SARS-CoV-2 Antibodies Detected in Mother's Milk Post-Vaccination

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Abstract

Background: The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has infected over 127 million people worldwide, with almost 2.8 million deaths at the time of writing. Since no lactating individuals were included in initial trials of vaccine safety and efficacy, research on SARS-CoV-2 vaccination in lactating women and the potential transmission of passive immunity to the infant through mother's milk is needed to guide patients, clinicians, and policy makers on whether to recommend immunization during the worldwide effort to curb the spread of this virus.
Research Aims: (1) To determine whether SARS-CoV-2 specific immunoglobins are found in human milk after vaccination, and (2) to characterize the time course and types of immunoglobulins present.
Methods: A longitudinal cohort study of lactating women (*N* = 7) who planned to receive both doses of the Pfizer-BioNTech or Moderna SARS-CoV-2 vaccine between December 2020 and January 2021 provided milk samples. These were collected pre-vaccination and at 11 additional timepoints, with the last sample at 14 days after the second dose of vaccine. Samples were analyzed for levels of SARS-CoV-2 specific immunoglobulins A and G (IgA and IgG).
Results: We observed significantly elevated levels of SARS-CoV-2 specific IgG and IgA antibodies in human milk beginning approximately 7 days after the initial vaccine dose, with an IgG-dominant response.
Conclusions: Maternal vaccination results in SARS-CoV-2 specific immunoglobulins in human milk that may be protective for infants.

Background

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has infected over 127 million people worldwide at the time of this writing, causing nearly 2.8 million deaths (Dong et al., 2020). Two novel mRNA vaccines encoding the viral spike protein were approved and administered to patients in the United States beginning in December 2020, with the potential to slow the rate of infection and decrease the incidence of severe cases that lead to hospitalization and death. As is common, initial trials for the Pfizer-BioNTech BNT162b2 and Moderna mRNA-1273 vaccines excluded pregnant and breastfeeding women (Baden et al., 2021; Polack et al., 2020). Although a few recent studies have been published on this topic (Gray et al., 2021; Perl et al., 2021), there is still a dearth of evidence to guide women, clinicians, and policymakers about whether to recommend immunization for these populations as the vaccines become available worldwide.

Newborns and infants rely on the antibodies, cytokines, enzymes, and leukocytes found in human milk as their main source of immunological enrichment (Palmeira & Carneiro-Sampaio, 2016). Infants 0–5 months old who are exclusively fed human milk have a 8.66-fold lower mortality risk from infections than formula-fed infants (Sankar et al., 2015) and a population-wide increase in percentage of human milk-fed

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infants resulted in a decline in rates of infant pneumonia and gastroenteritis (Wright et al., 1998). IgA is the most abundant antibody in human milk, conferring protection from pathogens by inhibiting binding to host cells in the respiratory and gastro-intestinal mucous membranes (Palmeira & Carneiro-Sampaio, 2016). However, recently it has been shown that IgG found in human milk also plays an important role in viral immunity (Fouda et al., 2011; Mazur et al., 2019).

Researchers have previously shown that women infected with SARS-CoV-2 produce antibodies in their milk against the virus. One group found a robust secretory-immunoglobulin A (IgA) dominant response in human milk from 15 women after confirmed infection with SARS-CoV-2 (Fox et al., 2020). In another study with 18 women who had confirmed COVID-19, 76% of milk samples contained SARS-CoV2 specific IgA, 80% had SARS -CoV-2 specific IgG and 62% of the milk samples were able to neutralize SARS-CoV-2 activity in vitro (Pace et al., 2021). Other researchers detected SARS-CoV-2 specific secretory IgA and IgG in banked milk samples collected in the United States during February–April 2020 (Demers-Mathieu et al., 2021), however, findings from that study were limited by lack of confirmed SARS-CoV-2 infection in the individuals who donated milk.

In recently published studies also conducted December 2020–February 2021, a group from Israel found IgG and IgA antibodies in human milk after vaccination with Pfizer vaccine (Perl et al., 2021), and researchers in the United States demonstrated SARS-CoV-2 specific antibodies in breastmilk following either Moderna or Pfizer vaccination (Gray et al., 2021). While our goal of identifying SARS-CoV-2 specific antibodies in human milk is similar, our study design with more frequent sample collection timepoints allows us to better elucidate the time course of the antibody responses. Our aims were (1) to determine whether SARS-CoV-2 specific immunoglobins are found in human milk after vaccination, and (2) to characterize the time course and types of immunoglobulins present.

Methods

Research Design

This was a longitudinal prospective one-group cohort study comparing the baseline (pre-vaccination) levels of human milk antibodies to levels after vaccination with Pfizer-BioNTech BNT162b2 or Moderna mRNA-1273 in a group of lactating women. This study design was chosen because it allows us to observe the outcome of interest (antibodies in human milk) in a sample of participants selected for the exposure of interest (vaccination). Sample collection was approved by the hospital's institutional review board (IRB #06-108A).

Setting

The study was conducted at a community hospital in Portland, Oregon, USA. Oregon has good access to

Key Messages

- Since no lactating individuals were included in initial trials, research on SARS-CoV-2 vaccination in lactating women is needed to guide patients, clinicians, and policy makers during the worldwide effort to curb the spread of this virus.
- In a cohort of seven lactating women, we observed significantly elevated levels of SARS-CoV-2 specific IgG and IgA antibodies in human milk beginning approximately 7 days after the initial vaccine dose, with an IgG-dominant response.
- Maternal vaccination results in SARS-CoV-2 specific immunoglobulins in human milk that may be protective for infants.

breastfeeding support and higher breastfeeding rates than the United States as a whole, with 84.8% of all mothers breastfeeding their infants at 8 weeks after delivery in 2017 (Centers for Disease Control and Prevention, 2020). Participants were all health care workers who were eligible to receive the vaccine through their employers starting in December 2020 or January 2021.

Sample

A total of seven participants were recruited from December 2020 to January 2021. Participants were eligible for the study if they were currently breastfeeding and were already scheduled to receive the SARS-CoV-2 vaccine. Participants were excluded if they had known previous exposure to the SARS-CoV-2 virus or had previously tested positive for the virus. All seven women received both doses of the vaccine and completed the study. This sample size ($N = 7$) was adequate to detect a difference in antibody concentrations of 80 units/ml with greater than 80% power for both IgA and IgG.

Measurement

Human milk antibody levels against both the SARS-CoV-2 viral spike protein and the spike receptor binding domain (RBD) were quantified by enzyme-linked immunosorbent assay (ELISA) per the following protocol. Ninety-six-well NUNC MAXISORP ELISA plates were coated with 1.0 μg/ml of SARS-CoV2 Spike protein prefusion-stabilized ecto-domain (LakePharma, #46328) or 1.0 μg/ml of SARS-CoV-2 Spike receptor-binding domain protein (LakePharma, #46438) overnight at 4 °C. The following day plates were washed with phosphate-buffered saline (PBS)/0.5% Tween and blocked with PBS/0.5%Tween containing 5% Blotting-Grade Blocker (Bio-Rad, #1706404) for 2 hr at 37 °C. Plates were washed, and titrated milk samples (4-fold dilutions; 1:10-1:640) were aliquoted in single wells and incubated for
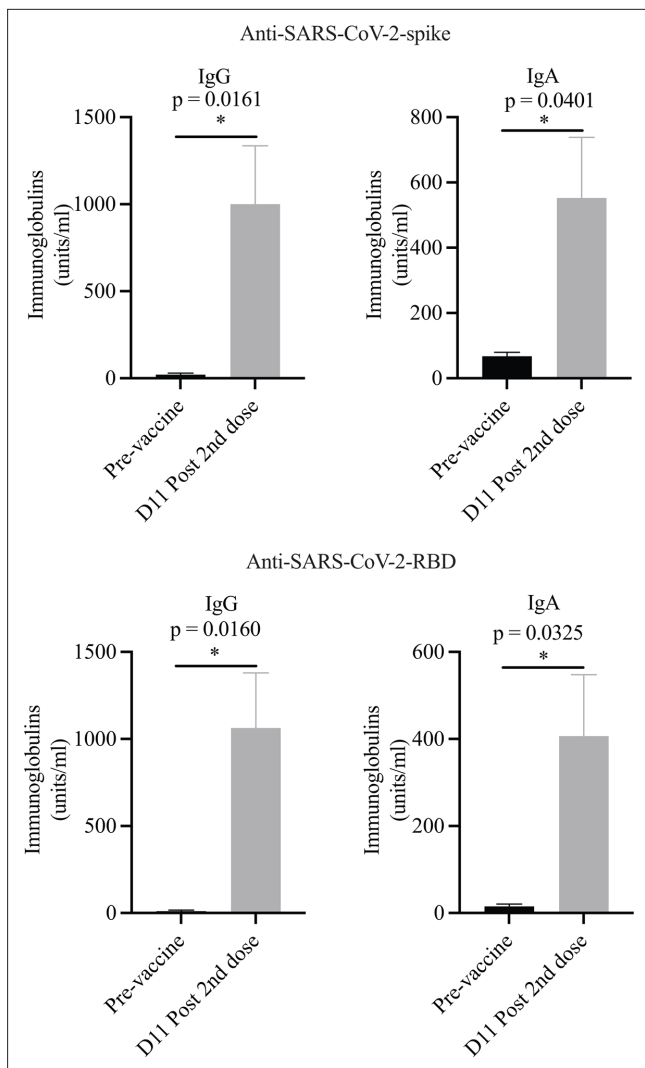
Figure 1. Bar Plot of Pre- and Post-Vaccination (Day 11 Post-Booster) Levels (in Units/ml) of Anti-SARSCoV-2 Spike and RBD IgG and IgA in Human Milk (*n* = 7).
Note. Error bars represent standard error of the mean.

1 hr at room temperature. Plates were washed with PBS/0.5% Tween, followed by incubation with either anti-IgG horseradish peroxidase (HRP; 1:10000, Fisher Scientific, #A18811) or anti-IgA HRP (1:8000, Jackson ImmunoResearch, #109-035-011) for 30 min at room temperature. Plates were washed with PBS/0.5% Tween and exposed to SureBlue 3,3',5,5'-tetramethylbenzidine (TMB) Peroxidase Substrate (VWR, #95059-286) for 45 min. The TMB reaction was stopped with 1M Phosphoric Acid, and the optical absorbance (450 nm, 1.0 sec) for each well was read on a Wallac Victor Microplate Reader.

To quantify sample spike-specific or spike RBD-specific IgG or IgA antibodies as well as normalize between ELISA plates, protein dilutions (250 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 15.6 ng/ml, 7.8 ng/ml, 3.9 ng/ml, and none) of

native human IgG protein (Abcam, ab91102) or native human IgA protein (Abcam, ab91025) were coated in the first column of wells on each ELISA plate. This column of wells was incubated with the same anti-IgG HRP (1:10000, Fisher Scientific, #A18811) or anti-IgA HRP (1:8000, Jackson ImmunoResearch, #109-035-011) as the rest of the ELISA plate as described above. A titration curve equation was determined by simple linear regression of the optical density at 450 nanometers (OD_{450}) readings from this column of wells, and had detection sensitivity of 3.9 ng/ml. To determine participant sample concentrations, OD_{450} readings from participant titrated samples lying within the linear portion of the titration curve were applied to the titration curve equation. These relative protein concentrations were multiplied by the dilution factor and assigned units/ml (1 U/ml = 1 ng/ml native IgG or IgA protein).

Data Collection

Recruitment and sample collection were conducted from December 2020 to March 2021. All participants provided oral and written consent for their milk samples to be used in this research. Sample collection was requested at the following timepoints: pre-vaccination; 1, 4, 7, 11, and 14 days post first vaccine dose; 1 day before the second dose; and 1, 4, 7, 11, and 14 days after the second dose. Participant 1 provided an additional milk sample at 80 days after the first dose. Human milk samples (2–4 ml) were collected by participants at home with personal clean electric breast pumps and transferred into investigator-provided sterile plastic containers stored immediately at −20 °C. Samples were dated and accepted if collected within 24 hr of the requested timepoint. Samples were kept frozen and transported on ice to the laboratory, where they were stored at −80 °C in an industrial freezer. The samples were rapidly thawed at 37 °C in a water bath and centrifuged at 1301 × g for 20 min at 4 °C. After removing the fat layer, the supernatant was collected, separated into aliquots, and stored at −80 °C until analysis. To test the durability of immunoglobulins to the standard processing protocol established by the Human Milk Bank Association of North America, we analyzed antibody levels in one sample from Participant 1 (Day 11 after the second dose) with and without Holder pasteurization of the whole milk sample—30 min of sustained heat at 62.5 °C, followed by rapid cooling and refreezing. Individually identifiable research data were confidentially maintained and saved to a secure hospital server.

Data Analysis

Antibody levels were reported as units/ml of IgG or IgA. Data were analyzed and graphed using Prism (GraphPad Software, La Jolla, CA). Immunoglobulin levels were compared using paired Student's *t*-test.
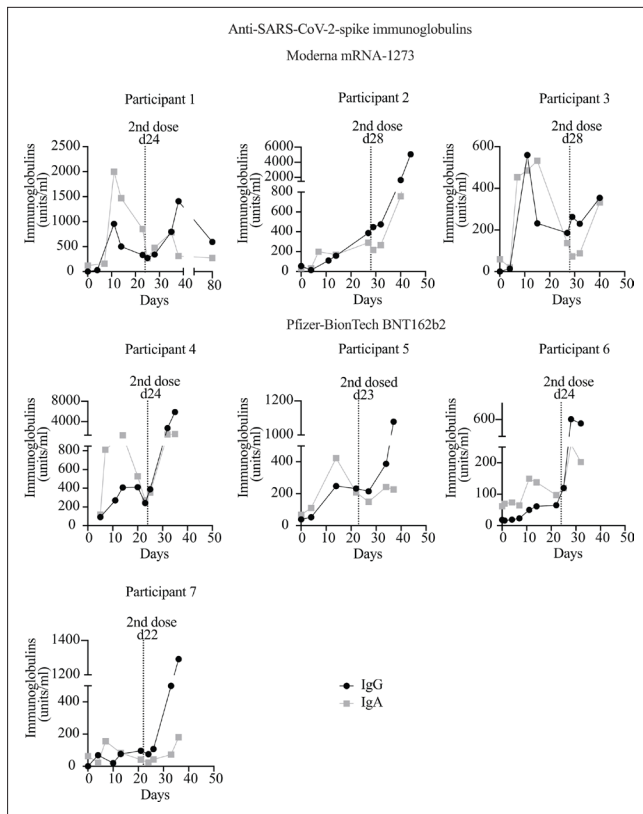
Figure 2. Levels (in Units/ml) and Kinetics of Anti-SARS-CoV-2 Spike IgG and IgA in Human Milk Post Vaccination.
Note. Participants 1, 2, and 3 received the Moderna vaccine, while participants 4, 5, 6, and 7 received the Pfizer-BioNTech vaccine. The dotted line represents the date of the second dose (booster) administration for each participant.

Figure 3. Levels (in Units/ml) and Kinetics of Anti-SARS-CoV-2 RBD IgG and IgA in Human Milk Post-Vaccination.
Note. Participants 1, 2, and 3 received the Moderna vaccine, while participants 4, 5, 6, and 7 received the Pfizer-BioNTech vaccine. The dotted line represents the date of the second dose administration for each participant.

Results

We received baseline (pre-vaccine) timepoint samples from all participants except Participant 4, and none of the participants tested had detectable levels of SARS-CoV-2 specific immunoglobulins in milk samples collected prior to vaccination. Levels of anti-spike IgG and IgA at Day 11 after the second dose were elevated relative to pre-vaccine baseline (18 units/ml vs. 1000 units/ml, $p = .0161$; 67 units/ml vs. 552 units/ml, $p = .0401$, respectively). Similarly, we found a significant increase in average levels of anti-RBD immunoglobulins (10 units/ml vs. 1063 units/ml, $p = .0160$; 15 units/ml vs. 406 units/ml, $p = .0325$; Figure 1). At peak, mean IgG levels in milk were roughly double the mean IgA levels for both anti-spike and anti-RBD immunoglobulins (1000 units/ml vs. 552 units/ml, 1063 units/ml vs. 406 units/ml, respectively). There was no detectable difference between the two vaccines in levels of antibody produced at this timepoint.

For all participants, levels of virus-specific IgG and IgA increased by Day 7 after vaccine (Figures 2 and 3). Levels of IgG and IgA broadly decreased prior to the administration of the second vaccine dose, and both IgG and IgA sharply
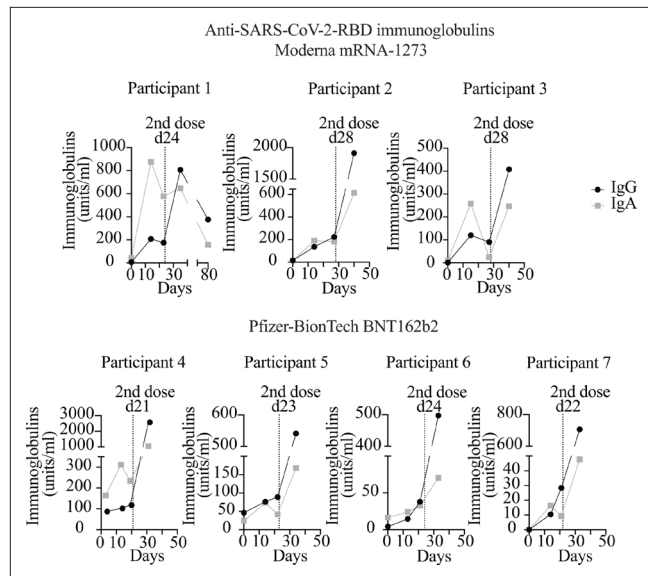
increased in the timepoints after the second dose. Both viral spike protein and RBD antibody titers remained elevated above baseline in one sample collected 80 days after vaccination (Figures 2 and 3, Participant 1); no other samples were collected at this timepoint. After pasteurization of one sample, there was a 6.30% decrease in anti-spike IgA antibody titer and a 6.67% drop in anti-spike IgG, while RBD-specific antibodies showed a 3.22% drop in IgG and a 0.68% decrease in IgA in pasteurized milk (Figure 4).

Discussion

Since the first confirmed case of novel coronavirus in November 2019, the SARS-CoV-2 virus has spread rapidly worldwide. Lactating women were excluded from initial vaccine trials, and at present only a few studies have been done in this population (Gray et al., 2021; Perl et al., 2021). Our group found a significant increase in IgA and IgG immunoglobulins in human milk specific to both the SARS-CoV-2 viral spike protein and its receptor binding domain starting between Day 7 and Day 14 after vaccination, slightly earlier than previously published. Perl et al. (2021) found a significant increase in SARS-CoV-2 specific antibodies at Day 14 and Gray et al. (2021) found a robust increase in Ig at the date of the second vaccine dose 3–4 weeks after baseline, however earlier timepoints were not reported. This time course also parallels the serum antibody response to these vaccines. In the Phase I trial of the Moderna mRNA-1273 vaccine, increased anti-spike serum IgG was seen at Day 15
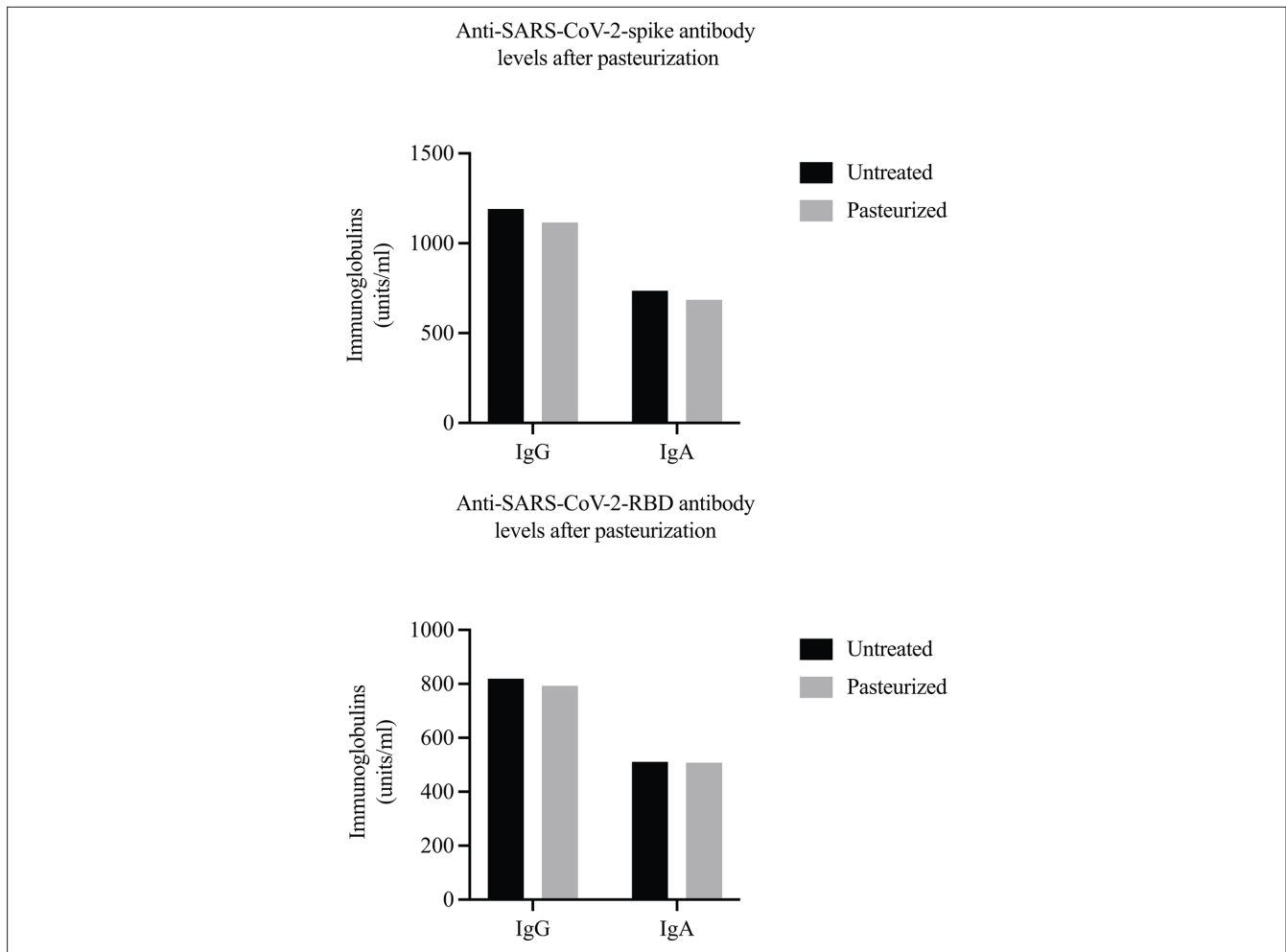
Anti-SARS-CoV-2-spike antibody
levels after pasteurization

Anti-SARS-CoV-2-RBD antibody
levels after pasteurization

Figure 4. Bar Plot of Antibody Titers (in units/ml) Before and After Holder Pasteurization of the Day 11 Post Second Dose Milk Sample From Participant 1.

(Jackson et al., 2020), while in preliminary analysis of BNT162b2 (Pfizer-BioNTech), IgG increase from baseline to convalescent-equivalent levels was seen between Day 8 and Day 21 (Sahin et al., 2020). The antibody specificity to the receptor binding domain of the spike protein we observed in our samples suggests that these antibodies are capable of blocking viral access to human cells.

In contrast to the IgA-dominant antibody response in the milk of women previously infected with SARS-CoV-2 (Fox et al., 2020), our results indicate that the post-vaccine antibody response in human milk is IgG dominant. This is consistent with previously published studies about SARS-CoV-2 vaccination (Gray et al., 2021; Perl et al., 2021) and has also been observed after Tdap vaccination in pregnancy (Orije et al., 2021). One potential explanation for this difference is that vaccinated mothers are exposed to viral antigen via intramuscular injection, whereas infected mothers have immunological education occurring in the mucosa, where IgA plays a greater role. While most researchers have focused on the role of human milk IgA in providing passive immunity, evidence is emerging that IgG is also an important

mediator of infant immunity to viruses such as HIV and RSV (Fouda et al., 2011; Mazur et al., 2019).

Banked human milk is an important resource for infants, and the relative stability of SARS-CoV-2 antibodies in the sample we tested before and after Holder pasteurization is promising. In recently published work, prevalence of IgA antibodies against SARS-CoV-2 in human milk from a large cohort of lactating mothers in the Netherlands (October 2020–February 2021) was 23.1% (Juncker et al., 2021). As vaccination rates continue to increase worldwide, donor milk prevalence of SARS-CoV-2 Ig is expected to increase further, and this will be an important source of SARS-CoV-2 immunity for infants who require supplementary feeding, or in cases when maternal vaccination is contraindicated.

Limitations

While establishment of a human milk antibody response to the SARS-CoV-2 vaccine is promising, our study was limited by small sample size in a relatively homogeneous cohort of healthcare workers and bears repeating in a more diverse

and randomly sampled cohort of participants as vaccines become more widely available to the public. The ELISA method we used to evaluate SARS-CoV-2 IgA and IgG showed that antibodies were present that will bind to the viral spike protein and receptor binding domain; however, we did not perform a blocking assay to test whether the affinity of these immunoglobulins was great enough to prevent viral binding to human cells. It is also important to note that our study did not include any testing of the infant immune response. Further research is needed on the longevity of the antibody response in mother's milk, as well as the magnitude and duration of effect on infant immunity to the virus.

Conclusion

Currently, little research is available to guide lactating women and their healthcare providers when deciding whether or not to get a SARS-CoV-2 vaccine. We provided evidence that mothers vaccinated against SARS-CoV-2 produce antibodies to this virus in their milk that may be protective for infants.

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