BREASTFEEDING MEDICINE
Volume 16, Number 5, 2021

DOI: 10.1089/bfm.2020.0369

High Levels of Interferon-Alpha Expressing Macrophages in Human Breast Milk During SARS-CoV-2 Infection: A Case Report

Jack C. Yu,[1] Hesam Khodadadi,[2,3] Évila Lopes Salles,[2,3] Quyen Pham,[4] Pinkal Patel,[4] and Babak Baban[2,3]

Abstract

Introduction: In addition to hand washing and wearing masks, social distancing and reducing exposure time to <15 minutes are the most effective measures against the spread of COVID-19. Unfortunately, three of these guidelines are very difficult, if not impossible, for nursing babies: they cannot wear masks, stay six feet away from the lactating breasts, nor consistently finish within 15 minutes while nursing. We report a case of a nursing mother with SARS-CoV-2 infection, documenting changes of immune cells and cytokines in breast milk with and without the infection.

Case Description: With Institutional Review Board (IRB) approval, we obtained expressed breast milk samples from a lactating mother before and during SARS-CoV-2 infection as documented by reverse transcription-PCR. Using flow cytometry analysis, we measured the immune cell profiles and expression of cytokines such as interferon alpha (IFNα) in milk leukocytes before and during infection.

Results: There was an eightfold increase in IFNα+ milk leukocytes, from 1% before SARS-CoV-2 infection to 8% when actively infected. The milk macrophages showed the highest increase in IFNα expression. Both T and B lymphocytes showed mild increase. Innate lymphoid cells, neutrophils, and natural killer cells showed no increase in IFNα expression and the dendritic cells actually showed a reduction.

Conclusion: We document the presence and high expression of IFNα in the breast milk macrophages of a lactating mother with confirmed COVID-19, compared with her milk before the infection.

Keywords: breast milk, cellular immunity, interferon alpha, COVID-19, SARS-CoV-2

Introduction

The COVID-19 pandemic continues to stress the health care system throughout the world. Hand washing, social distancing, mask wearing, and reduction in exposure time remain the four key components of countermeasures against viral spread. Breastfeeding is incompatible with social distancing—it cannot occur at 3.66 m, nor 1.83 m. Mask wearing and exposure time are also problematic. However, fortunately to this date, there are no clearly proven cases of vertical transmission of COVID-19 through human breast milk or breastfeeding. Even with that, there are reasonable concerns regarding possible transmission of SARS-CoV-2 during breastfeeding; further investigation is definitely required. Two important questions remain. (1) Does the immune composition of human milk change because of maternal COVID-19? (2) What are these alterations? Regarding soluble immune factors and composition of immune cells in the milk from mothers infected with COVID-19, there are several reports demonstrating the elevated level of IgA, IgG, and IgM.[1,2] To our knowledge, there have been no reports of any cellular analysis of innate or adaptive immune systems in human milk during maternal COVID-19 infection. Considering the significance of breastfeeding in the development of infant immunity, we report in this study, for the first time, evaluation of human expressed breast milk (EBM) from a lactating mother before and during COVID-19 (confirmed by reverse transcription-PCR of nasopharyngeal swab).

[1]Children's Hospital of Georgia and Medical College of Georgia, Augusta University, Augusta, Georgia, USA.
[2]Department of Oral Biology and Diagnostic Sciences, Dental College of Georgia, Augusta University, Augusta, Georgia, USA.
[3]Center for Excellence in Research, Scholarship and Innovation, Dental College of Georgia, Augusta, Augusta University, Augusta, Georgia, USA.
[4]Department of Pediatrics, Medical College of Georgia, Augusta University, Augusta, Georgia, USA.

Methods

We collected 30 mL of mature (7 months after delivery) EBM from a mother with confirmed COVID-19. EBM from the same subject taken in January 2020 (1 month after delivery), before the pandemic, served as control. EBM sample was obtained using breast pump and immediately transferred to the laboratory for analysis. We sorted leukocytes, lymphoid, and myeloid cells from the EBM. For total leukocytes, we used CD45. For resting and activated T cells, we used CD3/CD69. For B cells, we used CD19. For natural killer cells, we used CD16/CD56. For innate lymphoid cells, ILCs, we sorted for CD45/+ Lineage/− but + for IL12Rβ2/GATA3/RORγt/interferon γ (IFNγ)/interleukin (IL)9/IL13/IL17/IL22. For myeloid lineage, we used the following markers: neutrophils: CD16/CD49d, macrophages: CD11b/CD16/CD68, and dendritic cells CD1/MHCII. For each cell type we further measured the expression of four cytokines: IL-6, tumor necrosis factor α, IFNα, and IL-1β using flow cytometry as described previously.[3,4] This study was approved by the Institutional Review Board and institutional Biosafety Committee at Augusta University. The subject provided written informed consent and there was no financial compensation.

Results

There was no significant difference between total leukocytes (CD45$^+$) in milk from subject before and during infection with COVID-19 (320,000 cells/mL before COVID compared with 365,000 cells/mL during infection with COVID-19). The most striking difference (eightfold increase) was in the percentage of macrophages expressing IFNα (a type I interferon) (Fig. 1D). IFNα was substantially higher in the mothers' milk when she had COVID-19 compared with her milk before infection (Fig. 1C). Although the level of IFNα expression during COVID-19 was the highest in macrophages followed by dendritic cells and T cells, dendritic cells demonstrated the highest level of IFNα expression *before* COVID-19 infection with a reduction of IFNα+ dendritic cells during the infection (Fig. 2).

Discussion

The comparison between the cellular components of breast milk showed no significant quantitative difference before and during infection by SARS-CoV-2. There was a qualitative change with an eightfold increase in IFNα-producing macrophages. After viral entry into the host cell, one of the earliest nonstructural proteins, translated by the viral RNA suppresses the production of host IFNα. The finding of increased numbers of milk macrophages expressing IFNα suggests that either the virus did not infect the cells of the breast, or the infection has progressed beyond the stage of immune silencing. Oncologists know that even after administering massive doses of IFNα to patients with malignancy, the IFNα remains low in their breast milk. The high molecular weight of IFNα (19.5 kD) may limit the transfer of IFNα into the milk.[5] Importantly, reported in this study for the first time, increase in milk macrophages expressing IFNα from a mother with active COVID-19 may represent an effective strategy of elevating IFNα in breast milk level. Transferring cells capable of producing IFNα rather than IFNα itself bypasses the aforementioned problem. Given the important primary antiviral role of interferons, breastfeeding can provide immune protection for both mother and infant. There are three theoretical ways of

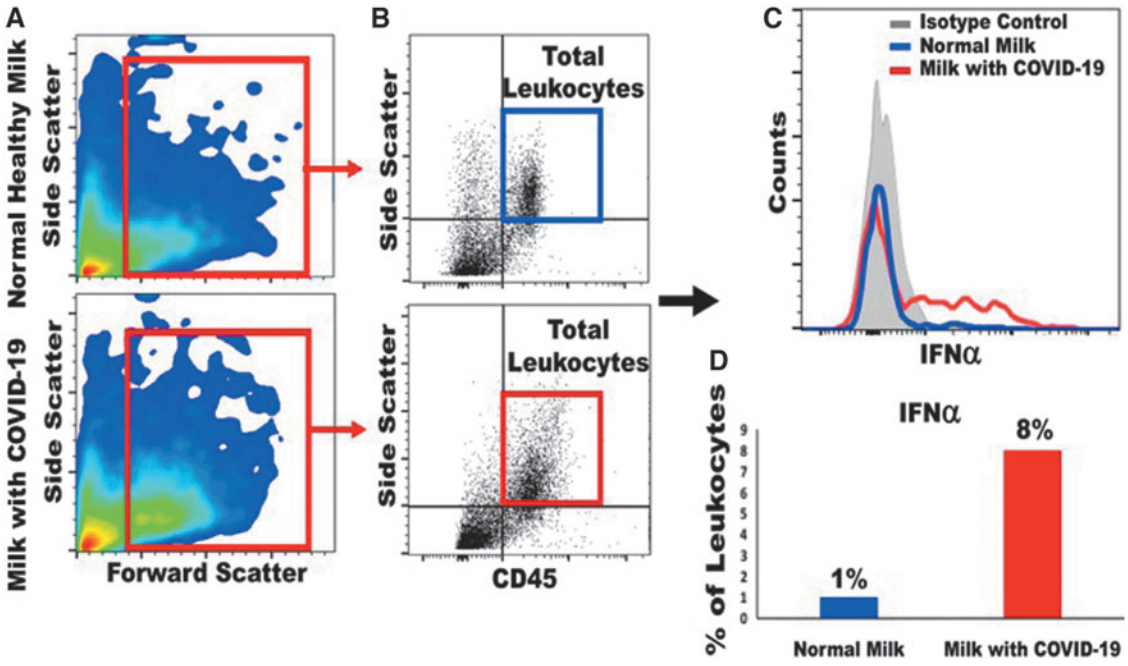

FIG. 1. High-level expression of IFNα in milk from lactating mother with active COVID-19. Flow cytometry analysis of milk from COVID-19 positive mother showed significant increase in the expression of IFNα by leukocytes (CD45$^+$ cells) compared with the normal milk sample before the infection **(A–C)**. **(D)** Displays the numeric values of expression before and during COVID-19 infection. Comparative analysis is based on a milk sample at each stage from one lactating mother before and after diagnosis with COVID-19 infection. IFNα, interferon alpha.

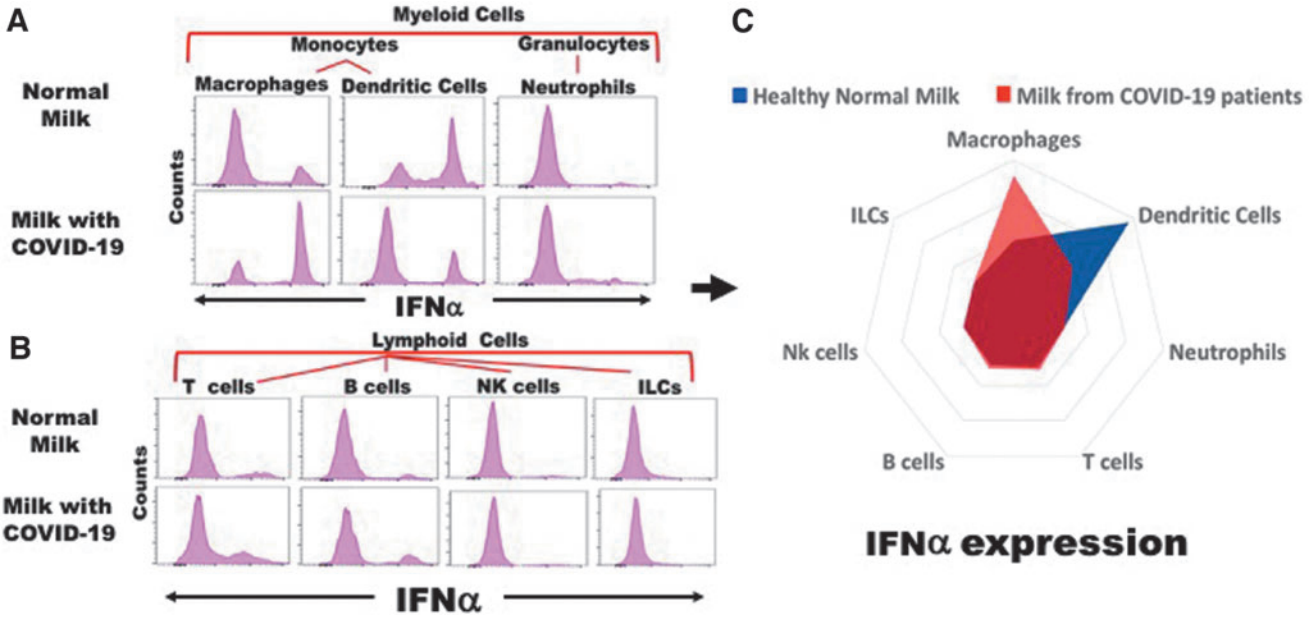

FIG. 2. Macrophages express the most level of IFNα in the milk from lactating mother with active COVID-19. Flow cytometry analysis of myeloid and lymphoid cells in the human milk demonstrated that macrophages express the most level of IFNα in the milk from lactating mother with active COVID-19, followed by dendritic cells, T cells, and B cells, respectively **(A, B)**. Analysis of normal milk from very same subject before COVID-19 showed the most level of IFNα expression by dendritic cells, followed by macrophages, T cells, and B cells **(A, B)**. The radar graph **(C)** depicts the distribution pattern of IFNα expression in major subpopulations of leukocytes in lactating mother before the disease and after being diagnosed with COVID-19.

increasing milk IFNα level: (1) from source outside the breast, (2) from immune cells in the breast, and (3) from milk-secreting tubule-alveolar cells of the breast. For reasons already stated, mechanism 1 is not likely. The third mechanism is also unlikely. If viral infection occurs in the tubule-alveolar cells, they may indeed increase the expression of IFNα, with likely actual viral presence in milk. In reality, there are limited observations supporting this. In fact, according to Center for Disease Control (CDC) although it is still unknown whether mothers with COVID-19 can transmit the virus through breast milk. The data available suggest that milk may test positive for SARS-CoV-2 by RT-PCR, but does not contain replicating competent virus.[6] The second possibility is the most likely: breast immune cells may produce elevated level of IFNα because of the active systemic infection of COVID-19. However, the origin and viral infection status of these cells are unclear. Fluctuation of residential and infiltrating innate immune cells (e.g., monocytes and macrophages) in the breast with the capability of IFNα production are likely responsible for elevated level of milk IFNα. Considering the antiviral role of IFNα, high level of IFNα in milk is not surprising. Similar to COVID-19 patients treated with IFNα,[7,8] the infants of mother infected with COVID-19 and producing higher levels of IFNα in breast milk may benefit from this natural antiviral agent. It is very possible that the maternal serum IFNα level in response to the viral infection is higher than that seen in the milk. Furthermore, the difference in the profile of IFNα expressing cells in the milk before and during COVID-19, with macrophages being the most abundant, signifies the important role of macrophages as the main cellular component of innate immunity in human milk.

Limitations of this study are (1) findings from only a single mother infected with COVID-19 and (2) samples from only one point in time before and one point in time after COVID-19 infection. Nonetheless, the results are in agreement with and supplement two important recent reports by Chambers et al.[6] and Marín Gabriel et al.[9] in that there was no documented infection transmitted to the infant by breast milk in this case and that observable changes occurred in the breast milk. Including this case, there are 26 cases of nursing mothers with COVID-19 and no vertical transmission to the infants, although one milk sample contained segments of viral RNA.[6] In a literature review of 50 articles by Deniz and Tezer, 17 newborns, 8 placental tissue samples, 3 milk samples, and 1 amniotic fluid sample tested positive by RT-PCR.[10]

Conclusions

We have identified in human milk from a COVID-19 positive woman, an increased amount of IFNα and an increased percentage of macrophages expressing IFNα in her breast milk. This may contribute some level of protection of breastfeeding infants from COVID-19 infection or symptomatic COVID-19 infection. Ongoing testing of human breast milk immune factors and bioactive factors in response to COVID-19 infection in breastfeeding mothers is needed; along with correlation with infants' COVID-19 status.

Authors' Contributions

All authors contributed to the study, commented on the article, and approved the final version.

Disclosure Statement

No competing financial interests exist.

Funding Information

This work was sponsored by institutional seed money of Dental College of Georgia (DCG) and Medical College of Georgia (MCG) jointly, as well as by National Institute of Health award (R01NS110378). The funding organizations had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

References

1. Demers-Mathieu V, Dung M, Mathijssen GB, et al. Difference in levels of SARS-CoV-2 S1 and S2 subunits- and nucleocapsid protein-reactive SIgM/IgM, IgG and SIgA/IgA antibodies in human milk. *J Perinatol* 2020:1–10.
2. Dong Y, Chi X, Hai H, et al. Antibodies in the breast milk of a maternal woman with COVID-19. *Emerg Microb Infect* 2020;9:1467–1469.
3. Khodadadi H, Salles ÉL, Jarrahi A, et al. Cannabidiol modulates cytokine storm in acute respiratory distress syndrome induced by simulated viral infection using synthetic RNA. *Cannabis Cannabinoid Res* 2020;5:197–201.
4. Baban B, Malik A, Bhatia J, et al. Presence and profile of innate lymphoid cells in human breast milk. *JAMA Pediatr* 2018;172:594–596.
5. Kumar AR, Hale TW, Mock RE. Transfer of interferon alfa into human breast milk. *J Hum Lact* 2000;16:226–228.
6. Chambers C, Krogstad P, Bertrand K, et al. Evaluation for SARS-CoV-2 in breast milk from 18 infected women. *JAMA* 2020;324:1347–1348.
7. Wang B, Li D, Liu T, et al. Subcutaneous injection of IFN alpha-2b for COVID-19: An observational study. *BMC Infect Dis* 2020;20:723.
8. Sallard E, Lescure FX, Yazdanpanah Y, et al. Type 1 interferons as a potential treatment against COVID-19. *Antiviral Res* 2020;178:104791.
9. Marín Gabriel MÁ, Malalana Martínez AM, Marín Martínez ME, et al. Negative transmission of SARS-CoV-2 to hand-expressed colostrum from SARS-CoV-2–positive mothers. *Breastfeed Med* 2020;15:492–494.
10. Deniz M, Tezer H. Vertical transmission of SARS CoV-2: A systematic review. *J Matern-Fetal Neonatal Med* 2020:1–8.

Address correspondence to:
Babak Baban, PhD, MPH, MBA, FAHA
Department of Oral Biology and Diagnostic Sciences
Dental College of Georgia
Augusta University
Augusta, GA 30912
USA

E-mail: bbaban@augusta.edu