

The Origin of Human Milk Bacteria: Is There a Bacterial Entero-Mammary Pathway during Late Pregnancy and Lactation?¹⁻⁴

Juan M. Rodríguez*

Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, Madrid, Spain

ABSTRACT

Human milk is a source of bacteria to the infant gut; however, the origin of milk bacteria, as well as their impact on neonatal gut microbiota establishment, remains largely unknown. In the past years, results provided by different research groups suggest that certain bacteria from the maternal gastrointestinal tract could translocate through a mechanism involving mononuclear immune cells, migrate to the mammary glands via an endogenous cellular route (the bacterial entero-mammary pathway), and subsequently colonize the gastrointestinal tract of the breast-fed neonate. If such findings are confirmed in the future, we could exert a positive influence on infant health by modulating the maternal gut microbiota. *Adv Nutr* 2014;5:779–784.

Introduction

In the past decade, some culture-dependent studies revealed that colostrum and milk from healthy women contain bacteria, including staphylococci, streptococci, corynebacteria, lactic acid bacteria, propionibacteria, and bifidobacteria (1). Later, the application of culture-independent techniques, including microbiome approaches, confirmed the presence of DNA from these and other bacterial genera (1). Therefore, such biologic fluids are continuous sources of live bacteria to the infant gastrointestinal tract and, in fact, different studies have shown that there is a mother-to-infant transfer of bacterial strains through breastfeeding (2–7). Traditionally, it was believed that any bacterial cell found in human milk was the result of contamination from the infant's oral cavity or the mother's skin (8). However, the detection of live bacterial cells and/or DNA from anaerobic species that are usually related to gut environments and that

cannot survive in aerobic locations has fueled a scientific debate on the origin of milk-associated bacteria.

Infant's Mouth and Maternal Skin as Potential Sources of Bacteria to Mammary Ducts and Milk

The microbiome of the different human body locations constitutes a dynamic network of interrelated communities (9). Therefore, the possibility that the infant's mouth or maternal skin may provide some bacteria to the milk is not incompatible with the role of human milk as a source of bacteria to the infant's mouth, maternal skin, and other infant/mother locations (Fig. 1).

Some bacteria from the infant's oral cavity may contaminate milk during suckling because of milk flow back into the mammary ducts (10); however, this mechanism does not explain why precolostrum secreted by some women before delivery (and obviously before any contact with the infant's mouth) already contains the microbiota that characterizes human milk (11). Although the human salivary microbiome is still widely unknown, *Streptococcus* species seem to be dominant both in adults (12–14) and in edentulous infants (15–18). Streptococci are also among the dominant phylotypes in human milk (3,19,20), suggesting a potential role in the shaping of the salivary microbiota. The origin of the oral microbiota is far from elucidated and deserves research attention because of its relevant implications for human health.

¹ This article is a review of the symposium "It's Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant" held 29 April 2014 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2014 in San Diego, CA. The symposium was sponsored by the American Society for Nutrition (ASN) and the ASN Lactation RIS and supported by an educational grant from Medela, Inc.

² A summary of the symposium "It's Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant" was published in the September 2014 issue of *Advances in Nutrition*.

³ Supported by project AGL2013-41980-P from the Ministry of Economy and Competitiveness, Spain.

⁴ Author disclosures: J. M. Rodríguez, no conflicts of interest.

* To whom correspondence should be addressed. E-mail: jmrodrig@ucm.es.

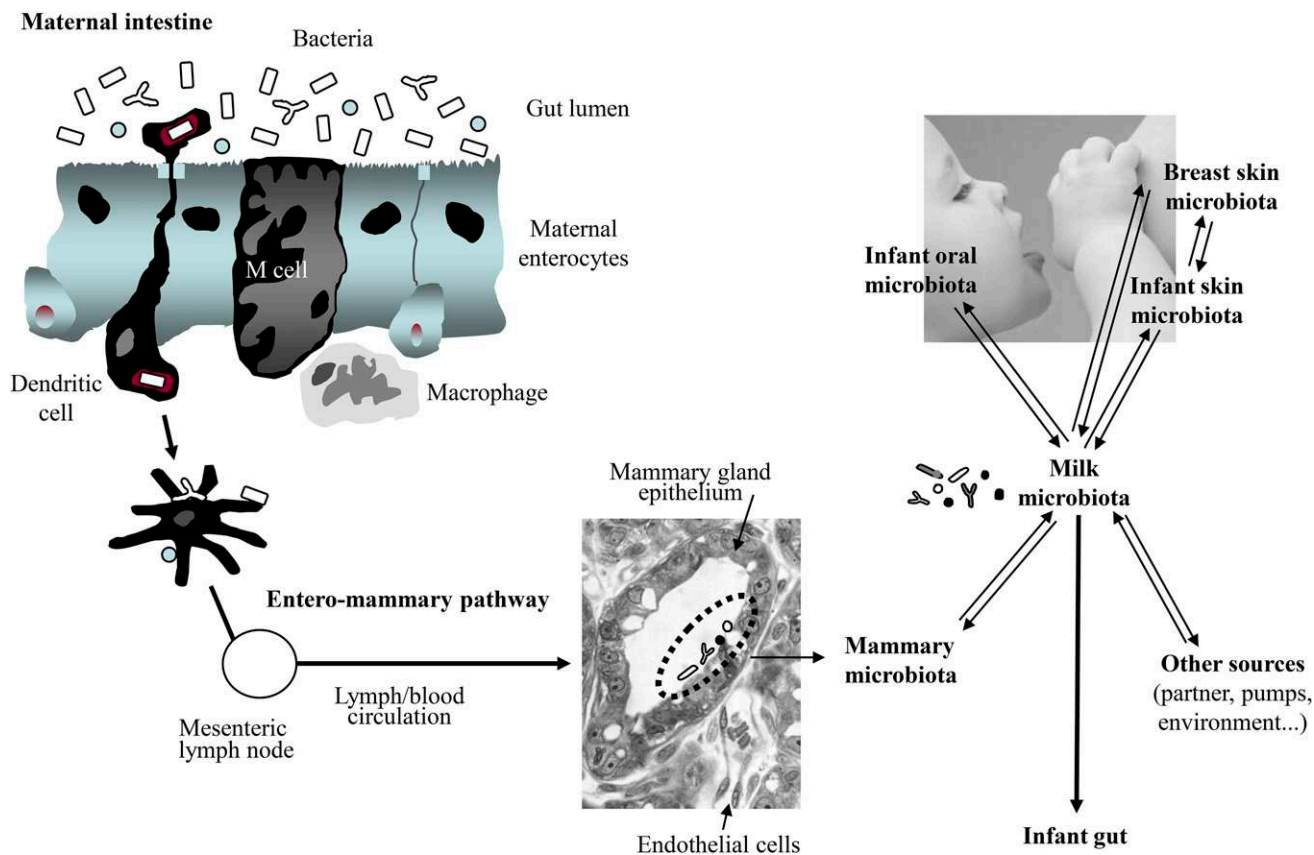


FIGURE 1 Sources of the bacteria present in human milk, including a model to explain how some maternal bacterial strains could be transferred to the infant gut through an entero-mammary pathway.

Some of the bacteria that are commonly isolated from skin, such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* (21,22), are also frequent in human milk. However, it should be highlighted that although staphylococci, corynebacteria, and propionibacteria have been traditionally associated with the skin, they are widespread in most, if not all, human mucosal surfaces; in fact, the populations of such bacterial groups reach their highest concentrations in the mucosal layers of the digestive and genitourinary tracts. In addition, such bacteria have been detected in samples of chorioamnion and amniotic fluid from pregnant women and in umbilical cord blood obtained from healthy neonates born either by vaginal or cesarean delivery (17,19,23,24); this suggests that they may colonize the fetal skin and digestive tract in utero and raises the possibility that the presence of bacteria in chorioamnion, amniotic fluid, colostrum, and milk may share a common or similar mechanism in healthy hosts.

Streptococci and staphylococci have received marginal attention regarding their role in the early colonization of the infant gastrointestinal tract despite being the dominant bacteria in human milk (3,6). Interestingly, an abundant presence of *Staphylococcus epidermidis* seems to be a differential feature of the feces of healthy breast-fed infants when compared with those of formula-fed infants (3,25–28). Such bacteria seem to have coevolved with mammary glands and usually display specific properties that favor their

growth in the mammary environment during lactation. For example, some staphylococci (e.g., *S. epidermidis* and *Staphylococcus aureus*) are typically associated with catheters and indwelling medical devices in hospital settings, and mammary glands develop an extraordinary complex net of “catheter-like” ducts during late pregnancy and lactation, providing an excellent physical support to these microorganisms. Second, lactose and galactose metabolism of staphylococci is highly efficient through the D-tagatose-6-phosphate pathway (29). Finally, these bacteria readily metabolize human milk oligosaccharides (30).

Indeed, it has been proposed that some coagulase-negative staphylococci and some streptococci from the *mitis* and *salivarius* groups may have a beneficial function by preventing colonization of the host by more severe pathogens, such as *S. aureus* (31–35). A previous study showed that cows’ udders that contained coagulase-negative staphylococci were less susceptible to mastitis after experimental challenge with *S. aureus* (36).

Despite sharing of some phylotypes, the comparison between the bacterial communities detected in milk and those found on breast skin reveals that there are major differences between them (20). As an example, *Bifidobacterium* is a strictly anaerobic genus and therefore skin is a highly improbable source of such microorganisms in milk (37). Sharing of *Bifidobacterium longum* DNA in maternal feces, human milk, and neonatal feces within the same mother-neonate

pair has been reported (38). More recently, pyrosequencing allowed identifying gut-associated obligate anaerobic genera, such as *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, and members of the Clostridia class (*Blautia*, *Clostridium*, *Collinsella*, and *Veillonella*), shared between maternal feces, human milk, and neonatal feces (7). Furthermore, several butyrate-producing members of Clostridia (*Coprococcus*, *Faecalibacterium*, *Roseburia*, and *Subdoligranulum*) were shared between maternal feces and human milk. A major drawback of culture-independent studies is the lack of information about the viability of the detected populations and the lack of possibility for strain-level discrimination, which is necessary for demonstrating that the same bacterial strain was shared between mother and neonate. Thus, without confirming the presence of these populations by culture, isolation, and strain level discrimination, it remains unclear whether human milk is a source of viable gut-associated obligate anaerobes or if dead cells or parts thereof are transferred to the breast-fed neonate (7). However, transfer of bifidobacteria, lactobacilli, and/or other bacteria at the strain level from the maternal gastrointestinal tract to the neonatal gut (39–41), from the maternal gastrointestinal tract to human milk (42–44), from milk to the neonatal gastrointestinal tract (3,6), and from the maternal gastrointestinal tract to milk and the infant gastrointestinal tract (5,7) has also been demonstrated by using culture and strain-level discrimination. Such studies reinforce the hypothesis that at least some bacteria, including obligate anaerobes, may be vertically transferred from mother to neonate via breastfeeding.

Gut Bacterial Translocation during Late Pregnancy and Lactation as a Physiologic Event

Recent findings suggest that selected bacteria of the maternal gastrointestinal microbiota can access the mammary glands through an entero-mammary pathway (11). Previous studies indicated that certain bacteria from the maternal digestive tract may spread to extradiigestive locations in healthy hosts (23,45–48).

Although this is a controversial issue, some studies have offered a scientific basis for such physiologic translocation [reviewed in (1)]. The mechanism would involve dendritic cells (DCs) and CD18⁺ cells (49–51), which would be able to take up nonpathogenic bacteria from the gut lumen and subsequently carry them to other locations, including the lactating mammary gland (52) (Fig. 1). It must be highlighted that there is an important efflux of intestinal immune cells to the mammary glands during late pregnancy and lactation (53) and that, in fact, the existence of an entero-mammary circulation of IgA-producing cells is long known (54).

Research carried out by 2 independent groups obtained in vitro and in vivo data reinforcing the hypothesis that at least some human milk bacteria may reach the mammary glands through an internal route, involving maternal DCs and macrophages (38,55,56). As an example, 2 lactobacilli strains isolated from human milk (*Lactobacillus salivarius*

CECT 5713 and *Lactobacillus gasseri* CECT 5713) were able to translocate across a Caco-2 cell monolayer through a DC-mediated mechanism (55) (Fig. 2). In addition, oral inoculation of pregnant mice with a genetically labeled *Enterococcus faecium* M1a strain led to the isolation and PCR detection of the labeled strain in the amniotic fluid (23) and milk (Jiménez E, Fernández L, Martín R, Rodríguez JM, 2005, unpublished results) of the inoculated animals. In contrast, it could not be detected in the respective samples obtained from a noninoculated control group. Similarly, oral administration of lactobacilli strains isolated from human milk led to their presence in the milk of >50% of the recruited women (42,44).

An increased bacterial translocation from the gut to mesenteric lymph nodes and mammary glands in pregnant and lactating mice was observed in another study (38). Bacteria could be observed histologically in the subepithelial dome and interfollicular regions of Peyer's patches, in the lamina propria of the small bowel, and associated with cells in the glandular tissue of the mammary gland. The Peyer's patches of pregnant and lactating mice were macroscopically larger than those of control mice and had a more prominent subepithelial dome and more dilated draining lymphatic vessels, containing mononuclear cells. The same study showed that human milk contains viable bacteria, including *Streptococcus*, *Lactobacillus*, and *Bifidobacterium*, whereas acridine orange staining of milk and blood cytopreparations identified bacterial cells in association with maternal mononuclear cells. Globally, these results strongly suggest the involvement

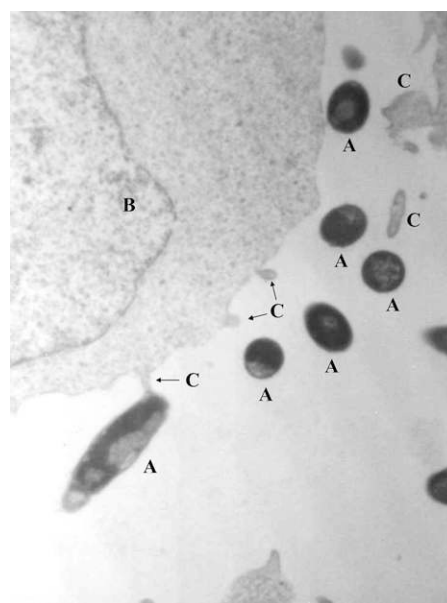


FIGURE 2 Specific interactions between cells of a *Lactobacillus gasseri* strain isolated from human milk ("A") and dendritic ("B") cells, as assessed by transmission electron microscopy (55). The interactions were studied by using trans-well bicompartmental assays in which bacterial cells and immature dendritic cells were initially separated by a monolayer of Caco-2 cells. C, dendritic cell dendrites.

of mononuclear cells in the transport of intestinal bacteria to the mammary glands in late pregnancy.

The passage of viable bacteria through the intact intestinal mucosa is known as bacterial translocation. This phenomenon was postulated >60 y ago (57), although the term “translocation” was first used to describe the passage of *Serratia marcescens* from the duodenum of rats, where it had been inoculated, to the lymph (58). Later, the term bacterial translocation was defined as the passage of viable bacteria from the gastrointestinal tract into the lamina propria and then to the mesenteric lymph nodes and other extraintestinal organs such as spleen, liver, kidneys, peritoneal cavity, or bloodstream (59). Traditionally, gut bacteria translocation has been associated with pathogenic conditions and therefore it has been mainly studied in patients (with, e.g., severe burns, transplants, pancreatitis, cardiopulmonary diseases, AIDS) in whom pathogenic bacteria had spread throughout the body causing sepsis, multiple organ failure, and, sometimes, death (60).

However, it is known that a low rate of bacterial translocation also occurs in healthy individuals without causing detrimental effects in the host (60–62). In a study involving 132 patients who underwent laparotomy, 5 showed positive culture results in their blood samples, but the isolated bacteria lacked pathogenic traits and were not related to the patient morbidity (63). Langa (55) reported that the rates of translocation of some lactic acid bacteria (0.002–0.009% after 2 h) through a trans-well system, involving interactions between immune cells and Caco-2 cells, were notably lower than those (>20%) reported for *Vibrio cholerae* (64) and invasive *Salmonella* (65). In fact, it has been suggested that

bacterial translocation to extraintestinal tissues is a beneficial physiologic event in healthy hosts because it may be associated with immunomodulation, including the initial maturation of the neonatal immune system (38,66,67).

Many transient anatomic and physiologic changes occur during pregnancy and lactation to provide a suitable framework for the development of the fetus first, and the neonate later. These changes affect virtually all systems, including the cardiovascular, respiratory, genitourinary, and digestive systems. Interestingly, such adaptations may favor an increased bacterial translocation during late pregnancy and early lactation (Fig. 3). Adequate cardiovascular adaptations must secure good placental development and appropriate fetal growth. Therefore, changes in the cardiovascular system are characterized by a progressive and generalized vasodilatation state and an increase in several variables or processes, including blood volume, stroke volume, cardiac output, heart rate, regional blood flow to various organs (e.g., uterus, kidneys, gastrointestinal tract, skin, breasts), angiogenesis, and blood concentration of coagulation factors and leukocytes.

The hormonal action induces relevant oral changes during pregnancy, affecting the mouth’s pH and microbiota; the gums become hyperemic and edematous and tend to bleed. The main effects of gestation on the gastrointestinal system are associated with the displacement of the abdominal organs by the progressive growth of the uterus and to a decreased motility, presumably because of the effect of progesterone on smooth muscle contractility. This causes an increase in the gastric emptying time, whereas a decreased gastric secretion results in a more basic gastric pH.

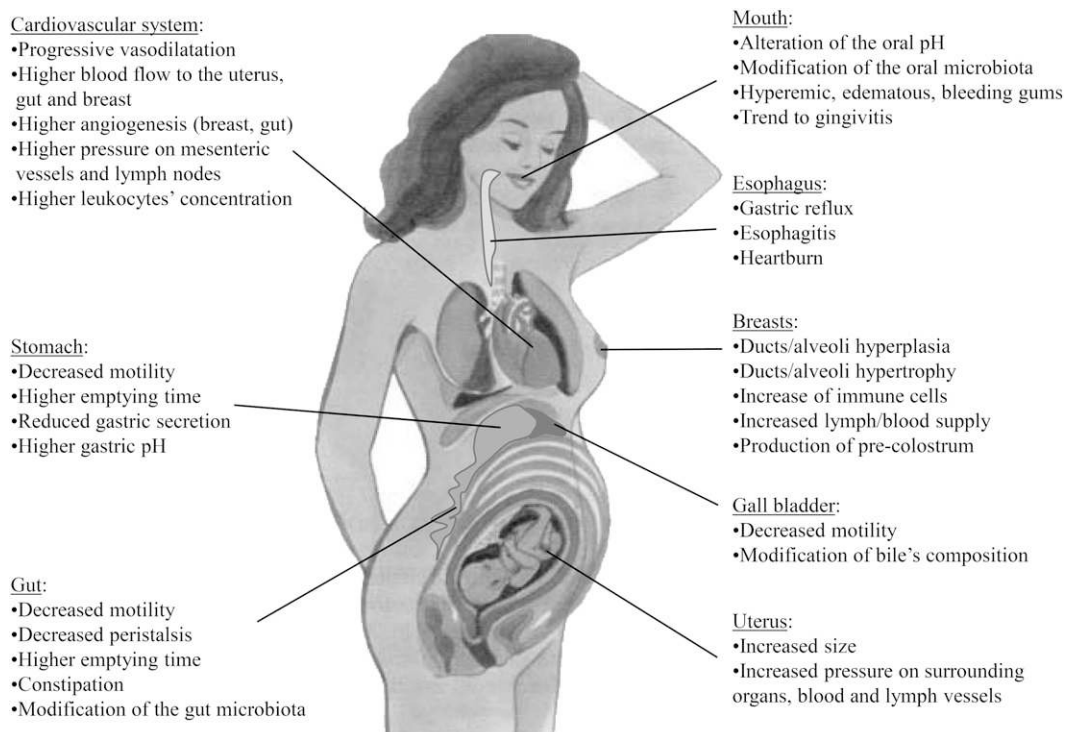


FIGURE 3 Physiologic adaptations of the body during pregnancy that may favor an increased bacterial translocation.

The decreased gut motility and peristalsis, particularly in the last trimester of pregnancy, along with the increased pressure of the uterus, can cause problems of constipation and hemorrhoids. In addition, the maternal mesenteric blood vessels are exposed to estrogens and to an increasing fetal pressure, leading to transient vascular engorgements and blood stasis. In addition, 1 of the body's most dramatic adaptations to late pregnancy and lactation is a large increase in the size and complexity of the maternal intestine (68). Globally, the digestive tract is characterized by weakened barriers against bacterial growth, increased permeability, and reduced peristalsis, 3 factors that are closely associated to bacterial translocation (61).

Finally, several anatomic and physiologic changes in the mammary system, including ducts, areola, and nipples (69), during pregnancy facilitate the formation of a specific mammary microbiota (1). Interestingly, it has been considered that there is an obvious functional relation between the intestinal tract and the mammary glands during late pregnancy and lactation (68).

If the existence of the bacterial entero-mammary pathway is confirmed, this novel form of mother-neonate communication could influence the current understanding of neonatal gut development and provide future opportunities for manipulating an aberrant microbiota establishment and reduce the risk of disease, such as by administering probiotics to the neonate and/or to the breastfeeding mother (70).

Acknowledgments

The sole author read and approved the final manuscript.

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