It’s Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant

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ABSTRACT

Human milk is the optimal source of nutrition for the nursing infant. Classically, the nutrients (water, protein, lipid, carbohydrate, vitamins, and minerals) were studied as the critical components of milk serving the growth needs of the infant for optimum growth. However, human milk contains factors other than the classically defined nutrients for which researchers are investigating potential roles in infant and maternal health, development, and well-being. The symposium addressed some of the exciting factors being studied, including microbes and maternal cells found within milk. Drs. Michelle McGuire and Juan M. Rodriguez addressed the presence of a bacterial community in human milk produced by healthy and mastitic mothers, potential sources of those bacteria, and the impact of milk-derived bacteria on the nursing infant. Drs. Donna Geddes, Peter Hartmann, and Foteini Hassiotou discussed the potential importance of maternal cells. For years, immune cells were known to be present in human milk, but recent evidence suggests that their impact is as much on the infant as on the health of the lactating mammary gland. Finally, the existence of highly plastic stem cells in human milk opens doors for previously unforeseen developmental “training” of the nursing infant.


Introduction

Human milk is “alive” and contains cells of both bacterial and host origin. Typically, the presence of bacteria in milk was considered an indication of infection. However, this notion may need revision because culture-dependent and -independent techniques more recently revealed that human milk produced by healthy women contains a diverse microbial community including >200 phylotypes. A core milk microbiome with a limited number of operational taxonomic units represents ~50% of the relative abundance. The other 50% of bacteria in human milk appears to be very personalized, suggesting that their community structure can be modified by the mother’s environmental exposure. How these bacteria reach the mammary gland and are incorporated into the milk remains unclear. Combined results of various studies suggest that some bacteria present in the maternal gastrointestinal tract could reach the mammary gland during late pregnancy and lactation through a mechanism involving intestinal immune cells. Thus, modulation of the maternal gastrointestinal microbiota during pregnancy and lactation could have a direct effect on infant health via their incorporation into milk. Conversely, some factors may cause a mammary dysbiosis, leading to infectious mastitis, a condition that represents 1 of the primary medical causes for early undesired weaning. Thus, altering human milk microbiota may offer novel ways to improve the health of the breastfed infant and potentially the breastfeeding mother. The symposium presentations by Drs. Michelle McGuire and Juan M. Rodriguez focused on bacterial cells and shared emerging research data from metagenomic, transcriptomic, and metabolomic studies focused on the role of human milk bacteria in health and disease. Dr. Donna Geddes’ symposium presentation focused on maternal immune cells because their numbers and composition rapidly respond to infections in the mammary gland and other maternal infections but also to infant infections. Although the maternal response to infant infection is not fully understood, it was...
proposed that the retrograde ductal flow associated with milk ejection during breastfeeding is a route for the transfer of infant-derived pathogens via the nipple, which may locally stimulate an immune response in the mother’s mammary gland. Clearly, this has major implications for both the mother and the infant. The symposium presentations by Drs. Peter Hartmann and Foteini Hassiotou focused on recently discovered human milk stem cells. New exciting advances show that these cells are viable and functional when ingested, suggesting potential functions in the breastfed infant. Moreover, their ability to differentiate into cells from all 3 germ layers makes them a candidate for stem cell–related therapies. The symposium presented the latest findings on milk-borne stem cell research and applications from the world-leading research group in this area. Overall, the timely symposium session put a new “live” spin on human milk research that spurred great interest for various groups of nutrition scientists within the ASN membership and beyond.

Milk: The First Probiotic Food?
The genesis of the concept of a “probiotic” food is generally attributed to Dr. Ilya Mechnikov, a Russian immunologist and microbiologist who was awarded the Nobel Prize for Physiology or Medicine in 1908. Indeed, >1 century ago, Mechnikov hypothesized that health could be enhanced and life prolonged by manipulating gastrointestinal microbial communities using probiotic foods, particularly yogurt. However, in 2001, a new and more restrictive definition for the term probiotic was proposed in a report of an expert committee assembled by the FAO and the WHO: “live micro-organism which, when administered in adequate amounts, confers a health benefit on the host.” As such, answering the fundamental question posed in this presentation, “Is human milk a probiotic?” requires first grappling with the meaning of the term probiotic. Additionally, researchers must establish whether human milk contains bacteria, a concept at odds with long-held dogma. To that end, there are now scores of publications providing ever-growing evidence of a paradigm shift in this regard. For instance, Hunt et al. (1) used 454 pyrosequencing and discovered that human milk contains a diverse bacterial community, that its membership varies substantially among lactating women, and that there exists a somewhat unique microbial fingerprint within a particular woman. Cabrera-Rubio et al. (2) showed that this microbial community structure may be influenced by maternal weight and mode of delivery. Khodaya-Pardo et al. (3) published evidence that lactation state, gestational age, and mode of delivery are all associated with variation in the milk microbiota, particularly in terms of abundance of Bifidobacterium species known to be highly prevalent in the feces of breast-fed infants. Limited evidence also exists that milk components, such as FAs and complex carbohydrates (oligosaccharides), might be related to the abundance of specific bacterial taxa in milk, such as Staphylococcus species. In summary, a growing literature suggests that human milk, like all other fluids secreted by the body, indeed contains bacteria that are likely to play important roles in the colonization of the infant’s gastrointestinal tract. As such, human milk should be considered a probiotic food, at least when respecting Mechnikov’s original intent of the concept. Determining factors that influence which bacteria are present in human milk and precisely how they influence the lactating mammary gland and the recipient infant’s health remain basic science and public health realms in which almost nothing is known.

Rise and Fall of the Human Milk Microbiota
The origin of the bacteria present in human milk has become a controversial and attractive issue in the past years (4). Traditionally, it was believed that milk harbored bacteria that were the result of contamination with bacteria from the mother’s skin or the infant’s oral cavity. However, the comparison of the bacterial communities detected in milk, breast skin, and infant mouth samples indicates that, although these communities share some phylotypes, major differences exist. Therefore, growing evidence demonstrates that bacterial communities in milk are not simply a result of skin contamination but constitute a site-specific microbiota. Different studies suggest that some bacteria present in the maternal gastrointestinal tract could reach the mammary gland during late pregnancy and lactation through a mechanism involving monocytes in the intestine. Dendritic cells (DCs) can penetrate the intestinal epithelium to “sample” bacteria directly from the lumen. More specifically, DCs are able to open tight junctions between intestinal epithelial cells, reach out through dendrites beyond the epithelium, and directly sample bacteria, while preserving the integrity of the epithelial barrier through the expression of tight-junction proteins. Once attached to DCs, bacteria may travel to other locations via the circulation of monocytes within the mucosal-associated lymphoid system. Antigen-stimulated cells move from the intestinal mucosa to colonize distant mucosal and epithelial surfaces, including those of the lactating mammary gland. Exposure of immature DCs to bacterial strains isolated from human milk leads to stimulation of 2 DC activation surface markers: 1) the class II major histocompatibility complex; and 2) the B7.2 protein. The translocation of bacteria from the lumen of the intestine to mesenteric lymph nodes and ultimately to the mammary gland in mice occurs during late pregnancy and lactation. In lactating women, 2 successive trials demonstrated that oral administration of lactobacilli strains (originally isolated from human milk) leads to their presence in human milk, confirming the existence of a bacterial entero-mammary pathway. Thus, maternal gastrointestinal bacteria during pregnancy and lactation could have a direct effect on infant health and that of the lactating mammary gland.

Immune Cells in Human Milk
Immune cells accessed via human milk protect the mammary gland from infection and are thought to provide active immunity and to promote development of immunocompetence in the infant. Immune cells are thought to exert these
benefits via phagocytosis, secretion of antimicrobial factors, and/or antigen presentation in the breasts of lactating mothers and in the gastrointestinal tract of the infant; these effects may also be extended to other tissues in infants via the systemic circulation. Recently, Hassiotou et al. (5) showed that a low proportion of human milk immune cells (0–2% of total cells) exists in the milk of healthy mother/infant dyads during established lactation. Immune cell numbers increase rapidly in response to infection of the mammary gland and other maternal infections, as well as infant infections, returning to baseline amounts during recovery. Although the response of the mammary gland to infant infection is not understood fully, it was proposed that the retrograde ductal flow associated with milk ejection during breastfeeding is a route for the transfer of infant pathogens via the nipple, which may locally stimulate an immune response in the breast. It is of note that the breastmilk of mothers exclusively breastfeeding their infants has higher baseline immune cell content than the breastmilk of mothers who do not exclusively breastfeed, further supporting the important protective role of human milk. As expected, the greatest human milk immune cell counts were measured during mastitis, and, when treatment was delayed, there was a corresponding delay in reduction of immune cell numbers to the normal baseline amounts. Interestingly, milder breast conditions, such as sore nipples and blocked ducts, had less dramatic responses, further supporting the notion that the maternal response is protective of additional infection of the breast. More importantly, the marked difference in the immune cell response between mild inflammatory conditions and mastitis highlights the potential for a new diagnostic test for mastitis. There is increasing frequency of reports of diminished and unrecoverable milk production as a result of severe mastitis. Clearly, this has major implications for the mother and infant, particularly if it is unexpected. The new data provide novel tools for the timely diagnosis of breast infections, which has the potential to prevent early undesirable cessation of breastfeeding, prolonging its benefits for both the infant and the mother.

Stem Cells in Human Milk

Stem cells are not only present in the embryo and adult tissues but also in body fluids, such as the blood. Recent advances showed that human milk also contains stem cells, adding a new dimension to the potential developmental benefits of human milk feeding to the infant (6). For many decades, it was thought that immune cells were dominating the cellular fraction of human milk, particularly as a result of the examination of colostrum or early lactation milk by most investigators and the lower specificity of older cellular characterization techniques. However, flow cytometric analyses of mature human milk revealed recently that, in healthy mother/infant dyads, non-immune cells comprise the majority of the cellular fraction. Stem cells were identified in the non-immune cell fractions. Human milk stem cells were shown to be highly plastic and to differentiate in culture into cells from the 3 germ layers (6). Importantly, Hassiotou and Hartmann (7) used mouse models to provide the first evidence that milk stem cells are transferred to different tissues of the breast-fed offspring, remaining viable and integrating within host tissue. These exciting new findings now suggest the possibility that human milk stem cells provide developmental benefits to the breast-fed infant. Harnessing the properties and natural fate of milk stem cells may provide new tools in treating infant diseases (8). In addition, the breast origin of some of these stem cells highlights their function(s) in the lactating mammary gland and their potential use as diagnostic tools in lactation pathologies. Finally, the natural ability of these stem cells to transfer from 1 organism (the mother) to another (the infant) and to survive and integrate in vivo within host tissue generates exciting new opportunities for the exploration of the use of human milk stem cells in regenerative medicine.

In conclusion, human milk is a complex fluid with a multitude of components, each of which may contribute substantially to infant and maternal health. The presence of bacteria and maternal cells in milk is only now realized as an important route of communication between mother and infant. Future work will address how presence and distribution of bacteria and maternal cells are regulated in human milk and will investigate potential benefits to the nursing infant and the breastfeeding mother.

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References