Proceedings of the Nutrition Society (2007), **66**, 384–396 DOI:10.1017/S0029665107005654

The Winter Meeting of the Nutrition Society, an international conference to mark the 100th anniversary of the birth of Elsie Widdowson, hosted by MRC Human Nutrition Research Cambridge jointly with the Neonatal Society supported by the Royal Society of Medicine was held at Churchill College, Cambridge on 11–13 December 2006

Symposium on 'Nutrition in early life: new horizons in a new century'

Session 1: Feeding and infant development
Breast-feeding and immune function

Lars Å. Hanson
Department of Clinical Immunology, Göteborg University, Göteborg, Sweden

The newborn receives, via the placenta, maternal IgG antibodies against the microbes present in its surroundings, but such antibodies have a pro-inflammatory action, initiating the complement system and phagocytes. Although the host defence mechanisms of the neonate that involve inflammatory reactivity are somewhat inefficient, this defence system can still have catabolic effects. Breast-feeding compensates for this relative inefficiency of host defence in the neonate by providing considerable amounts of secretory IgA antibodies directed particularly against the microbial flora of the mother and her environment. These antibodies bind the microbes that are appearing on the infant's mucosal membranes, preventing activation of the pro-inflammatory defence. The major milk protein lactoferrin can destroy microbes and reduce inflammatory responses. The non-absorbed milk oligosaccharides block attachment of microbes to the infant's mucosae, preventing infections. The milk may contain anti-secretory factor, which is anti-inflammatory, preventing mastitis in mothers and diarrhoea in infants. Numerous additional factors in the milk are of unknown function, although IL-7 is linked to the larger size of the thymus and the enhanced development of intestinal Tγδ lymphocytes in breast-fed compared with non-breast-fed infants. Several additional components in the milk may help to explain why breast-feeding can reduce infant mortality, protecting against neonatal septicaemia and meningitis. It is therefore important to start breast-feeding immediately. Protection is also apparent against diarrhoea, respiratory infections and otitis media. There may be protection against urinary tract infections and necrotizing enterocolitis, and possibly also against allergy and certain other immunological diseases, and tumours. In conclusion, breast-feeding provides a very broad multifactorial anti-inflammatory defence for the infant.

Breast-feeding: Immune function: Microbial exposure: Milk components

The microbial exposure of the infant from delivery onwards brings risk of early infections, but promotes development of the immune system

On leaving the protected intrauterine environment the newborn meets a world full of microbes, most of which are harmless and some are even protective, but many are potentially dangerous. It is vitally important that this early microbial exposure can be managed by the neonate. The high early-life mortality in poor socio-economic areas clearly illustrates this issue (Stoll, 2006).

During passage through the birth canal the offspring becomes exposed to microbes of maternal origin. Being delivered next to the mother's anus the newborn is subsequently colonized by the mother's microbial flora. This microflora is the least threatening, since the mother provides defence against these microbes primarily via breast-feeding, but also to some extent via her transplacentally-transferred IgG antibodies. These antibodies provide tissue defence that is pro-inflammatory by activating the complement system and phagocytes (Hanson, 2004). This form of defence is costly, because the

Abbreviation: SIgA, secretory IgA.
Corresponding author: Professor Lars Å. Hanson, fax +46 31 342 46 21, email lars.a.hanson@immuno.gu.se

inflammation will consume energy and will cause the common symptoms of disease, such as pain, tiredness, fever and loss of appetite, via the increase in leptin caused by the cytokines IL-1β, TNFα and IL-6 produced by the leucocytes that are activated by exposure to infectious agents. Furthermore, this form of defence, although necessary and vital, is clearly undesirable in the young infant because it can interfere with growth and development. The link between frequent infections in early life and impaired growth is commonly observed in poor socio-economic regions. In contrast, the host defence provided via the milk protects without inducing inflammation (Hanson, 2004); it can even counteract inflammation. The various modes of function of human milk components in relation to host defence will be described in some detail, but first the immune system in early life will be discussed.

The immune system of the neonate has several characteristics. First, it is of limited size, but relatively complete in terms of the presence of various forms of cells (Lewis, 2006). The lymphocytes are still of limited number and specificity. The neonate will only begin to enlarge its immune system when it is exposed to the numerous microbes present from delivery onwards. This enlargement process involves an increase in the lymphocytes with receptors that recognize the microbes that are in contact with the mucosal membranes; particularly those of the gastrointestinal tract where about 80% of the immune system is ultimately to be found. This very large number of lymphoid cells can be attributed to the very large surface area of the intestinal mucosa and the fact that it is more exposed to large numbers of microbes of a wider range of species than any other mucosal surface (Brandtzaeg *et al*. 2005). The rate of development of the immune system in early life is comparable with that of the nervous system.

Second, the immune system must be programmed to respond only to threatening microbes and not to the body's own tissues, foods and other harmless material. On the other hand, it must develop tolerance to such factors. The process is not fully understood, but obviously exposure to a normal intestinal microbial flora has a central role in the normal development of oral tolerance and builds on the appearance of regulatory T lymphocytes. There are suggestions, for instance, that the ongoing increase in allergic diseases in recent decades may be linked to inefficient development of oral tolerance. This situation might be a result of changes in delivery strategies that prevent the normal exposure to the mother's microflora, including the greater frequency of Caesarean section deliveries and other changes in the environmental hygiene of the family, with subsequent failure to fully develop oral tolerance. This explanation has been termed the 'microflora hypothesis' (Noverr & Huffnagle, 2005). A recent study in mice clearly demonstrates that exposure to Gram-negative bacteria present in the birth canal induces tolerance to such microbes by eliminating the Toll-like receptor 4 specific for Gram-negative bacteria on the intestinal epithelial cells, presumably to prevent inflammatory intestinal responses to the flora that subsequently colonizes the intestine (Lotz *et al*. 2006). However, the submucosal macrophages retain their Toll-like receptor 4 so that they can respond, activating the submucosal host defence on exposure to Gram-negative bacteria.

Early breast-feeding promotes the establishment of an optimal microbial gut flora in the infant from delivery onwards, counteracting potential pathogens (Adlerberth *et al*. 1999). Certain harmless strains, such as *Lactobacillus rhamnosus* and other lactobacilli, are promoted by human milk (Ahrne *et al*. 2005). Some bifidobacteria also seem to be favoured by breast-feeding (Salminen *et al*. 2005). The capacity of such micro-organisms to colonize the gut of the infant is promoted by breast-feeding, e.g. via the production of bacterial adhesins, which help the bacteria to attach to the mucosa and remain and multiply in the infant's gut. This process has been demonstrated in a recent study for certain non-pathogenic *E. coli* (Nowrouzian *et al*. 2005).

Components in human milk supporting the infant's host defence

It is apparent, therefore, that early life is a dangerous period, during which the neonate is exposed to the microbial flora of the environment, which may include many potential pathogens. Although the immune system of the neonate is immature, it has a high capacity to adapt and respond to microbial exposure, a process that takes much of the first year of life. After birth and during the first few months of life breast-feeding can contribute to the defence of the infant. Some of the major contributory factors and their functions will now be described.

Secretory IgA

The major human antibody is secretory IgA (SIgA), which comprises approximately 80% of all human Ig and is present in all exocrine secretions. It prevents microbes from reaching mucosal membranes, so that they cannot cause infections. SIgA was first discovered in, and isolated from, human milk (Hanson, 1961; Hanson & Johansson, 1962), and comprises 80–90% of the Ig in colostrum and milk. Its concentration is ≤ 12 g/l in colostrum and approximately 0·5–1 g/l in mature milk (Hanson, 2004).

The specificity of SIgA antibodies in milk is based on the incorporation of the microbes into the numerous aggregates of lymphoid cells in the mother's gut (the Peyer's patches). After exposure to these microbes the lymphoid cells migrate from the Peyer's patches to various mucosal membranes and exocrine glands, where they settle and produce the SIgA antibodies (Fig. 1). These antibodies are primarily directed against the microflora in the mother's gut. Thus, the mother's milk will contain SIgA antibodies against the microflora in her gut, i.e. the microbes that normally colonize the infant from delivery onwards; the process is termed the entero-mammaric link. Thus, if there are potential pathogens in the mother's gut flora, protection is provided by the milk SIgA. This process is illustrated by an observation made by Mata *et al*. (1969) in the village of Santa Maria Cauque' in Guatemala, where they were investigating the microbial flora of mothers and their infants. It was reported that the breast-fed infants of two mothers with diarrhoea as a result of *Shigella* did not

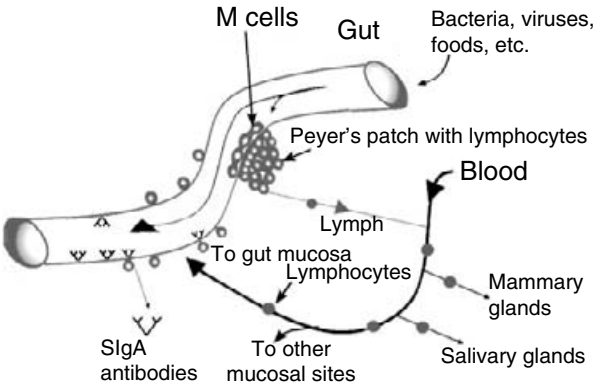

Fig. 1. Microbes are taken up in the gut mucosa by the numerous Peyer's patches, which contain specialized lymphocytes. They respond by producing secretory IgA (SIgA) antibodies specific for those microbes. These lymphocytes from the Peyer's patches then migrate to mucosal membranes, i.e. in the gastrointestinal and respiratory tracts, as well as to exocrine glands such as the mammary glands. As a result of this 'enteromammaric link' the milk will contain SIgA antibodies against the mother's intestinal microflora, to which the infants are exposed from delivery onwards. (From Hanson, 2004, 2007; reproduced with permission from Hale Publishing, Amarillo, TX, USA.)

show any symptoms of diarrhoea, even though the *Shigella* strain infecting the mothers was also found in their infants' stools. The presence of this homing mechanism has been established in man and in experimental animals (Goldblum *et al*. 1975; Roux *et al*. 1977) by showing that specific SIgA antibodies appear in the milk soon after oral exposure of the lactating woman or experimental animal.

The major mode of action of SIgA antibodies, which are relatively resistant to enzymic degradation, is to bind micro-organisms, thus preventing them from reaching, attaching to and entering through mucosal membranes. This protective capacity remains in some of the fragments after enzymic degradation of the antibody molecule. Thus, SIgA can prevent activation of defence mechanisms such as the IgM and IgG antibodies in the blood and tissues of the infant, which activate the complement system and establish large numbers of granulocytes and other phagocytes that produce the pro-inflammatory cytokines IL-1β, TNFα, IL-6 and IL-8. The SIgA is more resistant to enzymic degradation than serum IgA; SIgA binds and neutralizes common bacterial enzymes that can degrade serum IgA (Plaut, 1978).

In clinical studies of breast-feeding it has been demonstrated that the levels of SIgA antibodies in the milk relate to protection against infections caused by enterotoxigenic *E. coli*, *Vibrio cholerae*, *Campylobacter*, *Shigella* and *Giardia lamblia* (Glass *et al*. 1983; Cruz *et al*. 1988; Ruiz-Palacios *et al*. 1990; Hayani *et al*. 1992; Walterspiel *et al*. 1994; Long *et al*. 1999).

In addition to the anti-bacterial effects of the milk SIgA, it appears that its carbohydrate side chains may function as promoters of the growth of *E. coli* with type 1 pili (F Nowrouzian, I Adlerberth, AE Wold and V Friman, unpublished results). These *E. coli* are generally of low virulence and may compete for space and nutrients with potentially more-pathogenic bacteria in the breast-fed infant's gut. It has also been suggested that human SIgA supports the formation of a thin biofilm on the epithelial surface of the gut, which might promote the normal microbial colonization of the gut (Bollinger *et al*. 2003).

Against this background it obviously makes sense to start breast-feeding directly after delivery, thus providing the neonate with SIgA-mediated protection. Indeed, a recent study in Ghana has shown that when breast-feeding is started within 1 h of delivery 22% of neonatal deaths are prevented, while the effect of starting breast-feeding within 1 d of birth is a 16% reduction as compared with starting at 3 d of age (Edmond *et al*. 2006). This finding may reflect the protective capacity not only of milk SIgA antibodies, but also of additional protective components in the milk, some of which will be described.

Lactoferrin

This major milk protein is present in colostrum at a concentration of about 5–7 g/l, which decreases to 1–3 g/l in mature milk (Goldman *et al*. 1982). It is a member of the transferrin family and consists of two lobes, each of which binds Fe. It is present in all exocrine secretions and also in granulocytes. There is a specific receptor in the gut for lactoferrin and lactoferrin fragments (Kawakami & Lonnerdal, 1991).

Lactoferrin is relatively resistant to degradation by trypsin and chymotrypsin, and stools of breast-fed babies contain considerable amounts of lactoferrin. Both lactoferrin and its fragments are bactericidal for many Gram-positive and Gram-negative bacteria. This effect does not seem to be related to the binding of Fe, which is needed by most bacteria; rather, it is the result of a destabilizing effect on the outer cell membrane of bacteria, making them more sensitive to lysozyme, which is also present in the milk (Ellison, 1994; Leitch & Willcox, 1998). Lactoferrin promotes growth of the strictly anaerobic Bifidobacteria, but it also cleaves colonization factors on the potential pathogen *Haemophilus influenzae* and hinders the mucosal adherence of enteropathogenic *E. coli* (Hendrixson *et al*. 2003; Ochoa *et al*. 2003). Furthermore, it has antiviral effects and acts against fungi such as *Candida albicans* (Nikawa *et al*. 1994). Lactoferrin, like SIgA, seems to have the capacity to decrease the risk of infections caused by bacteria, viruses and possibly fungi without the use of inflammatory mechanisms.

In addition, lactoferrin has the capacity to enter the nuclei of leucocytes and block the transcription factor NF-κB, which otherwise induces the production of the pro-inflammatory cytokines IL-1β, TNFα, IL-6 and IL-8. These cytokines cause inflammation, tenderness, tiredness, fever and loss of appetite by increasing leptin, while giving rise to many more leucocytes at the site of the infection that has initiated this host response. The inflammation associated with dextran sulphate-induced colitis in mice is reduced by human lactoferrin and some of its fragments (Haversen *et al*. 2003). This effect is paralleled by reduced levels of IL-1β in the blood and fewer TNFα-producing cells. It seems advantageous for breast-fed infants to have their host defence supported by a milk protein that helps

prevent infections and at the same time may suppress the infant's inflammatory response, which would otherwise disturb their health, appetite and growth (Togawa *et al*. 2002).

Lactoferrin and fragments of lactoferrin are taken up by a receptor in the gut and appear in the urine of breast-fed infants (Goldblum *et al*. 1989). This process may explain why human lactoferrin and certain active fragments of lactoferrin given perorally to mice can protect against experimental urinary tract infections (Haversen *et al*. 2000). Clinical studies reported by Mårild *et al*. (2004) also suggest that breast-feeding protects against urinary tract infections in children. Furthermore, some experimental studies (Edde *et al*. 2001; Gomez *et al*. 2001) suggest that lactoferrin may act against intestinal infections caused by *E. coli* and *Shigella flexneri*.

Carbohydrate components in milk interfering with microbial attachment to mucosal membranes

Human milk contains substantial amounts of oligosaccharides, glycoproteins and glycolipids. The oligosaccharides constitute the third-largest solid fraction in milk, after lactose and fat (Kunz & Rudloff, 1993). There are more than ninety different oligosaccharides, which are produced in the mammary glands. Only about 1% is absorbed and appears in the urine (Rudloff *et al*. 1996). These oligosaccharides seem to affect the composition of the gut microflora and may partly explain why breast-fed children carry potentially pathogenic *E. coli*, *Klebsiella* and other *Enterobacteriacae* strains less often than non-breast-fed children (Gothefors *et al*. 1975; Uauy & Araya, 2004).

To be able to infect a host via the mucosal membranes, where most infections occur, the microbes must attach to the mucosal cells. This process is specific, with different microbes binding to different carbohydrate structures on the cell surface (Newburg *et al*. 2005). The carbohydrate components of human milk function as receptor analogues and can therefore prevent microbes from binding to the carbohydrate moiety on the mucosal epithelium to which they are specifically adapted to bind. Certain bacterial toxins can be blocked in a similar way. Such specific anti-adhesion effects of milk oligosaccharides on the binding of microbes to mucosal epithelium have been demonstrated for: diarrhogenic *E. coli*, *Campylobacter jejuni*, *V. cholerae* and a *Salmonella*; otitis-causing *Streptococcus peumoniae* and *H. influenzae*; HIV-1 and numerous other microbes (Korhonen *et al*. 1985; Andersson *et al*. 1986; Newburg, 1999; Ruiz-Palacios *et al*. 2003; Coppa *et al*. 2006).

A similar capacity to prevent microbial attachment to specific mucosal carbohydrate structures has been demonstrated for glycoconjugates in human milk. The milk glycolipid Gb3 prevents binding of *Shigella dysenteriae* and a Shiga-like toxin from enterohaemorrhagic *E. coli* (Newburg, 1997). Similarly, the ganglioside GM1 blocks adhesion of *V. cholerae* and binds to the *E. coli*, *V. cholerae* and *C. jejuni* enterotoxins (Holmgren *et al*. 1983; Otnaess *et al*. 1983; Ruiz-Palacios *et al*. 1983). Respiratory syncytial virus (Laegreid *et al*. 1986) and hepatitis A virus (Zajac *et al*. 1991) are each neutralized by a different milk glycoprotein.

Another mechanism is illustrated by a Mac-2 protein, plentiful in milk, that supports the defence system by helping macrophages to bind to microbes as the initial step before engulfing and destroying them (Fornarini *et al*. 1999).

Mucin, and the fat globules in which they are mainly found, block the binding of *E. coli* to epithelial cells and inhibit replication of rotavirus (Schroten *et al*. 1992). Lactadherin, which is a mucin-associated glycoprotein, inhibits rotavirus and prevents its replication, presumably reducing the risk of this very common infectious agent (Newburg *et al*. 1998). Similarly, casein components seem to reduce cellular binding by *Actinomyces* and streptococci, whereas κ-casein inhibits mucosal attachment by *Helicobacter pylori* (Neeser *et al*. 1988; Strömqvist *et al*. 1995).

Anti-secretory factor

This human peptide, which is anti-inflammatory and anti-secretory, is produced in response to bacterial enterotoxins and also as a result of eating a specially-treated cereal (Lange & Lonnroth, 2001). It appears in human milk and has been shown in a placebo-controlled study to prevent clinical mastitis (Svensson *et al*. 2004). In a double-blind placebo-controlled study of acute or prolonged diarrhoea in 240 Pakistani children (Zaman *et al*. 2007) it was found to be protective. When giving the anti-secretory factor orally together with oral rehydration it was found that recovery within 3 d is attained in an additional 30% of the children.

α-Lactalbumin

After a specific reorganization of the molecule a major milk protein, α-lactalbumin, has been found to have a striking effect on human tumour cell lines (Svanborg *et al*. 2003) and has been termed 'human α-lactalbumin made lethal to tumour cells'. *In vivo* effects on human papilloma and *in vitro* effects on numerous human tumour cell lines, as well as on a human glioblastoma cell line in an animal model, have been demonstrated (Pettersson *et al*. 2006). It is not known whether 'α-lactalbumin made lethal to tumour cells' can be the basis for the claims that breast-feeding may reduce childhood leukaemia and maternal breast cancer.

Additional potentially-protective and anti-inflammatory factors and signals

It has been suggested that the milk lysozyme, in cooperation with lactoferrin and SIgA, might be involved in the elimination of *E. coli* (Adinolfi *et al*. 1966). These claims have yet to be confirmed. Anti-microbial effects of lipid components and a lipase in human milk have also been studied (Gillin *et al*. 1985; Hernell *et al*. 1986). The presence of the β-defensin LBD-1 has been demonstrated in human milk (Tunzi *et al*. 2000). For all these and several additional factors in milk there is a need for further research in order to define their potential biological role in

the breast-fed infant. This position is certainly relevant to the many cytokines, hormones and growth factors present in human milk.

It has been proposed that the presence of IL-7 in milk is linked to the fact that the central organ in the immune system, the thymus, is twice as large in breast-fed infants compared with non-breast-fed infants (Hasselbalch *et al*. 1999; Ngom, 2004). Studies in Africa have shown that a smaller thymus at birth predicts a higher infant mortality from infections, independent of other factors known to reduce the size of the thymus such as birth weight and malnutrition (Aaby *et al*. 2002). IL-7 seems to enhance not only the size of the thymus, but also has a stimulating effect on its output of the Tγδ lymphocytes aggregated in the cryptopatches in the intestinal mucosa (Laky *et al*. 2003). Among the many cytokines also present in milk is transforming growth factor-β, which like IL-10 down regulates inflammation (Hvas *et al*. 2007). In a mouse study (Letterio *et al*. 1994) transforming growth factor-β from milk was found to be absorbed in the gut and to depress the inflammation that occurs in mice lacking the gene for transforming growth factor-β. Human milk contains soluble receptors for IL-1β and for TNFα, resulting in blockage of the pro-inflammatory effects of these cytokines (Garofalo & Goldman, 1999). Numerous other milk components are also anti-inflammatory, involving many different mechanisms: lactoferrin, as mentioned earlier, blocks cytokine production via NF-κB in leucocyte nuclei (Haversen *et al*. 2003); complement is inhibited by lysozyme, lactoferrin, α-lactalbumin, soluble complement inhibitors and complement regulatory factors (Ogundele, 1999); PG in milk inhibit neutrophil enzymes and are cytoprotective; several anti-proteases in milk, such as α1-antitrypsin, α1-antichymotrypsin and elastase inhibitor, block potentially-tissue-damaging enzymes (Garofalo & Goldman, 1999). It should be added that the large amounts of SIgA antibodies in the colostrum and milk also act as an anti-inflammatory agent by binding microbes, thus preventing them from attaching to and invading the mucosal membranes in the respiratory and gastrointestinal tracts, where they would bind to and activate Toll-like receptors on leucocytes in those sites.

Furthermore, there are numerous antioxidants in human milk, such as catalase, lactoferrin, α-tocopherol, β-carotene, L-histidine and ascorbic acid, which prevent hydroxyl radical formation and lipid peroxidation, degrade superoxide and scavenge oxygen radicals (Garofalo & Goldman, 1999).

Colostrum and milk contain high levels of soluble CD14; more than twenty times the concentration in serum (Labeta *et al*. 2000). This component helps phagocytes in the gut to be activated, via their Toll-like receptor 4 receptors, by Gram-negative bacteria for which these receptors are specific. Gram-positive bacteria will in a corresponding manner bind to their specific receptor, the Toll-like receptor 2, on phagocytes. Again, this binding is enhanced by the CD14 from the milk (Vidal *et al*. 2001). Such a function may help to defend the mammary gland against infections, but on the other hand may bring about clinical and/or subclinical mastitis caused by inflammation in the mammary glands (Filteau, 2003).

Human milk also contains many more signals from the mother to her offspring, such as additional cytokines, chemokines, hormones, growth factors, factors blocking cytokine receptors, complement-inhibiting factors, factors blocking cytokine receptors, maturation factors, soluble Toll-like receptors etc. Little is known about their possible effects in relation to breast-feeding.

Breast-feeding and protection against diseases in the infant

Numerous studies have been performed to determine whether breast-feeding protects against infections and immunological diseases such as asthma and allergy, autoimmune diseases and also tumours. A problem with such studies is that for ethical reasons it is difficult to undertake completely controlled studies, since breast-feeding and non-breast-feeding cannot easily be randomized and blinded. However, there are several studies that provide useful information, although variations in living conditions, dietary intake and exposure to infectious agents in different populations make it important to evaluate the outcomes with caution. The following is a brief review of such studies.

Reduction in infant deaths by breast-feeding

In developing countries that have very high infant mortality and high fertility breast-feeding serves to diminish both factors, which together provide a strong protective effect (Hanson *et al*. 1994; Labbok *et al*. 1997, 2004). Several studies have demonstrated protection by breast-feeding in early life via the numerous anti-infectious and anti-inflammatory components in the colostrum and mature milk. This effect is illustrated in a study in Brazil (Victora *et al*. 1987), in which it was found that exclusive breast-feeding reduces the risk of dying as a result of diarrhoea by 14·2-fold, whereas partial breast-feeding is associated with a reduction of 4·2-fold compared with no breast-feeding. A recent WHO report (Bahl *et al*. 2005), based on combined results from Ghana, India and Peru, has shown that there is no significantly different risk of death when comparing infants who are exclusively and predominantly breast-fed. However, the hazard ratios obtained when comparing infants who are not breast-fed with those predominantly breast-fed and those partially breast-fed respectively are 10·5 ($P<0·001$) and 2·46 ($P<0·001$). Based on the evident protective capacity of breast-feeding, it has been suggested that not breast-feeding may be the most common immunodeficiency in infancy (Hanson, 1998).

A recent important investigation in Ghana, which was part of the WHO study (Bahl *et al*. 2005), has demonstrated, as mentioned earlier, that starting breast-feeding within 1 h of delivery reduces infant mortality by 22 % compared with starting after 3 d, which is the most common practice in rural Ghana (Edmond *et al*. 2006). Initiating breast-feeding from day 1 was reported to result in a reduction in mortality of 16 %, illustrating how supportive the breast-milk defence components are in handling the

early microbial colonization in a region of Ghana that has heavy microbial exposure. A careful analysis with robust statistics has claimed that promoting breast-feeding could prevent as many as 720 postneonatal deaths per year in USA (Chen & Rogan, 2004).

However, a recent critical review of the protective capacity of breast-feeding against early infections in preterm infants indicates that many, if not most, previous studies do not fulfill the strict criteria required for critical evaluation (de Silva *et al*. 2004).

Breast-feeding and protection against neonatal infections

Several earlier reports have claimed to show protection against neonatal septicaemia and meningitis (Hanson, 2007). In a planned prospective study Narayanan *et al*. (1982) fed expressed breast milk to low-birth-weight babies and noted significant protection compared with feeding formula ($P<0{\cdot}001$). Cases of moderate to severe diarrhoea caused by *Campylobacter* and calicivirus were found to occur less often if the mother's milk is high in 2-linked fucosylated oligosaccharides. This effect may be associated with the capacity of such milk components to function as analogues to the specific mucosal receptors to which these microbes have to bind to achieve contact with the host (Morrow *et al*. 2004). It has been suggested that breast-feeding may also support host defence in the neonate by preventing hypoglycaemia and hypothermia (Huffman *et al*. 2001). A recent analysis of late-onset septicaemia in extremely-premature infants has indicated that the lower mortality in Norway compared with studies in other regions may be linked to the practice of very early full enteral feeding with human milk (Ronnestad *et al*. 2005). Without establishing such feeding within the second week of life the adjusted relative risk of late-onset septicaemia was found to be 3·7 (95% CI 2·0, 6·9).

Breast-feeding and necrotizing enterocolitis

This condition presumably illustrates how bacteria colonize the newborn and are not prevented from reaching the intestinal mucosa where the infant has some pro-inflammatory tissue defence, but little or no mucosal defence of its own. Attaching to the mucosa and even infiltrating it because of the lack of an efficient defence, the microbes will initiate an unbalanced inflammatory response that may result in local necrosis and even penetration of the gut mucosa (Lucas & Cole, 1990; Schanler *et al*. 1999; McGuire & Anthony, 2003). This condition has a high mortality, but breast-feeding reduces the risk, presumably by providing mucosal protection and suppression of inflammation. This protection is probably mediated, for example, by the milk SIgA antibodies, which through the enteromammaric link described earlier are directed against the microbes of the mother and her environment, and can bind them to prevent them from attaching to and infiltrating the mucosa (Fig. 1). In addition, numerous other milk components may be protective in this situation, including the many milk oligosaccharides functioning as receptor analogues and thus preventing the microbes from reaching the infant's gut mucosa. The milk contains an enzyme that degrades the pro-inflammatory platelet-activating factor (Furukawa *et al*. 1993). The milk also contains the anti-inflammatory cytokines IL-10 (Fituch *et al*. 2004) and transforming growth factor-β. The milk defensin might be supportive. In addition, breast-feeding seems to promote a probiotic-like microflora that may also be protective by reducing the number of potentially-dangerous microbes by competition for nutrients and space, as discussed earlier.

Breast-feeding and sudden death in infancy

There is evidence that breast-feeding provides protection against this condition, but the effect is weak (Alm *et al*. 2002). Other protective factors are more important, especially not letting the infant sleep on its stomach.

Breast-feeding and diarrhoea

Diarrhoea, as a common cause of death in infancy, is a major contributor to infant mortality, and it is obviously important to determine the protective capacity of breast-feeding. Numerous studies have confirmed that breast-feeding is associated with a marked reduction in morbidity and mortality (Glass & Stoll, 1989; Howie *et al*. 1990; Victora, 1990). In a large randomized study in Belarus it was found that breast-feeding protects against gastroenteritis during the first year of life (Kramer *et al*. 2001).The study has also shown that exclusive breast-feeding for 6 months results in fewer cases of gastroenteritis than exclusive breast-feeding for 3 months (Kramer *et al*. 2003). A UK study (Quigley *et al*. 2006) has shown, after adjusting for confounders, that breast-feeding protects against gastroenteritis when comparing no milk with any milk (OR 2·74; $P<0{\cdot}005$), or not exclusive breast-feeding with exclusive breast-feeding (OR 3·62; $P<0{\cdot}006$). The protection reached an OR of 5 in less-privileged areas when comparing no milk with any milk and an OR of 17·66 when comparing no breast-feeding with exclusive breast-feeding.

The protection against diarrhogenic bacteria and their toxins appears to be mediated via the milk SIgA antibodies, according to studies of infections with enterotoxigenic *E. coli*, *Shigella*, *V. cholerae* and *G. lamblia* (Glass *et al*. 1983; Cruz *et al*. 1988; Ruiz-Palacios *et al*. 1990; Hayani *et al*. 1992; Long *et al*. 1999). The human peptide anti-secretory factor, which can be induced in human milk (Svensson *et al*. 2004), has recently been shown to give protection against acute diarrhoea as well as prolonged diarrhoea (Zaman *et al*. 2007). A Mexican study (Morrow *et al*. 1992) has shown that breast-feeding is associated with a 5-fold lower risk of diarrhoea caused by *G. lamblia* compared with no breast-feeding, and a 1·8-fold reduction when comparing partial breast-feeding with no breast-feeding. In relation to protection against rotavirus infections by breast-feeding, a lack of clear protection has been noted or, alternatively, a postponement of the disease or asymptomatic infections (Duffy *et al*. 1986; Clemens *et al*. 1993). A delaying effect on rotavirus appearance in the stool associated with higher levels of SIgA antibodies has been reported (Espinoza *et al*. 1997). However, another

study of nosocomial rotavirus infections has demonstrated protection by breast-feeding (Gianino *et al*. 2002).

Breast-feeding and respiratory tract infections

Investigations of the protective capacity of breast-feeding against otitis media have usually shown efficient protection against acute and prolonged infections (Cushing *et al*. 1998; Dewey *et al*. 1995; Duncan *et al*. 1993). It has been suggested that breast-feeding may protect against upper respiratory infections, but this role needs confirmation (Howie *et al*. 1990). In deprived areas breast-feeding is strongly protective against pneumonia (Victora *et al*. 1987; Cesar *et al*. 1999). However, the previously-mentioned well-controlled Belarus study (Kramer *et al*. 2003) has shown no difference in the prevalence of pneumonia when comparing infants exclusively breast-fed for 3 and 6 months. Other studies from the USA and Australia (Ford & Labbok, 1993; Oddy *et al*. 2003) have found that breast-feeding reduces the risk of developing pneumonia. Another US study (Wright *et al*. 1989) has described protection by breast-feeding against wheezing respiratory tract infections during the first 4 months of life. A Norwegian investigation (Nafstad & Jaakola, 2003) has shown that protection by breast-feeding against respiratory tract infections is more obvious if the mother smokes. A study has been conducted in USA to compare the effects of exclusive breast-feeding for 4 and 6 months on the appearance of pneumonia and otitis media, after adjustment for confounding factors (Chantry *et al*. 2006). A significant reduction in infections was found in the 6-month group compared with the 4-month group, decreasing the risk of attacks of pneumonia (OR 4·27 (95% CI 1·27, 14·35)) and of having up to three attacks of otitis media (OR 1·95 (95% CI 1·06, 3·59)). A recent investigation indicates that breast-feeding protects against clinical measles infection (Silfverdal & Montgomery, 2007).

Breast-feeding and urinary tract infections

In addition to an Italian study (Pisacane *et al*. 1992), a recent more-extensive prospective case–control study in Sweden has been reported (Mårild *et al*. 2004). Both studies show protection, but the Swedish study has demonstrated that longer duration of breast-feeding reduces the risk of infection among girls, with a similar trend among boys. Breast-feeding until 7 months results in enhanced protection for ≤ 2 years of age.

A Spanish group (Talayero *et al*. 2006) has investigated the effect of breast-feeding on hospitalization; cases of perinatal infections were excluded. The study was based on 1385 infants during the years 1996 and 1999. It was estimated that 30% of the seventy-eight hospital admissions caused by infections would have been avoided for each additional month of full breast-feeding. The authors suggest that full breast-feeding at 4 months of age would have prevented 56% of hospital admissions among the infants before 1 year of age.

The American Academy of Pediatrics (Gartner *et al*. 2005) has recently made a policy statement (Breast-feeding and the use of human milk) that summarizes the evidence for the benefits of breast-feeding, including protection against infections.

Long-term effects on the offspring of breast-feeding

Vaccine responses

It is well known that the transplacentally-transferred maternal IgG antibodies can inhibit the infant's own immune response, e.g. against measles vaccine. For this reason it is preferable, when possible, to start vaccination against measles after 12 months of age.

Breast-feeding, on the other hand, does not inhibit vaccine responses, with one exception; peroral vaccination with live poliovirus will fail if the infant is breast-fed too close before or after the dose is given. The milk antibodies will otherwise neutralize the live vaccine virus (World Health Organization, 1995). Some studies have shown that breast-feeding may enhance vaccine responses. A long-lasting enhancing effect was noted for the IgG2 antibody response to vaccination against *Haemophilus influenzae* type b (Silfverdal *et al*. 2002). This result has been confirmed and the study expanded to include similar effects by breast-feeding on the antibody response against pneumococci (Silfverdal *et al*. 2007). Exclusive breast-feeding for ≥ 90 d provides a higher proportion of infants with vaccine responses above protective levels. Previously, it has been shown that breast-feeding may enhance vaccine responses to tetanus, diphtheria, live poliovirus and *Haemophilus influenzae* type b (Hahn-Zoric *et al*. 1990; Pabst & Spady, 1990). However, some studies of vaccine responses to tetanus toxoid and *Haemophilus influenzae* type b vaccines have shown no such enhancing effects (Stephens *et al*. 1984; Watemberg *et al*. 1991; Decker *et al*. 1992). Such differences could be a result of, for example, variations in levels of transplacentally-transferred maternal antibodies against the vaccines used and possibly to differences in the amounts of relevant components in the mothers' milk.

Protection against infections

There are several studies that indicate that breast-feeding may enhance long-term protection against certain infections, e.g. gastroenteritis, respiratory tract infections, skin infections, urinary tract infections and severe complications to measles infections. The data has recently been reviewed (Hanson, 2004, 2007).

Effects against inflammatory diseases in the infant–child–adult

There have been many recent studies that have investigated the possibility that breast-feeding may affect the risk of developing the many common diseases for which it is known that inflammatory processes play a central role in the pathogenesis. As there are a multitude of components that affect the development and function of the immune system, and various forms of inflammation in the growing individual, many factors in the milk could be involved in such effects. There are very many studies of the possible role of breast-feeding in various forms of allergic diseases,

several showing protection, but some the reverse. The literature is very extensive and the results are not easy to summarize briefly; however, they have been the subject of recent reviews (Hanson, 2004, 2007). Positive effects of breast-feeding on inflammatory autoimmune diseases such as diabetes mellitus types 1 and 2, rheumatoid arthritis, coeliac disease, ulcerative colitis and Crohn's disease have been published and also summarized recently, as indicated earlier. Further studies are needed to confirm many of the results obtained.

Numerous investigators have studied the possibility that breast-feeding may prevent overweight and obesity in children and adults. Protective effects against obesity have been demonstrated in some large European studies (von Kries *et al*. 1999; Toschke *et al*. 2002). A dose–dependent protective effect has been noted in meta-analyses (Arenz & von Kries, 2005; Harder *et al*. 2005). The effect may remain into adolescence (Gillman *et al*. 2006). A similar observation has been reported in a Norwegian study, but with less effect in adulthood (Kvaavik *et al*. 2005). In large investigations from South and North America protective effects have been noted in some population groups, but not in others; it has also been proposed that the effect may be temporary (Bogen *et al*. 2004; Grummer-Strawn & Mei, 2004; Burke *et al*. 2005; Kersey *et al*. 2005; Araujo *et al*. 2006). Unmeasured confounding factors may explain the positive effects published (Nelson *et al*. 2005). It is possible that breast-feeding can protect against overweight and obesity, but at a rather low level at higher ages, with the effect impaired by many factors (Owen *et al*. 2005).

There have been claims that breast-feeding has protective effects against CVD, including hypertension, dyslipidaemia, obesity and/or insulin resistance (Singhal *et al*. 2004; Singhal & Lucas, 2004). Additional studies support the existence of such effects (Lawlor *et al*. 2004; Martin *et al*. 2005*b*). These effects may be related to the many components in milk, e.g. leptin, grehlin and insulin-like growth factor, which may affect appetite and metabolism (Savino *et al*. 2005; Hanson, 2007).

A meta-analysis has shown that breast-feeding protects against tumours in childhood such as acute lymphoblastic leukaemia, Hodgkin's disease and neuroblastoma (Martin *et al*. 2005*a*); ≤5% of childhood acute leukaemia and lymphoma may be prevented by increasing breast-feeding from 50% to 100%.

Tumours in adulthood, e.g. prostate, colo-rectal and gastric cancers are not reduced by breast-feeding, but there is a reduction in premenopausal breast cancer (Martin *et al*. 2005*c*). Each pregnancy diminishes the risk of breast cancer by 7% and this risk is reduced by another 4·3 years for each year of breast-feeding (Martin *et al*. 2005*c*). The recent discovery of the anti-tumour effect of 'α-lactalbumin made lethal to tumour cells', the reorganized milk α-lactalbumin, suggests that it might be involved in some of these anti-tumour effects of human milk (Svanborg *et al*. 2003; Pettersson *et al*. 2006).

Recent studies suggest that feeding human milk may also support the developmental outcome of extremely-low-birth-weight infants (Vohr *et al*. 2006). Other studies have debated whether breast-feeding can enhance IQ development, but a recent extensive investigation has found little or no effect (Der *et al*. 2006). It has been proposed that breast-feeding may enhance the resilience of children to later psychosocial stress (Montgomery *et al*. 2006).

Have the mammary glands developed from the innate immune system?

The skin glands that produce milk are unique to mammals. In a recent review their morphological and functional origin have been discussed in relation to their double function of providing the offspring with both nutrients and defence against infectious agents (Vorbach *et al*. 2006).

Most animal species use innate immune responses for their defence against infectious agents (Kimbrell & Beutler, 2001). In contrast to the acquired specific immune system based on specific antibodies and lymphocytes primarily directed against infectious agents, the non-specific host defence provides broad defence of lesser specificity (Hanson, 2007). Innate defence is very complex and includes many different components and mechanisms. Vorbach *et al*. (2006) suggest that lactation has evolved in incremental steps from providing protection to also offering nutrients. They highlight that the importance of milk for the offspring both for defence and nutrition is exemplified particularly by the unique evolution of two anti-microbial enzymes, xanthine oxidoreductase and lysozyme, both expressed in and secreted from the lactating mammary epithelium (Shahani *et al*. 1973). These two components are directly involved in the evolution of the nutritional capacity of milk as well as being antimicrobial.

Vorbach *et al*. (2006) thus argue that the mammary gland and its product, the milk, initially developed from the innate immune system and that this origin could explain why there are so many components in the milk that play more than one role for defence and nutrition. Against this background it becomes easier to see how and why so many defence strategies have developed in the mammary glands and the milk, all defending without inducing inflammation in the offspring; some components, like lactoferrin, even actively counteract inflammation. Thus, appetite is not disturbed, as it is when tissue defence is initiated; a process that always induces inflammation, which is consistently costly, particularly for a growing individual, and also affects appetite, i.e. via pro-inflammatory cytokines increasing leptin levels.

Acknowledgements

Support for research forming the basis of part of this review was obtained from the Swedish International Development Agency, the Bergvall and the Philipsson Foundations and the Royal Society for Arts and Sciences in Göteborg, Sweden.

References

Aaby P, Marx C, Trautner S, Rudaa D, Hasselbalch H, Jensen H & Lisse I (2002) Thymus size at birth is associated with infant mortality: a community study from Guinea-Bissau. *Acta Paediatrica* **91**, 698–703.

Adinolfi M, Glynn AA, Lindsay M & Milne CM (1966) Serological properties of gamma-A antibodies to Escherichia coli present in human colostrum. *Immunology* **10**, 517–526.

Adlerberth I, Hanson LÅ & Wold AE (1999) Ontogeny of the intestinal flora. In *Development of the Gastrointestinal Tract*, pp. 279–292 [IR Sanderson and WA Walker, editors]. Hamilton, Ontario: BC Dexter Inc.

Ahrne S, Lonnermark E, Wold AE, Aberg N, Hesselmar B, Saalman R, Strannegard IL, Molin G & Adlerberth I (2005) Lactobacilli in the intestinal microbiota of Swedish infants. *Microbes and Infection* **7**, 1256–1262.

Alm B, Wennergren G, Norvenius SG, Skjaerven R, Lagercrantz H, Helweg-Larsen K & Irgens LM (2002) Breast feeding and the sudden infant death syndrome in Scandinavia, 1992–95. *Archives of Disease in Childhood* **86**, 400–402.

Andersson B, Porras O, Hanson LÅ, Lagergård T & Svanborg Edén C (1986) Inhibition of attachment of Streptococcus pneumoniae and Haemophilus influenzae by human milk. *Journal of Infectious Diseases* **153**, 232–237.

Araujo CL, Victora CG, Hallal PC & Gigante DP (2006) Breastfeeding and overweight in childhood: evidence from the Pelotas 1993 birth cohort study. *International Journal of Obesity* **30**, 500–506.

Arenz S & von Kries R (2005) Protective effect of breastfeeding against obesity in childhood. Can a meta-analysis of observational studies help to validate the hypothesis? *Advances in Experimental Medicine and Biology* **569**, 40–48.

Bahl R, Frost C, Kirkwood BR, Edmond K, Martines J, Bhandari N & Arthur P (2005) Infant feeding patterns and risks of death and hospitalization in the first half of infancy: multicentre cohort study. *Bulletin of the World Health Organization* **83**, 418–426.

Bogen DL, Hanusa BH & Whitaker RC (2004) The effect of breast-feeding with and without formula use on the risk of obesity at 4 years of age. *Obesity Research* **12**, 1527–1535.

Bollinger RR, Everett ML, Palestrant D, Love SD, Lin SS & Parker W (2003) Human secretory immunoglobulin A may contribute to biofilm formation in the gut. *Immunology* **109**, 580–587.

Brandtzaeg P, Carlsen HS & Farstad IN (2005) The human mucosal B-cell system. In *Mucosal Immunology*, 3rd ed., pp. 617–654 [J Mestecky, J Bienenstock, M Lamm, W Strober, J McGhee and L Mayer, editors]. Amsterdam: Elsevier Academic Press.

Burke V, Beilin LJ, Simmer K, Oddy WH, Blake KV, Doherty D, Kendall GE, Newnham JP, Landau LI & Stanley FJ (2005) Breastfeeding and overweight: longitudinal analysis in an Australian birth cohort. *Journal of Pediatrics* **147**, 56–61.

Cesar JA, Victora CG, Barros FC, Santos IS & Flores JA (1999) Impact of breast feeding on admission for pneumonia during postneonatal period in Brazil: nested case-control study. *British Medical Journal* **318**, 1316–1320.

Chantry CJ, Howard CR & Auinger P (2006) Full breastfeeding duration and associated decrease in respiratory tract infection in US children. *Pediatrics* **117**, 425–432.

Chen A & Rogan WJ (2004) Breastfeeding and the risk of postneonatal death in the United States. *Pediatrics* **113**, e435–e439.

Clemens J, Rao M, Eng M, Ahmed F, Ward R, Huda S *et al.* (1993) Breast-feeding and the risk of life-threatening rotavirus diarrhea: Prevention or postponement? *Pediatrics* **92**, 680–685.

Coppa GV, Zampini L, Galeazzi T, Facinelli B, Ferrante L, Capretti R & Orazio G (2006) Human milk oligosaccharides inhibit the adhesion to Caco-2 Cells of diarrheal pathogens: Escherichia coli, Vibrio cholerae, and Salmonella fyris. *Pediatric Research* **59**, 377–382.

Cruz JR, Gil L, Cano F, Caceres P & Pareja G (1988) Breast-milk anti-Escherichia coli heatlabile toxin IgA antibodies protect against toxin-induced infantile diarrhoea. *Acta Paediatrica Scandinavica* **77**, 658–662.

Cushing AH, Samet JM, Lambert WE, Skipper BJ, Hunt WC, Young SA & McLaren LC (1998) Breastfeeding reduces risk of respiratory illness in infants. *American Journal of Epidemiology* **147**, 863–870.

Decker MD, Edwards KM, Bradley R & Palmer P (1992) Comparative trial in infants of four conjugate Haemophilus influenzae type b vaccines. *Journal of Pediatrics* **120**, 184–189.

Der G, Batty GD & Deary IJ (2006) Effect of breastfeeding on intelligence in children: prospective study, sibling pairs analysis, and meta-analysis. *British Medical Journal* **333**, 945–951.

de Silva A, Jones PW & Spencer SA (2004) Does human milk reduce infection rates in preterm infants? A systematic review. *Archives of Disease in Childhood* **89**, F509–F513.

Dewey KG, Heinig MJ & Nommsen-Rivers LA (1995) Differences in morbidity between breast-fed and formula-fed infants. *Journal of Pediatrics* **126**, 696–702.

Duffy LC, Byers TE, Riepenhoff-Talty M, La Scolea LJ, Zielezny M & Ogra PL (1986) The effects of infant feeding on rotavirus-induced gastroenteritis: A prospective study. *American Journal of Public Health* **76**, 259–263.

Duncan B, Ey J, Holberg CJ, Wright AL, Martinez FD & Taussig LM (1993) Exclusive breast-feeding for at least 4 months protects against otitis media. *Pediatrics* **91**, 867–872.

Edde L, Hipolito RB, Hwang FF, Headon DR, Shalwitz RA & Sherman MP (2001) Lactoferrin protects neonatal rats from gut-related systemic infection. *American Journal of Physiology* **281**, G1140–G1150.

Edmond K, Zandoh C, Quigley M, Amenga-Etego S, Owusu-Agyei S & Kirkwood B (2006) Delayed breastfeeding initiation increases risk of neonatal mortality. *Pediatrics* **117**, e380–e386.

Ellison RT (1994) The effects of lactoferrin on gram-negative bacteria. In *Advances in Experimental Medicine and Biology. Lactoferrin: Structure and Function*, pp. 71–90 [TW Hutchens, SV Rumballand and B Lönnerdal, editors]. New York: Plenum Press.

Espinoza F, Paniagua M, Hallander H, Svensson L & Strannegård Ö (1997) Rotavirus infections in young Nicaraguan children. *Pediatric Infectious Disease Journal* **16**, 564–571.

Filteau S (2003) The influence on mastitis on antibody transfer to infants through breast milk. *Vaccine* **21**, 3377–3381.

Fituch CC, Palkowetz KH, Goldman AS & Schanler RJ (2004) Concentrations of IL-10 in preterm human milk and in milk from mothers of infants with necrotizing enterocolitis. *Acta Paediatrica* **93**, 1496–1500.

Ford K & Labbok M (1993) Breast-feeding and child health in the United States. *Journal of Biosocial Science* **25**, 187–194.

Fornarini B, Iacobelli S, Tinari N, Natoli C, De Martino M & Sabatino G (1999) Human milk 90K (Mac-2 BP): possible protective effects against acute respiratory infections. *Clinical and Experimental Immunology* **115**, 91–94.

Furukawa M, Narahara H, Yasuda K & Johnston JM (1993) Presence of platelet-activating factor-acetylhydrolase in milk. *Journal of Lipid Research* **34**, 1603–1609.

Garofalo RP & Goldman AS (1999) Expression of functional immunomodulatory and anti-inflammatory factors in human milk. *Clinics in Perinatology* **26**, 361–377.

Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ & Eidelman AI (2005) Breastfeeding and the use of human milk. *Pediatrics* **115**, 496–506.

Gianino P, Mastretta E, Longo P, Laccisaglia A, Sartore M, Russo R & Mazzaccara A (2002) Incidence of nosocomial

rotavirus infections, symptomatic and asymptomatic, in breast-fed and non-breast-fed infants. *Journal of Hospital Infection* **50**, 13–17.

Gillin FD, Reiner DS & Gault MJ (1985) Cholate-dependent killing of Giardia lamblia by human milk. *Infection and Immunity* **47**, 619–622.

Gillman MW, Rifas-Shiman SL, Berkey CS, Frazier AL, Rockett HR, Camargo CA Jr, Field AE & Colditz GA (2006) Breast-feeding and overweight in adolescence. *Epidemiology* **17**, 112–114.

Glass RI & Stoll BJ (1989) The protective effect of human milk against diarrhoea: a review of studies from Bangladesh. *Acta Paediatrica Scandinavica* **351**, 131–136.

Glass RI, Svennerholm AM, Stoll BJ, Khan SR, Hassain KMB, Huq MI & Holmgren J (1983) Protection against cholera in breast-fed children by antibodies in breast-milk. *New England Journal of Medicine* **308**, 1389–1392.

Goldblum R, Ahlstedt S, Carlsson B, Hanson L, Jodal U & Lidin-Janson G (1975) Antibody-forming cells in human colostrum after oral immunization. *Nature* **257**, 797–799.

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, Garza C, Nichols BL & Goldblum RM (1982) Immunologic factors in human milk during the first year of lactation. *Journal of Pediatrics* **100**, 563–567.

Gomez HF, Herrera-Insua I, Siddiqui MM, Diaz-Gonzalez VA, Caceres E, Newburg DS & Cleary TG (2001) Protective role of human lactoferrin against invasion of Shigella flexneri M90T. *Advances in Experimental Medicine and Biology* **501**, 457–467.

Gothefors L, Olling S & Winberg J (1975) Breast feeding and biological properties of faecal E. coli strains. *Acta Paediatrica Scandinavica* **64**, 807–812.

Grummer-Strawn LM & Mei Z (2004) Does breastfeeding protect against pediatric overweight? Analysis of longitudinal data from the Centers for Disease Control and Prevention Pediatric Nutrition Surveillance System. *Pediatrics* **113**, e81–e86.

Hahn-Zoric M, Fulconis F, Minoli I, Moro M, Carlsson B, Böttiger M, Räihä N & Hanson L (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.

Hanson L, Ashraf R, Zaman S, Karlberg J, Khan SR, Lindblad B & Jalil F (1994) Breastfeeding is a natural contraceptive and prevents disease and death in infants, linking infant mortality and birth rates. *Acta Paediatrica* **83**, 3–6.

Hanson LÅ (1961) Comparative immunological studies of the immune globulins of human milk and blood serum. *International Archives of Allergy and Applied Immunology* **18**, 241–267.

Hanson LÅ (1998) Non-breastfeeding – The most common immunodeficiency. *Hong Kong Journal of Paediatrics* **3**, 5–8.

Hanson LÅ (2004) *Immunobiology of Human Milk. How Breast-feeding Protects Babies*. Amarillo, TX: Pharmasoft Publishing.

Hanson LÅ (2007) The role of breastfeeding in the defense of the child. In *Textbook of Breastfeeding* [T Hale and P Hartmann, editors]. Amarillo, TX: Hale Publishing (In the Press).

Hanson LÅ & Johansson BG (1962) Immunological characterization of chromatographically separated protein fractions from human colostrum. *International Archives of Allergy and Applied Immunology* **20**, 65–79.

Harder T, Bergmann R, Kallischnigg G & Plagemann A (2005) Duration of breastfeeding and risk of overweight: a meta-analysis. *American Journal of Epidemiology* **162**, 397–403.

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

Haversen LA, Baltzer L, Dolphin G, Hanson LÅ & Mattsby-Baltzer I (2003) Anti-inflammatory activities of human lactoferrin in acute dextran sulphate-induced colitis in mice. *Scandinavian Journal of Immunology* **57**, 2–10.

Haversen LA, Engberg I, Baltzer L, Dolphin G, Hanson LÅ & Mattsby-Baltzer I (2000) Human lactoferrin and peptides derived from a surface-exposed helical region reduce experimental Escherichia coli urinary tract infection in mice. *Infection and Immunity* **68**, 5816–5823.

Hayani KC, Guerrero ML, Morrow AL, Gomez HF, Winsor DK, Ruiz-Palacios GM & Cleary TG (1992) Concentration of milk secretory immunoglobulin A against Shigella virulence plasmid-associated antigens as a predictor of symptom status in Shigella-infected breast-fed infants. *Journal of Pediatrics* **121**, 852–856.

Hendrixson DR, Qii J, Shewry SC, Fink Dl, Petty AG, Baker EN, Plat AG & St Gem JW III (2003) Human milk lactoferrin is a serine protease that cleaves Haemophilus surface proteins at arginine-rich sites. *Molecular Microbiology* **47**, 607–617.

Hernell O, Ward H, Blackberg L & Pereira ME (1986) Killing of Giardia lamblia by human milk lipases: an effect mediated by lipolysis of milk lipids. *Journal of Infectious Diseases* **153**, 715–720.

Holmgren J, Svennerholm AM & Lindblad M (1983) Receptor-like glycocompounds in human milk that inhibit classical and El Tor Vibrio cholerae cell adherence (hemagglutination). *Infection and Immunity* **39**, 147–154.

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

Huffman SL, Zehner ER & Victora C (2001) Can improvements in breast-feeding practices reduce neonatal mortality in developing countries? *Midwifery* **17**, 80–92.

Hvas CL, Kelsen J, Agnholt J, Hollsberg P, Tvede M, Moller JK & Dahlerup JF (2007) Crohn's disease intestinal CD4+ T cells have impaired interleukin-10 production which is not restored by probiotic bacteria. *Scandinavian Journal of Gastroenterology* **42**, 592–601.

Kawakami H & Lonnerdal B (1991) Isolation and function of a receptor for human lactoferrin in human fetal intestinal brush-border membranes. *American Journal of Physiology* **261**, G841–G846.

Kersey M, Lipton R, Sanchez-Rosado M, Kumar J, Thisted R & Lantos JD (2005) Breast-feeding history and overweight in Latino preschoolers. *Ambulatory Pediatrics* **5**, 355–358.

Kimbrell DA & Beutler BB (2001) The evolution and genetics of innate immunity. *Nature Reviews Genetics* **2**, 256–267.

Korhonen TK, Valtonen MV, Parkkinen J, Vaisanen-Rhen V, Finne J, Orskov F, Orskov I, Svenson SB & Makela PH (1985) Serotypes, hemolysin production, and receptor recognition of Escherichia coli strains associated with neonatal sepsis and meningitis. *Infection and Immunity* **48**, 486–491.

Kramer MS, Chalmers B, Hodnett ED, Sevkovskaya Z, Dzikovich I, Shapiro S *et al.* (2001) Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *Journal of the American Medical Association* **285**, 413–420.

Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP *et al.* (2003) Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. *American Journal of Clinical Nutrition* **78**, 291–295.

Kunz C & Rudloff S (1993) Biological functions of oligosaccharides in human milk. *Acta Paediatrica* **82**, 903–912.

Kvaavik E, Tell GS & Klepp KI (2005) Surveys of Norwegian youth indicated that breast feeding reduced subsequent risk of obesity. *Journal of Clinical Epidemiology* **58**, 849–855.

Labbok MH, Clark D & Goldman AS (2004) Breastfeeding: Maintaining an irreplaceable immunological resource. *Nature Reviews Immunology* **4**, 565–573.

Labbok MH, Hight-Laukaran V, Peterson AE, Fletcher V, von Hertzen H & Van Look PF (1997) Multicenter study of the Lactational Amenorrhea Method (LAM): I. Efficacy, duration, and implications for clinical application. *Contraception* **55**, 327–336.

Labeta MO, Vidal K, Nores JE, Arias M, Vita N, Morgan BP *et al.* (2000) Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. *Journal of Experimental Medicine* **191**, 1807–1812.

Laegreid A, Otnaess AB & Fuglesang J (1986) Human and bovine milk: comparison of ganglioside composition and enterotoxin-inhibitory activity. *Pediatric Research* **20**, 416–421.

Laky K, Lewis JM, Tigelaar RE & Puddington L (2003) Distinct requirements for IL-7 in development of TCR gamma delta cells during fetal and adult life. *Journal of Immunology* **170**, 4087–4094.

Lange S & Lönnroth I (2001) The antisecretory factor: synthesis, anatomical and cellular distribution, and biological action in experimental and clinical studies. *International Review of Cytology* **210**, 39–75.

Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S & Davey Smith G (2004) Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. *Circulation* **110**, 2417–2423.

Leitch EC & Willcox MD (1998) Synergic antistaphylococcal properties of lactoferrin and lysozyme. *Journal of Medical Microbiology* **47**, 837–842.

Letterio JJ, Geiser AG, Kulkarni AB, Roche NS, Sporn MB & Roberts AB (1994) Maternal rescue of transforming growth factor-b1 null mice. *Science* **264**, 1936–1938.

Lewis DB (2006) Developmental immunology and role of host defenses in fetal and neonatal susceptibility to infection. In *Infectious Diseases of the Fetus and the Newborn Infant*, pp. 87–210 [JS Remington, JO Klein, CB Wilson and CJ Baker, editors]. Philadelphia, PA: Elsevier Saunders.

Long K, Vasquez-Garibay E, Mathewson J, de la Cabada J & DuPont H (1999) The impact of infant feeding patterns on infection and diarrheal disease due to enterotoxigenic Escherichia coli. *Salud Publica de Mexico* **41**, 263–270.

Lotz M, Gutle D, Walther S, Menard S, Bogdan C & Hornef MW (2006) Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *Journal of Experimental Medicine* **203**, 973–984.

Lucas A & Cole TJ (1990) Breast milk and neonatal necrotising enterocolitis. *Lancet* **336**, 1519–1523.

McGuire W & Anthony MY (2003) Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. *Archives of Disease in Childhood* **88**, F11–F14.

Mårild S, Hansson S, Jodal U, Oden A & Svedberg K (2004) Protective effect of breastfeeding against urinary tract infections. *Acta Paediatrica* **93**, 164–168.

Martin RM, Gunnell D, Owen CG & Smith GD (2005*a*) Breastfeeding and childhood cancer: A systematic review with metaanalysis. *International Journal of Cancer* **117**, 1020–1031.

Martin RM, Gunnell D & Smith GD (2005*b*) Breastfeeding in infancy and blood pressure in later life: systematic review and meta-analysis. *American Journal of Epidemiology* **161**, 15–26.

Martin RM, Middleton N, Gunnell D, Owen CG & Smith GD (2005*c*) Breast-feeding and cancer: the Boyd Orr cohort and a systematic review with meta-analysis. *Journal of the National Cancer Institute* **97**, 1446–1457.

Mata LJ, Urrutia JJ, Garca RF & Béhar M (1969) Shigella infection in breast-fed Guatemalan Indian neonates. *American Journal of Diseases of Children* **117**, 142–146.

Montgomery SM, Ehlin A & Sacker A (2006) Breastfeeding and resilience against psychosocial stress *Archives of Disease in Childhood* **91**, 990–994.

Morrow AL, Reves RR, West MS, Guerrero ML, Ruiz-Palacios GM & Pickering LK (1992) Protection against infection with Giardia lamblia by breast-feeding in a cohort of Mexican infants. *Journal of Pediatrics* **121**, 363–370.

Morrow AL, Ruiz-Palacios GM, Altaye M, Jiang X, Guerrero ML, Meinzen-Derr JK, Farkas T, Chaturvedi P, Pickering LK & Newburg DS (2004) Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. *Journal of Pediatrics* **145**, 297–303.

Nafstad P & Jaakola JJK (2003) Breastfeding, passive smoking, and asthma and wheeze in children. *Journal of Allergy and Clinical Immunology* **112**, 807–808.

Narayanan I, Prakash K, Prabhakar AK & Gujral VV (1982) A planned prospective evaluation of the anti-infective property of varying quantities of expressed human milk. *Acta Paediatrica Scandinavica* **71**, 441–445.

Neeser JR, Chambaz A, Del Vedovo S, Prigent MJ & Guggenheim B (1988) Specific and nonspecific inhibition of adhesion of oral actinomyces and streptococci to erythrocytes and polystyrene by caseinoglycopeptide derivatives. *Infection and Immunity* **56**, 3201–3208.

Nelson MC, Gordon-Larsen P & Adair LS (2005) Are adolescents who were breast-fed less likely to be overweight? Analyses of sibling pairs to reduce confounding. *Epidemiology* **16**, 247–253.

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 (1999) Human milk glycoconjugates that inhibit pathogens. *Current Medicinal Chemistry* **6**, 117–127.

Newburg DS, Peterson JA, Ruiz-Palacios GM, Matson DO, Morrow AL, Shults J *et al.* (1998) Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet* **351**, 1160–1164.

Newburg DS, Ruiz-Palacios GM & Morrow AL (2005) Human milk glycans protect infants against enteric pathogens. *Annual Review of Nutrition* **25**, 37–58.

Ngom PT, Collinson A, Pido-Lopez J, Henson S, Prentice A & Aspinall R (2004) Improved thymic function in exclusively breast-fed babies is associated with higher breast milk IL-7. *American Journal of Clinical Nutrition* **80**, 722–728.

Nikawa H, Samaranayake LP, Tenovuo J & Hamada T (1994) The effect of antifungal agents on the in vitro susceptibility of Candida albicans to apo-lactoferrin. *Archives of Oral Biology* **39**, 921–923.

Noverr MC & Huffnagle GB (2005) The 'microflora hypothesis' of allergic diseases. *Clinical and Experimental Allergy* **35**, 1511–1520.

Nowrouzian FL, Monstein HJ, Wold AE & Adlerberth I (2005) Effect of human milk on type 1 and P-fimbrial mRNA expression in intestinal Escherichia coli strains. *Letters in Applied Microbiology* **40**, 74–80.

Ochoa TJ, Noguera-Obenza M, Ebel F, Guzman CA, Gomez HF & Cleary TG (2003) Lactoferrin impairs type III secretory

system function in enteropathogenic Escherichia coli. *Infection and Immunity* **71**, 5149–5155.
Oddy WH, Sly PD, de Klerk NH, Landau LI, Kendall GE, Holt PG & Stanley FJ (2003) Breast feeding and respiratory morbidity in infancy: a birth cohort study. *Archives of Disease in Childhood* **88**, 224–228.
Ogundele MO (1999) Inhibitors of complement activity in human breast-milk: a proposed hypothesis of their physiological significance. *Mediators of Inflammation* **8**, 69–75.
Otnaess AB, Laegreid A & Ertresvag K (1983) Inhibition of enterotoxin from Escherichia coli and Vibrio cholerae by gangliosides from human milk. *Infection and Immunity* **40**, 563–569.
Owen CG, Martin RM, Whincup PH, Davey-Smith G, Gillman MW & Cook DG (2005) The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *American Journal of Clinical Nutrition* **82**, 1298–1307.
Pabst HF & Spady DW (1990) Effect of breast-feeding on antibody response to conjugate vaccine. *Lancet* **336**, 269–270.
Pettersson J, Mossberg AK & Svanborg C (2006) alpha-Lactalbumin species variation HAMLET formation, and tumor cell death. *Biochemical and Biophysical Research Communications* **345**, 260–270.
Pisacane A, Graziano L, Mazzarella G, Scarpellino B & Zona G (1992) Breastfeeding and urinary tract infection. *Journal of Pediatrics* **120**, 87–89.
Plaut AG (1978) Microbial IgA proteases. *New England Journal of Medicine* **298**, 1459–1463.
Quigley MA, Cumberland P, Cowden JM & Rodrigues LC (2006) How protective is breastfeeding against diarrhoeal disease in infants in 1990s England? A case-control study. *Archives of Disease in Childhood* **91**, 245–250.
Ronnestad A, Abrahamsen TG, Medbo S, Reigstad H, Lossius K, Kaaresen PI, Egeland T, Engelund IE, Irgens LM & Markestad T (2005) Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. *Pediatrics* **115**, e269–e276.
Roux M, McWilliams M, Phillips-Quagliata J, Weisz-Carrington P & Lamm M (1977) Origin of IgA-secreting plasma cells in the mammary gland. *Journal of Experimental Medicine* **146**, 1311–1322.
Rudloff S, Pohlentz G, Diekmann L, Egge H & Kunz C (1996) Urinary excretion of lactose and oligosaccharides in preterm infants fed human milk or infant formula. *Acta Paediatrica* **85**, 598–603.
Ruiz-Palacios GM, Calva JJ, Pickering LK, Lopez-Vidal Y, Volkow P, Pezzarossi H & West MS (1990) Protection of breast-fed infants against Campylobacter diarrhea by antibodies in human milk. *Journal of Pediatrics* **116**, 707–713.
Ruiz-Palacios GM, Cervantes LE, Ramos P, Chavez-Munguia B & Newburg DS (2003) Campylobacter jejuni binds intestinal H(O) antigen (Fuca1,2Galb1,4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. *Journal of Biological Chemistry* **278**, 14112–14120.
Ruiz-Palacios GM, Torres J, Torres NI, Escamilla E, Ruiz-Palacios BR & Tamayo J (1983) Cholera-like enterotoxin produced by Campylobacter jejuni. Characterisation and clinical significance. *Lancet* **ii**, 250–253.
Salminen SJ, Gueimonde M & Isolauri E (2005) Probiotics that modify disease risk. *Journal of Nutrition* **135**, 1294–1298.
Savino F, Fissore MF, Grassino EC, Nanni GE, Oggero R & Silvestro L (2005) Ghrelin, leptin and IGF-I levels in breast-fed and formula-fed infants in the first years of life. *Acta Paediatrica* **94**, 531–537.
Schanler RJ, Shulman RJ, Lau C, Smith EO & Heitkemper MM (1999) Feeding strategies for premature infants: randomized trial of gastrointestinal priming and tube-feeding method. *Pediatrics* **103**, 434–439.
Schroten H, Hanisch FG, Plogmann R, Hacker J, Uhlenbruck G, Nobis-Bosch R & Wahn V (1992) Inhibition of adhesion of S-fimbriated Escherichia coli to buccal epithelial cells by human milk fat globule membrane components: a novel aspect of the protective function of mucins in the nonimmunoglobulin fraction. *Infection and Immunity* **60**, 2893–2899.
Shahani KM, Herper WJ, Jensen RG, Parry RM Jr & Zittle CA (1973) Enzymes in bovine milk: a review. *Journal of Dairy Science* **56**, 531–543.
Silfverdal SA, Bodin L, Ulanova M, Hahn-Zoric M, Hanson LA & Olcen P (2002) Long term enhancement of the IgG2 antibody response to Haemophilus influenzae type b by breastfeeding. *Pediatric Infectious Disease Journal* **21**, 816–821.
Silfverdal SA, Ekholm L & Bodin L (2007) Breastfeeding enhances the antibody response to Hib and Pneumococcal serotype 6B and 14 after vaccination with conjugate vaccines. *Vaccine* **25**, 1497–1502.
Silfverdal SA & Montgomery SM (2007) Breastfeeding protects against more severe manifestations of clinical measles infection. *Proceedings of the 12th International Conference of the International Society for Research of Human Milk and Lactation (ISRHML), Cambridge 2004*. (In the Press.)
Singhal A, Cole TJ, Fewtrell M & Lucas A (2004) Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet* **363**, 1571–1578.
Singhal A & Lucas A (2004) Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet* **363**, 1642–1645.
Stephens S, Kennedy CR, Lakhani PK & Brenner MK (1984) In vivo immune responses of breast- and bottle-fed infants to tetanus toxoid antigen and to normal gut flora. *Acta Paediatrica Scandinavica* **73**, 426–432.
Stoll B (2006) Neonatal infections: a global perspective. In *Infectious Diseases of the Fetus and the Newborn Infant*, pp. 27–57 [JS Remington, JO Klein, CB Wilson and CJ Baker, editors]. Philadelphia, PA: Elsevier Saunders.
Strömqvist M, Falk P, Bergström S, Hansson L, Lönnerdal B, Normark S & Hernell O (1995) Human milk kappa-casein and inhibition of Helicobacter pylori adhesion to human gastric mucosa. *Journal of Pediatrics* **21**, 288–296.
Svanborg C, Ågerstam H, Aronson A, Bjerkvist R, Duringer C, Fischer W *et al.* (2003) HAMLET kills tumor cells by an apoptosis-like mechanism – cellular, molecular and therapeutic aspects. *Advances in Cancer Research* **88**, 1–29.
Svensson K, Lange S, Lönnroth I, Widström A-M & Hanson LÅ (2004) Induction of anti-secretory factor in human milk may prevent mastitis. *Acta Paediatrica* **93**, 1228–1231.
Talayero JMP, Liza'n-Garcia M, Puime AO, Muncharaz MJB, Soto BB, Sanchez-Palomares M, Serrano LS & Rivera LL (2006) Full breastfeeding and hospitalization as a result of infections in the first year of life. *Pediatrics* **118**, e92–e99.
Togawa J, Nagase H, Tanaka K, Inamori M, Umezawa T, Nakajima A, Naito M, Sato S, Saito T & Sekihara H (2002) Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. *American Journal of Physiology* **283**, G187–G195.
Toschke AM, Vignerova J, Lhotska L, Osancova K, Koletzko B & Von Kries R (2002) Overweight and obesity in 6- to 14-year-old Czech children in 1991: protective effect of breast-feeding. *Journal of Pediatrics* **141**, 764–769.
Tunzi CR, Harper PA, Bar-Oz B, Valore EV, Semple JL, Watson-MacDonell J, Ganz T & Ito S (2000) Beta-defensin expression in human mammary gland epithelia. *Pediatric Research* **48**, 30–35.

Uauy R & Araya M (2004) Novel oligosaccharides in human milk: understanding mechanisms may lead to better prevention of enteric and other infections. *Journal of Pediatrics* **145**, 283–285.

Victora CG (1990) Case-control studies of the influence of breast-feeding on child morbidity and mortality: methodological issues. In *Human Lactation 4. Breastfeeding Nutrition Infection and Infant Growth in Developed and Emerging Countries*, pp. 405–418 [SA Atkinson, LA Hanson and RK Chandra, editors]. St John's, Newfoundland: ARTS Biomedical Publisher.

Victora CG, Smith PG, Vaughan JP, Nobre LC, Lombardi C, Teixeira AM, Fuchs SM, Moreira LB, Gigante LP & Barros FC (1987) Evidence for protection by breast-feeding against infant deaths from infectious diseases in Brazil. *Lancet* **2**, 319–322.

Vidal K, Labeta MO, Schiffrin EJ & Donnet-Hughes A (2001) Soluble CD14 in human breast milk and its role in innate immune responses. *Acta Odontologica Scandinavica* **59**, 330–334.

Vohr BR, Poindexter BB, Dusick AM, McKinley LT, Wright LL, Langer JC & Poole WK for the NICHD Neonatal Research Network (2006) Beneficial effects of breast milk in the neonatal intensive care unit on the developmental outcome of extremely low birth weight infants at 18 months of age. *Pediatrics* **118**, e115–e123.

von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V & von Voss H (1999) Breast feeding and obesity: cross sectional study. *British Medical Journal* **319**, 147–150.

Vorbach C, Capecchi MR & Penninger JM (2006) Evolution of the mammary gland from the innate immune system? *BioEssays* **28**, 606–616.

Walterspiel JN, Morrow AL, Guerrero ML, Ruiz-Palacios GM & Pickering LK (1994) Secretory anti-Giardia lamblia antibodies in human milk: protective effect against diarrhea. *Pediatrics* **93**, 28–31.

Watemberg N, Dagan R, Arbelli Y, Belmaker I, Morag A, Hessel L, Fritzell B, Bajard A & Peyron L (1991) Safety and immunogenicity of Haemophilus type b-tetanus protein conjugate vaccine, mixed in the same syringe with diphtheria-tetanus-pertussis vaccine in young infants. *Pediatric Infectious Diseases* **10**, 758–761.

World Health Organization (1995) Factors affecting the immunogenicity of oral poliovirus vaccine: a prospective evaluation in Brazil and the Gambia. WHO Collaborative Study Group on Oral Poliovirus Vaccination. *Journal of Infectious Diseases* **95**, 1097–1106.

Wright AL, Holberg CJ, Martinez FD, Morgan WJ & Taussig LM (1989) Breast-feeding and lower respiratory tract illness in the first year of life. *British Medical Journal* **299**, 946–949.

Zajac AJ, Amphlett EM, Rowlands DJ & Sangar DV (1991) Parameters influencing the attachment of hepatitis A virus to a variety of continuous cell lines. *Journal of General Virology* **72**, 1667–1675.

Zaman S, Mannan J, Lange S, Lönnroth I & Hanson LÅ (2007) Efficacy of anti-secretory factor in reducing severity and duration of acute and prolonged diarrhoeal illness in children 6–24 months of age – a placebo controlled trial. *Acta Paediatrica* (In the Press).