HIV transmission through breastmilk: the science behind the understanding of current trends and future research

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SUMMARY

Breastmilk protects the infant from many diseases and many short- term and long- term benefits accrue. At the same time it is also known that breastfeeding acts as a vehicle for some infective agents. It is now accepted that breastmilk transmission of Human Immunodeficiency Virus-1 (HIV-1) is an important   mode of paediatric infection . Despite this fact, many researchers have observed that corresponding to the volume of milk consumed by the infant, maternal transmission via breastmilk   is   still comparatively low. Some have noted the long latency period of breastmilk HIV transmission with evidence of numerous anti-HIV factors in breastmilk.

Although there are accepted standard guidelines on infant feeding in mothers who are HIV positive in many countries, it maybe equally important to realize gaps in our knowledge of   mother- to -child HIV transmission.   From an evolutionary perspective, the role of the mammary epithelial cell (MEC) and of breastmilk , in contributing to and possibly in influencing HIV-1 transmission is intriguing.   The presence of HIV-1 or of other viruses in maternal milk seem to be a requisite to spur immunological defenses   to optimize necessary   protection to the infant. This article reviews some aspects of the science of HIV transmission through breastmilk   and   reflects the concept -based understanding of current policies on HIV and breastfeeding. At the same time, it highlights uncertainties in this field and the urgency for future research in this direction. Accepting current notions of breastmilk HIV transmission, greater deliberation by research   may throw more light on why breastfeeding with its abundant advantages is fraught with the hazards of transmission of a deadly disease.

KEY WORDS:
Breastmilk, human immunodeficiency virus (HIV),   mammary epithelial cell

INTRODUCTION

Perinatal transmission of Human Immunodeficiency Virus (HIV-1) by avoidance of breastfeeding significantly reduces mother-to-child transmission [1].   The spread of HIV-1 by breastfeeding is avoided by substitution with artificial formula milk [1,2] In Malaysia, with a prevalence of between 0.1 and 1 percent ,HIV transmission rate during breastfeeding is estimated to be about 15% depending on the duration of breastfeeding [3,4]. Hence in Malaysia the current policy is that HIV positive mothers do not breastfeed if replacement feeding is acceptable, feasible, affordable,

sustainable and safe (AFASS)[5,6]. In some other parts of Asia, the majority of women do not know their HIV status and the risks of increased morbidity and mortality from unsafe feeding practices from unclean water supply have been well documented[1,2,7]. In Africa, the latest evidence suggests that, in the context of highly active antiretroviral therapy (HAART) provision for HIV-positive women or antiretroviral therapy (ARV) prophylaxis to breastfeeding infants, avoidance of breastfeeding leads to worse outcomes [8]. Also noteworthy is that exclusive breast milk feeding is less perilous than mixed breast milk and solid food feeding[2]. The new WHO guidelines for HIV and infant feeding is that countries should choose one infant-feeding strategy that health services can advise for HIV-positive mothers [8]. As per WHO guidelines, in mothers known to be HIV positive, commercial infant   formula, should only be initiated when specific conditions are met [9].

The lactating mammary gland and infectious agents

The lactating mammary gland has been implicated in the transmission of infectious agents to the suckling infant [10]. This is in striking contrast to its well recognized distinctive role of immunological protection of its young. Breastmilk is well recognized as vehicle for the Moloney Leukaemia Virus, Sarcoma Virus, and Mammary Tumour Virus[10]. Cytomegalovirus passes through milk and Rubella, Herpes Simplex, and Hepatitis B affect the suckling infant in the event of active   maternal infection [10]. Epstein Barr Virus (EBV) and Human Herpes Virus-6 (HHV6) although found in human milk, seldom infect the breastfed neonate [10]. Infection rate in milk of Hepatitis C is low unless maternal viral load is high [10].   In Malaysia, in maternal hepatitis B, mothers may continue breastfeeding provided the infant receives Hepatitis B vaccine as soon as possible preferably within the first 48 hours of birth.   In maternal Hepatitis C there is no contraindication to breastfeeding [11].

In man the RNA retroviruses including HIV-1, Human T Lymphocyte Virus-1 (HTLV-1), and Human T Lymphocyte Virus -2 (HTLV-2) are all transmitted through breastmilk [10]. Of these the HIV-1 is the major human pathogen found free in solution and within breastmilk monocytes comprising half the number of cells in healthy milk [12]. Although the significance of the cell- free and cell - associated forms of HIV in breastmilk transmission remains uncertain, both cell free and cell associated forms of HIV play a role in breastmilk transmission.

It is of particular interest to refer to the ample evidence signifying the role of the lactating mammary gland in immunoprotection before its   function as a reservoir of

PRACTICAL CLINICAL POINTERS
Table I: Current concepts and practices of breastfeeding in HIV positive mothers in Malaysia and in some other parts of the world

Breastfeeding policy in some other parts of the world
A breastfeeding mother with HIV may breastfeed her child if:
formula milk is not available
formula milk not available continuously for 6 months
safe water supply is not available
formula milk available and mother treated with HAART*
Breastfeeding policy in Malaysia
A breastfeeding mother with HIV does not breastfeed her child whether or not
mother is treated with HAART *
mother is not treated with HAART *

*Highly Active Anti Retroviral Therapy

nutritive vigour in the young[13]. The developmental immaturities in the fetus are compensated by transplacental and amniotic fluid transfer of maternal factors in utero[13]. Augmentation of mucosal barriers and as observed by Goldman (2002) changes of the permeability of the intestinal tract in the breast-fed infant is an example of beneficial developmental sequences conferred to the suckling young[13] Immaturities of the naive immune system are partially overcome by numerous immune factors in milk[13].

Innate and adaptive immunity in breastmilk
Milk is a cellular medium throughout lactation with 4×10^9/L in the colostrum , with a fall to approximately 10^8–10^9/L in mature milk[14] Parri passu with this cellular guard is the innate armour of defenses within milk – a spectrum of substances the mother provides in her breastmilk prior to the activation of a form of 'immune intelligence' which eventually harnesses more specific protective mechanisms. The innate system, confers dual advantages of protection as well as nutrition to the suckling infant[15].

Metal binding proteins, lactoferrin,a pleuripotent protein and transferrin have concentrations impressively higher in milk than in plasma[9,10,12,13,14]. Lactoperoxidase in milk catalyses host defenses at mucosal surfaces[13]. Mucins , glycoproteins shield from infections and are key components of mucus[9,13,14]. An adhesive glycophosphoprotein in breastmilk, osteopontine, is involved in T-helper-1(TH-1) type immune responses[13, 16]. Peptides of casein and milk triacyglycerols are equally important defense to the suckling infant[15]. The complement system in breastmilk aids identification and exclusion of important pathogens[17].

Against the HIV too, an array of innate factors capable of inhibiting mainly the cell free form of the virus in breastmilk

exists[18]. Some anti-viral factors influenced by genetic polymorphism like beta defensins are expressed in mammary epithelial cells and have notable anti – HIV activity[19] Elevated α-defensins linked to HIV viral load in breast milk may have a role to decrease mother- to- infant transmission by reducing the infectivity of the virus in breast milk or by increasing infant resistance where an analogy is made to passive immunization[20].

More specific defenses of adaptive immunocompetence in breastmilk need time and recognition of antigen for their functions. The adaptive responses are immune specific and dynamic, responding appropriately to maternal antigen exposure[19, 21]. Exposure of T-cells and B-cells to specific antigens with proliferative T cell responses and immunological memory, are the hall marks of this arm of immunity[22]. The secretory component (SC) found in milk as a surface epithelial receptor or a soluble form shields sIgA (sIgA), the prime immunoglobulin in mucosal immunity, from proteolytic digestion and are useful developmental strategies between the lactating mother and her infant[15,22].

The beneficial role of breastmilk
The beneficial role of breastfeeding in the prevention of many diseases must be reiterated. The consequences of not breastfeeding in maternal HIV must certainly be weighed against its immense advantages in the protection of other diseases.

Its positive role in respiratory tract infections, otitis media, and in gastrointestinal infections and sepsis compared to formula milk is well known[23]. In the preterm infant breastfeeding not only confers a significant reduction in incidence in NEC, its feeding benefits are time tested in the NICU pertaining both to inpatient stay as well as to hospital

Table II: Breastmilk : innate and adaptive factors

Breastmilk factors and HIV	
Innate factors : with or without maternal HIV	Adaptive factors : with maternal HIV
Lymphocytes and macrophages	specific mucosal sIgA
Lactoperoxidase	specific mucosal IgG
Alpha Defensins	specific sIgM
Lactoferrin	natural CCR5 antibodies
Lysozyme	cytolytic HIV-specific CD8+ T cells
Mucin	CCL28 chemokines
Glycoproteins	RANTES
Lipases	specific interleukins
Other immunonutritive factors	memory T cells

readmissions[23]. Sudden infant death syndrome (SIDS) rates, clinical asthma, atopic dermatitis, and eczema are also favourably influenced by breastfeeding[23]. Better neurodevelopmental outcome is another impressive advantage conferred to the breastfed particularly important in the preterm infant[23].

In the context of HIV, the potential of the mammary gland for genetic interactions is pertinent. In coeliac disease breastmilk plays a role via possible immunomodulation vis-a-vis the genetic susceptibility of the infant to reduce the risk of the disease[23]. A gene involved in the genetic control of fatty acid pathways, Fatty acid desaturase 2, (FADS2), moderates the link between breastfeeding and IQ implying its potential to unmask new genes in complex phenotypes[24]. Breastfeeding reduces the incidence of diseases with genetic links like insulin dependant diabetes mellitus (IDDM), autoimmunity and atopy[23, 25]. In childhood leukaemias and lymphomas there are protective factors in breastmilk[23,25]. Overall there is robust evidence to support equally the immediate and enduring benefits of breastfeeding .

The mammary epithelial cell (MEC) and HIV transmission in breastmilk
The role of the MEC which is the principal cell type in the healthy, uninfected milk and the primary milk secreting cell of the mammary gland is important[26,27]. It is most likely the first cells a pathogen encounters on entry into the lactating mammary gland .The RNA retroviruses infect the mammary epithelial cells antenatally[10]. In addition, Pattern Recognition Molecules and Receptors (PRMs and PRRs) expressed by the MEC also participate in immunity; these molecules mediate secretion of innate immune factors and possibly bridge innate to adaptive immunity[15]. Hence the protective role of the mammary gland.
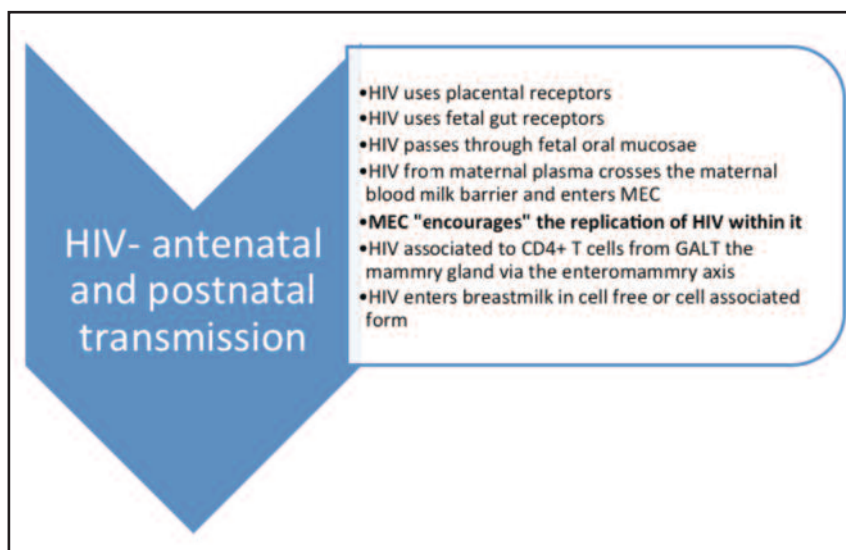
It merits mention in this context of the physiology of synchronized functions of the MEC. Exocytosis, lipid synthesis, transmembrane secretion of ions and water and transcytosis of extra-alveolar proteins like immunoglobulins, hormones and albumin from the interstitial space occur[26,27]. Lymphocytes, monocyte and macrophages can also enter the MEC into the milk duct[26,27]. In the fully lactating gland, MEC functions as a barricade between milk and the interstitium[26,27]. It must be highlighted that this barrier becomes ineffective in mastitis; possibly then, leading to passive emergence of HIV-1 virions into milk.

While the source and the genetics of the HIV transmitted in milk are albeit debatable, many possibilities have been considered. The reduced transmissibility of the virus in breastmilk may be the consequence of the selective blood – milk barrier discerning viral load-with a lesser transmission in maternal milk compared to plasma. This concept is confirmed by the detection of groups of identical viruses in milk and plasma[28].

Despite the protective role of the MEC, the mammary gland as such seems to actively contributes to breast milk transmission of HIV-1. The innate capacity of MEC to endosomally acquire HIV-1 aiding in virus infection and replication in CD4(+) target cells is adequate proof of the vibrant role of the MEC[29]. Thus MEC serves both as a pool for HIV-1 and promotes the spread of the virus[29]. However,it could be argued that MEC lacks CD4 receptors and therefore may not be a significant source of HIV in breastmilk[30].

Intestinal mucosa and placenta are implicated in mucosal differentiation of phenotypes consequent to expression of specific receptors for HIV[31] The lactating mammary gland ,an integral part of the mucosal immune system may have similar functions in determining HIV viral phenotype. The origin of T cells in breastmilk via the enteromammry axis from the Gut Associated Lymphoid Tissue (GALT) is known. In the HIV positive mother, these T cells that enter the mammary gland during breastfeeding could be infected by HIV[30]. These HIV infected T cells could be different from the HIV variants present in the peripheral blood of the breastfeeding mother[30].

• HIV uses placental receptors
• HIV uses fetal gut receptors
• HIV passes through fetal oral mucosae
• HIV from maternal plasma crosses the maternal blood milk barrier and enters MEC
• **MEC "encourages" the replication of HIV within it**
• HIV associated to CD4+ T cells from GALT the mammry gland via the enteromammry axis
• HIV enters breastmilk in cell free or cell associated form

HIV- antenatal and postnatal transmission

Antenatal and postnatal transmission of HIV from mother to child
Fig. 1: Antenatal and postnatal transmission of HIV from mother to child

While this is so there are factors that restrict viral production; the mammary cells also express APOBEC3 and APOBEC3G genes, proteins of a family of retroviral restriction factors that reduce viruses produced in them, limiting their infectivity [32].

The entry point of HIV transmission in breastmilk remains not fully elucidated. Tonsillar tissue may well be the portal of entry but defensins, lysozyme, thrombospondin and salivary secretory leukocyte protease inhibitor (SLPI) are anti-HIV factors present in the oral cavity [33]; although the extent of the maturity of these factors in the suckling infant is questionable. Tonsillar mucosa with M cells adjacent to CD4 lymphocytes is vulnerable to infection with HIV-1. Amongst other factors, this susceptibility is due to the viral coreceptor CXCR4 [30,34] Subsequent to ingestion of HIV-1 infected breast milk, infant gut mucosal surfaces are involved in transmission of the virus. Cell free or cellular HIV may be found in the submucosa via mucosal breaches or even via the passage through intact mucosae [35]. There is little doubt that mucosal trauma facilitates entry of the HIV-1; further many inflammatory factors increase expression of HIV receptors and augment spread of cell associated HIV [35]. Direct HIV transmission from mother –to- child that may occur via HIV infected blood from cracked nipples through abrasions in the mucous membranes of the infant's mouth is known. However, HIV transmission from mother –to- child may also occur across intact fetal oral and intestinal epithelia [36]. Both cell-free HIV infection as well as cell associated viruses do not seem to require mucosal breach for their passage [36].

Breastmilk responses to HIV
In humans breast milk antibodies do not enter the infant circulation via the gut [37]. Protective immunoglobulins excreted in milk are mainly secretory IgA(sIgA)[37]. The specificity of maternal milk sIgA is mostly driven by the immunophysiological cascade of the entero-mammary axis[37]. Specific secretory IgA or IgM in the breastfed infant inhibit enterocyte transcytosis [37]. While IgA is the predominant breastmilk immunoglobulin, local mucosal immune responses mediated by IgG are more important

against HIV [38]. Protection against HIV at the mucosae is possibly due to several factors; some of these factors include blocking viral or infected cell adhesion to epithelial cells, interference of transepithelial transport, viral neutralization in the mucosa and exclusion of infected cells through antibody-dependent cell-mediated cytotoxic reactions [39]. Amongst antibodies, those directed against the ELDKWA epitope had higher neutralising activity than serum antibodies against the HIV-1 [37,40]. Others postulate that plasma-derived IgG antibodies mediate many of the low-level HIV neutralization and Antibody Dependant Cell mediated Cytotoxic (ADCC) activity in breast milk [41]. Additionally, the presence of anti-idiotypic antibodies capable of enhancing infant antibody responses have been reported in breastmilk[37,42]. Whether these antibodies play a role in protection against the HIV is uncertain.

Uninfected infants exposed to milk fromHIV-1-infected mothers have HIV-1 gp160-specific IgA antibodies in salivary secretions at one month of birth, and were still uninfected over twelve months [43]. The relevance of the HIV exposure in triggering such antibodies is evident as such a response was absent in those unexposed [43]. In this study the authors conclude that their findings "provide some evidence that natural HIV-1 exposure via the oral route can stimulate a humoral immune response in infants younger than 6 months of age and this may enhance understanding of how children are protected against mucosal exposure to HIV-1" [43]. It would also not seem unreasonable to question how such a humoral immune response would benefit the infant when faced with other mucosal pathogens. Could these responses augment mucosal surface protection in the suckling infant?

The presence of sIgA and sIgM in the breastmilk of women infected with HIV-1 during the whole lactation period was linked to lower mother-to-infant transmission of HIV[37]. Even here, relevant secretory immunoglobulins confer some protection to the suckling infant without elimination of the virus.
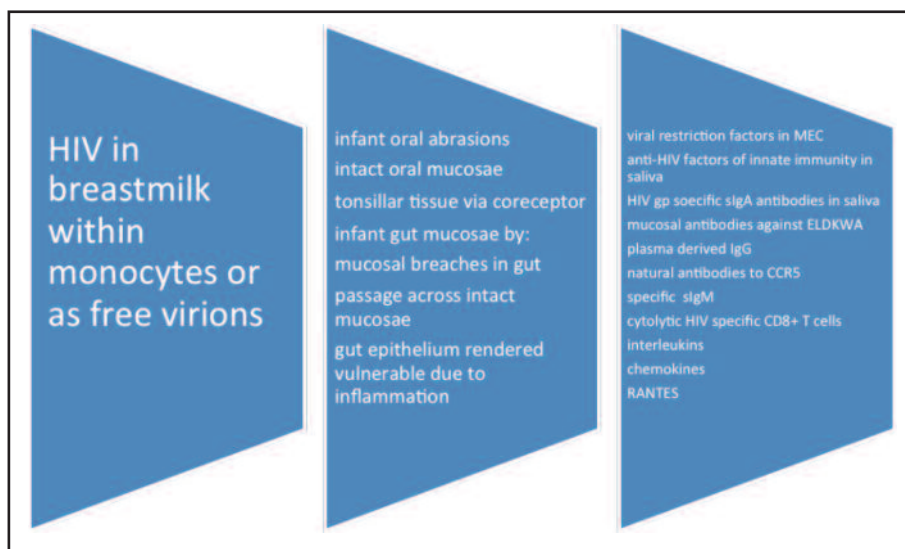

Fig. 2: HIV in breastmilk- transmission and "protection"

Table III: This table illustrates the known and postulated consequences of breastfeeding in a setting of maternal HIV
Consequences of breastfeeding in maternal HIV

Transmission to the suckling infant and an important cause of paediatric HIV infection
MEC and CD4 [+] T cells in breastmilk act as persistent "sources " or "reservoirs " of HIV
Some anti-HIV factors of uncertain function in breastmilk
Stimulates low level of antigenecity without inducing disease–comparable to :   a) passive immunization   b) an arm of immune education of the naïve immune system
Stimulates mucosal protective factors to strengthen immune defenses at mucosae which may be relevant geodemographically to: a) HIV   b) other mucosal viruses (that use mucosae as portal of entry)
Activates and modulates immature T cells for uncertain function
Potential to stimulate memory T cells for prolonged protection or for uncertain rationale

The predominant lymphocytes in the breast milk are CCR5-expressing memory CD4 T-cells [30,44] CCR5 is the main HIV coreceptor, involved in virus entry and cell-to-cell spread[30,44,45]. Natural antibodies to CCR5 inhibit infection of macrophages and dendritic cells with HIV and may limit the transmission of HIV through breastfeeding [45]. Via various possible mechanisms viruses other than HIV can potentially also induce the formation of anti-CCR5 Abs [46]. It may be postulated that the breastfeeding mother who is not HIV positive could be exposed to other such viruses with capacity to induce anti-CCR5 antibodies in host cells; such environmental exposure could thus lead to the passage of these viruses into breastmilk potentially provoking the production of blocking antibodies against HIV in breastmilk, a mechanism that could be taken advantage of.This reemphasises the need for exclusive breastfeeding in all mothers not infected by HIV.

Transfer of maternal T-cell reactivity to tuberculin protein from mother to the neonate via breast-feeding is possible [47]. Breastmilk T cells have unique functional capacity for activation, mucosal affinity via homing resceptors and the potential for immune memory[48].

Adoptive T cell recruitment could function locally to reduce the HIV viral load in breast milk, potentially decreasing the intensity of transmission[49,50]. Deficiencies in early life related to T cell integrity and function are partially overcome by more immuno competent maternal T cell activity[50]. The presence of cytolytic HIV-specific CD8^{+} T cells in breast milk help protect HIV transmission at mucosal sites; this implies that the mucosal immune response is critical in breastmilk HIV transmission[50]. It would be of interest to know how these cytolytic cells function in response to other mucosal pathogens.

The immature cytokine profile of the neonate is skewed towards a T-helper type 2 (TH2) response to antigen [51]. However, useful cytotoxic reactions in infancy induced by CD8 cell interferon (IFN)-γ to autologous envelope (Env) peptides in mother –to child HIV infections have been noted[51]. Neonatal macrophage derived dendritic cells (MDDCs) require a higher level of activation than adult dendritic cells (DCs) but, once activated, are capable of effector responses[51,52]. In infants an appropriate antigen exposure potentially augments useful effector responses [52].

On the other hand, it is equally important to recognize that the specific functional characteristics of breastmilk T cells alluded to earlier is a haven for HIV-breastmilk transmission[48]. HIV finds a "stable population of CD4 T cells associated reservoir" which may challenge "even efficient antiretroviral therapy" [48]. T cells in breastmilk pertaining to HIV transmission although having some useful function, seem to favour the HIV virus in breastmilk.

In the lactating mammary gland,migration of IgA-secreting plasma cells into the mucosal lamina propria correlates with specific chemokines and chemokine receptors[53]. CCL28, a chemokine also known as mucosae-associated epithelial chemokine mediates mucosal immunity in HIV exposure and infection[53]. CCL28 concentration in breast milk correlates with longer survival in infants vertically-infected by HIV [54]. Breast milk levels of chemokines, macrophage inflammatory protein-1 and regulated upon activation, normal T-cell expressed, and secreted (RANTES) are immunologically important ingredients. They are stimulatory to the activity of memory T lymphocytes [54]. The transmitters among the HIV-1-infected mothers expressed higher levels of breast milk RANTES levels than non-transmitters [54].

Chronic infections,perhaps via sensitisation, can modulate some aspects of immunity in the newborn through the development of a more appropriate T cell repertoire [55]. In this context one may hypothesise that low grade infections transmitted from mother-to-child bear a similar objective. Maternally derived interleukin-7, when passed on to the suckling infant, crosses the intestinal epithelium of the suckling infant [56]. Its role in the development and functions of T lymphocytes, enhancing T cell production in the thymus are deemed important in immunoprotection [56]. Such regulatory activity of interleukin (IL)-7 on lymphopoiesis and T-cell homeostasis in breastmilk could influence the risk of transmitting HIV to the breastfed infant[57,58,59]. Interleukin-15(IL-15) concentrations are linked to a decreased risk of HIV transmission and IL-15–mediated immunity may also have a role in protecting the infant against HIV transmission during breast-feeding[60].

CONCLUSION
The risks and benefits of breastfeeding in HIV endemic areas and in breastfeeding by HIV positive mothers are important considerations in the implementation of breastfeeding policies in mothers who are HIV positive. WHO recommendations in rural Africa is that in the context of HAART provision for HIV-positive women or ARV prophylaxis to breastfeeding infants, avoidance of

breastfeeding leads to worse outcomes. Hence exclusive breastfeeding for 6 months in HIV positive mothers on HAART is the recommended feeding choice in some regions.

It is also evident that in breastmilk transmission of HIV, there are many factors that operate to mitigate the transmission hazard to the suckling infant. Certain forms of the virus, cell free or cell associated, are found reduced in the breastmilk of some HIV positive mothers. Immune responses including those of mucosal immunity in an infant exposed to the milk of maternal HIV positivity clearly is in dire need of different vehicles of immunoprotection .Regardless of the viral forms, there is adequate proof of some success in lowered transmission in breastmilk. In addition, other anti HIV factors in one way or the other also participate in varying degrees in mother–to–child breastmilk transmission.

Furthering this, the milieu of the breastfed infant whose mother is HIV negative but who is exposed to many other viruses also poses unique immune challenges to the immature immune system. The 'mimicry' of other viruses to HIV in stimulating specific natural antibodies to the CCR5 neutralising epitope also blocks HIV replication is of much significance; because should the breastfeeding mother with such 'mimicry' antibodies become HIV positive, her milk should not transmit the HIV to her suckling infant. These conjectures which by no means are infallible direct our attention for serious consideration.

The risks of transmission of HIV via breastmilk are coupled with some innate protection in the lactating mammary gland. Reactive triggers of adaptive immunity occur due to the occurrence of the virus in maternal milk. These reactive responses seem to have a role in protection and for viral persistence.

Undeniably breastmilk is a vehicle for HIV transmission. In the wake of this dismal consequence, there are factors which confer protection and even some form of immune direction to the naïve immune system - the rationale of which are hitherto unexplored and unexplained.

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Multiple Choice Questions

1. The following statements are correct regarding breastfeeding
   a) In Malaysia maternal HIV is a contraindication to breastfeeding
   b) Hepatitis B is an absolute contraindication for breastfeeding
   c) HIV is found in breastmilk
   d) Mixed feeding is a risk factor for HIV infections
   e) Hepatitis C is an absolute contraindication to breastfeeding

2. Innate protection in breastmilk include
   a) lipases
   b) mucin
   c) specific sIgA
   d) anti CCR5 antibodies
   e) lactoferrin

3. Pertaining to mucosal immunity in breast milk
   a) it is vital defense against mucosal pathogens
   b) immunoglobulins excreted in milk are mainly sIgA
   c) T cells in breastmilk are mainly from gut associated lymphoid tissue (GALT)
   d) milk sIgA is driven by the entero-mammary axis
   e) adaptive immune factors do not play a role

4. Methods of paediatric HIV infection through breastfeeding include :
   a) intact oral mucosae
   b) tonsillar tissue via coreceptor
   c) infant gut mucosae
   d) mucosal breaches of gut
   e) inflammation of the gut

5. Adaptive responses to breastmilk HIV include :
   a) specific interleukins
   b) RANTES
   c) cytolytic specific CD8+ cells
   d) lactoperoxidase
   e) specific antibodies to CCR5