dewey KG & Lonnerdal B (1998) Stress during labour and delivery and early lactation performance. *American Journal of Clinical Nutrition* **68**, 335–344.

Connor AE (1979) Elevated levels of sodium and chloride in milk from mastitic breast. *Pediatrics* **63**, 910–911.

Coutsoudis A, Pillay K, Spooner E, Kuhn L & Coovadia HM (1999) Influence of infant-feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa: a prospective cohort study. *Lancet* **354**, 471–476.

Crago SS, Prince SJ, Pretlow TG, McGhee JR & Mestecky J (1979) Human colostral cells. I. Separation and characterization. *Clinical and Experimental Immunology* **38**, 585–597.

Defrance T, Vanbervliet B, Briere F, Durand I, Rousset F & Banchereau J (1992) Interleukin 10 and transforming growth factor-β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *Journal of Experimental Medicine* **175**, 671–682.

Dignass AU & Podolsky DK (1993) Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor-β. *Gastroenterology* **105**, 1323–1332.

Donovan SM & Odle J (1994) Growth factors in milk as mediators of infant development. *Annual Review of Nutrition* **14**, 147–167.

Dunn DT, Newell M-L, Ades AE & Peckham CS (1992) Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *Lancet* **340**, 585–588.

Filteau SM (2000) Milk components with immunomodulatory potential. *Advances in Nutritional Research* (In the Press).

Filteau S & Tomkins A (1994) Infant feeding and infectious disease. In *Infant Nutrition: Issues in Nutrition and Toxicology*, pp. 143–162 [AF Walker and BA Rolls, editors]. London: Chapman and Hall.

Filteau SM, Leitz G, Mulokozi G, Bilotta S, Henry CJK & Tomkins AM (1999*a*) Milk cytokines and subclinical breast inflammation in Tanzanian women: effects of dietary red palm oil and sunflower oil supplementation. *Immunology* **97**, 595–600.

Filteau SM, Rice AL, Ball JJ, Chakraborty J, Stoltzfus R, de Francisco A & Willumsen JF (1999*b*) Breast milk immune factors in Bangladeshi women supplemented postpartum with retinol or β-carotene. *American Journal of Clinical Nutrition* **69**, 953–958.

Garofalo R, Chheda S, Mei F, Palkowetz KH, Rudloff HE, Schmalstieg FC, Rassin DK & Goldman AS (1995) Interleukin-10 in human milk. *Pediatric Research* **37**, 444–449.

Goldblum RM, Schanler RJ, Garza C & Goldman AS (1989) Human milk feeding enhances the urinary excretion of immunologic factors in low birth weight infants. *Pediatric Research* **25**, 184–188.

Goldman AS (1993) The immune system of human milk: antimicrobial, antiinflammatory and immunomodulatory properties. *Pediatric Infectious Disease Journal* **12**, 664–671.

Gonzalez-Cossio T, Habicht J-P, Rasmussen KM & Delgado HL (1998) Impact of food supplementation during lactation on infant breast-milk intake and on the proportion of infants exclusively breast-fed. *Journal of Nutrition* **128**, 1692–1702.

Goto K, Chew F, Torun B, Peerson JM & Brown KH (1999) Epidemiology of altered intestinal permeability to lactulose and mannitol in Guatemalan infants. *Journal of Pediatric Gastroenterology and Nutrition* **28**, 282–290.

Grazioso CF & Buescher ES (1996) Inhibition of neutrophil function by human milk. *Cellular Immunology* **168**, 125–132.

Hahn-Zoric M, Fulconis F, Minoli I, Moro G, Carlsson B, Bottiger M, Raiha N & Hanson LA (1990) Antibody responses to parenteral and oral vaccines are impaired by conventional and low protein formulas as compared to breast-feeding. *Acta Paediatrica Scandinavica* **79**, 1137–1142.

Hamosh M (1998) Protective function of proteins and lipids in human milk. *Biology of the Neonate* **74**, 163–176.

Harmsen MC, Swart PJ, de Bethune M-P, Pauwels R, De Clercq E, The TH & Maijer DKF (1995) Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vitro. *Journal of Infectious Diseases* **172**, 380–388.

Hasselbalch H, Engelmann MDM, Ersboll AK, Jeppesen DL & Fleischer-Michaelsen K (1999) Breast-feeding influences thymic size in late infancy. *European Journal of Pediatrics* **158**, 964–967.

Hasselbalch H, Jeppesen DL, Engelmann MDM, Michaelsen KF & Nielsen MB (1996) Decreased thymus size in formula-fed infants compared with breastfed infants. *Acta Paediatrica* **85**, 1029–1032.

Hawkes JS, Neumann MA & Gibson RA (1999) The effect of breast feeding on lymphocyte subpopulations in healthy term infants at 6 months of age. *Pediatric Research* **45**, 648–651.

Herias MV, Cruz JR, Gonzalez-Cossio T, Nave F, Carlsson B & Hanson LA (1993) The effect of caloric supplementation on selected milk protective factors in undernourished Guatemalan mothers. *Pediatric Research* **34**, 217–221.

Howie PW, Forsyth JS, Ogston SA, Clark A & Florey CV (1990) Protective effect of breast feeding against infection. *British Medical Journal* **300**, 11–16.

Ishizaka S, Kimoto M, Tsujii T & Saito S (1994) Antibody production system modulated by oral administration of human milk and TGF-β. *Cellular Immunology* **159**, 77–84.

Keeney SE, Schmalstieg FC, Palkowetz KH, Rudloff HE, Le B-M & Goldman AS (1993) Activated neutrophils and neutrophil activators in human milk: increased expression of CD11b and decreased expression of L-selectin. *Journal of Leukocyte Biology* **54**, 97–104.

Khin-Maung-U, Nyunt-Nyunt-Wai, Myo-Khin, Mu-Mu-Khin, Tin-U & Thane-Toe (1985) Effect on clinical outcome of breast feeding during acute diarrhea. *British Medical Journal* **290**, 587–589.

Kling PJ, Sullivan TM, Roberts RA, Phillips AF & Koldovsky O (1998) Human milk as a potential enteral source of erythropoietin. *Pediatric Research* **43**, 216–221.

Kumar RM & Shahul S (1998) Role of breast-feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. *Journal of Hepatology* **29**, 191–197.

Leroy V, Newell M-L, Dabis F, Peckham C, Van de Perre P, Bulterys M, Kind C, Simonds RJ, Wiktor S & Msellati P (1998) International multicentre pooled analysis of late postnatal mother-to-child transmission of HIV-1 infection. *Lancet* **352**, 597–600.

Letterio JJ, Geiser AG, Kulkarni AB, Roche NS, Sporn MB & Roberts AB (1994) Maternal rescue of transforming growth factor-β1 null mice. *Science* **264**, 1936–1938.

Lonnerdal B, Forsum E, Gebre-Medhin M & Hambraeus L (1976) Breast milk composition in Ethiopian and Swedish mothers. II. Lactose, nitrogen and protein contents. *American Journal of Clinical Nutrition* **29**, 1134–1141.

Lonnerdal B, Zavaleta N, Kusunoki L, Lanata CF, Peerson JM & Brown KH (1996) Effect of postpartum maternal infection on proteins and trace elements in colostrum and early milk. *Acta Pediatrica* **85**, 537–542.

Lundin BS, Karlsson MR, Svensson LA, Hanson LA, Dahlgren UIH & Telemo E (1999) Active suppression in orally tolerized rats coincides with *in situ* transforming growth factor-beta expression in the draining lymph nodes. *Clinical and Experimental Immunology* **116**, 181–187.

Lunn PG, Northrop-Clewes CA & Downes RM (1991) Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. *Lancet* **338**, 907–910.

Miotti PG, Taha TET, Kumwenda NI, Broadhead R, Mtimavalye LAR, Van der Hoeven L, Chiphangwi JD, Liomba G & Biggar RJ (1999) HIV transmission through breastfeeding: a study in Malawi. *Journal of the American Medical Association* **282**, 744–749.

Miranda R, Saravia NG, Ackerman R, Murphy N, Berman S & McMurray DN (1983) Effect of maternal nutritional status on immunological substances in human colostrum and milk. *American Journal of Clinical Nutrition* **37**, 632–640.

Morton JA (1994) The clinical usefulness of breast milk sodium in the assessment of lactogenesis. *Pediatrics* **93**, 802–806.

Narayanan I, Murthy NS, Prakash K & Gujral VV (1984) Randomised controlled trial of effect of raw and holder pasteurised human milk and of formula supplements on incidence of neonatal infection. *Lancet* **ii**, 1111–1113.

Newburg DS (1997) Do the binding properties of oligosaccharides in milk protect human infants from gastrointestinal bacteria? *Journal of Nutrition* **127**, 980S–984S.

Newburg DS, Linhardt RJ, Ampofo SA & Yolken RH (1995) Human milk glycosaminoglycans inhibit HIV glycoprotein gp120 binding to its host cell CD4 receptor. *Journal of Nutrition* **125**, 419–424.

Nicoll A, Newell M-L, Van Praag E, Van de Perre P & Peckham C (1995) Infant feeding policy and practice in the presence of HIV-1 infection. *AIDS* **9**, 107–119.

Northrop-Clewes CA, Lunn PG & Downes RM (1997) Lactose maldigestion in breast-feeding Gambian infants. *Journal of Pediatric Gastroenterology and Nutrition* **24**, 257–263.

Nube M & Asenso-Okyere WK (1996) Large nutritional differences in nutritional status between fully weaned and partially breast fed children beyond the age of 12 months. *European Journal of Clinical Nutrition* **50**, 171–177.

Pabst HF & Spady DW (1990) Effect of breast-feeding on antibody response to conjugate vaccine. *Lancet* **336**, 269–270.

Peaker M (1974) Recent advances in the study of monovalent ion movements across the mammary epithelium: relation to onset of lactation. *Journal of Dairy Science* **58**, 1042–1047.

Perez-Escamilla R, Cohen RJ, Brown KH, Rivera LL, Canahuati J & Dewey KG (1995) Maternal anthropometric status and lactation performance in a low-income Honduran population: evidence for the role of infants. *American Journal of Clinical Nutrition* **61**, 528–534.

Peterson JA, Patton S & Hamosh M (1998) Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. *Biology of the Neonate* **74**, 143–162.

Pickering LK, Granoff DM, Erickson JR, Masor ML, Cordle CT, Schaller JP, Winship TR, Paule CL & Hilty MD (1998) Modulation of the immune system by human milk and infant formula containing nucleotides. *Pediatrics* **101**, 242–249.

Planchon SM, Martins CAP, Guerrant RL & Roche JK (1994) Regulation of intestinal epithelial barrier function by TGF-β1. *Journal of Immunology* **153**, 5730–5739.

Prentice A (1987) Breast feeding increases concentrations of IgA in infants' urine. *Archives of Disease in Childhood* **62**, 792–795.

Prentice A, Prentice AM, Cole TJ & Whitehead RG (1983) Determinants of variations in breast milk protective factor concentrations of rural Gambian mothers. *Archives of Disease in Childhood* **58**, 518–522.

Prentice A, Prentice AM & Lamb WH (1985) Mastitis in rural Gambian mothers and the protection of the breast by milk antimicrobial factors. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 90–95.

Rasmussen KM (1992) The influence of maternal nutrition on lactation. *Annual Review of Nutrition* **12**, 103–117.

Sazawal S, Bhan MK & Bhandari N (1992) Type of milk feeding during acute diarrhea and the risk of persistent diarrhea: a case control study. *Acta Paediatrics* Suppl. 381, 93–97.

Schroten H, Bosch M, Nobis-Bosch R, Kohler H, Hanisch F-G & Plogmann R (1999) Secretory immunoglobulin A is a component of the human milk fat globule membrane. *Pediatric Research* **45**, 82–86.

Semba RD, Kumwenda N, Hoover DR, Taha TE, Quinn TC, Mtimavalye L, Biggar RJ, Broadhead R, Miotti PG, Sokoll LJ, van der Hoeven L & Chiphangwi JD (1999*a*) Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. *Journal of Infectious Diseases* **180**, 93–98.

Semba RD, Kumwenda N, Taha TE, Hoover DR, Lan Y, Eisinger W, Mtimavalye L, Broadhead R, Miotti PG, van der Hoeven L & Chiphangwi JD (1999*b*) Mastitis and immunological factors in breast milk of lactating women in Malawi. *Clinical and Diagnostic Laboratory Immunology* **6**, 671–674.

Shuster DE, Kehrli ME & Baumrucker CR (1995) Relationship of inflammatory cytokines, growth hormone, and insulin-like growth factor-1 to reduced performance during infectious disease. *Proceedings of the Society for Experimental Biology and Medicine* **210**, 140–149.

Shuster DE, Kehrli ME & Stevens MG (1993) Cytokine production during endotoxin-induced mastitis in lactating dairy cows. *American Journal of Veterinary Research* **54**, 80–85.

Shuster DE, Lee EK & Kehrli ME (1996) Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows in mid-lactation. *American Journal of Veterinary Research* **57**, 1569–1575.

Silfverdal SA, Bodin L, Hugosson S, Garpenholt O, Werner B, Esbjorner E, Lindquist B & Olcen P (1997) Protective effect of breastfeeding on invasive *Haemophilus influenzae* infection: a case-control study in Swedish preschool children. *International Journal of Epidemiology* **26**, 443–450.

Thomsen AC, Espersen T & Maigaard S (1984) Course and treatment of milk stasis, noninfectious inflammation of the breast, and infectious mastitis in nursing women. *American Journal of Obstetrics and Gynecology* **149**, 492–495.

Uauy R, Stringel G, Thomas R & Quan R (1990) Effect of dietary nucleosides on growth and maturation of the developing gut in the rat. *Journal of Pediatric Gastroenterology and Nutrition* **10**, 497–503.

Van de Perre P (1995) Postnatal transmission of human immunodeficiency virus type 1: the breast-feeding dilemma. *American Journal of Obstetrics and Gynecology* **173**, 483–487.

Victora CG, Vaughan JP, Martines JC & Barcelos LB (1984) Is prolonged breast-feeding associated with malnutrition? *American Journal of Clinical Nutrition* **39**, 307–314.

Vochem M, Hamprecht K, Jahn G & Speer CP (1998) Transmission of cytomegalovirus to preterm infants through breast milk. *Pediatric Infectious Disease Journal* **17**, 53–58.

Waller KP, Colditz IG, Flapper P & Seow H-F (1997) Leukocyte and cytokine accumulation in the ovine teat and udder during endotoxin-induced inflammation. *Veterinary Research Communications* **21**, 101–115.

Warden RA, Noltorp RS, Francis JL, Dunkley PR & O'Loughlin EV (1997) Vitamin A deficiency exacerbates methotrexate-induced jejunal injury in rats. *Journal of Nutrition* **127**, 770–776.

Willumsen JF, Filteau SM, Coutsoudis A, Uebel KE, Newell M-L & Tomkins AM (2000) Subclinical mastitis as a risk factor for mother-infant HIV transmission. In *Short and Long Term Effects of Breast Feeding on Child Health* [B Koletzko, editor]. London and New York: Kluwer Academic/Plenum Publishing.

World Health Organization (1993) *Breast-feeding: The Technical Basis and Recommendations for Action*. Geneva: WHO.

World Health Organization Working Group on Infant Growth (1994) *An Evaluation of Infant Growth*. Geneva: WHO.

Wright AL, Bauer M, Naylor A, Sutcliffe E & Clark L (1998) Increasing breastfeeding rates to reduce infant illness at the community level. *Pediatrics* **101**, 837–844.

Zavaleta N, Lanata C, Butron B, Peerson JM, Brown KH & Lonnerdal B (1995) Effect of acute maternal infection on quantity and composition of breast milk. *American Journal of Clinical Nutrition* **62**, 559–563.

https://doi.org/10.1017/S002966510000080X Published online by Cambridge University Press