Invited Review

The human milk microbiota: Origin and potential roles in health and disease

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A R T I C L E   I N F O

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A B S T R A C T

Human milk has traditionally been considered sterile; however, recent studies have shown that it represents a continuous supply of commensal, mutualistic and/or potentially probiotic bacteria to the infant gut. Culture-dependent and -independent techniques have revealed the dominance of staphylococci, streptococci, lactic acid bacteria and bifidobacteria in this biological fluid, and their role on the colonisation of the infant gut. These bacteria could protect the infant against infections and contribute to the maturation of the immune system, among other functions. Different studies suggest that some bacteria present in the maternal gut could reach the mammary gland during late pregnancy and lactation through a mechanism involving gut monocytes. Thus, modulation of maternal gut microbiota during pregnancy and lactation could have a direct effect on infant health. On the other hand, mammary dysbiosis may lead to mastitis, a condition that represents the first medical cause for undesired weaning. Selected strains isolated from breast milk can be good candidates for use as probiotics. In this review, their potential uses for the treatment of mastitis and to inhibit mother-to-infant transfer of HIV are discussed.

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1. Bacterial diversity of human milk

Human milk is a complex species-specific biological fluid adapted to satisfy the nutritional requirements of the rapidly growing infant; additionally, it educates the infant immune system and confers a certain degree of protection against pathogens [1]. These effects reflect the synergistic action of many bioactive molecules, present in colostrum and milk, including immunocompetent cells, immunoglobulins, fatty acids, polymamines, oligosaccharides, lysozyme, lactoferrin and other glycoproteins, and antimicrobial peptides [2], which inactivate pathogens individually, additively, and synergistically [3].

More recently, several studies have revealed that colostrum and breast milk are continuous sources of commensal, mutualistic and potentially probiotic bacteria to the infant gut [4–11](Table 1). This is a relevant finding since intramammary human milk was traditionally considered to be sterile. In fact, human milk constitutes one of the main sources of bacteria to the breastfed infant gut since a baby consuming approximately 800 mL/day of milk would ingest between $1 \times 10^5$ and $1 \times 10^7$ bacteria daily [5]. This may explain
Table 1
Main bacterial genera or species isolated from human milk or which DNA sequences have been retrieved from this biological fluid.

<table>
<thead>
<tr>
<th>Method</th>
<th>Main species/genera*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial isolation</td>
<td>L. acidophilus, L. fermentum, S. epidermidis, Str. mitis, Str. salivarius</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>L. plantarum, S. epidermidis, Streptococcus spp.</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>E. faecium, L. fermentum, L. gasseri</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>E. faecalis, L. crispatus, L. rhamnosus, Lc. lactis, Leuc. mesenteroides, R. mucilaginosus</td>
<td>[5,6]</td>
</tr>
<tr>
<td></td>
<td>S. aureus, S. capitis, S. epidermidis, S. hominis, Str. mitis, Str. oris, Str. paracasei, Str. salivarius</td>
<td>[11]</td>
</tr>
<tr>
<td>DNA detection</td>
<td>L. reuteri</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>[7,8]</td>
</tr>
<tr>
<td></td>
<td>E. durans, E. faecalis, E. faecium, E. hirae, E. mundtii, L. animalis, L. brevis, L. fermentum, L. gasseri, L. helveticus, L. oris, L. plantarum, Peptostreptococcus, Propionibacterium, R. pentosaceus, Streptococcus, Streptococcus galolyticus, Streptococcus, Streptococcus, Streptococcus, Streptococcus, Streptococcus</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>B. longum</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>E. faecalis, E. faecium, L. fermentum, L. gasseri, L. rhamnosus, Lc. lactis, Leuc. citreum, Leuc. fallax, Prop. acnes, S. epidermidis, S. hominis, Str. mitis, Str. paracasei, Str. salivarius, W. cibaria, W. confusa</td>
<td>[27,28]</td>
</tr>
<tr>
<td></td>
<td>B. longum, Clostridium spp., Lactobacillus spp., Staphylococcus spp., Streptococcus spp.</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>B. adolescentis, B. animalis, B. bifidum, B. breve, B. catenolactum, B. longum</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>Bifidobacterium spp., Clostridium spp., Enterococcus spp., Lactobacillus spp., Staphylococcus spp., Streptococcus spp.</td>
<td>[121]</td>
</tr>
<tr>
<td></td>
<td>B. adolescentis, B. bifidum, B. breve, B. longum</td>
<td>[11]</td>
</tr>
</tbody>
</table>

*Abbreviations: B., Bifidobacterium; E., Enterococcus; K., Kocuria; L., Lactobacillus; Lc., Lactococcus; Leuc., Leuconostoc; P., Peptococcus; Prop., Propionibacterium; R., Rothia; S., Streptococcus; Str., Streptococcus; W., Weissella.

why the bacterial composition of the gut microbiota of breastfed infants is closely related to that found in the breast milk of their respective mothers and, also, why the development of a more diverse microbiota coincides with the weaning period [12]. It must be highlighted that human milk oligosaccharides (HMOs) also play a key role in driving the diversity of the infant gut microbiota [13]. HMOs are the third-largest solid component of milk and, while their structural complexity renders them non-digestible to the host, they are liable to hydrolytic enzymes of the infant colonic microbiota [14]. It has been previously demonstrated that selected bifidobacterial and lactobacilli phylotypes use specific HMOs secreted early in the lactation cycle [15]; more recently, the genome sequences of two bifidobacterial strains have revealed several adaptations for milk utilization within the infant microbiome [16,17].

The first descriptions of the bacterial diversity of breast milk in healthy women were based on the use of culture media and showed the predominance of staphylococci, streptococci, lactic acid bacteria (LAB), propionibacteria and closely related Gram-positive bacteria [4,5,18,19] (Table 1), including new bacterial species, such as Streptococcus lactarius [20]. Human milk has also been shown to be a source of live bifidobacteria to the infant gut [11]. The fact that bacteria belonging to such genera can be isolated from fresh breast milk of healthy women from distant countries suggests that their presence in this substrate is a common event. Therefore, they should be considered as components of the natural microbiota of human mammary gland, instead of mere contaminant bacteria. The mammary microbiota is somehow peculiar since it has a transient nature: its development starts during the last third of pregnancy, reaches the highest complexity at the end of such period, remains quite constant throughout lactation, declines sharply at weaning, and rapidly disappears when there is no milk in the mammary gland and the apoptosis process responsible for mammary involution begins (Fig. 1).

Several studies have shown that there is a mother-to-infant transfer of bacterial strains belonging, at least, to the genera Lactobacillus, Staphylococcus, Enterococcus, and Bifidobacterium through breastfeeding [4,7,11,21–24]. Independently of the original source of such bacteria, human milk is the source of hundreds of bacterial phylotypes to the infant gastrointestinal tract. It has been suggested that exposure of the breast-fed infant to such a wealth of bacterial phylotypes may exert beneficial effects against diarrheal and respiratory diseases, and may reduce the risk of developing other diseases, such as diabetes or obesity [25,26].

The application of culture-independent molecular techniques, and particularly those based on 16S rRNA genes, allowed a complementary biodiversity assessment of the human milk microbiome. The use of such techniques confirmed the dominance of staphylococci and streptococci and the presence of LAB, propionibacteria and bifidobacteria, and revealed the existence of DNA belonging to other bacterial groups, such as some Gram-negative bacteria [11,27–29]. In addition, the application of the “-omics” approach (genomics, metagenomics, transcriptomics, proteomics, and metabolomics) to the study of the human mammary microbiota is already in progress and there is no doubt that the results provided by such techniques will open new perspectives to understand the initiation and development of the infant gut microbiota [30].

Recently, the first microbiome study focused on human milk was published [25]. The authors used microbial identification techniques based on pyrosequencing of the V1–V2 region of the bacterial 16S rRNA gene to characterize the bacterial communities present in milk samples collected from 16 women self-described as healthy at three time-points over 4 weeks. Results indicated that milk bacterial communities were generally complex and, although a few genera (Streptococcus, Staphylococcus, and Serratia) represented greater than 5% of the relative community abundance, eight other genera represented ≥1% of the communities observed across samples. Among the hundreds of operational taxonomic units (OTUs) detected in the milk of every woman, only 9 (Streptococcus, Staphylococcus, Serratia, Pseudomonas, Corynebacterium, Ralstonia, Propionibacterium, Sphingomonas, and Bradyrhizobiaceae) were present in every sample from every woman. These 9 “core” OTUs represented approximately half of the microbial community observed, although their relative abundance varied greatly between subjects. The remaining half of the community was not conserved across women. These findings are in contrast with those of the gut microbiome, where only a low set of OTUs is shared among individuals [31], or the vaginal microbiome which comprises several different core groups [32]. On the other hand, milk bacterial community was generally stable over time within an individual. Similarly to the mammary microbiota, previous studies of various sites of the human microbiome have also revealed that the
bacterial communities associated with a particular individual over time are often stable and highly personalized [33].

The results of a second study on the breast milk microbiome involving 18 mothers indicated that milk bacteria are not contaminants and suggested that this site-specific microbiome is influenced by several factors that significantly skew its composition [34]. More recently, our research group analyzed the metagenome of human milk samples obtained from healthy, mastitis-suffering or obese women, and observed that the bacterial community of breast milk may differ depending on the individual and on the health status of the lactating women [unpublished results].

2. Functions of human milk bacteria in the infant gut

In the last years, some studies have shown that human milk bacteria may play several roles in the infant gut. First of all, they can contribute to the reduction of the incidence and severity of infections in the breastfed infant by different mechanisms, such as competitive exclusion [35], production of antimicrobial compounds [6,9,10,35], or improvement of the intestinal barrier function by increasing mucus production and reducing intestinal permeability [35]. Recently, the administration of a human milk *Lactobacillus* strain to infants during 6 months led to 46%, 27%, and 30% reductions in the incidence rates of gastrointestinal infections, upper respiratory tract infections, and total number of infections, respectively [36]. Commensal coagulase-negative staphylococci and viridans streptococci provided by breast milk can be particularly useful to reduce the acquisition of undesired pathogens by infants exposed to hospital environments. In fact, some *Staphylococcus epidermidis* strains that inhibit in vivo colonization by *Staphylococcus aureus* have been postulated as a future strategy to eradicate such pathogen from the mucosal surfaces [37,38]. Similarly, it has been shown that viridans streptococci inhibit oral colonization by methicillin-resistant *S. aureus* in high-risk newborns exposed to hospital environments [39].

Breast milk bacteria may also participate in the correct maturation of the infant immune system since some strains are able to modulate both natural and acquired immune responses in mice and humans [40–42]. Their function seems to have a certain degree of flexibility depending on the conditions found in the gut environment. As an example, *Lactobacillus salivarius* CECT 5713 and *Lactobacillus fermentum* CECT 5716 enhanced macrophage production of Th1 cytokines, such as IL-2 and IL-12 and the inflammatory mediator TNF-α, in the absence of an inflammatory stimulus. However, both strains led to a reduction of Th1 cytokines when cells were incubated in the presence of lipopolysaccharide [40]. A recent study confirmed that *L. fermentum* CECT 5716 and *L. salivarius* CECT 5713 have a broad array of effects on the immune system [43]. They behaved as potent activators of NK cells and moderate activators of CD4+ and CD8+ T cells and regulatory T cells. Thus, they had an impact on both innate and acquired immunity. They strongly induced a wide range of pro- and anti-inflammatory cytokines and chemokines. The authors compared these strains with others belonging to the same species but isolated from sources different to breast milk and found some milk strain-specific effects, such as a higher induction of IL-10 and IL-1 production.

In relation to allergic conditions, the human milk strain *Lactobacillus gasseri* CECT 5714 reduced the incidence and severity of the allergic response in an animal model of cow’s milk protein allergy [44]. It is also interesting to note that the presence of viridans streptococci, one of the dominant bacterial groups in human milk, seems to be a feature of the healthy infant gut in contrast to that of the atopy-suffering infants [45].

Finally, human milk bacteria have a remarkable potential to play metabolic roles in the infant. The glycoconjugate of some lactobacilli and bifidobacteria, including that of species that have been isolated from human milk, may help to create a specific “healthy” microbiota in the infant gut [14,46]. These microorganisms might also contribute to infant digestion through the breakdown of sugars and proteins; this possibility is particularly attractive having in account that transit of food through the gastrointestinal tract is shorter in infants than in adults and, also, that the pH of the infant’s stomach is higher than that of the adult. In this context, human milk lactobacilli strains are metabolically active in the infant gut and increase the production of functional metabolites such as butyrate, which is the main energy source for colonocytes and a relevant compound in the modulation of intestinal function [47,48]. As a result, they improve the intestinal habit, with an increase in fecal moisture, and in stool frequency and volume [47,48].

3. Origin of the bacteria isolated from breast milk: is there a bacterial entero-mammary pathway?

The origin of the bacteria present in breast milk has become a controversial issue in the last years. Traditionally, it was believed that milk harbored bacteria that were just the result of contamination with bacteria from the mother’s skin or the infant’s oral cavity [19].

![Schematic representation of the acquisition and development of the human mammary microbiota.](image-url)
Infrared photography [49] has shown that a certain degree of retrograde flow back into the mammary ducts can occur during suckling. Obviously, such back flow may provide an ideal route for the exchange of bacteria from the infant’s mouth into the mammary gland (Fig. 2). But it is also obvious that breast milk can also be a source of bacteria to the infant’s mouth. Ecological niches in the human microbiome are not thought to be isolated environments, but rather a network of interrelated communities experiencing constant exchange [33]. Therefore, it is very likely that milk or mammary bacterial communities are no the exception, and that they are constantly influenced by exposure to other microbial populations associated with the mother and her infant. Little is known about the infant human salivary microbiome but investigations on adults have revealed that Streptococcus species are the dominant phylotype in this fluid [50–52] while such dominance is even higher in edentulous infants [53]. Streptococcus species are among the most abundant phylotype in colostrum and milk samples [7,8,25], supporting the hypothesis that the maternal milk bacteria may play an important role in establishing salivary bacterial communities and/or vice versa.

Some bacterial phylotypes usually present on adult skin, such as Staphylococcus, Corynebacteria, and Propionibacteria [54,55], are also common in human milk. This presents the possibility that interactions with the maternal skin microbiota may also contribute to shape the composition of milk microbiota (Fig. 2). However, a comparison of the bacterial communities detected in milk to those of the sebaceous skin found on the breast indicates that although the two communities share some phylotypes, major differences also exist [25]. Such relevant differences between the two communities indicate that bacterial communities in milk are not simply a result of skin contamination. Sampling of breast milk for microbiological analysis must take into account that skin contamination is almost unavoidable and that, logically, doubts on the original location (internal mammary gland or skin) of the isolated bacteria may arise. However, independent studies have shown that lactobacilli or bifidobacteria could not be isolated from breast skin swabs obtained from women that provided milk samples from which such bacteria were isolated [11,29]. Previously, it has been reported that lactobacilli and enterococcal isolates present in human milk are genotypically different from those isolated in the skin, within a same bacterial species and a same host [4]. In addition, bifidobacteria belongs to a strictly anaerobic genus and, therefore, skin is, at least, a very unlikely source [29]. These and other findings suggest that at least some of the bacteria present in the maternal gut could reach the mammary gland through an endogenous route [56].

Although the pathway and mechanisms that some bacteria could exploit to cross the intestinal epithelium and reach the mammary gland and other locations has not been elucidated yet, some works have offered a plausible scientific basis. It has been demonstrated that dendritic cells (DCs) can penetrate the gut epithelium to take up non-pathogenic bacteria directly from the gut lumen. DCs are able to open the tight junctions between intestinal epithelial cells, send dendrites outside the epithelium and directly sample bacteria, while preserving the integrity of the epithelial barrier through the expression of tight-junction proteins [57]. Using such mechanism, a Salmonella typhimurium strain that was deficient in invasion genes encoded by Salmonella pathogenicity island 1 (SPI1) was still able to reach the spleen after oral administration to mice [57]. This mechanism may not be exclusive to DCs, as CD18+ cells, including macrophages, have been shown to be essential for extra-intestinal dissemination of non-invasive Salmonella [58].

It has also been shown that intestinal DCs can retain small numbers of live commensal bacteria for several days in the mesenteric lymph nodes [59]. Once inside DCs and/or macrophages, gut bacteria could spread to other locations since there is a circulation of lymphocytes within the mucosal associated lymphoid system. Antigen-stimulated cells move from the intestinal mucosa to colonize distant mucosal surfaces, such as those of the respiratory and genitourinary tracts, salivary and lachrymal glands, and, most significantly, that of the lactating mammary gland [60]. In addition, it is known that, during the lactation period, colonization of the mammary gland by cells of the immune system is a selective process regulated by the lactogenic hormones [61]. This process is responsible for the abundance of such cells in human milk. A hypothetical model to explain how some maternal bacteria could be transferred to the neonatal gut is shown in Fig. 2.

The suggestion that the origin of the live bacteria found in breast milk could be the maternal gut and that the bacteria would arrive to the mammary gland through an endogenous route, involving maternal DCs and macrophages, has been confirmed recently by independent research groups. Initially, our group showed that the exposure of mouse immature DCs to two bacterial strains isolated from human milk led to a high stimulation of two DC activation surface markers: the class II major histocompatibility complex and
the B7.2 protein [62,63]. It seems clear that bacterial induction of DC maturation is an active process since it has been shown that dead bacteria or inert particles, such as latex beads, cannot activate DCs even if they are rapidly phagocytosed [64]. On the other hand, L. gasseri CECT 5715, other strain isolated from breast milk, showed a high level of binding to DCs and ability to translocate across a Caco-2 cell monolayer through a DC-mediated mechanism [62] (Fig. 3).

Later, other study showed that bacterial translocation from the gut to mesenteric lymph nodes and mammary gland occurred during late pregnancy and lactation in mice [65]. In addition, this work revealed that human breast milk cells contain a number of viable bacteria and a range of bacterial DNA signatures, which are also found in maternal peripheral blood mononuclear cells. Those peripheral blood mononuclear cells showed greater biodiversity than did those obtained from control women. Taken together, their results suggest that intestinally derived bacteria and bacterial components are transported to the lactating breast within mononuclear cells. The authors speculated that this programs the neonatal immune system to recognize specific bacterial molecular patterns and to respond appropriately to pathogenic and commensal organisms [65]. More recently, two successive studies that focused on the oral administration of three lactobacilli strains isolated from human milk (L. salivarius CECT 5713, L. gasseri CECT 5714, and L. fermentum CECT 5716) provided new evidences that show the existence of a bacterial entero-mammary pathway during lactation [66,67].

The mammary gland prepares for lactation through a series of developmental steps that occur during adolescence and pregnancy. The principal feature of mammary growth in pregnancy is a great increase in ducts and alveoli under a multi-hormonal influence. At the end of this period, the lobules of the alveolar system are maximally developed and small amounts of colostrum may be released for several weeks prior to delivery. Additionally, the nipple and areola markedly enlarge and the sebaceous glands within become more prominent [68]. The increased lymph and blood supply to the mammary gland and the oxytocin release that causes contraction of the mioepithelial cells that invest the mammary alveoli may also facilitate the presence of endogenous bacteria in breast milk. These changes provide good conditions for biofilm formation on the mammary areola and/or in the mammary duct system, leading to the formation of a specific and transitory mammary microbiota.

At the same time, the whole body (including immune, cardiovascular, respiratory, digestive and genitourinary systems) experiences diverse physiological adaptations during pregnancy and lactation, and most of them are compatible with an increase in the bacterial translocation rate in the gut.

It has to be highlighted that the presence of live LAB (similar to those found in human milk) in the bloodstream of healthy human hosts is not an uncommon event [69–72]. It is interesting to note that 125 of the 485 lactobacilli strains deposited in the PROSAFE collection were originally isolated from human blood, and it is also illustrative that these 125 strains belong to 16 different Lactobacillus species [69]. A study investigating the influence of the oral microbiota composition on pregnancy outcome in 300 pregnant women revealed that some bacteria, such as Actinomyces naeslundii, were linked to lower birth weight and earlier delivery while others, including Lactobacillus casei, were associated with a slightly higher birth weight and normal delivery [73]. These authors concluded that oral bacteria can enter the uterine environment through the bloodstream. Probably, in addition to the digestive and genitourinary tracts, LAB and other bacteria can inhabit, permanent or transiently, other body locations in healthy hosts, where they could contribute to extraintestinal probiotic effects. Additionally, human clinical studies have also indicated that oral intake of specific bacterial strains at defined doses play a key role in the prevention or improvement of extraintestinal conditions, and such probiotic effects would be hardly explainable if they were exclusively confined to the gut [74]. Globally, these studies indicate that certain intestinal bacteria may have a rather underrated ability to spread from the gut to extra-intestinal locations in healthy hosts.

4. Human milk bacteria as biotherapeutic agents

In recent years, the problems associated to the spread of clinical antibiotic resistances among pathogenic bacteria and to the rise of allergic or inflammatory diseases in developed countries have led to a new interest in probiotics, which are defined as live microorganisms that confer a health benefit on the host when administered in adequate amounts [75]. Obviously, probiotic bacteria that are originally isolated from human milk are particularly attractive organisms since they would fulfill some of the main criteria generally recommended for human probiotics, such as human origin, a history of safe prolonged intake by a particularly sensitive population (newborns, infants), and adaptation to mucosal and dairy substrates [76].

Among the bacteria isolated from human milk, species like L. gasseri, L. salivarius, Lactobacillus reuteri, L. fermentum or Bifidobacterium breve are considered among those with probiotic potential and enjoy the Qualified Presumption of Safety (QPS) status conceded by the European Food Safety Authority (EFSA). In contrast to other bacteria, these seem to be uniquely adapted to reside in the human digestive tract and to interact with us in symbiosis from the time we are born. Additionally, some of the strains isolated from this biological fluid have been shown to play antiinfectious, anti-inflammatory, immunomodulatory and metabolic roles, both in vitro and in vivo, including human studies (see Section 2). The recent genome sequencing of some of these strains [77–80] is providing some new clues to understand the relationship between phenotypic properties and their subjacent molecular basis [63]; in the future, such approach may contribute to a more rational selection, design and/or application of probiotic bacteria.

Exclusive breastfeeding during the first month of life has been linked to lower asthma [81] and atopic dermatitis [82] rates during childhood and, as a result, this practice has been strongly recommended to mothers with a family of atopy, as a possible means to prevent atopic eczema. Human milk LAB might play a role in...
this protective effect since it has been described that probiotic lactobacilli can be effective to prevent atopy and atopic diseases through a variety of mechanisms [83]. Gut bacteria are considered the earliest and most important stimulus for development of gut-associated lymphoid tissue and they can promote anti-allergic processes [83]. Therefore, milk of healthy women can be considered as a source of potentially probiotic or biotherapeutic bacteria with a role in protecting mothers and/or infants against a variety of allergic, inflammatory or infectious diseases.

4.1. Specific targets for human milk probiotic bacteria: lactational mastitis

Mastitis is a common disease during lactation since it has an incidence of up to 33% of the lactating mothers [84,85]. This inflammation of one or more lobules of the mammary gland usually has an infectious origin [86], involving staphylococci, streptococci and/or corynebacteria [85]. Traditionally, S. aureus has been considered as the main etiologic agent of acute mastitis although S. epidermidis is emerging as the leading cause of subacute and chronic mastitis both in human and veterinary medicine [87–90]. Multiresistance to antibiotics, formation of biofilms and mechanisms for evasion of the host immune response are common among clinical isolates of these two staphylococcal species [91–95]. This explains why this condition uses to be elusive to antibiotic therapy, and why it constitutes one of the main reasons to cease breastfeeding [85]. In this context, the development of new strategies for mastitis management based on probiotics, as an alternative or complement to antibiotic therapy, is particularly appealing.

For this purpose, our research group selected some lactobacilli strains on the basis of specific properties required for success in mastitis treatment after oral administration: a high survival rate during transit through the gastrointestinal tract, specific interactions with DCs, ability to colonize the mammary gland and, once there, mechanisms for competitive exclusion of mastitis-causing staphylococci and streptococci. Initially, a pilot trial highlighted the potential of L. salivarius CECT 5713 and L. gasseri CECT 5714, two strains isolated from breast milk, for the treatment of staphylococcal mastitis [66]. After 30 days, probiotics led to a significant reduction (~2 log10 CFU/mL) in the mean staphylococcal counts of the milk cultures, in contrast, the values achieved in the placebo group remained unchanged at the end of the study. This fact was correlated with the clinical evolution since, while no clinical signs of mastitis were observed in women assigned to the probiotic group at day 14, they persisted throughout the study in the placebo group.

More recently, the efficacy of L. fermentum CECT 5716 or L. salivarius CECT 5713, two lactobacilli strains isolated from breast milk, to treat lactational mastitis when administered orally was evaluated and compared to antibiotic therapy [67]. A total of 352 women with infectious mastitis were randomly divided in three groups. Those in groups A (n = 124) and B (n = 127) ingested daily 9 log10 CFU of L. fermentum CECT 5716 or L. salivarius CECT 5713, respectively, for 3 weeks while those in group C (n = 101) were submitted to antibiotic therapy prescribed in their respective Primary Care Centres. On day 0, the mean bacterial counts in milk samples of the three groups were similar (4.35–4.47 log10 CFU/mL) and lactobacilli could not be detected. On day 21, the mean bacterial counts in the probiotic groups (2.61 and 2.33 log10 CFU/mL) were lower than that of the control group (3.28 log10 CFU/mL). The probiotic treatment led to a significant reduction (1.7–2.1 log10 CFU/mL) in the milk bacterial count and to a rapid improvement of the condition. The final bacterial count was approximately 2.5 log10 CFU/mL, an acceptable bacterial load in milk of healthy women [56]. After the probiotic treatment, L. salivarius CECT 5713 and L. fermentum CECT 5716 could be isolated from the milk samples of women of the probiotic groups A and B, respectively. In contrast, the effectiveness of antibiotics prescribed to group C women differed significantly, both in the reduction of bacterial counts and in the improvement of the pain score.

4.2. Specific targets for human milk probiotic bacteria: anti-HIV activity

Although breastfeeding is a source of new pediatric HIV-1 infections worldwide [96,97], most breastfed infants of HIV-positive women remain uninfected despite repeated exposure of their oral and gastrointestinal mucosal surfaces to the virus. Therefore, breast milk has been recognized both as a vector of transmission and as vehicle of protection against HIV-1 [96].

Most breast milk-transmitted HIV infections take place in developing countries where breastfeeding usually remains the best election for providing optimal infant nutrition and protection against morbidity and mortality associated with diarrheal and lower respiratory infections [98,99]. In such countries, the choice of the most suitable infant feeding option for an HIV-infected mother should depend on her individual circumstances, including her health status and the local situation, and should also consider the health services available and the counseling and support she is likely to receive [100].

As a general rule, exclusive breastfeeding has been recommended for HIV-infected women during the first 6 months of life unless replacement feeding is acceptable, feasible, affordable, sustainable and safe [97,100]. Such feeding option may offer HIV-1-infected women in developing countries an affordable, culturally acceptable, and effective means of reducing mother-to-child HIV-1 transmission while maintaining the benefits of breastfeeding [101]. In fact, recent studies confirm that exclusive breastfeeding can be successfully supported in HIV-infected women [102,103]. In contrast, there seems to be an association between mixed breastfeeding and increased HIV transmission risk [104].

Several factors may modulate the risk of HIV-1 transmission via breastfeeding but the results of different studies focused on the potential role of specific milk components are not conclusive [105–107]. Recently, it was shown that breast milk contains innate factors that blocked infection of CD4+ cells with cell-free HIV, but did not affect infection with cell-associated virus [107]. On the other hand, humoral mucosal immunity to HIV does not appear to be a protective factor against viral transmission through breast milk [105,108]. Therefore, factors associated to protection against vertical transmission of HIV-1 through breastfeeding remain poorly understood [96].

Interestingly, the potential of some human milk strains to inhibit the infectivity of HIV with tropism to different co-receptors has been described recently [109]. A total of 38 bacterial strains isolated from breast milk were investigated for their ability to inhibit HIV-1 infection in vitro. The heat-killed bacteria and cell-free supernatants obtained from some strains inhibited HIV-1 infection; the highest levels corresponded to killed bacteria from Lactobacillus curvatus VM25 (55.5%), L. fermentum VM31 (52.5%), Pediococcus pentosaceus VM95 (49.0%), and P. pentosaceus VM21 (45.5%). HIV-inhibition was observed with supernatants from eight strains, although the most effective were L. salivarius VM5 (42%) and L. gasseri VM22 (40%) supernatants. Four strains (L. salivarius VM5, L. gasseri VM22, Lactococcus lactis VM17, and Streptococcus salivarius VM18) were selected for further evaluation and demonstrated distinct inhibition patterns against R5-, X4- and R5/X4-tropic HIV-1. These results demonstrate for the first time that LAB from human breast milk can significantly inhibit HIV-1 infection in vitro, and suggest a possible role for these bacteria in mucosal protection against HIV-1 in breastfeed infants.

Human milk bacteria may exert an anti-HIV action in the infant gut through a variety of mechanisms (Fig. 4), including
interactions with and modulation of DCs immunological functions [110–112]. DC–HIV interaction is critical for the infection outcome. Although DCs are able to induce specific immune responses against a broad variety of pathogens, HIV-1 has evolved ways to exploit DCs as cellular reservoirs, therefore facilitating viral dissemination, persistence in lymphoid tissues and evasion of antiviral immunity [113,114]. Additionally, oral lactobacilli isolated from healthy humans can capture HIV-1 in vitro through lectin-like interactions between mannose residues on the viral envelope glycoprotein gp120 and the bacteria [115]. This physical interaction (the so-called “HIV-1 trap”) may prevent viral attaching to cellular receptors on mucosal tissues.

Recently, it was observed that the genome of L. salivarius CECT 5713 contains genes encoding four proteins (1184, 1230, 1306, and 1686) potentially involved in human molecular mimetism [63]. Among them, protein 1230 was considered of particular relevance because of its similarity with proteins related to DC functionality, such as CD209 antigen (DC-SIGN) and the C-type lectin domain family 4 member M (DC-SIGN2). In addition, protein 1230 contains a recognition motif for high mannose N-linked oligosaccharides present in a variety of pathogen antigens, including HIV gp120. Consequently, it might have the potential to block gp120 from binding to target cells and, therefore, to inhibit HIV infectivity. To test such predictions, the ability of L. salivarius CECT 5713 to stimulate the maturation of immature DCs and to inhibit the in vitro HIV-1 infectivity was assessed [63]. The heat-killed cells led to a reduction in viral infectivity of TZM-bl target cells with R5 (HIV-1_R1), CXCR4 (HIV-1_R4) and R5/X4 (HIV-1_C786) of 42.3, 58.9, and 49.8%, respectively (mean values). Although to a lesser extent, cell-free conditioned supernatant from L. salivarius CECT 5713 cultures also inhibited HIV-1_R1 (23.5%), HIV-1_R4 (21.4%) and HIV-1_C786 (17.6%) infectivity. The results of this work suggest that molecular mimetism displayed by L. salivarius CECT 5713 may have an antagonistic role toward HIV gp120 and, therefore, may explain, at least partly, its activity against HIV infectivity in vitro.

Intestinal permeability decreases faster in breast-fed infants than those given formula [116], suggesting that some components in breast milk influence the maturation of the gut mucosal epithelium. Introduction of food proteins or enteric pathogens during formula or mixed replacement feeding may increase the likelihood of HIV transmission by stimulating intestinal inflammatory responses in the neonatal gut, leading to increased permeability and to facilitation of virus penetration. In fact, gastrointestinal tract impairment in HIV-positive patients is present in the early phases of HIV disease, and this impairment is associated with alterations in gut microbiota and intestinal inflammatory parameters [117]. These findings support the hypothesis that gastrointestinal tract alterations are a key factor in HIV pathogenesis, and may explain why infants who are exclusively breastfed have a significantly lower risk of being HIV-infected than infants who are bottle-fed or mixed-fed [118,119].

5. Conclusion

Human milk is a source of bacteria to the infant gut, where they may play a variety of antiinfectious, immunomodulatory, and metabolic roles. In fact, recent studies indicate that the mammary gland contains its own microbiota during late pregnancy and lactation. This bacterial community may differ depending on the individual and the health status of the lactating women. It seems that certain bacteria from the maternal gut can use mononuclear immune cells to colonize, first, the mammary gland and, later, the infant gut through breast-feeding. If further studies confirm these findings, they would have practical consequences since it would imply that modulation of the maternal intestinal microbiota can have a direct effect on her infant’s health, opening new perspectives for bacteriotherapy and probiotics.

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