DECIPHERING THE ENTEROMAMMARY CIRCUIT

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SUMMARY

Breast milk is a source of bacteria for the infant gut; however, the origin of milk bacteria, as well as their impact on the establishment of the neonatal gut microbiota, remains largely unknown. In recent years, results provided by different research groups suggest that certain bacteria from the maternal gastrointestinal tract could translocate through a mechanism involving mononuclear cells (dendritic cells and macrophages), migrate to the mammary glands through an endogenous cellular route (the enteromammary pathway) and subsequently colonize the infant gastrointestinal tract. Consequently, the infant gut microbiota could be acted upon by modulating the maternal gut microbiota.

KEY WORDS: *lactation, human milk, microbiota, bacteria, translocation, dendritic cells*

INTRODUCTION:

In recent years, numerous studies have revealed that human milk contains its own microbiota, which is transferred to the infant (Fernández et al., 2020). Traditionally, any bacterial cells present in human milk were considered to be the result of contamination originating either from the infant's oral cavity or from the mother's skin. However, comparisons between the bacterial communities detected in milk and those found on the skin of the breast or in the child's mouth have revealed that there are some shared phylotypes between the different niches but that there are also major differences between them (Hunt et al. 2011, Cabrera-Rubio et al. 2012, Jost et al. 2013, Jimenez et al. 2015). On the other hand, the detection of live bacterial cells and/or DNA corresponding to anaerobic species that are generally related to the intestinal ecosystem and cannot survive in aerobic places, such as the skin, has fueled a scientific debate on the origin of bacteria associated with milk and has served to hypothesize that some of the bacteria in human milk may originate in the maternal digestive tract (mouth, gastrointestinal tract) and reach the mammary gland through an endogenous route (Rodríguez 2014). In fact, the results of various studies indicate that at least some members of the human milk microbiota may come from the mother's digestive tract.

The term bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal tract into the lamina propria and subsequently into the mesenteric lymph nodes and other extra-intestinal organs, such as the spleen, liver, kidneys, peritoneal cavity or bloodstream. Traditionally, the translocation of intestinal bacteria
had been associated with pathological conditions and, therefore, had been studied
mainly in patients in whom pathogenic bacteria had disseminated causing sepsis, failure in various organs and, sometimes, even the death of the affected individuals.

However, it is known that a physiological process of bacterial translocation exists in
healthy individuals, without harmful effects on the host (Rodríguez et al. 2001). In fact, it has been suggested that bacterial translocation to extra-intestinal tissues is a beneficial physiological event in healthy children, as it may be associated with the initial maturation and learning of the neonatal immune system (Perez et al. 2007).

During pregnancy and lactation, many transient anatomical and physiological changes occur that involve virtually all systems, including the cardiovascular, respiratory, genitourinary and digestive systems, and that provide an adequate framework for the development of the fetus and the newborn. Such adaptations favor an increase in bacterial translocation during late pregnancy and lactation. Bacterial translocation during the final period of gestation has already been described in mice, where oral inoculation of a genetically marked strain led to its isolation and detection by PCR in amniotic fluid and milk (Jiménez et al., 2005). Subsequent work has confirmed bacterial translocation from the intestine to mesenteric lymph nodes and mammary glands in pregnant and lactating mice (Perez et al., 2007; Treven et al., 2015). Bacteria were observed histologically in the subepithelial dome and intercellular regions of Peyer 's patches, in the lamina propria of the small intestine, and in mammary glandular tissue. Peyer 's patches in these mice were macroscopically larger and had a more prominent subepithelial dome than those in control animals. In the same study, mononuclear immune cells present in milk and peripheral blood samples from several healthy women were stained with acridine orange, allowing the identification of bacteria in close association with these cells (Perez et al., 2007). Consequently, it has been suggested that certain bacteria selected from the maternal digestive microbiota may access the mammary glands through an enteromammary route (Rodríguez, 2014).

Previous studies have indicated that certain bacteria from the maternal digestive tract can spread to extra-digestive sites in healthy hosts (Vankerckhoven et al., 2004, Dasanayake et al., 2005). Subsequently, other works have offered a scientific basis for such physiological translocation. The mechanism would involve dendritic cells and CD18+ cells (Vázquez-Torres et al., 1999, Rescigno et al., 2001), which would be able to capture viable non-pathogenic bacteria present in the lumen of the intestine and subsequently carry them to other sites, including the lactating mammary gland.t should be remembered that there is an intense flow of intestinal immune cells to the mammary glands during pregnancy and lactation (Bertotto et al., 1991; Roitt et al., 2001). The process of bacterial translocation during late pregnancy and lactation may be favored by three different factors: (a) the state of immunological privilege for fetal tolerance; (b) the intense vascularization of the mammary glands during these periods due to large processes of angiogenesis and vasculogenesis ; and (c) the pre-colostrum begins to fill the mammary duct during the last third of pregnancy providing a rich nutritive environment for the bacteria, thus facilitating their growth.

To demonstrate this process in vivo , our group investigated the possibility that oral administration of *Lactococcus lactis y Ligilactobacillus salivarius* led to their in vivo translocation during gestation, transferring from the digestive tract to the mammary gland (de Andrés et al., 2018). To reveal this possible enteromammary route, genetic and phenotypic marking *lux genes* of the strains was used and then their oral administration to pregnant mice. This marking method constitutes an excellent tool for the *in vivo* detection of specific bacteria in animal models. Both strains could be isolated from milk samples or mammary gland biopsies after oral administration to mice. It could be argued that their presence in milk could be the result of superficial fecal contamination; however, such a route can hardly explain their isolation and detection from deep mammary biopsies. Furthermore, in a previous study, oral administration of *L. salivarius* to pregnant women led to the presence of the strain in the milk of some of the women after delivery (Fernández et al., 2016). It should be noted that, in contrast to female mice, contamination of human milk with fecal material is highly unlikely (Mediano et al., 2017).

Further studies are required to elucidate the exact molecular mechanisms by which some bacterial strains can physiologically translocate in certain hosts or life stages. Nevertheless, the existence of such entero-mammary bacterial pathways would provide new opportunities to manipulate altered maternal-fetal microbiota, reducing the risk of premature birth, mastitis or certain childhood diseases. In this context, it has already been shown that oral administration of some strains isolated from human milk to women with ongoing mastitis or with a history of mastitis after previous pregnancies leads both to their presence in the milk and to the prevention or cure of that pathology (Arroyo et al., 2010; Fernandez et al., 2016).

CONFLICT OF INTEREST: No

REFERENCES:

1.Arroyo R, Martín V, Maldonado A, Jiménez E, Fernández L, Rodríguez JM. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of lactobacilli isolated from breast milk. Clin Infect Dis. 2010;50(12):1551-1558. doi:10.1086/652763

2.Bertotto A, Gerli R, Castellucci G, Scalise F, Vaccaro R. Human milk lymphocytes bearing the gamma/delta T-cell receptor are mostly delta TCS1-positive cells. Immunology. 1991;74(2):360-361.

3.Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr. 2012;96(3):544-551. doi:10.3945/ajcn.112.037382

4.Dasanayake AP, Li Y, Wiener H, Ruby JD, Lee MJ. Salivary Actinomyces naeslundii genospecies 2 and Lactobacillus casei levels predict pregnancy outcomes. J Periodontol. 2005;76(2):171-177. doi:10.1902/jop.2005.76.2.171

5.de Andrés J, Jiménez E, Chico-Calero I, Fresno M, Fernández L, Rodríguez JM. Physiological translocation of lactic acid bacteria during pregnancy contributes to the composition of the milk microbiota in mice. Nutrients. 2017;10(1):14. doi:10.3390/nu10010014

6.Fernández L, Cárdenas N, Arroyo R, et al. Prevention of Infectious Mastitis by Oral Administration of Lactobacillus salivarius PS2 During Late Pregnancy. Clin Infect Dis. 2016;62(5):568-573. doi:10.1093/cid/civ974

7.Fernández L, Pannaraj PS, Rautava S, Rodríguez JM. The microbiota of the human mammary ecosystem. Front Cell Infect Microbiol. 2020; 10: 586667. doi:10.3389/fcimb.2020.586667

8.Jiménez E, de Andrés J, Manrique M, et al. Metagenomic analysis of milk of healthy and mastitis-suffering women. J Hum Lact. 2015;31(3):406-415. doi:10.1177/0890334415585078

9.Jiménez E, Fernández L, Marín ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005;51(4):270-274. doi:10.1007/s00284-005-0020-3 Jimenez et al. 2015

10.Jost T, Lacroix C, Braegger C, Chassard C. Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. Br J Nutr. 2013;110(7):1253-1262. doi:10.1017/S0007114513000597

11.Mediano P, Fernández L, Jiménez E, et al. Microbial diversity in milk of women with mastitis: potential role of coagulase-negative staphylococci, viridans group streptococci, and corynebacteria. J Hum Lact. 2017;33(2):309-318. doi:10.1177/0890334417692968 Mira y Rodríguez, 2017

12.Perez PF, Doré J, Leclerc M, et al. Bacterial imprinting of the neonatal immune system: lessons from maternal cells?. Pediatrics. 2007;119(3):e724-e732. doi:10.1542/peds.2006-1649

13.Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol. 2001;2(4):361-367. doi:10.1038/86373

14.Rodríguez JM. The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? Adv Nutr. 2014;5(6):779-784. doi:10.3945/an.114.007229

15.Rodriguez AV, Baigorí MD, Alvarez S, Castro GR, Oliver G. Phosphatidylinositol-specific phospholipase C activity in Lactobacillus rhamnosus with capacity to translocate. FEMS Microbiol Lett. 2001;204(1):33-38. doi:10.1111/j.1574-6968.2001.tb10858.x

16.Roitt, I., 2001. Essential Immunology. Blackwell Scientific Publications, Oxford, 2001.

Treven P, Mrak V, Bogovič Matijašić B, Horvat S, Rogelj I. Administration of probiotics Lactobacillus rhamnosus GG and Lactobacillus gasseri K7 during pregnancy and lactation changes mouse mesenteric lymph nodes and mammary gland microbiota. J Dairy Sci. 2015;98(4):2114-2128. doi:10.3168/jds.2014-8519

17.Vankerckhoven, V. V., Autgaerden, T. V., Huys, G., Vancanneyt, M., Swings, J., & Goossens, H. Establishment of the PROSAFE collection of probiotic and human lactic acid bact. Microb Ecol Health Dis 2004; 16: 131-136.doi: 10.1080/08910600410032349

18.Vazquez-Torres A, Jones-Carson J, Bäumler AJ, et al. Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. Nature. 1999;401(6755):804-808. doi:10.1038/44593