Published in final edited form as:

J Hum Lact. 2019 August ; 35(3): 535–537. doi:10.1177/0890334419851489.

The Impact of Technological Advances on our Understanding of the Dynamic Nature of Human Milk Cells: A Commentary About “Characterization of Stem Cells and Immune Cells in Preterm and Term Mother’s Milk” (Li et al., 2019)

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Keywords

breastfeeding; human milk; milk composition; neonatology

In their article entitled “Characterization of stem cells and immune cells in preterm and term mother’s milk,” Li, et al. (2019) categorized stem and immune cells on the basis of their phenotypes but also on the basis of gestational age at birth, and other maternal factors. Specifically, the authors found that the percentages of hemopoietic stem cells and major immune cells in human milk dynamically changed at different lactation stages—variations seen in colostrum compared to transitional and mature milk—and were influenced by gestational age, maternal body mass index (BMI), and mode of delivery. Other investigators previously had identified differences in human milk stem cell phenotype and gene expression on the basis of gestational age at delivery (Briere, Jensen, McGrath, Young, & Finck, 2017). This report corroborates and extends this earlier scientific work in the evaluation of how these stem and immune cells vary by stage of lactation as a function of gestational age.

Li et al. (2019), in their analyses of milk cells, utilized flow cytometry. What exactly is flow cytometry? It is a technology that allows separation and identification of cells within fluids in limited numbers that can be sorted on the basis of many characteristics, including their cell surface receptors, and which has allowed us to make advances in milk research that had been stymied in years past. For example, flow cytometry allows the measurement of multiple physical characteristics of a single cell often treated with fluorescent dyes that can distinguish one cell type from another and the frequency of that given cell type in a specific fluid (in this case, human milk). Flow cytometry is used in various applications based on the detection of membrane, cytoplasmic, and nuclear antigens; therefore, it is not only whole cells that can be studied, but also their components within: Organelles, nuclei, DNA, RNA,

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

chromosomes, cytokines, and proteins can be investigated utilizing flow cytometry (Adan, Alizada, Kiraz, Baran, & Nalbant, 2017). Thus, this technology allows for “immunophenotyping” of cells, analysis of cell viability and apoptosis, and the detection of various cytokines within the cell or associated with the cell membrane, to name just some examples (Adan et al., 2017).

To better appreciate the findings of Li et al. (2019), a further explanation on how flow cytometry works is needed. Flow cytometry typically utilizes laser beam technology to induce light scattering and fluorescent emission. Fluorescent-labeled antibodies targeting specific cell surface receptors are selectively used. Light scattering and fluorescent emissions occur after the light energy strikes the moving particles or cells. Other applications of flow cytometry include cell sorting: Specifically, fluorescent activated cell sorters (referred to as FACS) are flow cytometers with the ability to sort fluorescent-labeled cells from a mixed population or complex fluid structure, typically through electrostatic separation (Adan et al., 2017; Lian, He, Chen, & Yan, 2019; Shields, Reyes, & Lopez, 2015), so important when studying human milk, with its various compartments—aqueous, fat (and its milk fat globules encased by a membrane—the milk fat globule membrane), and cells. More recent advances include separation of physical or microfluidic properties of the cells to achieve the separation of targeted components (Adan et al., 2017; Lian et al., 2019; Shields et al., 2015).

As with any technology, however, caution has to be shown when interpreting results when, for example, there is a narrow sampling of subjects (e.g., including women from one locality or from one racial/ethnic group only, and not others), thus limiting generalizability of the findings; when variable methods between laboratories and studies are used to isolate and treat cells; or if conclusions are made based on small sample sizes (Alvarez, Helm, Degregori, Roederer, & Majka, 2010). There is a need for a detailed description of the study or experimental design, the participants who are providing the samples being tested, to include their sociodemographic and clinical characteristics and a rationale of why they were studied; the number of days postpartum (to ascertain if the milk is in the colostrum, transitional, or mature milk stage); the time of day of expression, and how the milk was obtained—pumped from one breast or both breasts until emptied—and stored; and specifics of how the cell suspension—in this case human milk—samples were prepared, how they were treated chemically, what fluorescent antibodies were used to identify targeted cells types, and specifics on the computational software that was used. With this in mind for the future, with the establishment of standardized methods to facilitate data comparisons, progress will occur in the study of human milk.

Focusing again on the article by Li et al. (2019), the investigators described the methodology in detail such that their experiments may be reproduced, and they used established and validated statistical methods to analyze their data. Utilizing markers specific for hematopoietic stem cells (CD34+), and markers specific for mesenchymal stem cells (CD9+, CD 105+, CD 44+), Li et al. (2019), were able to show differences in their longitudinal study of milk samples from mothers of varying gestational ages focusing on the percentages of these cells as milk progressed from colostrum through to the mature stage of lactation. The authors suggest that the control or pathway leading to the expression of mesenchymal

stem cells and pluripotent stem cells in human milk differs from that of hematopoietic stem cells, but what those mechanisms of expression are remains unknown.

Stage of lactation was associated with the greatest differences in the studied phenotypes of milk samples. This parallels the dynamic changes of the neonatal immune system as well as the gastrointestinal tract. The immune naivety of neonates, together with the increased gut permeability or “open-ness” of their GI tracts, makes them particularly vulnerable to infections and inflammatory changes. Breastfeeding and the provision of human milk to the recipient infant have long been known to improve survival and to decrease mortality from infections when compared to formula-fed infants (Bartick et al., 2017). This is particularly true for preterm infants, with their greater risk for feeding intolerance and necrotizing enterocolitis, particularly in extremely low birth weight infants (Kantorowska et al., 2016; Sisk, Lovelady, Dillard, Gruber, & O’Shea, 2007).

Looking at human milk under the microscope or at the cellular level allows us to appreciate the dynamic interplay between mother and infant. That stem cells found in a mother’s milk should differ by stage of lactation and by gestational age with a greater number of cells and certain cell types in colostrum, corresponding with the greatest time of vulnerability in the neonate, is a pivotal point in evolution, affording greater survival of infants with this exogenous source of immunocompetent cells from the mother. The changes surrounding the milk cell phenotype with progression of lactation underscores the importance of fresh mother’s milk as opposed to frozen milk, as the process of freezing destroys those cells. Further, pasteurization, the time-honored yet obsolete method of milk sterilization (that we have yet to improve upon) to reduce the risk of bacterial and viral transmission to the recipient infant, destroys not only certain nutritional components in human milk but also its very cells.

When Drs Smith and Goldman (Smith & Goldman, 1968) discovered human milk macrophages, now over 50 years ago, it was the start of a new era in our ability to understand the elegance of human milk and to remind us that structure begets function. We continue on this journey of discovery through careful studies that depend on technological advancement to inform our understanding about the structure and function of human milk. As was the case decades ago, there remains the question of what purpose these hematopoietic and mesenchymal stem cells in human milk serve. Do these cells confer immunity to the recipient infant directly, or in an indirect manner through the immune mediators that they secrete into the milk? Are there multiple purposes for these cells in their interactions with other milk cells, with the milk fat globule membrane, and with the neonate directly from the moment the milk is ingested?

Given that the neonate has increased gut permeability at birth, and that there are cells in the mother’s milk, it has long been thought that milk cells migrate from the lumen of the gastrointestinal tract that begins in the mouth, to the tonsils and adenoids—vital lymphoid tissue in development—and to the multiple layers in the gut, including Peyer’s patches, eventually to access the lymphatic and circulatory systems. Studies from the past decade support this premise. There are stunning results from mouse models that demonstrate the ability of milk cells to migrate into the circulation and to the brain (Aydin, Yigit,

Vatandaslar, Erdogan, & Ozturk, 2018; Cabinian et al., 2016; Moles et al., 2018). Yet, our understanding of how this happens remains obscure. It is only through careful observation and well-designed studies that we will continue the process of more fully understanding the complexity and elegance of mother's milk. Li et al. (2019), through their published findings, are part of that process.

Acknowledgments

Funding

The author disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Funded in part by the South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, NIH/NCAT Grant number UL1 TR000062.

References

- Adan A, Alizada G, Kiraz Y, Baran Y, & Nalbant A (2017). Flow cytometry: Basic principles and applications. *Critical Reviews in Biotechnology*, 37(2), 163–176. doi: 10.3109/07388551.2015.1128876 [PubMed: 26767547]
- Alvarez DF, Helm K, Degregori J, Roederer M, & Majka S (2010). Publishing flow cytometry data. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 298(2), L127–L130. doi: 10.1152/ajplung.00313.2009 [PubMed: 19915158]
- Aydin MS, Yigit EN, Vatandaslar E, Erdogan E, & Ozturk G (2018). Transfer and integration of breast milk stem cells to the brain of suckling pups. *Scientific Reports*, 8(1), 14289. doi:10.1038/s41598-018-32715-5
- Bartick MC, Jegier BJ, Green BD, Schwarz EB, Reinhold AG, & Stuebe AM (2017). Disparities in breastfeeding: Impact on maternal and child health outcomes and costs. *Journal of Pediatrics*, 181, 49–55 e46. doi:10.1016/j.jpeds.2016.10.028 [PubMed: 27837954]
- Briere CE, Jensen T, McGrath JM, Young EE, & Finck C (2017). Stem-like cell characteristics from breast milk of mothers with preterm infants as compared to mothers with term infants. *Breastfeed Medicine*, 12, 174–179. doi:10.1089/bfm.2017.0002
- Cabinian A, Sinsimer D, Tang M, Zumba O, Mehta H, Toma A, . . . Laouar A (2016). Transfer of maternal immune cells by breastfeeding: Maternal cytotoxic t lymphocytes present in breast milk localize in the Peyer's patches of the nursed infant. *PLoS One*, 11(6), e0156762. doi:10.1371/journal.pone.0156762
- Kantorowska A, Wei JC, Cohen RS, Lawrence RA, Gould JB, & Lee HC (2016). Impact of donor milk availability on breast milk use and necrotizing enterocolitis rates. *Pediatrics*, 137(3), e20153123. doi:10.1542/peds.2015-3123
- Li Shujuan, Zhang Lan, Zhou Qi, Jiang Siyan, Yang Yi, & Cao Yun. (2019). Characterization of stem cells and immune cells in preterm and term mother's milk. *Journal of Human Lactation*, 35(3), 528–534. [PubMed: 31026180]
- Lian H, He S, Chen C, & Yan X (2019). Flow cytometric analysis of nanoscale biological particles and organelles *Annual Review of Analytical Chemistry*, Palo Alto, CA: Annual Reviews. doi:10.1146/annurev-anchem-061318-115042
- Moles JP, Tuailon E, Kankasa C, Bedin AS, Nagot N, Marchant A, . . . Van de Perre P (2018). Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant. *Pediatric Allergy and Immunology*, 29(2), 133–143. doi:10.1111/pai.12841 [PubMed: 29197124]
- Shields CW 4th, Reyes CD, & Lopez GP (2015). Microfluidic cell sorting: A review of the advances in the separation of cells from debulking to rare cell isolation. *Lab on a Chip*, 15(5), 1230–1249. doi: 10.1039/c4lc01246a [PubMed: 25598308]
- Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, & O'Shea TM (2007). Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. *Journal of Perinatology*, 27, 428–433. [PubMed: 17443195]

Smith CW, & Goldman AS (1968). The cells of human colostrum. I. In vitro studies of morphology and function. *Pediatric Research*, 2, 103–109. [PubMed: 4173318]

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