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Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant

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Abstract

Initiating breastfeeding within the first hour of life confers an important benefit in terms of child mortality and severe morbidity. Intestinal permeability to ingested macromolecules and immunoglobulins is limited to the first days of human life. These exchanges cease in the very early post-partum period but may increase beyond the neonatal period in response to local inflammation or introduction of a weaning food. From animal- and limited human-based observations, compelling evidence points out to breastmilk cells also trafficking from mother to infant mucosal tissues and participating to the maternal microchimerism. The precise nature of breastmilk cells that are involved is presently not known but likely includes progenitor/stem cells—representing up to 6% of breastmilk cells—with possible contribution of mature immune cells. Stem cell microchimerism may induce tolerance to non-inherited maternal antigens (NIMAs), breastfeeding generating regulatory T cells (T_{reg}) that suppress antimaternal immunity. Therefore, in complement to pregnancy-induced microchimerism, breastfeeding-induced microchimerism may be pivotal in infant immune development, intestinal tissue repair/growth and protection against infectious diseases. As a continuum of the gestational period, the neonatal gut may be considered as a temporary, but important developmental extension of the role played by the placenta during intrauterine life; breastmilk playing the role of maternal blood by delivering maternal soluble factors (macromolecules, Ig, cytokines) and immunologically active milk cells. A better understanding of breastfeeding-induced maternal microchimerism would provide further evidence in support of public health messages that reinforce the importance of early initiation of breastfeeding.

KEYWORDS

breastfeeding, gut closure, maternal microchimerism, maturation of neonatal immune system

Jean-Pierre Molès and Edouard Tuillon contributed equally to this work.

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1 | INTRODUCTION

The nutritional and immunologic benefits of human milk consumption through exclusive breastfeeding (EBF) on child health, development, and survival have led to the universal recommendation of EBF as the “gold standard” for early infant feeding worldwide.¹ Breastfeeding is associated with a lower incidence of globally important infectious diseases like infectious diarrhea and pneumonia, as well as increasingly important chronic diseases such as diabetes.^{2,3} As a result of potential health impacts, it is estimated that up to 15 million child deaths could be averted in 10 years⁴ and 20 million disability-adjusted life years prevented⁵ with EBF practiced. A more recent Lancet series on breastfeeding concluded that 823 000 child deaths and 20 000 maternal deaths could be prevented each year by scaling up breastfeeding (summarized in an editorial in *The Lancet*, Vol 390, August 5, 2017). The current WHO guidelines in support of the practice of EBF are to (i) provide only breastmilk for the first 6 months, with no other non-medically prescribed foods or fluids until complementary foods are introduced at 6 months of age; (ii) initiate breastfeeding within 1 hour of delivery; (iii) breastfeed on demand; and (iv) avoid the usage of bottle, teats, or pacifiers.¹

These recommendations have been based on literature focused on the practice of breastfeeding or the consumption of human milk. However, frequently studies have used the act of breastfeeding as a direct proxy for infant breastmilk consumption. Understanding this distinction is important as the act of breastfeeding by the mother and infant dyad, and the consumption of breastmilk by the infant may have distinct mechanistic implications. Untangling the maternal and infant mechanistic factors that contribute to the infant health outcomes associated with EBF and/or exclusive breastmilk consumption will be important to advance our understanding and rationale of immune development during early infancy.

An example of this mechanistic complexity underlies the recommendation to initiate breastfeeding within 1 hour of delivery. Evidence associated with this is that early initiation of breastfeeding is associated with a greater likelihood of EBF being practiced, and practiced for a longer duration.⁶ These better EBF practices may in fact be due to a stronger maternal-infant bond that develops with the early maternal-infant skin-to-skin contact when breastfeeding occurs within 1 hour. In a recent meta-analysis of almost 100 000 infants, initiation of breastfeeding within 1 hour and practice of EBF was independently associated with significantly lower neonatal and early infancy mortality.⁶ While EBF practices according to current recommendations would ultimately contribute to a greater overall volume of breastmilk consumed over the EBF period, it may also be important that the timing of early initiation of the practice of breastfeeding also ensures that the infant has access to unique early benefits of breastmilk. It is well established that breastmilk composition varies throughout lactation stages, both nutritionally and immunologically. Of the human milk immune factors reported thus far, immunoglobulins (Ig) and immune cells are in greatest concentration in colostrum compared to mature milk.⁷⁻⁹ During the first 2 weeks after birth, breastmilk leukocyte content

decreases dramatically to reach a concentration that is thereafter maintained in mature milk throughout lactation.¹⁰

While immunologic variation in milk composition during lactation has been studied on a limited basis,^{11,12} this has not yet been considered closely in conjunction with the timing of the infant intestinal development, both in terms of the structure and function of the infant mucosa and maturation of mucosal immunology. The human neonatal intestine is characterized by increased permeability to large molecules, including Ig and other macromolecules,¹³ and breastfeeding is associated with earlier gut closure of intestinal permeability.¹⁴ Thus, we hypothesize that the early infant intestinal mucosa is an open route to milk immune cells. This open gate closes, whether by maternal-mediated factors through variation in milk composition and/or by infant-mediated factors that impact intestinal mucosal changes, in part dependent on infant food source, in the very early post-partum period. A better understanding of this window of opportunity for human milk immune cell transfer may have important implications for early post-partum care of mothers and their newborns and for longer term child's health.

2 | (RE)DEFINING NEONATAL GUT CLOSURE

The intestinal mucosa forms a physical, biochemical, and immune barrier that blocks or regulates the transfer of the gut luminal contents to the interstitial submucosal tissue. The neonatal intestinal mucosa barrier is distinct in many known ways from a healthy adult intestinal mucosal barrier. A significant difference from a barrier perspective is the greater, but temporary, permissiveness of the neonatal mucosa. “Gut closure” closely relates to neonatal gut permeability which, in turn, is directly linked to two physiologic mechanisms.

The first mechanism is most classically associated with the concept of gut closure. It consists of the passage of macromolecules from the lumen of the infant's gut via paracellular space to blood. In the intestinal epithelium, the intercellular junctions are made up of at least three distinct elements: tight junctions, adherens junctions, and desmosomes.¹⁵ The most apical structures, the tight junctions, are the rate-limiting factor in epithelial paracellular permeability. M cells are highly differentiated epithelial cells associated with Peyer's patches (PP) and tonsils. They have antigen transport functionalities that are interlocked with adjacent cells with similar intercellular junctions as enterocytes.

The intestinal permeability to macromolecules can be estimated in vivo by non-invasive methods. A common method is the lactulose/mannitol test that assesses urinary excretion of orally ingested macromolecules. Lactulose permeates across the gut surface only in case of epithelial cell damage or intercellular junction defects, whereas mannitol, being threefold smaller in size, is readily absorbed through the normal gut epithelial barrier. These inert sugars are excreted in urine, after being filtered freely at the glomerulus level. Hence, an elevated lactulose/mannitol ratio in urine is a useful indirect indicator

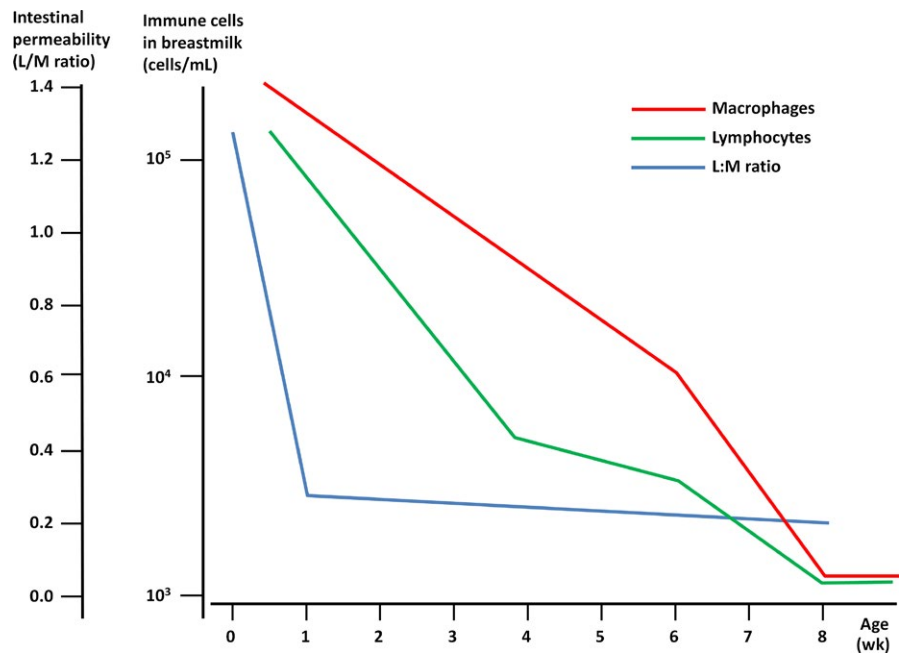


FIGURE 1 Timing of changes in the degree of infant intestinal permeability and corresponding breastmilk immune cell concentration among young infants. In human neonates, intestinal permeability (lactulose/mannitol ratio, blue line) drops rapidly after birth and reaches near adult values in a few days or weeks. In parallel, immune cell content (lymphocytes: green line; macrophages: red line) in breastmilk drops by approximately $1.5 \log_{10}$ during the same period. Adapted from Goldman⁸ and Catassi¹⁴ [Colour figure can be viewed at wileyonlinelibrary.com]

of gut permeability. In humans, intestinal permeability to macromolecules is very high shortly after birth and then drops dramatically within the first 7 days of life (Figure 1). Interestingly, this period of increased permeability is longer in non-breastfed infants.¹⁴ It is likely that gut permeability temporarily increases again at the introduction of a weaning food or in the case of gut inflammation for any reason. More recently, stool calprotectin concentration has been identified as a marker of intestinal permeability in healthy neonates. Calprotectin is secreted from stimulated neutrophils, eosinophils, and monocytes and expressed in some mucosal epithelial cells. Calprotectin is stable in stool and is eliminated intact in the feces. The level of calprotectin that can be measured in routine practice using CE/FDA-approved assays decreases over the first week of life. Another surrogate marker of intestinal maturation is the intestinal fatty acid-binding protein (I-FABP), present in mature enterocytes, and filtered in urine after being released in blood when enterocytes detach from villi. The observed post-natal increase in urinary I-FABP concentration may reflect increasing gut maturation.^{16,17}

While gut permeability drops rapidly during the first days of life, a very gradual process of gut closure is observed over the first few years of life.^{18,19} It is currently unknown whether this second phase of prolonged, but diminished gut permeability has a significant physiologic role. Studies measuring intestinal permeability by lactulose/mannitol ratio have shown prolonged intestinal permeability in formula-fed infants compared to breastfed infants during the first 2 weeks,^{14,20} with similar gut permeability observed at 1 month. Human milk, especially colostrum and early milk, contains several gut trophic factors such as epidermal growth factor. These factors play roles in tissue repair/growth and in this context may contribute to the “closure” of intercellular junctions.²¹⁻²³ The maturation of intercellular junctions is also regulated by microRNA (miRNA), such as miR-192, which modulates the stability and translation of mRNAs encoding tight junction proteins and plays an important role in the control of intestinal epithelial barrier function.²⁴

Breastmilk is a rich source of miRNA for the infant.²⁵ We have recently detected the presence of miR-192 in mature human breastmilk (Molès JP, unpublished data), while the identification of these miRNAs in colostrum and early breastmilk remains under investigation.

The second mechanism of intestinal permeability is related to the active transport of IgG and immune complexes (IC) from breastmilk to the infant intestinal submucosae. Transcytosis of Ig allows protection from degradation through the binding to neonatal-Fc-receptor (FcRn). The duration of FcRn expression on the neonatal gastrointestinal tract varies between mammalian species and, for many, occurs at weaning—but, by contrast, much earlier in human infants. Rodents and many other mammals depend exclusively on this transport mechanism for prolonged periods to maintain adequate levels of IgG in blood.^{13,26,27} For example, maternal immunity is mediated exclusively by colostral Ig in ruminants.²⁸ FcRn are present on the luminal surface of proximal small intestine enterocytes. FcRn-mediated uptake in rat pups accounted for approximately 80% of serum IgG levels.²⁹ The presence of this receptor on the fetal enterocyte surface is responsible for in utero IgG transport from amniotic fluid to the fetal blood.³⁰ In human neonates, FcRn is expressed on the luminal surface of enterocyte for a very short period, only a few hours or days after birth. In contrast, FcRn persists for the whole life on the contra-luminal surface of those cells and contributes to the transport of Ig, including secretory IgA (SIgA), from the submucosae and blood to the mucosal surfaces.^{31,32} This receptor is also present on the surface of syncytiotrophoblastic cells where it ensures active transport of maternal IgG across the placenta to fetal blood.³³⁻³⁶

Glycosylation of maternal IgG evolves over gestational time and likely also during lactation.³⁷ Hyperglycosylated IgG has potent anti-inflammatory properties.³⁸ It is therefore possible that breastmilk IgG transferred to the nursing infant contributes to the support of the anti-inflammatory microenvironment at the neonatal gut surface and thus to the neonatal maturation and regulation of the immune response.

While these mechanisms support the global benefits of breastfeeding, they do not fully explain why breastfeeding initiation within the first hour of life has such tremendous impact on the reduction in infant mortality.⁶ We propose a third mechanism involving the immune cells from early milk at this time of high infant intestinal permeability. Specifically, this mechanism termed maternal microchimerism (MMC) is the transfer and persistent nesting of maternal cells from early breastmilk to infant intestinal mucosal tissue, and from there, perhaps to other infant immune tissues. Data and physiology support the idea that these maternal milk cells are transmitted to the breastfed infant especially during the early breastfeeding phase, a period during which the highest concentration of milk leukocytes and stem cells is present in colostrum and early breastmilk (Figure 2). We hypothesize that breastmilk cell transfer occurs before gut closure is completed and that this would favor a more efficient and effective maturation of the mucosal and/or systemic immune system, with short-term implications on infectious disease outcomes.

During the first weeks of life, a uniquely hospitable infant intestinal environment exists for maternal cells in breastmilk to cross intact

through the neonatal intestinal barrier. This is due to the presence of diminished digestive enzyme activity, infant stomach hypochlorhydria, and loose intestinal intercellular junctions (with access to paracellular space), together allowing for the transfer and survival of maternal milk cells. As an example, milk macrophages survive several hours in the gastrointestinal tract of infant mice after oral administration.³⁹ Cellular complexes produced during milk-saliva interactions may play a key role in protecting breastmilk cells from acidity during gastric transit.⁴⁰ To date, a comprehensive investigation to clearly identify which milk cell types transit intact through the stomach environment has not been conducted. However, early experiments performed more than 30 years ago clearly demonstrated an active uptake of living lymphocytes through the neonatal gastric mucosa.⁴¹

In rodents and non-human primates with a prolonged period of gut permeability, nesting of breastmilk cells into pup's tissues has been largely demonstrated, either as a long-lived cell population⁴² or as a transient homing in neonatal tissues.⁴³ The most likely mechanism of cell transfer involves paracellular passage of breastmilk cells by means of weakened interepithelial junctions.⁴⁴ Demonstration in humans is

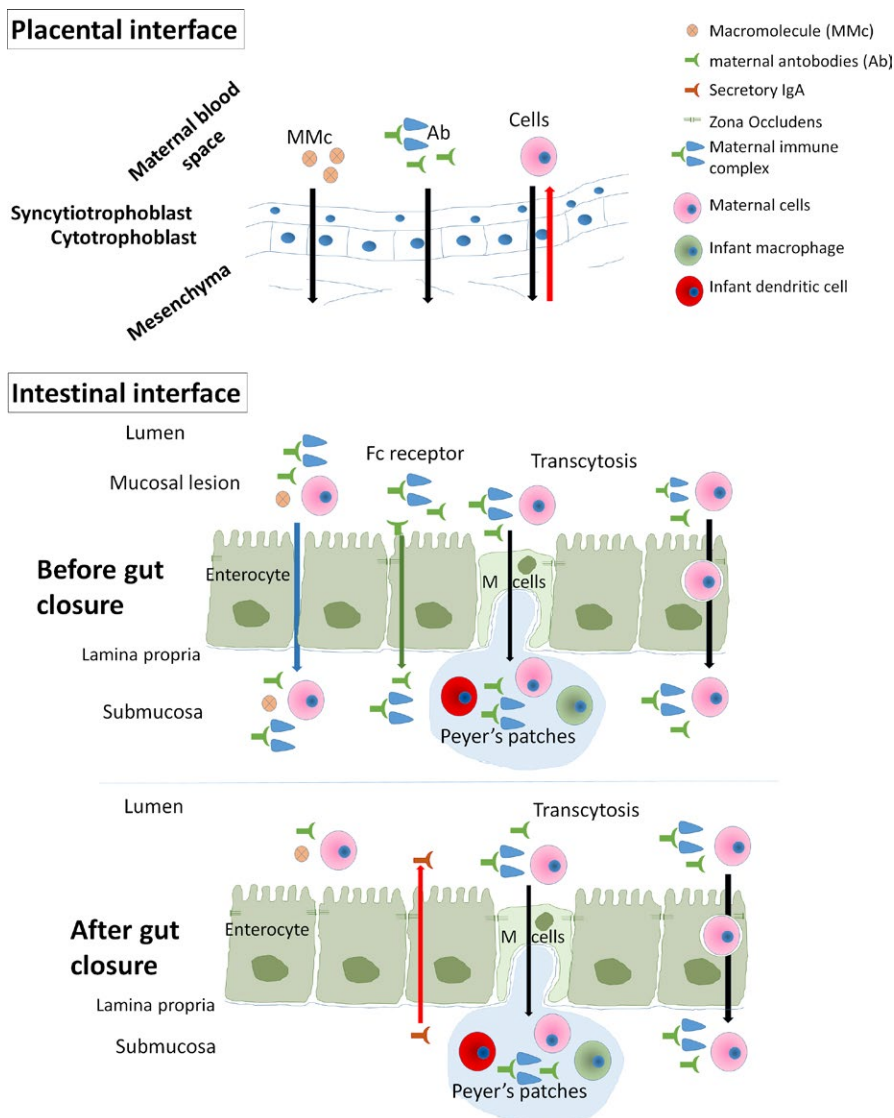


FIGURE 2 Schematic overview of the mother to fetus/infant cellular exchanges at the placental and the intestinal levels. Passive and active processes are indicated, as well as the nature of the exchange

hampered by the much shorter duration of gut permeability and an inability to obtain infant intestinal tissue samples in a non-invasive manner for research purposes.

Intriguingly, the first few weeks of human life is also a period of rapid maturation of the neonatal cellular immune response through the maturation of the Toll-like receptor (TLR) system, a phenomenon that is considerably enhanced by breastfeeding.⁴⁵ Again, the temporal association between increased gut permeability and the timing of the maturation of the infant TLR system suggests that the breastfed neonate may be using biologically active milk cells in an effective and developmentally appropriate way.

3 | THE NEONATAL INTESTINAL MUCOSAL IMMUNE SYSTEM: IN NEED OF MILKY HELP FROM MOM?

A common perspective is that humans are born with an incompetent and immature immune system. This view is in fact grossly exaggerated and perhaps, conceptually inappropriate. The ontogeny of the human immune system evolves rapidly, so much so that by 14 weeks of gestation, most fetal immune cells (NK cells, $\gamma\delta$ T cells, B cells, dendritic cells, and $\alpha\beta$ T cells) are mature and display potential effector functions.⁴⁶ However, it is the capacity to mount an appropriate and therefore effective immune response that depends not only on the functional capacities of each of its cellular components, but also on the adaptation and priming of the neonatal immune system the evolving environment. With this respect, microbial colonization of the gut occurring early in life plays a crucial role in the immune response development.⁴⁷ Breastfeeding and breastmilk consumption, through a variety of synergistic mechanisms, contribute to the appropriate selection of commensal bacterial populations in the infant gastrointestinal system and to the final maturation of the neonatal immune system.³²

Breastfeeding promotes a favorable intestinal commensalism in the infant.³² In addition to direct exposure of the newborn gastrointestinal tract to maternal gut and vaginal microbiome flora during delivery, the breastmilk microbiome plays an indirect role in transfer of maternal gut bacteria through an existing maternal entero-mammary axis.⁴⁸⁻⁵⁰ Human milk oligosaccharides play also a crucial role for pathogen defense, selective neonatal microbiota, and immune modulation of the neonatal intestine.^{51,52} Also, Ig, both transported on infant's mucosal surface from the blood and submucosal areas by FcRn, and provided by ingested breastmilk, plays a major role in controlling pathogens and selecting favorable gut microbiota. In turn, this affects local inflammation level, gut closure, antigen transfer, and antigen-specific immune response.

Very few studies have investigated the role of maternal immune cells in breastmilk on infant immunologic maturation and regulation. Comparisons of breastfed vs formula-fed children have consistently demonstrated an enhanced antibody immune response to vaccines and a longer vaccine protection in breastfed babies.⁵³ This is even more evident for vaccine responses strictly dependent on T-cell functionality, such as BCG response.⁵⁴ Mother and infant characteristics,

and particularly maternal and infant infections may modulate breastmilk cells gene expression and cytokines secretions.^{10,55,56}

In animal models, trans-epithelial transfer of immune cells from breastmilk modulates delayed-type hypersensitivity in the recipient.⁵⁷ This is consistent with the lower frequency of allergic diseases, such as atopy or celiac disease, in breastfed in comparison with formula-fed infants. A careful study of factors associated with neonatal immune response demonstrated that breastfeeding is the major determinant of such response in strong association with a better TLR7-mediated IL-10 neonatal cell production.⁵⁸ These observations suggest that functional milk immune factors are transferred from the mother to the breastfed baby. These breastmilk factors may be cells able to traffic across intestinal epithelium,⁵⁷ exosomes containing miRNA,^{59,60} cytokines, or other soluble factors such as oligosaccharides.⁵²

4 | IMMUNOLOGIC COMPOSITION OF HUMAN MILK: DISTINCT FROM HUMAN BLOOD

The first milk that is excreted, colostrum, has highly concentrated maternal cells of various types including epithelial cells, T and B lymphocytes, NK cells, dendritic cells, macrophages, and others. In the weeks following birth, the immune cell concentration of breastmilk decreases rapidly.⁸ For example in the first 4 weeks, the drop of immune cell concentration is in the order of one log-fold. This drop closely parallels the timing of the decrease in intestinal permeability (see Figure 1) and the expression of FcRn on infant enterocytes, as well as the improved acidity regulation in the infant stomach. As for protein and lipid composition, the cells contained in breastmilk considerably evolve over time in terms of absolute concentration and relative frequency and are considered to be matched to infant nutritional needs. The fact that milk compositional change parallels the progressive maturation of intercellular junctions in the infant's gut epithelium suggests that these active cells of maternal origin may be transferred to the infant and contribute to early infant immune development. Alternatively, it may simply reflect a co-evolution or co-adaptation between intercellular junction properties and breastmilk cellular composition.

In humans, breastmilk lymphocytes consist mainly of an extralymphoid memory cell population.⁶¹ The majority of CD4 and CD8 T cells are effector memory cells.^{62,63} The proportion of activated immune cells is also much higher in breastmilk than in blood.⁶⁴ The mammary gland is an effector site of the maternal mucosal immune system. The vast majority of maternal immune cells present in breastmilk have been primed in inducing sites of the maternal mucosal immune system such as the PP in the gut, or the tonsil in the human pharynx, and then migrate from these inducing sites to the mammary gland. It is therefore not surprising that most breastmilk immune cells harbor homing markers indicative of their mucosal origin.^{61,64}

Innate lymphoid cells (ILC), although being truly innate immune cells, have striking functional similarities with T cells and play an important role in tissue protection by regulating local immunity and

Study	Animal species	Key findings
Cabinian A, <i>PLoS One</i> 2016 ⁷⁴	Mouse	Localization of maternal milk cytotoxic T cells in pup Peyer's patches
Tuboly S, <i>Acta Veterinaria Hungarica</i> 1995 ⁴³	Sheep-lamb dyad	Milk lymphocytes from a sheep immunized against tetanus deposited on the gut surface of offspring evoked an enhanced response to tetanus vaccine in lamb
Hanson LA, <i>Pediatr International</i> 2002 ⁷³	Multiple species	Breastmilk lymphocytes are taken up into the gut mucosa and found in mesenteric lymph glands
Seelig LL Jr, <i>J Reprod Immunol</i> 1987 ⁴¹	Mouse	Radiolabeled milk lymphocytes cross the gastric epithelium; heat inactivation inhibits transfer
Ma LJ, <i>PLoS One</i> 2008 ⁵⁷	Mouse	Most trans-epithelial migrated cells derived from the 3%-4% of milk cells positive for T lymphocyte markers. Cells homed to the spleen and thymus, with maximal accumulation at 3-4 wk. Cells can transfer T cell-mediated delayed-type hypersensitivity response
Jain L, <i>Archives of disease in childhood</i> 1989 ⁹⁴	Baboons	Breastmilk immune cells cross intestinal mucosa by diapedesis, enter bloodstream, and migrate to organs (liver, spleen, bone marrow, gastrointestinal tract) providing active immunity
Sheldrake RF, <i>Res Vet Sci</i> 1985 ⁹⁵	Rats and lambs	Transport of milk lymphocytes across intestinal surface, through lymph ducts to the mesenteric lymph nodes
Tuboly S, <i>Advances in experimental medicine and biology</i> 2002 ⁴⁴	Piglet and lamb maternal-offspring dyads	Colostrum lymphocytes transported across intestinal surface via the lymphatic vessel and then transported to the mesenteric lymph nodes. Electron microscopy revealed intercellular absorption, and absorbed lymphoid cells remain immunologically active
Weiler IJ, <i>Am J Reprod Immunol</i> 1983 ⁹⁶	Mouse	Approximated that at least 0.1% of consumed cells infiltrate young mouse tissues
Miller SC, <i>J Reprod Immunol</i> 1981 ⁷²	Mouse	Trans-epithelial migration of intact colostrum cells not observed
Silvers WK, <i>J Immunol</i> 1975 ⁹⁷	Mouse and rat	Foster nursing does not impact survival and immunologic competence of mice or rats
Schnorr KL, <i>J Reprod Immunol</i> 1984 ⁹⁸	Lamb	Intestinal absorption of milk leukocytes, followed by detection in blood
Zhou L, <i>Immunology</i> 2000 ⁹⁹	Mouse	GFP(+) leukocytes infiltrated through the digestive tract wall and mainly localized in neonatal liver
Arvola M, <i>Biol Reprod</i> 2000 ¹⁰⁰	Mouse	Functional maternal immunoglobulin-secreting cells (or B cells) can be transferred to neonate via breastfeeding
Hughes A, <i>Immunology</i> 1988 ³⁹	Mouse	Labeled macrophages fed to newborn mice survived for at least 4 h in the gastrointestinal tract and, in some cases, localized in the mucosal tissue. In one case, a labeled cell was found in the spleen

TABLE 1 Maternal breastmilk cell trafficking to offspring or fostered animals in animal models

GFP, green fluorescent protein.

inflammation.⁶⁵ Different ILC subsets characterized by different homing markers populate a variety of human tissues.⁶⁶ MR1-restricted mucosal-associated invariant T (MAIT) cells are another recently identified population of immune cells which can be activated by bacterial subproducts in case of tissue bacterial infection, and singularly mycobacterial infections. They are involved in antigen presentation, inflammation control, and tissue response to viral, bacterial, and fungal infections.⁶⁷ In the last few months, our group has been able to consistently identify ILC and MAIT cells in breastmilk samples of healthy donors, confirming the effector nature of the mammary gland in the mucosal-associated lymphoid tissue (MALT), and notably, some subsets of these cells were present in much higher proportions in breastmilk than in blood (Tuaille E, unpublished).

Some years ago, the demonstration of stem cells of hemopoietic, mesenchymal, and neuro-epithelial lineages in breastmilk of healthy women came as a surprise.^{68,69} Although this discovery was welcomed as a new source of readily accessible stem cells for future biotherapy given their comparatively lower frequencies in blood, the physiologic role played by these milk stem cells in the mother-infant dyad remains poorly understood.⁷⁰ These cells with progenitor characteristics can be identified more easily in fresh breastmilk samples. Some of them harbor characteristics of mesenchymal stem cells (CD90, CD44, CD271, CD146), some of embryonic stem cells (TRA 60-1, Oct4, Nanog, Sox2), and some have markers of luminal mammary epithelial cells (cytokeratin 18).⁷¹ Given their potential, breastmilk stem cells are good candidates for MMC in the infant intestinal tissues. Indeed, it is likely that infants may become tolerant to stem cells of maternal origin as these cells do not express MHC antigens.

5 | BREASTMILK CELL TRAFFICKING COULD DRIVE NEONATAL IMMUNE SYSTEM MATURATION/REGULATION

Compelling evidence exists from many animal models that confirm an effector role of immune cell trafficking from the mammary gland to the infant's mucosal surfaces or tissues. Table 1 summarizes the

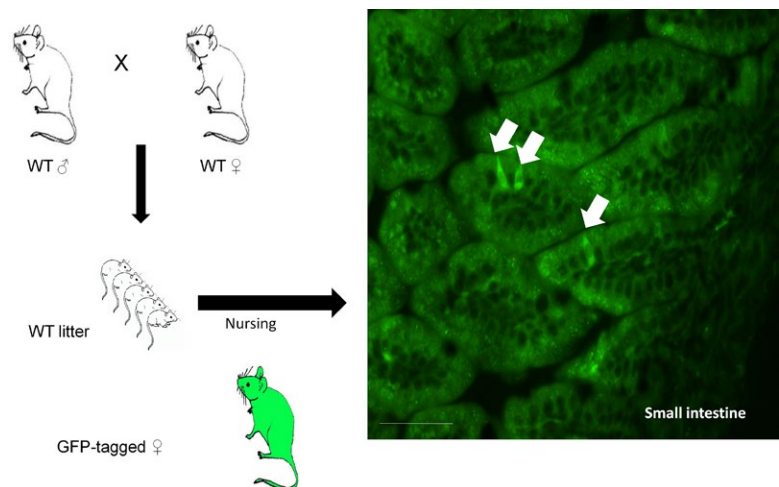
major findings related to breastmilk cell trafficking in animals. With very few exceptions,⁷² all experiments on animal models were conducted during the period of intense gut permeability suggesting that milk lymphocytes are not only taken up by the breastfed offspring but that this process results in a transfer of immunologic information and enhances the early immune response.⁷³ Interestingly, the experiments conducted in the study from Cabinian et al⁷⁴ were reproducible in MHC-mismatched animals, suggesting that breastmilk leukocytes may target infant PP independently of the MHC haplotype.

Figure 3 shows a mouse experiment demonstrating MMC of GFP-tagged breastmilk cells in the gut mucosae of fostered mice pups (Moles JP, unpublished).

Stem cells or progenitors from breastmilk transferred to the infant may play a role in immune maturation/regulation, immune tolerance to maternal MHC antigens as stem cells are not harboring MHC class 1 and class 2 antigens, mucosal ontogeny, and tissue repair and growth.⁷⁰ In a rat model of necrotizing enterocolitis (NEC), intraperitoneal administration of stem cells from amniotic fluid induced these cells to home to areas of mucosal injury in the intestine. This experimental stem cell therapy reduced the incidence of NEC and improved the survival of rat pups. Amniotic fluid stem cells also homed to intestinal villi and induced increased stromal cell COX-2 expression.⁷⁵ Similarly, bone marrow-derived mesenchymal stem cells administered either intraperitoneally or intravenously homed to sites of NEC-like injury and improved outcomes in a rat model of NEC.^{24,76}

During pregnancy, maternal and fetal cells transfer are exchanged across the placenta, and this has been best described for cells involved in tissue repair or to a lesser extent for cells that are involved in cancer and, potentially, in viral transmission. These microchimeric cells may persist for a long time; in fact, circulating fetal cells have been detected up to 27 years post-partum in the mother⁷⁷ and maternal PBMCs up to 62 years after birth in the offspring.⁷⁸ Maternal stem cell microchimerism in infants may induce tolerance to non-inherited maternal antigens (NIMA), breastfeeding generating Foxp3⁺ regulatory T cells (T_{reg}) that suppress

FIGURE 3 Microchimerism of GFP-tagged breastmilk cells in gut mucosae of fostered mice pups. Litter from wild-type C57Bl6 mice were immediately breastfed after delivery by a tagged mouse (GFP tag under actin promoter). The nursing mice gave birth on the same day also. After 4 wk of nursing, sections of different tissues were analyzed for GFP fluorescence. Discrete positive cells (marked by an arrow) can be observed inserted in the villi of small intestinal epithelium (bar = 50 μ m) [Colour figure can be viewed at wileyonlinelibrary.com]



Specific feature	Pregnancy-induced MMC	Breastfeeding-induced MMC
Cell trafficking direction	Bidirectional; from mother to fetus and from fetus to mother	From mother to child
Duration of cell exchange	Several months	<ul style="list-style-type: none"> • First few days/weeks (gut permeability) • At the introduction of a weaning food • Associated with intestinal inflammation
Involved cell types	Mature leukocytes (T cells, macrophages, dendritic cells), stem cells (ref 42)	Stem cells and progenitors, leukocytes (including ILC, MAIT?)
Destination of maternal cells	All tissues via umbilical blood stream	Infant mucosae (intestinal, pharyngeal, tonsillar, respiratory)
Origin of maternal cells	Maternal blood (and possibly amniotic fluid)	Colostrum and breastmilk
Functional consequences of MMC	<p>In mothers:</p> <ul style="list-style-type: none"> • Reproductive efficiency • Immune tolerance • Tissue repair? <p>In children:</p> <ul style="list-style-type: none"> • Immune tolerance • Tissue repair? • Immune maturation/regulation? 	<p>In infants:</p> <ul style="list-style-type: none"> • Immune tolerance • Tissue repair? • Immune maturation/regulation?
Disease susceptibility impacted	Cancer, degenerative diseases, autoimmune diseases, transplant rejection	Needs further investigation (Infectious diseases? autoimmune? transplant rejection? others?)

TABLE 2 Features of pregnancy- vs breastfeeding-induced maternal microchimerism (MMC)

antimaternal immunity and persist until adulthood.^{42,79} Indeed, MMC may explain the better acceptance of maternal transplants among individuals that were previously breastfed as infants.⁷⁹⁻⁸² Microchimerism has also been implicated in tolerance in hematopoietic stem cell transplantation.⁸³

Compelling evidence also points to the likelihood that maternal-fetal bidirectional cell exchange during pregnancy, and microchimeric seeding may promote the success of future pregnancies.⁸⁴ The fetus is exposed to genetically foreign NIMAs during in utero life, and it is hypothesized that this phenomenon can prime tolerogenic responses.⁸⁴ Indeed, in response to NIMAs stimulation, fetal CD4⁺ T cells undergo mainly T_{reg} differentiation that suppresses the NIMA-specific effector T cells.⁸⁵ A recent study established the cross-generational reproductive benefit conferred by maternal microchimeric cells retained in female offspring by accumulation of immune suppressive T_{reg} with NIMA specificity.⁸⁶ Importantly, in this study, there was a direct quantitative relationship between the microchimeric maternal cells and the cross-generational reproductive fitness. We can therefore hypothesize that, if tolerance and reproductive fitness (and maybe other consequences such as tissue healing or immune modulation) are quantitative functions of pregnancy-induced MMC, breastfeeding-induced MMC can only increase these beneficial functions in the child in an additive or synergistic fashion (see Table 2).⁸⁷

Breastmilk cells seem to transfer immune regulatory and tolerance signals to the nursing infant as illustrated by renal allograft tolerance

modulated by previous infant feeding practice and by the familial link to the donor. In a study performed in 55 recipients of maternal-donor-related renal transplant, a history of breastfeeding was associated with a more favorable post-transplant course, as 82% of previously breastfed recipients remained free of rejection at 1 year post-transplant vs 57% in previously non-breastfed recipients.⁸⁸ Similar association was not carried out when the father was the organ donor.⁸⁸ However, in recipients of kidney from sibling donors, the graft outcome was also more favorable in previously breastfed recipients, suggesting that breastfeeding effect is not entirely specific for maternal antigens.⁸⁹ In renal transplantation from sibling donors who are mismatched with the recipient for HLA haplotype, graft survival is higher when the donor has maternal HLA antigens not inherited by the recipient than when the donor has paternal HLA antigens not inherited by the recipient.⁹⁰ This strongly suggests that exposure to NIMAs by breastfeeding—as the only conceivable source of exposure—may play a beneficial role in renal graft tolerance.

6 | CONCLUSIONS

As the gestational period transitions to the early neonatal period, the infant gut may be considered as a temporary, but important developmental extension of the role played by the placenta during intrauterine life. At the same time, breastmilk plays the role of maternal blood

involved in mediating and delivering maternal soluble factors (macromolecules, Ig, cytokines) and immunologically active milk cells.

It is now evident that the period of intestinal permeability for macromolecules and immunoglobulins is particularly short in humans compared with other mammalian species. However, it is perhaps time to consider expanding the concept of "gut closure" to breastmilk cells including immune, stem, and progenitor cells. This hypothesis is supported by ample evidence of immune cell trafficking from maternal milk to neonatal tissues in animal models, by some indirect evidence in human beings that corresponds to simultaneous changes that alter breastmilk immune cells and gut permeability, and by the maturation of the TLR system early in life that is modulated by breastfeeding.

Transfer of biologically active maternal immune and/or stem/progenitor cells from breastmilk likely supports the developing neonatal immune system and facilitates its regulation. Trafficking of maternal milk stem cells, progenitors, and immunologically active cells from breastmilk may favor implantation of these living cells of maternal origin in the infant's tissues that may be involved in the effector immune response, in tissue repair and in the regulation of the neonatal immune response and immune tolerance. Long-term MMC may be one of the major mechanisms used to perpetuate the functions of cells of maternal origin in infant tissues. It may tentatively involve stem cells that are highly plausible candidates due to their absence of expression of surface MHC class 1 and class 2 determinants, immune cells, and other immune cell types in breastmilk. As this microchimerism mechanism implies prolonged infant immune tolerance to maternal cells, MMC may confer lifelong benefits such as immune protection against infectious agents, inflammation, cancer, and favor transplant tolerance.⁸⁸

However, MMC from breastmilk cells or breastfeeding-induced MMC may also be responsible for transmission of cell-associated maternal infections like HIV, CMV, EBV, or flaviviruses to their breastfed infants,⁹¹ with the resulting establishment of a long-term viral reservoir in infants and children. As an example, some progenitor cells, such as CD4(+) T memory stem cells, likely present in breastmilk may serve as reservoirs for HIV-1⁹² and may therefore be involved in transmission events and in the establishment of long-term viral reservoirs in infant's tissues. Further studies should be conducted in order to verify this hypothesis taking into account that cells involved in microchimerism may not necessarily harbor markers of HIV-1 functional reservoirs identified in blood.⁹³

Recent meta-analysis from very large cohorts suggests that early initiation of breastfeeding immediately after birth confers an important benefit to the neonate by reducing severe morbidity and mortality. The traffic of maternal immune cells from breastmilk to the infant mucosal surfaces and tissues may be the explanatory mechanism associated with such benefit. This would provide further evidence in support of public health messages that reinforce the importance of breastfeeding. While EBF for 6 months is the universal recommendation, for various reasons not all infants are fed this way. "Early and any" breastfeeding initiated in the maternity ward may be better than no breastfeeding at all, even if for any reason, the mother later chooses to feed her infant differently.

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