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The Importance of Breastfeeding in Rotaviral Diarrhoeas

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ABSTRACT

Globally, rotaviral vaccines in use today have contributed to the reduction of the incidence of rotaviral diarrhoeas. Despite the substantial protection conferred by the current vaccines against the rotaviral strains, it is only prudent to recognise that other protective factors, like breastfeeding, also provide some degree of protection against this disease. This article has attempted to review some important mechanisms of protection in breast milk against the rotaviruses and highlight the oft forgotten non-immunoglobulin fraction in breast milk as an additional tool of protection against rotavirus disease. The adaptive capacity of breast milk to environment is another compelling reason to continue breastfeeding as it can usefully complement and be significant in the use of many vaccines. Vital immunoprotective constituents in breast milk beneficially protect the infant by initiating and strengthening many immune responses and should be borne in mind as essential tools of defence even in an era where vaccines play a pivotal role in the combat against certain diseases. It is impressive that besides nutritive advantages, the suckling infant enjoys appreciable immunoprotection via exclusive breastfeeding.

Keywords: Breastfeeding, rotavirus, vaccines, immunoprotection

INTRODUCTION

Breast milk is dynamic in its nutritive and immunological responses and has the potential to respond to the individual infant's needs. The dynamic nutritional responses in breast milk are evidenced by the milk of the mother of the preterm infant being qualitatively different from that of a full term mother (Walker, 2004). Immunologically, the higher levels of rotavirus specific maternal antibodies in the breast milk of mothers in developing countries where socio-economic and environmental factors contribute to the increased prevalence of rotavirus diarrhoeas reflect active and beneficial protective responses in breast milk to the suckling infant (María, Martínez-Costa & Javier, 2006; Moon *et al.*, 2010). This form of unique adaptive capacity in breast milk renders it distinct from any form of vaccination available.

Historically, the emergence of new strains of viruses, due to environmental and host factors causing mutation has occurred in many viral diseases (Huang & Lok, 2011). Though viruses differ in their genetic make-up, they all possess the potential capacity to mutate (Graff *et al.* 2008). This may also be the case with rotaviruses in the post-vaccine era. Following mutation, new strains not

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incorporated in the available vaccines, may pose new threats and open up vistas for further development of newer vaccines.

Additionally, the present day vaccines consist of two-dose and three- dose regimes with a gap between the doses *(http://www.cigna.com/customer_care/healthcare_professional/coverage_positions/pharmacyph_6114_coveragepositioncriteria_rotavirus_vaccine_rotateq.pdf*, 2011). Logically, it would be a fair assumption that these dosage regimes have to be completed for adequate and safe protection. During the interim between doses, it behoves us to take advantage of the protective potential of breast milk. This protection, provided by the inherent constituents in breast milk could be timely as various dynamic arms in breast milk come into force both naturally and via maternal exposure.

BREASTMILK PROTECTION AGAINST ROTAVIRUSES

There is ample evidence displaying a relationship between breastfeeding and the prevention of diarrhoeal diseases (Qadri *et al.*, 2005). In diarrheal diseases caused by *Vibrio cholera* and the entertoxigenic *E.coli*, breastfeeding has proven to be important in protection (Qadri *et al.* 2005). In Campylobacter diarrhoeas, too, useful defences in breast milk have been demonstrated (Morrow *et al.*, 2004). Components in breast milk with specific antitoxin activity against the stable toxin of *E.coli* have been detected (Newburg, 2009), possibly contributing to a reduction in the incidence of these diarrhoeas in the breastfed infant.

However, the link between viral diarrhoeas and breastfeeding is less clear. The production of specific factors against specific viral components or their products are, in many instances, not as definitely correlated to the reduction in the incidence of virus-induced diarrhoea. In rotaviral diarrhoeas, many studies indicate a protective role of breast milk (Newburg, 2009). This observation is challenged by others who fail to demonstrate a significant correlation but acknowledge that background environmental and sanitation factors may also be pivotal in contributing to rotaviral diarrhoeas (Wobudeya *et al*., 2011).

In Malaysia, recent breastfeeding programmes have been shown to be effective in improving the prevalence of "ever breastfeeding, timely initiation of breastfeeding and continued breastfeeding for up to two years" (Fatimah *et al*., 2010); earlier studies in relation to breastfeeding and the rotavirus indicated that the duration of breastfeeding was important in protection (Yap, Sabil & Muthu, 1984). When 587 children with gastroenteritis were examined for the presence of rotaviruses, rotavirus was detected in 46.3 %, with children from 1-2 years of age having the highest infection rate. There was no gender predilection. Highest infection rate was observed in Chinese children with Indian children having the lowest (Yap *et al.*, 1984).

It has been shown that maternal antibodies, mainly secretory immunoglobulin A (sIgA) have been detected in the breast milk of mothers in some developing countries (Moon *et al.*, 2010). Further evidence to support that these immunoglobulins could be protective is reflected by the presence of strain specific anti-rotaviral activity expressed in breast milk (María *et al*., 2006). The anti-rotaviral activity exhibited in breast milk was mainly antibody mediated neutralisation of the rotavirus or components of the rotaviral particle (Moon *et al.*, 2010). The high prevalence of rotavirus specific neutralising antibodies in breast milk could interfere with the efficacy of some oral rotavirus vaccines in developing countries (Chan *et al.*, 2011).

Cytokines, immunological components in breast milk, have anti-rotaviral activity (Chirico *et al.*, 2008). Cytokines in breast milk can reach the neonatal intestines intact as they are largely shielded from digestion by protease inhibitors, mainly

alpha$_1$-antichymotrypsin and alpha$_1$-antitrypsin (Chirico *et al.,* 2008). Although the protease inhibitor activity of α_1-antitrypsin and α_1-antichymotrypsin may have relevance for the reduced rates of digestibility of these proteins, other reviews suggest that this may only delay their eventual breakdown (Lonnerdal, 2003). There is also a suggestion that some cytokines in breast milk may be sequestered until they reach the intestines to exert their functions (Field, 2005). Cytokines, in general, work together with other cytokines or with other substances in breast milk to exert their effects. The review of cytokine action in breast milk does not generally indicate that other immunological molecules can potentially interfere with their action or significantly alter their mechanism of action (Field, 2005; Hanson, 2000). Their anti-inflammatory activity indicates their role in immune modulation (Chirico *et al.,* 2008). However, cytokine-mediated additive, synergistic and modulatory activities in breast milk have been described and suggest that their interaction with other components of breast milk can be beneficial to the breastfed infant (Lawrence & Pane 2007). Contributory maternal features and their impact on some immune components in breast milk is yet another arena that has been explored (Thibeau & D'Apolito 2011). Amongst cytokines, osteopontin (OPN) is a sialated phosphoprotein found in tissues and secreted into body fluids including breast milk. (Rollo *et al.,* 2005). It is a multi-functional integrin ligand and a cytokine that is active in cell signalling and has activity against the rotavirus (Rollo *et al.,* 2005).

Studies have also documented the importance of the non-immunoglobulin fraction of breast milk in conferring protection against the rotavirus (Liu *et al*., 2009). The non-immunoglobulin fraction, a part of innate immunity takes little time to be produced and as such is readily available to the suckling infant (Newburg, 2009). Whether increasing the concentration of these factors in breast milk could augment their anti-rotaviral activity *in vitro* or *in vivo* needs elucidation. Mechanisms to enhance their levels in breast milk are not clear either. Extrinsic factors generally seem not to interfere with their action in breast milk.

ANTIBODY PROTECTION

In the neonate, the less severe outcome of rotaviral diarrhoeas is partly due to transplacental immunoglobulins, transferred from mother-to-infant consisting both of rotavirus specific IgG and IgA (Newburg, 2009; van den Berg *et al.* 2011). IgG is preferentially transferred due to placental receptor specificity and these immunoglobulins offer temporary protection to the immature neonatal immune system. IgG antibodies passively received by the foetus from the mother may not only be protective, but may also actively prime the immune system of the neonate (Newburg, 2009; van den Berg *et al.,* 2011). The neonatal immune system also depends on factors such as exposure to maternal flora via the birth canal and breast milk to confer immune direction for its optimal maturation (Hanson *et al*., 2003). Despite this immunological immaturity, there is convincing evidence that the foetus is also capable of mounting immune responses via anti-immunoglobulins and other mechanisms (Hanson *et al.,* 2003). These anti-idiotypic antibodies are also passed on to actively prime the naïve immune system. The effects of anti-idiotypic antibodies through the placenta or via milk act against viral antigens and are deemed important in priming the naïve immune system (Hanson *et al*., 2003). Additionally in neonatal virus infections, rotaviral strains may evolve through a process of immune selection; as in this age group, these strains may be novel and not usually encountered in the community (Ray *et al,* 2007). Furthermore, it maybe postulated that the lactating mother who is exposed to environmental antigens such as these

unusual strains of the rotaviruses could potentially actively produce specific secretory immunoglobulin A (sIgA) in the mammary gland via intestine to mammary gland activation. This is fortified by recognition of brisk breast milk immune responses to different maternal environmental challenges (Moon *et al.*, 2010; Chan *et al.*, 2011). The protective capacity of breast milk constituents lends itself to a reasonable assumption that many more infections by unusual strains could manifest as rotaviral-induced diarrhoeal diseases if breastfeeding were not instituted early enough.

Further testimony to beneficial mucosal protection of the gastrointestinal tract is borne out by the production of focused antibodies in breast milk as apt reactions to relevant maternal exposure (Brandtzaeg, 2008; Brandtzaeg, 2010). These antibodies are the consequence of the integration between the infants gut associated lymphoid tissue (GALT) and the lactating mammary gland (Brandtzaeg, 2008; Brandtzaeg, 2010); and are highest in colostrum with gradual reduction in levels seen in transitional milk (Korhonen, Marnila & Gill, 2000), fortifying the value in establishing breastfeeding at its earliest.

The precise mode of reduced viral infectivity in the "framework of the diverse roles of the functions of general mucosal immunity of breast tissue and breast milk in boosting vital immune responses" needs further clarification (Prameela & Mohamed, 2010). Generally, some recognised mechanisms include viral neutralisation by interference with viral attachment and virus-receptor interactions, inductions of conformational changes by neutralising antibodies, neutralisation by antibody coating and viral escape (Klasse *et al.* 2002). The current concerns of neutralising antibodies in breast milk that could interfere with certain oral rotavirus vaccines, pose an issue of clinical concern in the 'take' of these vaccines, which are found to be useful in reducing the incidence of rotavirus diarrhoeas in infancy (Czerkinsky & Holmgren, 2009; Walker & Black, 2011). These issues need to be addressed separately and cannot undermine the importance of breast milk as an immunological tool to combat rotaviral disease.

Also notable are responses of rotavirus non-structural glycoprotein (NSP4), a rotaviral enterotoxin, and the non-neutralising antibodies against the inner capsid protein VP6 of the rotaviral particle. NSP4 is a rotavirus enterotoxin which induces secretory diarrhoea (Vizzi *et al.*, 2005). In a study evaluating serum antibody responses against the NSP4 after rotavirus vaccination and natural infection, serum antibodies against NSP4 were found to protect against rotaviral diarrhoeas (Vizzi *et al.*, 2005). Other studies have indicated that protection by the inner capsid proteins of rotavirus required transcytosis of mucosal immunoglobulins, and further encourages the development of mucosal vaccination strategies to optimise defence of the intestine through secretory immunoglobulins (Schwartz-Cornil *et al.*, 2002). The significance of mucosal immunity in protection of rotaviral diarrhoeas is highlighted. In this context it should be re-emphasised that the lactating mammary gland is an integral part of the common mucosal immune system which stands as a sentinel in combating mucosal pathogens, like the rotavirus, that enter the body via the mucosal route (Prameela & Mohamed, 2010). Furthermore, as already mentioned, despite the problems with high levels of maternal milk antibodies in developing countries and their potential interference with the efficacy of oral rotaviral vaccines (Chan *et al.*, 2011), it may equally be postulated that in selected settings, it may be possible that mucosal vaccines and breastfeeding may well interact to facilitate or even augment each other in their overall protection against rotaviral diseases.

CYTOKINE PROTECTION AGAINST ROTAVIRUSES

An array of cytokines including interleukins, interferon gamma and growth stimulating factors are found adequately in breast milk though they are deficient in the neonate especially in preterm infants (Parreño *et al.*, 2010). By various mechanisms, the cytokines reach the neonatal intestines intact to exert their biological activities. In rotavirus diarrhoeas, various cytokine responses are found in different phases of the illness (Jiang *et al*., 2003). Documented race related variations of some cytokines among the breast milk of Asian, European and African mothers are a manifestation of inherent immune versatility in breast milk (Chirico *et al*., 2008), again exhibiting the dynamic responses within the immunoprotection of breast milk.

Epithelial barrier integrity, known to be superior in the breastfed infant as compared to the bottle-fed infant, is also due to the action of certain cytokines such as IL-10 and IFN-γ, while others namely TGF-α and epidermal growth factor strengthen epithelial barrier development (Brandtzaeg, 2008). It may be hypothesised that in one way or other, the integrity of the intestinal mucosa maybe a significant factor that may protect from potentially invasive rotaviral disease. Rotavirus-infected intestinal epithelial cells (IECs) can 'sense' rotavirus infection and 'signal' production of interferons like IFN-β by means of genetic conduits (Frias *et al.*, 2012). These could determine the extent of rotavirus replication in the intestinal epithelium (Frias *et al*., 2012).

OPN, a cytokine with diverse biological function exerts its antirotaviral activity by interrupting cellular sialic acids and integrin receptors (Maeno *et al*. 2009; Rollo *et al*., 2005). In breast milk sialated forms of OPN, found in significant concentrations, have anti-rotaviral activity (Rollo *et al.*, 2005). Anti-viral mechanisms of OPN include interfering with the attachment of viruses prior to attachment and penetration to the epithelium, seen as important in infectivity (Rollo *et al.*, 2005). There is also a suggestion that rotaviruses induce intestinal expression of OPN (Rollo *et al.*, 2005); a mechanism that could well be strategic in rotaviral defence. It is unclear if other cytokines in breast milk cooperate in their role in enhancing intestinal production of OPN. Noteworthy too, milk OPN seems contributory to host resistance via the enhancement of Th1 (Thelper-1) responses (Renkl *et al.*, 2005); akin to augmented T cell proliferation in the breastfed in response to BCG vaccination (Pabst *et al.*, 1989).

PROTECTION BY THE NON-IMMUNOGLOBULIN FRACTION OF BREAST MILK AGAINST ROTAVIRUSES

In breast milk, rotaviral protection is also conferred by the non-immunoglobulin fraction (Liu *et al.*, 2009). These components are mainly constituents of the innate immune system (Liu *et al*., 2009). The innate system, amongst others, has lipids enveloped within the plasma membrane and secreted from the apical mammary epithelial cell producing the milk-fat globule as well as certain proteins and growth factors sequestered and embedded within it (Lonnerdal 2003). Elegant early experiments indicate that breast milk mucin, probably the most important contributor to this fraction in breast milk, possesses antirotaviral activity, and exerts competitive inhibitory binding of the virus to the cellular receptor (Yolken, 1992; Isa 2006) The mucin is present as a mucin complex with a high molecular weight aggregate of several mucin-like glycoproteins linked also to the milk fat globule (Kvistgaard *et al*., 2004). The carbohydrate side chains and sialic acid present in the milk mucins are essential for the expression of breast milk anti-rotaviral activity (Isa, 2006). Part of the milk-mucin complex is Glycosylation-dependent Cell Adhesion Molecule 1 (GlyCAM-1) mucin

which is expressed by the lactating mammary gland (Hou *et al*., 2000).

It is now known that the rotavirus, like many other viruses, has the capacity to evade innate immune mechanisms (Liu *et al.*, 2009). The potential in breast milk in defending against immunological evasion by the rotavirus is unknown. Interestingly, however, within breast milk lies "a capacity to influence neonatal microbial recognition by modulating TLR-mediated responses specifically and differentially, implying the existence of novel mechanisms regulating soluble pattern recognition receptors or toll like receptors (TLRs)" (LeBouder *et al.*, 2006). It may be extrapolated that this novel recognition pattern could also play a role in strategies against evasive ploys by the rotavirus. Further, it could be postulated that control of the extent of the immunological reactions to limit the infection could be contributed by beneficial breast milk immunomodulation.

Anti-adhesive activity of breast milk oligosaccharides has been described for various gut pathogens (Isa, Arias & López, 2006) Sialic acid contained in oligosaccharides, glycolipids and glycoproteins in milk are important for protection via enhancement of vital physiological functions (Kvistgaard *et al*., 2004). Oligosaccharides and glycoproteins contribute to interfering with rotaviral adherence, an early step of infection (Isa, Arias & López, 2006).

Lactadherin, like mucin, generally resistant to gastric acid degradation, is a component of milk innate immune protection (Kvistgaard *et al.*, 2004). This 46-kDa sialylated mucin associated glycoprotein, present in varying concentrations on the milk fat globule membrane, shows inhibitory activity to a number of rotaviral strains and prevents symptomatic infection in infants (Kvistgaard *et al.*, 2004). Lactadherin possibly acts through a mechanism involving protein-virus interactions hindering the attachment of the virus to the host cell (Kvistgaard *et al*., 2004). The decoy activity exploits differences in its protein structure (Kvistgaard *et al*., 2004).

Lactoferrin, a proteolysis resistant dominant whey glycoprotein as well as a carrier protein, protects in the early phase of rotavirus infection by preventing entry of virus in the host cell (van der Strate *et al.*, 2001). It probably acts by either blocking cellular receptors or by direct binding to the virus particles (Superti *et al.*, 2011). Both the unsaturated and the saturated forms have anti-viral activity. (Superti *et al.*, 2011; Lonnerdal, 2003). It is possible that desialysation of lactoferrin enhances its activity against the rotavirus (Superti *et al.*, 2011).

CONCLUSION

Notwithstanding the proven efficacy of the present-day rotaviral vaccines against rotaviral diarrhoeas, it is clear that breast milk still justifiably has a role against rotaviruses. In areas of the world where rotaviral vaccines have not been fully implemented for financial or logistic reasons, breastfeeding coupled with environmental and sanitation factors, should indeed be the mainstay of protection to prevent these diarrhoeas.

Admittedly, breastfeeding alone is insufficient as a tool to entirely protect against these viruses; the dynamic nature of breast milk is partly expressed by the brisk geo-demographic variability of immunologically mediated protection. This unique potential in breast milk could enhance the capacity of vaccinations which generally require time and where relevant, dosage completion in a multi dose regime, to express expected and effective clinical protection. This period of suboptimal immunoprotection before completion of vaccination or before the vaccine achieves desired protection, could be covered by exclusive and sustained breastfeeding.

The dawn of exciting knowledge of the immunological potential in breast milk makes it not unreasonable to hypothesise

that certain hitherto unknown mechanisms to mitigate the complexities of viral resistance could be initiated by sustained and exclusive breastfeeding. In breast milk may lie yet untold immunonutritive prowess pivotal to the nutrition and the clinician alike.

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