

Review

Transfer of antibody via mother's milk

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Received 8 December 2002; accepted 25 March 2003

Abstract

Differing from humans, IgG from breast milk in many animal species (rodents, bovines, cats, ferrets, etc.) are transported across the intestinal epithelium into the neonatal circulation. This transport is located at the duodenal and jejunal level where enterocytes express a surface membrane receptor able to bind Fc of IgG and to facilitate transcytosis of these immunoglobulins. Fc γ -R, which is very similar to the placenta receptor responsible for active transplacental transfer of IgG in humans, binds IgG but not other isotypes. Maternal milk antibodies represent an important part of circulating IgG in these animals, as they are involved in the negative feedback of endogenous IgG synthesis. This phenomenon stops abruptly as soon as weaning takes place. Neonatal calves that have a defect in such transfer of maternal immunoglobulins are at high risk of systemic infectious diseases.

In humans, in whom gut closure occurs precociously, breast milk antibodies do not enter neonatal/infant circulation. A large part of immunoglobulins excreted in milk are IgA that protect mainly against enteric infections. The specificity of maternal milk IgA is driven by an entero-mammary cell circulation. Human milk also contains anti-idiotypic antibodies capable of enhancing infant antibody response. Maternal milk antibodies coat infant mucosal surfaces and some have a clear protective role. This has been studied extensively in infectious disease models such as rotavirus, *E. coli*, poliovirus, and retroviruses. In the rotavirus model, antirotaviral IgA can be detected in stools of breast-fed but not bottle-fed neonates. In a large cohort of lactating women infected with HIV-1 in Rwanda, anti-HIV milk antibodies of the IgG isotype were more frequently detected followed by secretory IgM. Surprisingly, anti-HIV-1 SIgA were less frequently found. The presence of milk SIgA at 15 days as well as the persistence of a SIgM response during the whole lactation period was associated with lower risk of HIV transmission from the mother to the infant. Recently, HIV-1 antibodies from maternal milk have been shown to block transcytosis in vitro in a monolayer enterocyte model. Among these antibodies, those directed against the ELDKWA epitope had higher neutralising activity than serum antibodies.

In humans, milk excreted antibodies play a major role in protecting infants from infection by pathogens having a mucosal portal of entry.
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Keywords: Mother's milk; HIV; Fc receptor

In many mammalian species, newborns are hypogammaglobulinaemic and milk immunoglobulins are the principal source of antibodies [1]. Indeed, milk IgG are recognised by a jejunal and duodenal Fc receptor situated on the enterocyte membrane. It is transcytosed intact and delivered to the sub mucosa and then into the blood. Fc γ R binds and transports IgG, preferentially IgG2a and 2b but not other immunoglobulin isotypes. These milk immunoglobulins represent an important proportion of circulating IgG in these animals and are also involved in a negative feedback system, depressing endogenous synthesis of IgG. This transport mechanism stops abruptly at the weaning time.

As an example, IgG molecules are not present in the blood-stream of newborn calves because they cannot cross the placenta during pregnancy. Neonatal calves that suffer failure of passive transfer of maternal immunoglobulins may be at increased risk for diseases. Without feeding with a

sufficient amounts of colostrum and milk, mortality of the newborn calves increases dramatically in the first few days and weeks of life. There is a direct relationship between the amounts of colostrum fed and serum IgG in the calves. If feeding is delayed by 12 h, not only is the newborn calf agammaglobulinaemic in the first 12 h of life but the effect of feedings on serum IgG concentration is considerably mitigated, suggesting that the transport mechanism is maximal only if initiated early.

In humans, breast milk antibodies do not enter the neonatal/infant circulation in substantial amounts. The most important immunoglobulin in human milk is secretory IgA (SIgA) which serves as a first line defence in mucosal areas [2]. M cells are present in inductive sites in both the intestinal and respiratory tract. They represent about 2% of the epithelial cells in Peyer's patches and are also represented in the nasal-associated lymphoid tissue, the tonsils and

adenoids. M cells play an important role in antigen processing and in the induction of antigen-specific immunity in mucosal effector sites. T cells as well as SIgA+ B cells primed by adequately processed bacteria, viruses or vaccines in these inductive sites migrate through the thoracic duct and blood stream into mucosal effector sites, such as the mammary gland.

Therefore, two major inductor/effector axes, the entero-mammary axis and the broncho (or respiratory)-mammary axis that can translate the maternal mucosal encounter of agents or immunization into breast-milk-related protection of newborns and infants, can be delineated.

Contrary to serum IgA which is predominantly monomeric, mucosal IgA is secreted as dimers with an adjoining peptide, the J chain which stimulates dimerisation. Dimeric IgA producing B cells migrate from inductive sites and home into the mammary gland. There, dimeric IgA binds to the polymeric immunoglobulin receptor (pIgR) situated at the basolateral surface of epithelial cells [3,4]. Recognition of immunoglobulins by this pIgR is strongly related to the presence of J chain [5]. Pentameric IgM has a higher affinity for pIgR than dimeric IgA. The dimeric IgA is actively transported across the epithelial cell. At the apical surface of the cell membrane, the external domain of the polymeric immunoglobulin receptor is cleaved and the complex is released into the milk. The remainder of the receptor that binds covalently to the dimeric IgA is the secretory component. The complex formed by the dimeric IgA and the secretory component is secretory IgA (SIgA) that is released in the milk. SIgA represents the major immunoglobulin in breast milk, followed by secretory IgM and IgG. Taken together they represent 88–94% of total milk immunoglobulins. The concentration of SIgA is much higher in colostrum (12 mg/ml) than in mature milk (1 mg/ml). On average, a fully breastfed infant ingests from 0.5 to 1.0 g of SIgA per day [2].

Colostrum and transition milk also contains cytokines that could possibly impact on SIgA milk content [6]: Interleukin 6 is known to enhance IgA production, TNF- α enhances secretory component production and TNF- β can trigger isotype switching toward IgA producing B cells. However, these cytokine effects have never been clearly demonstrated in human milk *in vivo*.

Mucosal defence functions of SIgA are essentially of three orders: intracellular neutralisation, virus excretion and immune exclusion. Intracellular neutralisation occurs when SIgA transcytosed back from the basolateral epithelial cell surface encounters endosomes containing viral particles. This phenomenon has been first observed with Sendai virus and more recently with measles virus and HIV [2,7,8]. Viruses from the submucosal area entrapped in immune complexes can be transcytosed back from the basolateral surface of the cells and excreted into the lumen. Immune exclusion can be achieved by inhibiting adherence of a pathogenic micro-organism to the mucosal surface. SIgA can also agglutinate bacteria or viruses or interfere with bacterial motility by interacting with their flagella.

Examples of antibodies suggested or proven to be protective in breast milk include SIgA directed against bacterial pili, which have been identified [2]. SIgA capable of preventing adherence of pneumococci and *Haemophilus influenzae* have also been found in human milk. The neutralising capacity of milk anti-poliovirus antibodies has also been shown. All these studies suggest that human milk antibody transfer confers real protection to infants against many bacterial and viral infections.

Two experimental studies in mice showed that milk anti-idiotypic antibodies directed to *E. coli* and respiratory syncytial virus transferred in milk can prime the offsprings immune system against these antigens [2]. Anti-idiotypic antibodies to poliovirus have been found in human milk but it remains to be demonstrated that these antibodies can prime the neonatal or infant immune system [9].

The HIV model is somewhat particular in that breast milk both confers potential protection of babies through antibodies and cellular transfer, but is also a route of transmission of the virus from mother to child [10]. This gives us a unique opportunity to consider a balance between transmission and protection in a single host-virus relationship.

HIV is present in breast milk of infected mothers as both free viral particles and infected cells. The sources of HIV in breast milk of an infected mother are consistent with a clear compartmentalisation. Productively replicating and latently infected cells are both present in breast milk. These originate from the systemic compartment as well as from the mucosal compartment, consistent with the homing process from the entero-mammary and the broncho-mammary axes. Free viral particles can originate from the systemic compartment. This is particularly true in the case of an inflammatory process in the mammary gland compromising vascular and tissue permeability. It has been shown *in vitro* that HIV particles could also be released by ductal or alveolar epithelial cells [11]. This phenomenon is modulated by the action of several hormones like tri-iodothyronine, β -oestradiol or prolactin.

In a cohort study performed in Kigali, Rwanda, specific humoral response toward HIV-1 in milk was evaluated over time in 208 HIV-1 infected mothers [10]. Unexpectedly, anti-HIV-1 SIgA was not predominant in breast milk. Indeed, anti-HIV-1 IgG, probably originating at least partly from the systemic compartment, as well as SIgM were more frequently detected.

A specific SIgA response and/or a persistent specific SIgM response in breast milk during 18 months of lactation were inversely correlated with transmission, suggesting that local maternal defences against the virus may protect against transmission. When combining data on the detection of viral DNA and of anti-HIV-1 SIgM in the same samples, the highest risk of transmission was calculated from mothers whose milk harboured the virus but not HIV antibodies. This suggests that, as demonstrated in the experimental Feline Immunodeficiency Virus model, transmission of HIV-1 by breast feeding may be the result of the conjunction of the

presence of the virus in milk and an ineffective maternal mucosal immune response.

The precise portal of entry for HIV-1 across mucosal surfaces in infants is presently unknown. It is now believed that free HIV particles could cross the infant intestinal mucosa through mucosal lesions or transcytosed through M cells, as shown in a rabbit model. Transcytosis of viral particles entrapped in immune complexes involving anti-HIV facilitating antibodies and use of a cellular Fc receptor, has been hypothesised but never proven. HIV-infected cells could cross the epithelium through intercellular spaces in case of tight junctions defects.

Attachment of HIV virions on the apical cell surface of human enterocytes does not normally occur. However, Morgan Bomsel and colleagues have shown that an HIV-infected cell may be polarised and release HIV particles in close contact with human enterocytes [12]. After attachment on galactosyl ceramide receptors, transcytosis and release of virus in the vicinity of macrophages and lymphocytes of the lamina propria occurs, in a similar manner as in M cells. Moreover, in elegant experiments using monolayers of tight human enterocytes, she showed that SIgA and SIgM can inhibit transcytosis both by inhibiting viral entry and transport [8]. Exactly as in the Sendai virus and in the measles virus models, dimeric IgA and pentameric IgM could neutralise HIV-1 intracellularly and prevent transcytosis. The inhibition of transcytosis can be prevented by addition of anti-secretory component. This suggests that the secretory component plays a critical role in the protection conferred by antibodies against transepithelial viral transport. Amongst these antibodies, those directed against the well conserved Kattinger epitope gp41 (with the ELDKWA motif) were the most effective in intracellular neutralisation [13]. This epitope is involved in the recognition of the alternative receptor galactosyl ceramide on epithelial cells.

A reasonable model of the effect of milk antibodies on breast milk transmission of HIV-1, supported by these experiments may suggest that the humoral response in breast milk decreases the infectivity of breast milk by immune exclusion and intracellular neutralisation, and conversely, facilitating antibodies may increase infectivity.

A recent study performed by Pierre Becquart and co-workers evaluated DNA and RNA HIV env C2-V3 diversity in both the breast milk and blood of three infected women [14]. In two mothers, a major proviral variant was found in breast milk that was minor in blood. In addition, the predominant RNA variant in breast milk was not represented at all in the blood of two mothers. Quite unexpectedly, RNA variants in colostrum of the three mothers were different from proviral variants. This study strongly suggests that driving forces are selecting HIV variants dif-

ferently in blood and breast milk. Thus, milk antibodies may well contribute to viral selection and compartmentalisation.

In summary, breast milk anti-HIV-1 antibodies transferred from mother to child may have potential functions such as immune exclusion of HIV from the infant's mucosal surfaces, intraepithelial neutralisation and inhibition of HIV transcytosis. It may contribute to complement-dependent cell or particle lysis and perhaps to HIV quasi-species selection and compartmentalisation. A strong doubt remains regarding the existence of HIV facilitating antibodies in milk. Further studies are needed to confirm the role of milk antibodies in preventing HIV postnatal transmission. These studies may require creative and sophisticated designs in order to be conclusive.

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