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ARTICLE

# Bacterial Imprinting of the Neonatal Immune System: Lessons From Maternal Cells?

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## ABSTRACT

OBJECTIVE. We examined the presence of a natural bacterial inoculum in breast milk and its intracellular transport from the maternal intestine to the breast through the circulation.

METHODS. Breast milk and peripheral blood were collected aseptically from healthy donors at various times after delivery, and the presence of viable bacteria was determined through plating. Temporal temperature gradient gel electrophoresis was used to examine the bacterial ribosomal DNA content in milk cells, maternal peripheral blood mononuclear cells, and feces and in corresponding infant feces. Blood from nongravid nonlactating women served as control samples. Bacterial translocation to extraintestinal tissues was also evaluated in virgin, pregnant, and lactating mice.

RESULTS. Breast milk contained a low total concentration of microbes of $<10^3$ colony-forming units per mL. Temporal temperature gradient gel electrophoresis revealed that maternal blood and milk cells contained the genetic material of a greater biodiversity of enteric bacteria. Some bacterial signatures were common to infant feces and to samples of maternal origin. Bacterial translocation from the gut to mesenteric lymph nodes and mammary gland occurred during late pregnancy and lactation in mice.

CONCLUSIONS. Bacterial translocation is a unique physiologic event, which is increased during pregnancy and lactation in rodents. Human breast milk cells contain a limited number of viable bacteria but a range of bacterial DNA signatures, as also found in maternal peripheral blood mononuclear cells. Those peripheral blood mononuclear cells showed greater biodiversity than did peripheral blood mononuclear cells from control women. Taken together, our results suggest that intestinally derived bacterial components are transported to the lactating breast within mononuclear cells. We speculate that this programs the neonatal immune system to recognize specific bacterial molecular patterns and to respond appropriately to pathogens and commensal organisms.

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**Key Words**
bacterial translocation, breast milk, immunity, maternal and child health, lactation

**Abbreviations**
MLN—mesenteric lymph node
rDNA—ribosomal DNA
TTGE—temporal temperature gradient gel electrophoresis
DC—dendritic cell
PBMC—peripheral blood mononuclear cell
PCR—polymerase chain reaction

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MUCOSAL DENDRITIC CELLS (DCs), via pattern recognition receptors such as Toll-like receptors, sample and respond to microbes, which bombard the intestinal mucosa continuously.[1] Normally, this results in tolerance to the normal microbiota and protection against pathogenic attack. Successful simultaneous deployment of such divergent processes requires sophisticated control mechanisms, which are not expected of an inexperienced, neonatal, immune system. However, intestinal colonization and assembly of specific bacterial communities in the absence of adverse immune responses reflect robust regulatory mechanisms, which may already operate in utero. Moreover, differences between breastfed and formula-fed infants in intestinal bacterial colonization[2] and susceptibility to disease[3] suggest that additional regulation is acquired through breast milk.

There is accumulating evidence that bacteria are transmitted to the infant via milk.[4] Most studies of the microbiologic features of milk have addressed the transmission of pathogens or contaminating commensal organisms in samples meant for milk banks.[4] The majority of the latter arise from the mother's skin or the infant's mouth.[4,5] However, certain species are suggested to colonize the neonatal intestine and to provide protection.[6] The interesting observation that breast milk is not sterile, even when collected aseptically,[7] raises the possibility that breast milk harbors a natural bacterial inoculum, which may influence neonatal colonization.

Milk leukocytes are cells that have migrated from the gut- and bronchial-associated lymphoid tissue to lactating mammary glands via the lymphatic vessels and blood circulation.[8,9] If some microbial species are indeed intrinsic to breast milk, then this cellular circuitry may explain how microbes are conveyed to the breast without any deleterious effect on maternal health. To address this, we examined the presence of bacteria in human milk, blood, and feces during lactation; in a second study, we examined bacterial translocation in nonpregnant, pregnant, and lactating mice.

## METHODS

### Human Milk, Blood, and Fecal Samples

Breast milk was collected from healthy lactating mothers who delivered at term. After rejection of ~2 to 3 mL of foremilk, the breast was cleaned with antiseptic soap, rinsed with sterile distilled water, and dried with sterile gauze before aseptic collection with an electrical breast pump. As a control, a swab of the areola was taken before milk collection. Samples of whole milk were plated on de Man, Rogosa, and Sharpe medium containing cysteine, on Eugon tomato, Drigalski, or Shaedler Neo Vanco medium, or on blood agar (bioMérieux, Marcy l'Etoile, France) and were incubated aerobically or anaerobically at 37°C. Leukocytes were collected from the remaining milk through centrifugation and were suspended in sterile phosphate-buffered saline containing 1% gentamicin (10 minutes), to kill extracellular bacteria. Washed cells were then divided into aliquots and were used to make cytopreparations, were frozen in RPMI medium (Life Technologies, Basel, Switzerland) containing 10% dimethylsulfoxide and fetal calf serum for flow cytometric analysis, or were lysed with cold, sterile, distilled water passed through a sterile needle for plating on bacterial culture medium. Bacterial isolates were characterized on the basis of macroscopic and microscopic morphologic features, Gram staining, and culture characteristics.

Approximately 10 mL of venous blood were collected from lactating women at different times after delivery or from 5 age-matched, nongravid, nonlactating women. The blood was centrifuged over Ficoll-Hypaque medium (Sigma-Aldrich, St Louis, MO), washed, and then processed as for milk cells. Maternal and infant fecal samples were collected in sterile tubes, divided into aliquots, and stored frozen at −80°C until required. Written consent was obtained from volunteers, and protocols were approved by our institutional review board and by the Swiss authorities.

### Flow Cytometry

Myeloid and lymphoid DCs in peripheral blood mononuclear cells (PBMCs) were examined by using the FACSCalibur system and DC-Kit from Becton Dickinson (Basel, Switzerland). In separate tubes, cells were labeled with fluorescein isothiocyanate-anti-CD11c and phycoerythrin-anti-CD14 (Becton Dickinson), according to the manufacturer's instructions.

### Temporal Temperature Gradient Gel Electrophoresis

Total DNA was extracted from 200 mg of fecal samples and from milk cells and PBMCs as described previously for feces and biopsies, respectively,[10] except that DNA precipitation of cells was performed overnight and the pellets were centrifuged (1 hour, 4°C). Isolated DNA was then used to amplify the V6–V8 regions of 16S ribosomal DNA (rDNA), with primers U968-GC-F and L1392-R.[10] The polymerase chain reaction (PCR) product size was 468 base pairs. Several dilutions of template DNA were made if the presence of PCR inhibitors was suspected. PCR amplification and temporal temperature gradient gel electrophoresis (TTGE) were performed as reported previously,[10] and Gel Compar II software (Applied Maths, Kortrigk, Belgium) was used to compare TTGE profiles. A PCR amplicon mixture of 7 cloned rDNAs from different bacterial species was used as a migration marker. Some of the TTGE bands that comigrated in maternal and infant samples were excised from the gel and sequenced.

### Cloning of 16S rDNA

DNA from cells and feces at 4 weeks after delivery were PCR amplified with primers U350-F and L1392-R. The PCR product size was 1080 base pairs. Ligation and cloning were in the pGEM-T vector system I (Promega, Madison, WI),[11] except for milk cells, for which 10 PCRs were pooled to make a 16S rDNA library. Forty-eight clones per library were sequenced with the primers M13F and M13R and an equal portion of the SSU rDNA (*Escherichia coli* positions 350–1392, representing nearly the full-length gene). The sequences from this molecular inventory were longer than those excised from the TTGE gels.

Sequences were checked manually, and the contigs were made by using BioEdit software (Ibis Therapeutics, Carlsbad, CA). The sequences were submitted to GenBank, and the Blast and Megablast programs of the Ribosomal Database Project (East Lansing, MI) were used to identify close phylogenetic relatives. Sequences were tested for chimera structure by using the Ribosomal Database Project analysis service Check Chimera, as well as during manual inspection of alignment. Sequences were compared by using the Blast2sequences program (National Center for Biotechnology Information, Bethesda, MD).

### Bacteria Localization in Milk and Blood Cells

After fixation in absolute ethanol, cytopreparations of human milk and blood cells were incubated for 5 minutes with 100 mg/mL acridine orange,[12] washed extensively, mounted in fluorescent mounting medium (Dako Schwiez, Baar, Switzerland), and analyzed with epifluorescence microscopy.

### Fetal Liver Tyrosine Kinase-3 Ligand

Fetal liver tyrosine kinase-3 ligand in human serum (one-half dilution) was assayed with an enzyme-linked immunosorbent assay, according to the manufacturer's instructions (R&D Systems, Epalinges, Switzerland). The detection limit of the assay was 10 pg/mL.

### Mice

Conventional virgin and pregnant/lactating C57/BL6 mice (Charles River Laboratories, L'Arbresle, France) were killed ($n = 10$ per group) at 5 to 6 days before parturition or at 1 to 2 days, 3 to 4 days, or 14 to 15 days after parturition. Samples of blood, intestinal contents, mesenteric lymph nodes (MLNs), spleen, liver, and mammary gland were collected aseptically for microbiologic analysis, fixed in Bouin's fixative before being mounted in paraffin blocks, and/or mounted in OCT medium and frozen in liquid nitrogen. The experimental procedure was approved by our institutional review board and by the Swiss authorities.

### Bacteria in Mouse Tissue

Microorganisms were observed in tissue by using Gram stain. For microbiologic analysis, samples of mouse tissue were homogenized, suspended in sterile phosphate-buffered saline, plated onto blood agar (bioMérieux), and incubated aerobically or anaerobically at 37°C.

### Statistical Analyses

The proportions of pregnant and lactating animals with viable bacteria in their tissue were compared with that of control animals by using Fisher's exact test. The median percentages of DC populations in the blood of lactating and control women were compared by using the Mann-Whitney test. TTGE profiles were compared by using Gel Compar II software (Applied Maths). Similarity coefficients (Pearson correlation method) were then calculated for each pair of profiles, yielding a similarity matrix. A dendrogram was constructed from this matrix by using an unweighted pair group method using arithmetic averages algorithm.

## RESULTS

### Bacterial Signatures Are Transferred From Mother to Infant Through Breast Milk

Skin swabs, made after cleaning the breast with antiseptic soap, did not yield viable bacteria. Aseptically collected breast milk contained a total concentration of $<10^3$ colony-forming units of bacteria per mL, composed of *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Peptostreptococcus*, *Staphylococcus*, *Corynebacterium*, and/or occasionally *Escherichia* spp. We next used TTGE to examine bacterial rDNA contents in milk cells and maternal PBMCs during lactation, and we compared the contents with those in maternal and infant fecal samples.

Maternal fecal samples gave classic TTGE profiles that were specific for each individual and of greater biodiversity than those of infant feces (Fig 1A). Although milk cells had a less complex microbiota than maternal feces, TTGE revealed a greater biodiversity than observed previously with plating. Figure 1A shows one mother-infant couple analyzed over weeks 1 to 4 after delivery. Similar TTGE profiles were observed for 6 other mother-infant pairs (data not shown). Interestingly, some bacterial signatures (Fig 1A, arrows) were common in infant feces and in several samples of maternal origin. With excision from the gel and sequencing, the lowest of these milk bands, which was especially intense in infant feces and comigrated in maternal feces and blood, was identified as *Bifidobacterium longum* on the basis of 369 nucleotides. The presence of *B longum* was also confirmed in the milk and infant feces of 3 other mother-infant couples (data not shown). One mother also had *B longum* DNA in her blood cells (Fig 1A), whereas another had the same species in her blood and in her feces. Sequencing of

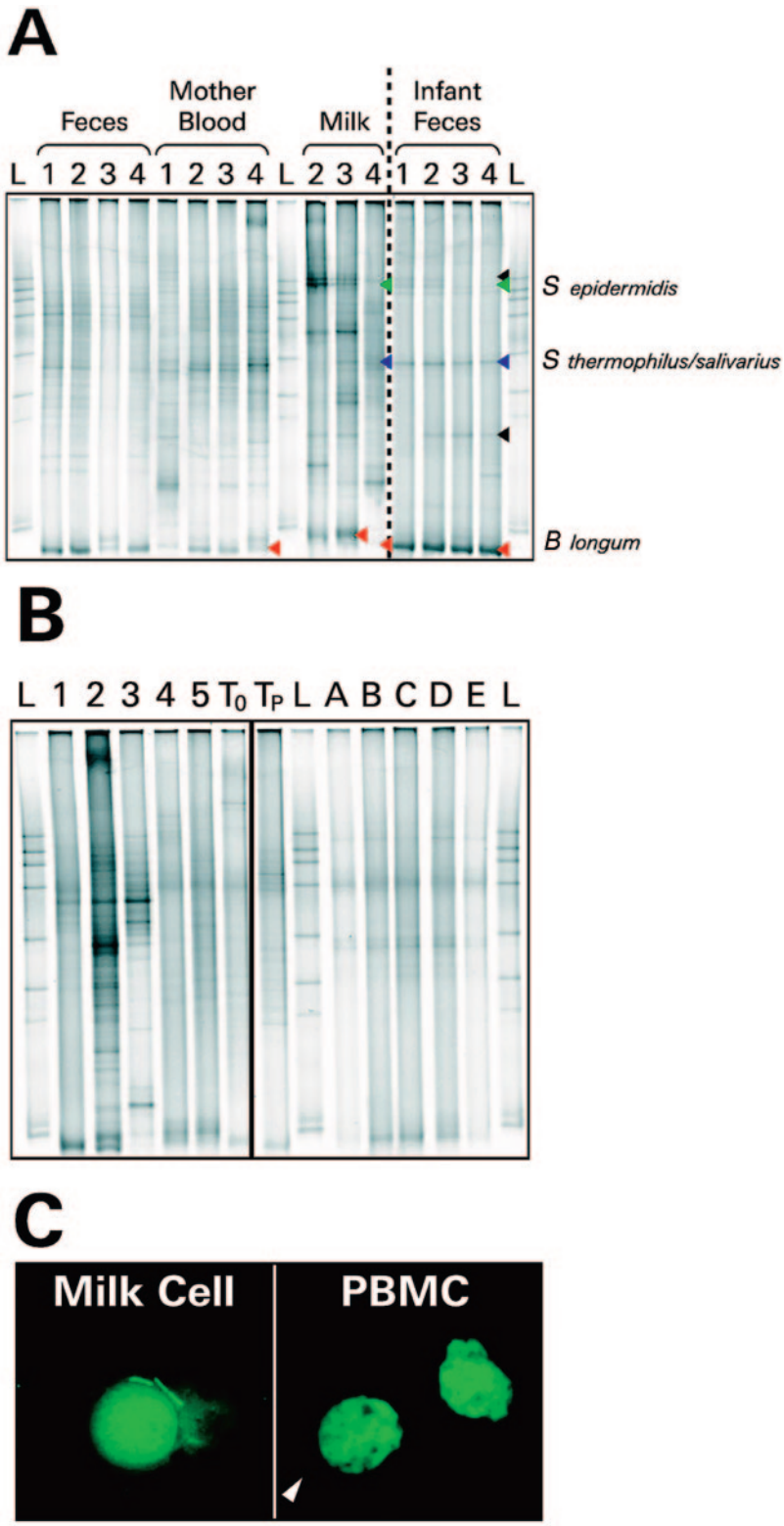


FIGURE 1

Bacterial signatures in maternal cells and infant feces. Profiling was performed by using TTGE-amplified fragments of rDNA. A, Infant feces, maternal PBMCs, milk cells, and feces (1–4 weeks after delivery). Duplicate PCRs were used for PBMCs and milk cells. A ladder (L) of PCR-amplified, cloned rDNA was used for gel normalization and image analysis. Arrowheads indicate signatures common to infant feces and maternal samples. Excision and sequencing of some bands identified *Bifidobacterium longum* (red arrowheads), *Streptococcus thermophilus/salivarius* (blue arrowheads), and *Staphylococcus epidermidis* (green arrowheads). B, PBMCs from mothers 4 weeks after delivery (lanes 1–5) and control women (lanes A–E). $T_0$ and $T_p$ represent PCR controls. C, Bacterial structures (arrowhead) in milk cells and PBMCs stained with acridine orange.

another band common to milk and infant feces identified DNA from *Streptococcus thermophilus/salivarius*.

Next, PCR products of milk cells were used to prepare rDNA libraries. Besides the species identified previously, sequencing of the clones revealed the presence of *Bacteroides*, *Clostridium*, and *Eubacterium* among a total of up to15 genera. Whereas the DNA from staphylococcal and streptococcal species were found in the milk cells of all mothers, DNA from clostridia and lactobacilli were found in the cells of 4 and 3 mothers, respectively. The presence of other genera was specific for each individual. The absence of milk cell genera in the PCR control samples shows that these bacterial DNA were not attributable to laboratory contamination. Lactose-degrading, lactic acid-producing bacteria together with *Staphylococcus* species were the most represented genera in infant feces. Of 23 sequences corresponding to bifidobacteria, 17 were related to *B longum*, 5 to *Bifidobacterium bifidum*, and 1 to *Bifidobacterium infantis*. Two identical rDNA sequences (99% identity of 1117 base pairs), corresponding to *S thermophilus* and *Staphylococcus epidermidis*, were identified in the milk cell clones and in the infant's feces.

PBMCs contained a restricted variety of bacterial rDNA sequences (Fig 1, A and B). Bacterial signals were present in cells of both lactating and nongravid nonlactating women, but the complexity of bacterial signatures was greater in the former (Fig 1B). Furthermore, although profiles for control women were similar, those for lactating women were specific for each individual. Acridine orange staining of milk and blood cytopreparations identified bacterial bodies in association with mononuclear cells (Fig 1C).

### DC Subsets Are Diminished in the Circulation of Lactating Women

The distribution of DC phenotypes in the PBMCs of lactating and nonlactating women was examined by using a commercial kit and flow cytometry. The frequencies of differentiated lymphoid DC (lineage$^-$CD14$^-$HLA-DR$^+$CD11c$^-$CD123$^+$) and myeloid DC (lineage$^-$CD14$^-$HLA-DR$^+$CD11c$^+$CD123$^-$) phenotypes tended to be lower in the circulation of lactating women during the first month after delivery than in that of control subjects (data not shown). This difference reached statistical significance for lymphoid DCs at 1 week after delivery ($P = .02$) and for myeloid DCs at 3 and 4 weeks after delivery ($P = .01$ and $P = .02$, respectively). The numbers of CD14$^+$CD11c$^+$ potential DC precursors were significantly lower throughout the first month after delivery (Fig 2).

### Increased Bacterial Translocation Occurs in Pregnant and Lactating Mice

Next, bacterial translocation to extraintestinal tissues was examined in conventional nonpregnant, pregnant, and lactating mice. Whereas 10% of control animals had positive MLN cultures, 70% of pregnant animals had bacteria in their MLNs (Fig 3A). Within 24 hours after delivery, fewer animals had positive MLN cultures but 80% of mice had viable bacteria in their mammary tissue. Although this value decreased to 50% by 3 to 4 days after delivery, it was still significantly different from that of control mice ($P < .005$). Both aerobic and anaerobic species translocated, and their numbers subsided gradually over time (Fig 3B).

During lactation, bacteria were observed histologically in the subepithelial dome and interfollicular regions of Peyer's patches (Fig 3C, left), in the lamina propria of the small bowel, and associated with cells in the glandular tissue of the mammary gland (Fig 3C, right). The Peyer's patches of pregnant and lactating mice were macroscopically larger than those of control animals and had a more prominent subepithelial dome and more dilated draining lymphatic vessels, containing mononuclear cells (Fig 4).

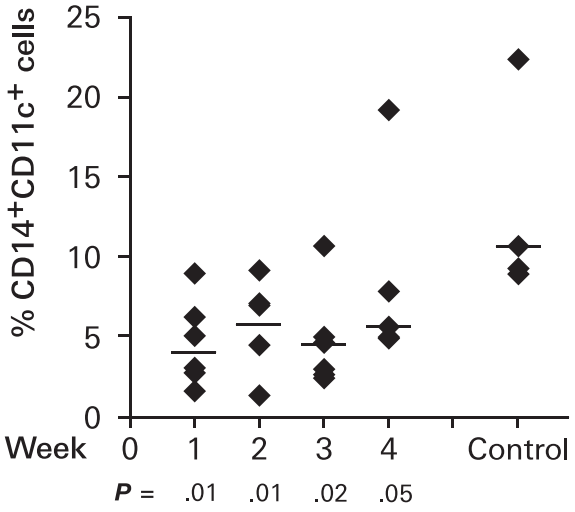


FIGURE 2
Flow cytometric analysis of CD14$^+$CD11c$^+$, potentially myeloid, DC precursors in PBMCs from lactating women ($n = 9$) at 1, 2, 3, and/or ≥4 weeks after delivery and from age-matched, nonlactating women ($n = 5$). Percentages of cells at different times in lactation were compared with those of control subjects by using the Mann-Whitney test.

## DISCUSSION

Aseptically collected breast milk contained a total concentration of microbes of $<10^3$ colony-forming units per mL, including *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Peptostreptococcus*, *Staphylococcus*, and/or *Corynebacterium*, with occasional *Escherichia* spp. This is less than the concentrations recently reported for breast milk[6] and may reflect elimination of organisms residing in the ducts or on the areola of the breast.[7] Therefore, the findings may give a better indication of bacteria that are intrinsic to milk. It is recognized that, despite every precaution, some of these isolates may still arise from contamination. Several studies have shown a similarity between the microflora of breast milk and that of the

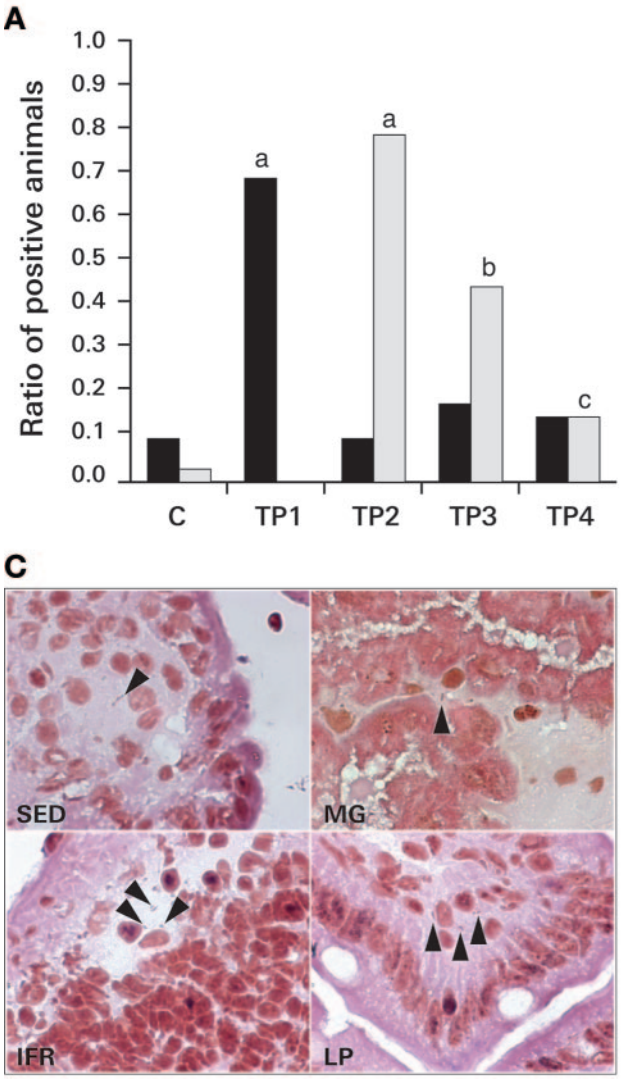


FIGURE 3
Bacterial translocation. A, Proportions of positive MLN (dark bars) and mammary gland (light bars) cultures from control mice (C), pregnant mice (TP1), and lactating mice at 0 to 1 day (TP2), 3 to 4 days (TP3), and 14 to 15 days (TP4) after delivery. [a]$P < .00005$; [b]$P < .005$; [c]$P < .05$, compared with control samples. B, Anaerobic (left) and aerobic (right) counts. The number of mice is given in parentheses. Values below the dotted line are <10 colony-forming units (CFU) per mL. MG indicates mammary gland. C, Gram staining, showing bacteria (arrowheads) in the subepithelial dome (SED) and interfollicular region (IFR) of Peyer's patches, in mammary gland, and in lamina propria (LP) of the distal small intestine.

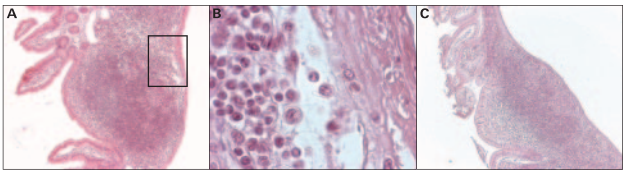


FIGURE 4
Hematoxylin and eosin staining of Peyer's patches in pregnant and control mice. Pregnant mice had reactive lymphoid tissue (A, ×100) and dilated lymphatic vessels containing mononuclear cells (B; represents framed area in A, ×1000), which were not seen in control animals (C, ×100).

nipple and areola.[4] The organisms most often in common were staphylococcal and streptococcal species. Increases in the number of staphylococci, streptococci, and *Lactobacillus acidophilus* species after feeding suggest that the infant's mouth is another potential source of bacteria.[5] Furthermore, a study reported that some strains of *Lactobacillus gasseri* and *Enterococcus faecium* in milk were identical to those in swabs of the areola and in oral swabs from the infant.[6] It might be argued that such sources of bacteria are also biologically relevant to neonates. Indeed, *Staphylococcus* species of the skin are common constituents of the early neonatal microbiota.[13] However, a bacterial presence in all of the milk samples we examined suggests that a discrete microbiota may exist naturally in breast milk. This prompted a subsequent investigation regarding its origin.

We considered that mononuclear phagocytes destined for the mammary gland capture components of the luminal microbiota before their departure from the gut and transfer them to the suckling infant through breast milk. In a first instance, we used TTGE to examine bacterial rDNA content in milk cells and maternal PBMCs and feces during lactation and then examined corresponding infant feces to address transfer of maternal bacteria through milk. Maternal feces yielded classic TTGE profiles that were specific for each mother and of greater biodiversity than those of infant feces. Although milk cells had a less-complex microbiota, TTGE revealed a greater biodiversity than the 2 or 3 genera observed

through plating and included genera corresponding to dominant autochthonous ileal and colonic organisms. These results confirmed the expected uptake of bacteria at these tissue sites and suggested that nonculturable bacteria or the DNA from dead bacteria may also be present intracellularly.

Interestingly, PBMCs contained a restricted variety of bacterial rDNA sequences that was more extensive during lactation. No viable bacteria were isolated. The reason for this is unknown, but perhaps the few, bacterially laden cells are diluted in the circulation. Alternatively, bacteria may be dead/quiescent because of intracellular antimicrobial effects.

Migration of bacteria within intestinally derived cells to the breast is supported by the observation that some rDNA bands were common to maternal feces, blood, and milk. Furthermore, because certain of these bands comigrated with those in infant feces, they may represent microbes transferred to the infant through the milk. Indeed, rDNA sequences corresponding to *S thermophilus*, *S epidermidis*, and *B longum* were identified in the milk cells and in the infant's feces. These 3 species were also detected in other milk samples and infant fecal samples. Moreover, *B longum* was detected in maternal blood and fecal samples.

Because we were aware that PCR amplification might have led inadvertently to false-positive results, we confirmed microscopically whether bacteria were associated with maternal cells. Unlike sepsis, in which translocating bacteria are associated with polymorphonuclear cells,[12] bacterial bodies were associated with a limited number (<0.1%) of milk and blood mononuclear cells. However, the possibility that bacterial components are also associated with polymorphonuclear cells cannot be excluded.

The observation that microbial components pass into the circulation of healthy individuals, albeit within an intracellular compartment, is potentially controversial and challenges the dogma that translocation of such material occurs only during sepsis. To verify such a phenomenon, we extended our study to conventional nonpregnant, pregnant, and lactating mice.

Although confined bacterial translocation to the MLNs was seen in control mice, heightened translocation to MLNs in the perinatal period was followed by colonization of the mammary gland in the immediate postpartum period. From this study, we cannot say whether additional "waves" of bacterial translocation occur earlier in pregnancy or later in lactation. Nevertheless, the increased translocation did not seem to be induced solely by parturition. Colonization of the breast coincided with an increased number of positive blood cultures and occasional translocation to the spleen and liver (data not shown). In contrast to pathologic conditions in which translocating microbes are mainly Gram-negative, penetrating species in pregnant and lactating mice included *Streptococcus*, *Lactobacillus*, and *Bifidobacterium*, whose numbers subsided gradually over time.

During lactation, bacteria were observed in the lamina propria of the small bowel and in the subepithelial dome and interfollicular regions of the Peyer's patches. Therefore, M cell-mediated uptake toward DCs in the Peyer's patch, direct sampling of luminal bacteria by dendrites of lamina propria DCs, and/or a low-level, physiologic leakiness of the epithelium may occur.[14] In healthy animals, a very limited number of bacteria cross the intestinal epithelium, evade uptake and killing by intestinal macrophages, and remain viable after phagocytosis by DCs.[15] Bacterially loaded DCs then migrate to the MLNs, where they initiate protective immune responses.[15] The more-prominent Peyer's patches observed in pregnant and lactating animals, with mononuclear cell exit through dilated lymphatic vessels, indirectly suggest that DCs may be implicated in the transport of intestinal microbial components to the breast, through the circuit used for induction of tolerance to soluble antigen.[16] Certainly, breast milk has a high proportion of phagocytes, which are also ineffective at killing ingested microbes.[17] Work demonstrating that $CD14^+$ milk mononuclear cells, which are normally considered to be macrophages, also express HLA-DR, CD86, CD83, and DC-specific intercellular adhesion molecule-3-grabbing nonintegrin suggests that these cells are partially differentiated DCs.[18] Moreover, because tissue macrophages are nonmigrating resident cells, milk DC-like cells derived from the maternal circulation are the most likely vehicles for intestinally derived microbial components. Therefore, we speculated that such cellular populations would be modulated during lactation. Indeed, we found that the frequencies of DC phenotypes and of $CD14^+CD11c^+$ intermediate DC-like cells were lower in the circulation during lactation. These findings agree with those of a study showing reduced numbers of circulating DC subsets in late pregnancy[19] and may reflect cellular trafficking toward the breast or intestine. We detected fetal liver tyrosine kinase-3 ligand, a stimulator of DC differentiation and mobilization,[20] in serum samples of 3 of 9 mothers (range: 13.9–71.7 pg/mL) and in 1 of 5 control samples.

Transfer of bacteria through milk may be a means by which maternal microbes colonize the neonatal gut.[4,6] Such a mechanism may provide a colonization advantage to bacteria of the mother's intestinal microbiota at a time when the low bacterial diversity in the neonatal intestine is permissive to colonization. In the present study, sequence homology between some strains in infant feces and milk suggests that this may indeed occur. However, we observed fewer viable organisms than reported previously[6] and, although a greater biodiversity of bacterial DNA was evident in milk cells, not all of those DNA bands comigrated with bands in the infant's feces. Clearly, there are more efficient routes through which maternal organisms colonize the neonatal gut.

We speculate that this phenomenon represents an education of the neonatal immune system by maternally derived bacterial molecular motifs.

Neonatal immune cells must learn to differentiate between self-antigens, dietary antigens, commensal organisms, and potential pathogens. We showed previously that human milk contains soluble pattern recognition receptors for bacterial motifs and that these may mediate different responses to Gram-negative and Gram-positive organisms and may modulate how neonatal cells perceive and respond to bacterial components.[21–23] In animal models, uptake of maternal leukocytes into neonatal tissues occurs during gestation and lactation.[24] Perhaps prolonged penetration of inconspicuous bacterial molecular patterns, via maternal DCs during pregnancy and lactation, induces tolerogenic responses that are analogous to those for self-antigens. Interestingly, osteoprotegerin, a DC survival factor that may also be important for maintaining immune tolerance,[25] demonstrates elevated levels in serum during pregnancy and lactation[26] and is present in significant quantities in human breast milk.[27]

Elevated translocation of bacteria or their components in the mother should certainly have some bearing on her immune status and may explain the physiologic activation of innate immunity that occurs during pregnancy.[28,29] Interestingly, bacterial DNA stimulates innate immunity in pregnant mice, improves maternal survival rates, and prevents pathogen transmission to the fetus.[30]

Our observations suggest a novel form of mother-infant communication, but they also highlight a potentially new mechanism of immune regulation in healthy individuals. As shown previously,[31,32] the blood of normal healthy subjects contains bacterial components. Some DNA may arise from human or microbial contamination. However, the greater number of bacterial DNA signatures in the PBMCs of healthy lactating women suggests that components of certain bacterial species are inherent to circulating cells. It is tempting to speculate that this represents an evolutionary strategy of immune surveillance and that such bacterial imprinting maintains tolerance to specific bacterial species and alerts distant anatomic sites of changes in local lymphoid tissues.

## CONCLUSIONS

Our study shows that human breast milk cells contain a limited number of viable bacteria and bacterial DNA that might have been transported from the mother's intestine to the mammary gland through an endogenous cellular route. An animal study suggests that this process begins in late pregnancy. The results suggest a novel form of mother-infant communication. However, additional studies are necessary to identify the underlying mechanisms of this heightened bacterial translocation and to elucidate the consequences of this phenomenon for pregnant and lactating women and for instruction of the neonatal immune system.


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