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Antibodies in Milk

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The immaturity of the infant's immune system and the rapid evolution of pathogens has created a demand for the mother to provide ready made specific defence factors to her offspring. This is achieved during the fetal period by transplacental transport of IgG antibodies, and after birth via IgA antibodies in the breast milk. The breast milk also contains a variety of nonspecific defence factors contributing to its antimicrobial effect. Breast feeding has been shown to decrease morbidity in gastroenteritis, septicemia, otitis media, urinary tract infection, encephalitis, pneumonia, and necrotizing enterocolitis. The antibody content in the mother's milk probably contributes not only to the immediate but also to the long term protection of the infant including both resistance to infection and development of immunological tolerance to harmless environmental antigens.

KEY WORDS: Antibodies; milk; IgA.

INTRODUCTION

Transfer of immunity from mother to offspring is phylogenetically a very old phenomenon that occurs in all species capable of antibody production. In non-mammalian species immunoglobulins are transferred from the egg yolk via the yolk sac to the developing embryo. At the other end of the evolutionary scale, in primates, the transfer of immunoglobulins to the circulation of the offspring is confined to the IgG isotype and takes place in the placenta. Other mammalian species show intermediates between yolk sac and intestinal transfer and in some species colostrum and milk provide all immunoglobulins transferred to the offspring and are hence essential for the survival of the offspring (Table I). The immunoglobulin content of colostrum and milk generally mirrors the needs of the offspring of a particular species. The most prominent variation is seen in the IgG and IgA content of colostrum. For example in ungulates (e.g., pigs, cows, sheep, and horses) colostrum contains more than 100g/L of IgG while IgA makes up 80% (12 g/L) of human colostral proteins (Table II). The IgA content of mature milk from most species is, however, comparable (≈0.5 g/L) except that ruminants (e.g., cows and sheep) have a relatively low level of IgA (0.02 g/L) and the dominating immunoglobulin is IgGl (0.5 g/L)(1).

IMMUNOGLOBULIN ISOTYPES AND ANTIBODIES IN HUMAN MILK

It has long been known that human milk contains antibodies. In the early sixties it was realized that, although serum-related antibodies were present in the milk, they differed from those in serum in that IgG showed a different electrophoretic mobility in milk and that IgA was the predominant immunoglobulin. IgA in milk also had additional structures compared with serum IgA (2). Later it was recognized that this special form of IgA, secretory IgA (sIgA)[3] was common to all external secretions. Human milk contains enormous amounts of sIgA about 1–2 g/L making up

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[3] Abbreviations: polymeric immunoglobulin receptor (pIg-R); secretory IgA (sIgA); ovalbumin (OvA).

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Table I. Transmission of Immunoglobulins from the Mother to the Circulation of the Offspring in Various Animal Species Including Humans[a]

Animal order		Transmission		Duration postnatal
		Prenatal	Postnatal	
Primates		placenta via Fcγ-receptor	trace amounts (small intestine)	?
Lagomorphs		yolk sac via Fcγ-receptor	small intestine (minor route)	?
Rodents		yolk sac via Fcγ-receptor (minor route)	proximal small intestine via Fcγ-receptor from milk	21 days
Carnivores		placenta/yolk sac Fcγ-receptor	small intestine (minor route)	?
Ungulates		none	entire small intestine all Ig isotypes from colostrum	24–30 hours
Avian		yolk sac	none	

[a] Time and route of transmission of immunoglobulins from mother to offspring.

25% of the total milk proteins. An adult produces approximately 0.04 g of IgA per kilo and day, or 2–3 grams per day, which is greater than the production of any other immunoglobulin class. The newborn infant produces very little sIgA, but is supplemented with 0.2–0.3 g/kg, equivalent to 0.5–1 g/day if fully breastfed. Thus, the breastfed infants receive five times more antibodies per area of mucosal membranes than the adult, possibly very important especially in the premature infant who has received insufficient amounts of IgG via transplacental passage.

THE ENTERO-MAMMARY LINK

Gastrointestinal exposure of the mother to antigens results in subsequent production of sIgA antibodies against these antigens. The preferential production of

Table II. Concentration (g/L) of the Three Major Immunoglobulin Isotypes in Colostrum/Milk and Serum from Normal Humans with the Calculated Ratios Between IgG and Either IgA or IgM[a]

Tested material	No. of samples	Immunoglobulin levels (g/L)				
		IgA	IgM	IgG	IgG:IgA	IgG:IgM
Serum	pool	3.28	1.32	12.30	3.750	9.32
Colostrum, 1–2 days	pool	12.34	0.61	0.10	0.008	0.16
Milk, 2–5 days	pool	0.99	0.34	0.08	0.081	0.24
Milk, 5–44 days	13	0.468	0.14	0.04	0.085	0.29
Milk, 55–147 days	3	0.89	0.14	0.05	0.057	0.36

[a] Immunoglobulins in serum and milk from healthy adults.

sIgA in the intestine is orchestrated by local populations of T-lymphocytes mainly of the Th2 (T-helper 2) phenotype producing interleukin 5 (3–5) and, in an additional set of T-cells, transforming growth factor β (6–8). Both these cytokines are the main factors that switch the IgM+ B lymphocytes initially activated to the production of IgA. During lactation these IgA producing B-cells originating in the intestine migrate to the mammary gland (9). This finding was part of the evidence that lymphoid cells leave the Peyer's patches after exposure to antigen and migrate to the various sites of local sIgA production in the body, such as mucosal membranes and exocrine glands, including the mammary glands (10–12) (see Fig. 1). As a consequence milk contains antibodies against numerous enterobacterial and other bacterial species, as well as viruses, parasites and even some food proteins. In addition there is evidence for a bronchomammary link resulting in milk sIgA against respiratory pathogens (13).

In cell transfer experiments in rats we noted that a selective homing occurred for cells producing not only IgA, but also IgM and IgG (14). The homing and maturation of plasma cells (15) and the expression of the polymeric immunoglobulin receptor (pIg-R) in the mammary gland is under hormonal control. Expression of the pIg-R has recently been shown to be inhibited by elevated progesterone-estradiol concentrations in the plasma during pregnancy. The subsequent increase at parturition is due to the concomitant decrease of the two circulating steroids and the increase of serum prolactin levels (16). Interestingly witch's milk also contains predominantly IgA presumably due to changes in maternal hormones at birth (17).

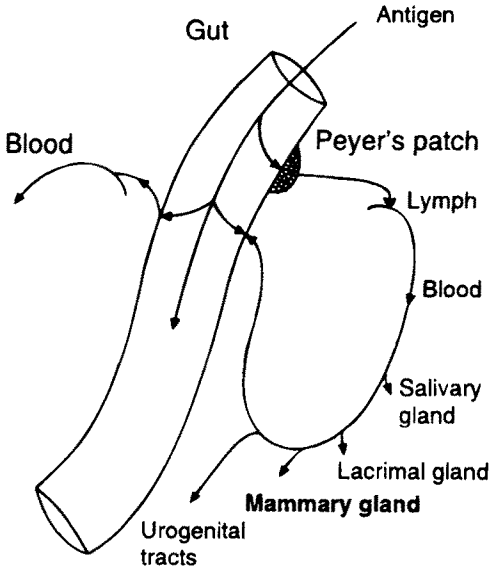

Fig. 1. Schematic diagram illustrating on the right side the homing of B-cells from the Peyer's patches, via the lymph and blood, to the exocrine glands, like the mammary gland, with local secretory IgA production.

There is evidence that some of the IgG antibodies in milk are produced locally in the mammary gland or selectively transferred through the mammary gland into the milk. For example the subclass distribution of IgG differs between milk and serum, with IgG2 and IgG4 being selectively enriched in the milk (18). Cell transfer studies in the rat show that IgG-producing blasts home to the mammary gland were they produce IgG locally (19, 20). Another indication of local production is the preferable usage of lambda light chains in milk antibodies. Mean lambda chains levels are consistently higher than these of kappa chains in milk, resulting in a reversed kappa/lambda ratio, significantly different from the classical 2:1 serum ratio (21). However, in humans IgM and IgG antibodies together make up only a few percent of the milk immunoglobulins of normal humans (Table II).

There is no detectable IgE in milk indicating that there is no local production or facilitated transport of IgE in the mammary gland. Even if transudation from serum parallels that for IgG then the chances of detecting IgE in milk would be minute.

Secretory IgA (Fig. 3) is formed by the combination of dimeric IgA with secretory component (SC) as a part of the polymeric immunoglobulin receptor (p-IgR) exposed on the basolateral aspect of the mammary gland epithelial cells (22, 23). The whole complex is internalized, and released to the milk as sIgA. The production of p-IgR mediated transport system exceeds that of the IgA production, especially during early lactation. Early milk, therefore, contains free secretory component (24) (Fig. 2). The p-IgR also binds and transports IgM and in IgA deficient mothers there is a compensatory elevation of IgM in the breast milk (25).

The homing of B-cell blasts both from mucosal surfaces and those participating in systemic immune responses (26) to the mammary gland ensure that milk contains a very broad range of antibodies, reflecting ongoing mucosal as well as systemic responses.

Not only antibodies, but also antigens either alone or in complex with antibodies are transferred to the infant via the breast milk (27, 28). Antigens can also be transferred as peptide fragments bound to soluble MHC class II molecules derived from the intestinal epithelium originating from the mother's diet (28–31). Antigens and/or specific antibodies delivered via the

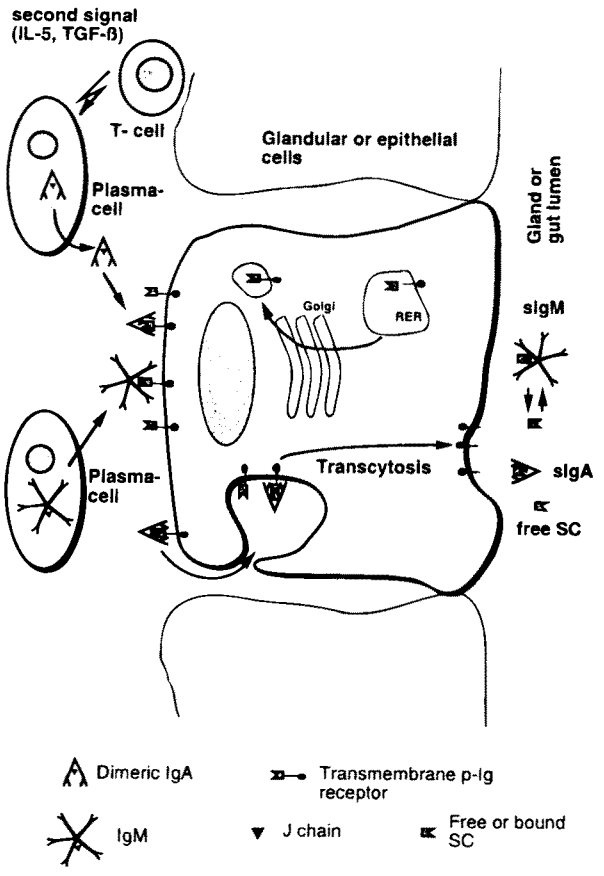

Fig. 2. Model for local generation of human secretory IgA and secretory IgM and transport through the epithelium in the mammary gland or gut. The epithelial cell produce the poly-Ig receptor with the secretory component (SC) necessary for transcytosis and release of the Ig's into the milk or gut lumen. (Modified from Brandzaeg *et al., Ann. N. Y. Acad. Sci.* **664**:61, 1992)

milk may either prime the offspring or render it tolerant. Sensitization to cow's milk proteins contained in human milk is one example (32). In isolator reared piglets receiving colostrum from sows that had been either immunized parenterally with ovalbumin (OvA) or fed OvA the hyperimmune colostrum primed the piglets for an OvA response. On the other hand the colostrum from the fed sows significantly suppressed the response to a subsequent immunization of the piglets with OvA (33) (Fig. 3). Similar effects have been observed in, various experimental animal models (34) but the significance in humans is unknown.

EVIDENCE THAT MILK ANTIBODIES ARE IMPORTANT FOR HOST DEFENCE

Numerous studies demonstrate that human milk prevents infections in breastfed babies. These findings

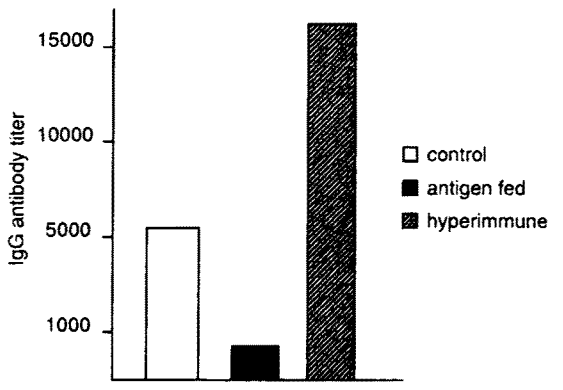

Fig. 3. Serum IgG antibody response in isolator reared piglets receiving colostrum at birth from groups of sows either fed ovalbumin (OvA) ■ or parenterally immunized with OvA ▨ or control colostrum □. The piglets were parenterally immunized at 4 w of age and antibody response assayed 4 w thereafter.

are especially striking under the poor hygienic conditions that occur in developing countries where there is a high risk of often lethal infections. The amount of sIgA antibodies against *Vibrio cholera, Campylobacter, Shigella, ETEC,* and *Giardia* in the milk has been shown to relate directly to the protection of the breastfed infant against these organisms (35). The protective effect of breastfeeding is particularly evident in diarrheal infections and it has been reported that the mortality from diarrhea in nonbreastfed infants compared to exclusively breastfed is 25 times higher (36). In some infections, e.g., *Giardia,* the amount of anti-Giardia sIgA in human milk is associated with prevention against diarrheal symptoms, but not against acquisition of the organism (37). It has also been demonstrated that mortality rates from lower respiratory tract infections can be decreased by breastfeeding (38). Breastfeeding appears to protect against otitis media (39) as well as necrotizing enterocolitis (40).

Breastfeeding has also been shown to protect against infections in developed countries. In a recent study Howie *et al.* (41) observed that breastfeeding produced good protection against gastroenteritis in a British community. Others have claimed protection by human milk IgA against *helicobacter pylori* infection in infancy (42). Protection by breastfeeding against acute lower respiratory infections has been demonstrated in Italy (43). Colonization of the oral cavity may also be controlled by breast milk as showed by the inhibited adherence of *C. albicans* to human oral epithelial cells by milk antibodies (44).

It is obviously important to start breastfeeding immediately after birth, especially in areas were other

foods or fluids carry a high risk of infection. Early suction at the breast most efficiently delivers the small early volumes of colostrum containing such high concentrations of antibodies that they can play an important role in controlling the intestinal flora and preventing infections (45, 46).

The protective capacity of IgA has been formally shown in an elegant model using mice into which hybridomas secreting a specific antibody were transplanted (47). Groups of mice bearing subcutaneous Sal4 hybridoma tumors secreting monoclonal sIgA into their gastrointestinal tracts were protected against oral challenge with *S. typhimurium* carrying the Sal4 epitope. Protection was shown to be dependent directly on specific recognition of the Sal4 epitope by the monoclonal IgA, since mice secreting Sal4 IgA from hybridoma tumors were not protected against a fully virulent mutant lacking that epitope. This experiment proves that specific milk antibodies are capable of providing protection against specific microorganisms.

OTHER PROTECTIVE AND IMMUNE FUNCTIONS OF BREAST MILK

In addition to specific antibody activity, milk immunoglobulins may interact with microorganisms by other mechanisms. For example, the N-linked oligosaccharide chains of sIgA can function as receptors for type 1-fimbriated *E. coli,* thereby reducing their attachment to colonic epithelial cells (45). These functions of the oligosaccharides are reviewed in the article by Newburg in this issue.

Two subclasses of IgA found in man, IgA1 and IgA2, occur in approximately equal proportions in breast milk. IgA1 possesses O-linked oligosaccharide chains, in addition to the N-linked chains found in all immunoglobulin classes. *Pseudomonas aeruginosa* and bacteria making up dental plaque are examples of bacteria that recognize the O-linked oligosaccharides of the structure Galβ1-3GalNAc, found in IgA1. The N-linked chains also differ slightly between the subclasses, with more completely glycosylated chains on IgA1 (46). The oligosaccharide chains of the p-IgR are extremely complex (48) and have been shown to inhibit the adherence of *Helicobacter pylori* to gastric eptithelium (49, 50).

Another important feature of sIgA is that it is relatively resistant to proteolytic degradation (51, 52). It can therefore persist and exert its action even in the small intestine of the breastfed infant where it otherwise would be rapidly degraded and nonfunctional.

IMMUNE EDUCATION BY IDIOTYPIC INTERACTION WITH MILK ANTIBODIES: EVIDENCE FOR ACTIVE PRIMING AND A POSSIBILITY OF LONG-TERM EFFECTS

Data is accruing showing that breastfeeding also actively stimulates and directs the immune response of the breastfed infant. The sIgA concentration increased more rapidly during the first 6 months after birth in exclusively breastfed infants than in those fed only formula (53).

Vaccine responses to oral polio virus vaccine and parenteral tetanus and diphtheria toxoid vaccines were enhanced by breastfeeding (54). The vaccines induced significantly higher responses in salivary IgA and stool IgM antibodies of the breastfed compared to a formula-fed group. Serum samples from the same children at the age of 21 to 40 months had significantly higher IgG antibodies to diphtheria toxoid and neutralizing serum antibodies to polio virus in the breastfed compared to the formula-fed controls. The response to *Haemophilus influenzae* type b polysaccharide capsule—protein carrier conjugate vaccine has also been studied. Again the conjugate vaccine gave a significantly elevated serum antibody response in breastfed compared to nonbreastfed infants (55).

We have shown that anti-idiotypic antibodies against polio virus are present in human milk (56). It is possible that such anti-idiotypic antibodies in milk may affect and enhance the immune response of the infant, priming them for better vaccine responses. Evidence that such responses are possible can be found in the study of Stein and Söderström (57) where anti-idiotypic antibodies given via the milk enhanced vaccine responses in neonatal mice against an *E. coli* capsular polysaccharide. Interestingly, monoclonal IgM anti-idiotypic antibodies administered orally have been shown to enhance the response to cholera toxin in another experimental animal model (58). This is still an unproven hypothesis but it provides the interesting idea of a prolonged education of the infants immune system by idiotypic interactions with antibodies present in mother's milk. With the possible vaccine response-enhancing activity of breastfeeding one might assume that the normal antibody responses to various microbes may also be stimulated by breastfeeding. This is in agreement with a recent study by Silfverdal *et al.* showing that breastfed babies were better protected against invasive *Haemophilus influenzae* type b infections, causing encephalitis, than non-breastfed even at 2–3 years of age (59). In addition

the study of Howie *et al.* (41) quoted earlier also showed protection against gastroenteritis beyond the period of breastfeeding itself. More recently it has been demonstrated that breastfeeding significantly reduces atopic disease up to 17 years of age (60). It is not clear at this time whether these effects result from stimulation of milk idiotypic determinants or other factors such as milk lymphocytes.

CONCLUSIONS

It is evident that antibodies along with other nonspecific factors in breast milk provide an immediate protection of the infant from infectious diseases most prominent in developing countries. The long-term effects of breastfeeding are still mostly unknown but a number of speculations are possible. Breast milk might secure the development of a stable and beneficial commensal bacterial flora which, along with anti-inflammatory factors in the milk itself, minimizes inflammatory reactions in the intestine. During the neonatal period when breast milk is the main source of environmental antigens but also serves as a protective "umbrella," the immune system of the infant might be educated in the difficult task of discriminating between harmless and harmful antigens in the environment, rendering the individual better prepared to fight infections and to avoid hypersensitivity reactions to harmless environmental antigens.

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