J. Dairy Sci. 101:1–11
https://doi.org/10.3168/jds.2017-13411

Breastfeeding-associated microbiota in human milk following supplementation with *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La-5, and *Bifidobacterium animalis* subspecies *lactis* Bb-12

Melanie Rae Simpson,*[1] Ekaterina Avershina,† Ola Storrø,* Roar Johnsen,* Knut Rudi,† and Torbjørn Øien*
*Department of Public Health and Nursing, NTNU – Norwegian University of Science and Technology, N-7030 Trondheim, Norway
†Department of Chemistry, Biotechnology and Food Science, University of Life Sciences, N-1432 Ås, Norway

ABSTRACT

Breastfeeding is one of the major factors affecting the early development of the infant gut microbiota, and weaning is associated with a shift in the gut microbiota toward a more adult composition. Through breastfeeding, infants receive bioactive components that shape their microbiota while also being exposed to the breast milk and breast surface microbial communities. Recent studies have suggested the possibility of an entero-mammary route of microbial transfer, opening the possibility of infant gut microbiota modulation through maternal probiotic supplementation. In this study, we have analyzed breast milk samples collected at 10 d and 3 mo postpartum from women participating in the Probiotics in the Prevention of Allergy among Children in Trondheim placebo controlled trial. Women who were randomized to the probiotic arm of the Probiotics in the Prevention of Allergy among Children in Trondheim trial received a fermented milk supplemented with *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La-5, and *Bifidobacterium animalis* ssp. *lactis* Bb-12, consuming this daily from 4 wk before their expected due date until 3 mo after birth. In total, 472 breast milk samples were assessed for the administered bacteria using quantitative real-time PCR and the microbiota transferred during breastfeeding was analyzed using 16S ribosomal RNA gene sequencing of 142 samples. We found that breastfeeding is unlikely to be a significant source of *L. rhamnosus* GG, *L. acidophilus* La-5, and *B. animalis* ssp. *lactis* Bb-12 for infants in the probiotic arm of the trial. Furthermore, maternal supplementation did not significantly affect the overall composition of the breast milk microbiota transferred during breastfeeding. We also present a descriptive analysis of this microbiota, which was largely dominated by *Streptococcus* and *Staphylococcus* genera at both 10 d and 3 mo postpartum. Samples collected at 3 mo postpartum had a statistically significant lower presence and relative abundance of the *Staphylococcus* genus. These samples also had a greater number of observed species and diversity, including more operational taxonomic units from the *Rothia*, *Veillonella*, *Granulicatella*, and *Methylbacterium* genera.

Key words: human milk, probiotics, atopic dermatitis, microbiota

INTRODUCTION

Breastfeeding is one of the major factors affecting the early development of the infant gut microbiota and weaning is associated with a shift in the gut microbiota toward a more adult-like composition (Wopereis et al., 2014; Bäckhed et al., 2015; Rodríguez et al., 2015). Multiple components of breast milk contribute to these effects, including human milk oligosaccharides, which promote the growth of some microbes, and lysozymes, lactoferrin, and antimicrobial peptides, which inhibit the growth of others (Cacho and Lawrence, 2017). Additionally, breastfeeding is a source of a diverse range of microbes that are found both on the breast surface and within the mammary glands of lactating women (Fitzstevens et al., 2017). Culture-dependent and -independent techniques have demonstrated a dominance of bacteria belonging to the *Staphylococcus*, *Streptococcus*, and *Propionibacterium* genera, as well as the presence of lactic acid bacteria and bifidobacteria in breast milk (Fitzstevens et al., 2017). The origin of these bacteria is thought to be a combination of the microbiotas associated with the mother's skin flora, the infant's oral mucosa, and the maternal gut. Recent studies have suggested the possibility of an entero-mammary route with selective trafficking of commensal bacteria from the maternal gut to the mammary glands via dendritic cells and macrophages (Rodríguez, 2014; Treven et al., 2015).

Received June 29, 2017.
Accepted October 17, 2017.
[1]Corresponding author: melanie.simpson@ntnu.no

In the Probiotics in the Prevention of Allergy among Children in Trondheim (**ProPACT**) placebo controlled trial, we found that maternal ingestion of 3 probiotic strains, while pregnant and breastfeeding, resulted in an almost 40% reduction in the cumulative incidence of atopic dermatitis among offspring at 2 yr of age (Dotterud et al., 2010). Women in the probiotic arm of the trial consumed fermented milk supplemented with *Lactobacillus rhamnosus* GG (**LGG**), *Lactobacillus acidophilus* La-5 (**La-5**), and *Bifidobacterium animalis* ssp. *lactis* Bb-12 (**Bb-12**), whereas women in the placebo group consumed heat-treated fermented milk without supplemented bacteria. Analysis of stool samples from the ProPACT study revealed that women in the probiotic arm had a higher prevalence and relative abundance of all 3 probiotic bacteria strains at 3 mo postpartum in their stool samples. A similar result was observed for the children of these women at 10 d and 3 mo of age, although only for the LGG (Dotterud et al., 2015). Breastfeeding may therefore be an ongoing source of LGG for these infants. Previous studies suggest that maternal supplementation with *L. rhamnosus* LC705 (Nasiraii et al., 2011), *Lactobacillus reuteri* (Abrahamsson et al., 2009), and *Lactobacillus fermentum* CECT5716 and *Lactobacillus salivarius* CECT5713 (Arroyo et al., 2010) may result in an increased presence of the administered bacteria in the breast milk of some, but not all, women. To our knowledge, no studies have investigated the transfer of LGG, *L. acidophilus,* or *Bifidobacterium* species through breastfeeding after maternal supplementation. *Lactobacillus rhamnosus* GG is of particular interest because it has been the most commonly administered bacteria in atopic dermatitis prevention studies and was observed to be transferred to infants in the ProPACT study.

In the present study, we investigated the bacteria transferred through breastfeeding using breast milk samples taken at 10 d and 3 mo postpartum from women participating in the ProPACT trial. The samples were collected without sterilization of the breast areola and are considered to give a more representative analysis of the bacteria ingested by suckling infants. We have therefore adopted the term "breastfeeding-associated microbiota" suggested by Sakwinska et al. (2016) to describe this bacterial community, which involves the breast milk and breast surface microbiotas in human milk ingested by the suckling infant. Our aim was to investigate whether maternal probiotic supplementation with LGG, La-5, and Bb-12 affected the presence of these strains among the bacteria transferred during breastfeeding at 10 d and 3 mo postpartum, and their association with the later development of atopic dermatitis. We also assessed the general microbiota associated with breastfeeding, considered temporal trends, and the relationships between the composition of the microbiota transferred during breastfeeding, probiotic supplementation, and atopic dermatitis.

MATERIALS AND METHODS

Participant Recruitment and Sample Collection

This study analyzed 472 breast milk samples collected from 252 women participating in the ProPACT trial. The design and clinical results from this randomized, placebo controlled trial have been described in detail elsewhere (Dotterud et al., 2010; Simpson et al., 2015). Briefly, 415 women, who intended to breastfeed, were randomized to receive a commercially available fermented milk (Biola, Tine AS, Oslo, Norway) containing 5×10^{10} cfu of LGG and Bb-12 and 5×10^{9} cfu of La-5 per 250 mL or a placebo fermented milk that contained no probiotic bacteria and was heat treated after fermentation. Participating women were to consume 250 mL per day of their allocated study milk from 36 wk gestation until 3 mo postpartum. Their infants did not receive any probiotic supplementation. The children were assessed for signs and symptoms of allergy-related diseases through questionnaires and clinical examination at 2 and 6 yr of age. Atopic dermatitis was diagnosed according to the UK Working Party diagnostic criteria (Williams et al., 1994) at the clinical examinations.

Participating women were provided with sterile sample tubes and were requested to collect breast milk at 10 d and 3 mo postpartum. The timing, with respect to time of day or whether fore- or hindmilk was collected, was not standardized. The women did not receive explicit instructions regarding washing or sterilization of the breast surface before sample collection. Samples were frozen in their home freezer until transportation to the laboratory where they were subsequently stored at −80°C. All available breast milk samples were included in the current study, provided that the child attended the 2-yr clinical follow-up (Figure 1).

Analysis of Microbiota

Breast milk samples (2 mL) were centrifuged at 215,000 × *g* for 30 min. The resulting pellet was resuspended in 100 μL of stool transport and recovery buffer and DNA was isolated using LGC Mag DNA extraction kit (LGC Genomics, Middlesex, UK) on a KingFisher FLEX magnetic particle processor (Thermo Fisher Scientific, Waltham, MA) according to the manufacturers' instructions. Samples were analyzed for total bacteria