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Specific IgA, But Not IgG, in Human Milk From COVID-19-Infected Mothers Neutralizes SARS-CoV-2

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Abstract: This study highlights the importance of human milk in providing anti-severe acute respiratory syndrome coronavirus 2 immunity to newborns. The highest protective activity of human milk against COVID-19 was found in colostrum from infected mothers. Neutralizing activity was associated with high levels of specific IgA. Depletion of IgA, but not IgG, from milk samples completely abolished the ability of human milk to neutralize severe acute respiratory syndrome coronavirus 2.

Key Words: COVID-19, human milk, breastfeeding, antibody, neutralization

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The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in millions of deaths and hundreds of millions of hospitalizations attributed to COVID-19 disease.[1] The SARS-CoV-2 pandemic has relatively spared children compared to adults. However, neonates and infants represent a vulnerable population, which will always be uniquely naïve to SARS-CoV-2 infection and will continuously be replenished over time as new babies are born. This highlights the importance and need for a specific approach to COVID-19 prevention in newborns. Breastfeeding during the first 6 months of life is the most effective way to prevent gastrointestinal and respiratory infections in infants[2] and may also be of paramount importance in preventing SARS-CoV-2 infection. Studies report that the number of infant deaths in low- and middle-income countries due to COVID-19 could be between 1800 and 2800 in 2020–2021.[1] If mothers with confirmed SARS-CoV-2 infection were separated from their infants and avoided or stopped breastfeeding, an additional 188,000 to 273,000 infant deaths would occur.[1] Human milk contains many compounds that contribute to its massive impact on preventing infant mortality and morbidity from infection. Some of these compounds do not have antigen specificity and do not require activation of the maternal immune system such as lactoferrin, lysozyme, peptides, human milk oligosaccharides or the secretory part of IgA.[3] In contrast, antigen-specific antibodies require the activation of the maternal immune system and allow antibodies to exert multiple functions, including epitope neutralization or antibody-dependent cell cytotoxicity.[3] While many studies have analyzed the presence of antibodies in human milk from infected[4] or vaccinated[5] mothers, very few have investigated the protective properties of human milk against SARS-CoV-2 infection.[4–6] In particular, no studies have formally demonstrated the contribution of maternal IgA and/or IgG to the SARS-CoV-2-specific neutralizing activity of human milk. Here, we provide evidence on the importance of the stage of lactation and more specifically, colostrum, as well as maternal infection for the best protective activity of human milk. Importantly, we demonstrate for the first time the need for IgA to mediate SARS-CoV-2 neutralizing activity.

METHODS

Population and Sample Collection

From March 2020 to July 2020, all pregnant women who underwent routine nasopharyngeal SARS-CoV-2 reverse transcription polymerase chain reaction (PCR) on the day of delivery were recruited at 3 hospitals located in Madrid, Spain. Term pregnant women with a positive reverse transcription-PCR test (n = 28) were categorized into asymptomatic and symptomatic disease groups. Symptoms were mild, the most frequent being fever, anosmia and cough. Only 1 patient was hospitalized for bilateral pneumonia. Healthy noninfected women (n = 24) were included as controls. Breast milk samples collected on day 3 (colostrum), day 15 (transitional milk) and day 27 (mature milk) were used for analyses (see Methods, Supplemental Digital Content 1, http://links.lww.com/INF/F439).

Sample Analysis

We determined the levels of anti-SARS-CoV-2 IgG and IgA by quantitative enzyme-linked immunosorbent assay using receptor binding domain (RBD) and Spike antigens (see Methods, Supplemental Digital Content 1, http://links.lww.com/INF/F439).

To investigate the human milk neutralizing effect, we performed an infectious virus focus reduction neutralization

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test (FRNT). Briefly, serial 2-fold dilutions of human milk were incubated with a fixed amount of virus and then applied to monolayers of Vero E6 cells. Foci formed by non-neutralized infectious virus were stained, counted for each human milk dilution, and compared to the positive virus only control. The percent reduction was calculated (see Methods, Supplemental Digital Content 1, http://links.lww.com/INF/F439).

To investigate the contribution of maternal antibodies in milk to virus neutralization activity, we performed FRNT of milk samples that had been depleted of IgA or IgG by a classical affinity purification using Peptide M and protein G, respectively (see Methods, Supplemental Digital Content 1, http://links.lww.com/INF/F439).

Statistical Analysis

Data were analyzed with the Kruskal–Wallis or Mann–Whitney test, using GraphPad Prism Software version 9.3.1 (La Jolla, CA). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Human Milk Anti-SARS-CoV-2 IgA and IgG Levels

Our first investigations focused on detecting IgA and IgG antibodies reacting against the SARS-CoV-2 Spike (S) and RBD proteins in human milk samples from infected (n = 28) and noninfected mothers (n = 24), collected at 3 different time points.

IgA antibodies binding to SARS-CoV-2 S protein were detected in more than 90% of human milk samples from both infected and noninfected mothers (Fig. 1A and Table, Supplemental Digital Content 2, http://links.lww.com/INF/F440). Upon infection, the levels of anti-S IgA and IgG were significantly increased in symptomatic mothers compared to asymptomatic mothers and uninfected mothers (Fig. 1A, B). The levels of anti-S IgA were 3 times higher in colostrum (day 3) compared to transitional and mature milk (day 15 and day 27) (Fig. 1C and Table, Supplemental Digital Content 2, http://links.lww.com/INF/F440). In contrast to IgA, anti-S IgG antibodies were only detected in 25% of noninfected mothers. Among infected mothers, 57% and 86% of asymptomatic and symptomatic mothers had detectable levels of anti-S

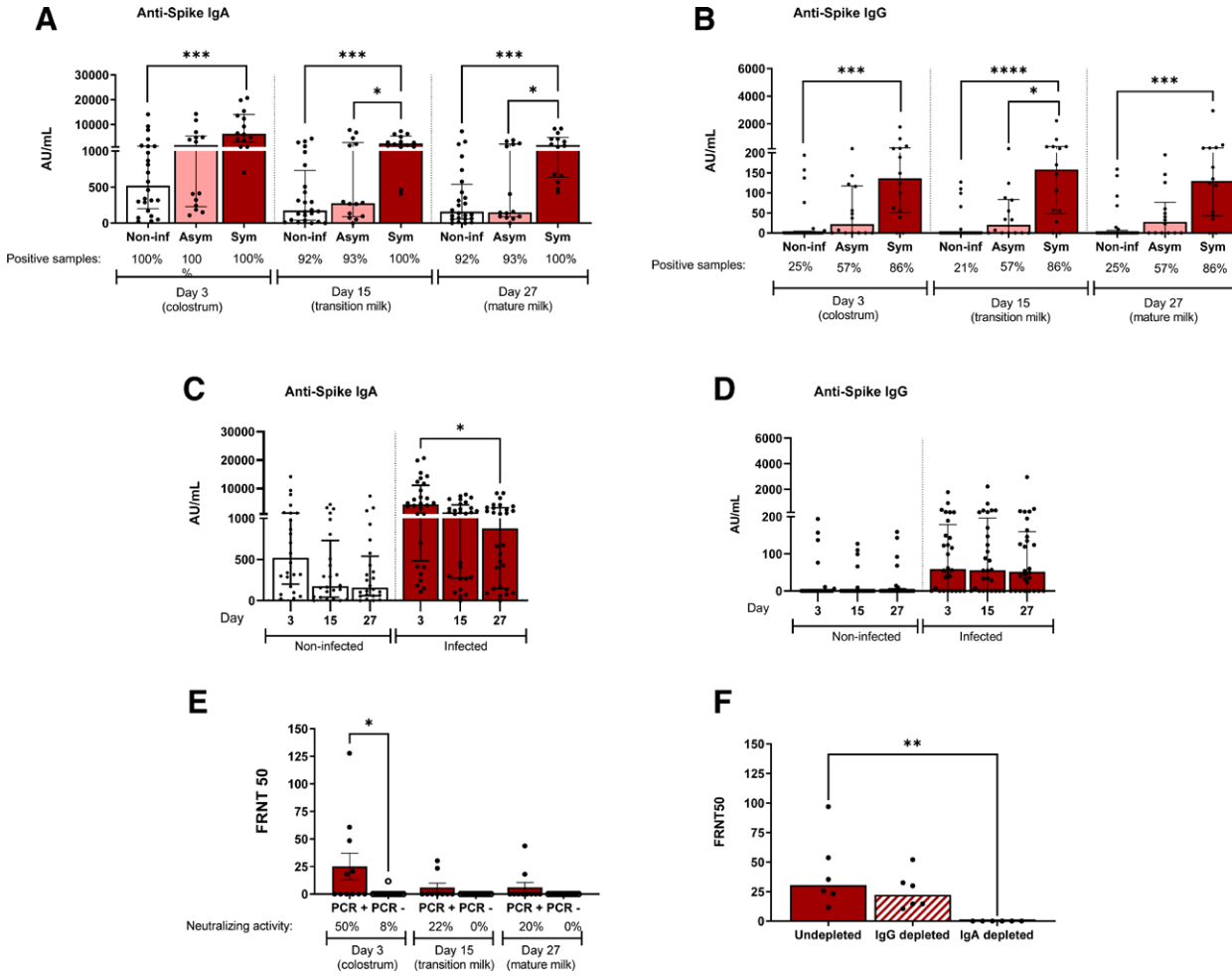

FIGURE 1. Comparison of antispike antibody levels and neutralizing activity in human milk from SARS-CoV-2 infected and noninfected mothers at 3 different time points. Human milk levels of antispike IgA (A and C), antispike IgG (B and D), FRNT50 values in infected versus noninfected (E) and FRNT50 values after IgG or IgA depletion (F). The bars represent the median and the error bars the interquartile range. White bars represent noninfected (noninf., PCR-neg) mothers and red bars represent infected (PCR-pos) mothers. Infected mothers are split into asymptomatic (Asym) and symptomatic (Sym) (A, B). *$P \leq 0.05$ **$P \leq 0.01$, ***$P \leq 0.001$ and ****$P \leq 0.0001$ [statistical test: Kruskal–Wallis (A, B, C, D, F) and Mann–Whitney test (E)]. full color online

IgG, respectively, and levels were higher in symptomatic mothers (Fig. 1B and Table, Supplemental Digital Content 2, http://links.lww.com/INF/F440). In contrast to IgA, anti-S IgG levels remained the same at all time points (Fig. 1D and Table, Supplemental Digital Content 2, http://links.lww.com/INF/F440). Similar observations on the frequency of detection and the levels of IgA and IgG antibodies in uninfected and infected mothers were made for anti-RBD IgA and IgG (Figure, Supplemental Digital Content 3, http://links.lww.com/INF/F441 and Table, Supplemental Digital Content 4, http://links.lww.com/INF/F440).

To determine whether the detection of IgA and IgG binding to SARS-CoV-2 proteins in human milk samples from noninfected mothers was due to nonspecific binding activity, we included the analysis of 30 prepandemic colostrum (day 1–2), transitional (day 7) and mature (day 30) milk samples collected in 2006. Except for anti-S IgA levels in colostrum that were higher in prepandemic samples compared to samples from noninfected mothers (most probably reflecting earlier colostrum stage), we found similar levels and percentages of human milk anti-S and anti-RBD specific IgA and IgG in prepandemic and noninfected mothers across all time points (Table, Supplemental Digital Content 2, http://links.lww.com/INF/F440, Table, Supplemental Digital Content 4, http://links.lww.com/INF/F440 and Figure, Supplemental Digital Content 5, http://links.lww.com/INF/F441), showing the non-epitope binding of milk IgA in all milk samples tested and in a small proportion of IgG.

Human Milk Virus Neutralizing Activity

To get insights into the capacity activity of human milk to protect against SARS-CoV-2 infection, we assessed the neutralization activity of a subset of 24 breast milk samples, comprising 12 from noninfected mothers and 12 from infected mothers. Among noninfected mothers, none of the samples showed neutralizing activity except for 1 from day 3 that was borderline (Fig. 1E). Among the samples from infected mothers, day 3 samples exhibited the highest frequency (50%) and level of neutralizing activity compared to transition and mature milk, 22% and 20%, respectively. Interestingly, neutralizing activity in human milk from infected mothers exhibited a positive correlation with the levels of SARS-CoV-2-specific spike IgA (Spearman $r = 0.62$, $P = 0.0006$; $r = 0.48$, $P = 0.01$ and $r = 0.45$, $P = 0.01$ at day 3, 15 and 27, respectively) and similar data were found with RBD IgA. No correlation was found with the levels of IgG. To confirm that SARS-CoV-2-specific IgA were required for the neutralizing activity of milk from infected mothers, we depleted IgA or IgG from milk from the 6 samples that showed the highest neutralizing activity. As illustrated in Figure 1F, milk depletion of IgA, but not IgG, resulted in the complete loss of neutralizing SARS-CoV-2.

DISCUSSION

The data presented here highlight that the highest protective activity of human milk against COVID-19 is found in the colostrum of infected mothers and depends on anti-SARS-CoV-2-specific IgA. Neutralizing activity in milk from noninfected mothers was rare (1/12) and at a low level, in agreement with a recent study.[6] We found that more than 90% of uninfected mothers have IgA in breast milk that binds to SARS-CoV-2 spike and RBD proteins. The level and frequency of detection of anti-SARS-CoV-2 IgA in noninfected mothers was similar to that found in prepandemic samples as previously described by,[7] highlighting the broad binding activity of breast milk IgA[8] or possible crossreactivity with seasonal coronaviruses.[7] However, the presence of specific antibody in milk from uninfected mothers was not able to neutralize viral activity and prevent it from infecting cells, reiterating the importance of performing functional neutralizing-antibody assays in addition to protein binding detection assays to assess the protective activity of human milk. Only a very small proportion of uninfected mothers had IgG that bound to SARS-CoV-2 spike protein. Infection resulted in increased levels of anti-SARS-CoV-2 IgA and the detection of SARS-CoV-2 IgG in the majority of samples. Further, the levels of anti-SARS-CoV-2 IgG and IgA increased with the severity of infection, consistent with previous studies.[9] Only 1 recent study has extensively investigated the functional properties of human milk anti-SARS-CoV-2 antibodies. Pullen et al[9] showed that milk from infected mothers had neutralizing antibodies and neutrophil phagocytosing antibodies, but had very low levels of other functional antibody activities, such as NK cell activating functions. However, no study has formally demonstrated yet that IgA is essential for neutralizing viral entry into cells. Here we showed that neutralizing activity was associated with high levels of specific IgA in human milk and the depletion of IgA, but not IgG, from milk samples completely abolished the ability of breast milk from infected mothers to neutralize SARS-CoV-2. This observation is of paramount importance because studies have shown that maternal vaccination elicits predominantly IgG rather than IgA, and only IgG tends to persist in breast milk.[10] This strongly suggests the importance of promoting maternal mucosal vaccination, instead of intramuscular, to promote specific IgA production in breast milk.[11] Supporting the importance of the IgA in the defense against SARS-CoV-2, a recent preclinical study demonstrated that nasal administration of IgA antibodies engineered from IgG monoclonal antibodies conferred protection and improved SARS-CoV-2 neutralization by up to 75-fold, compared to their parental IgG antibodies.[12] Finally, our study highlights the importance of the lactation period in providing anti-SARS-CoV-2 immunity to neonates, with neutralizing activity being the highest in colostrum. This could have a dramatic impact on the prevention of COVID-19 infection in newborns, knowing that at least 1 in 3 newborns is not fully colostrum-fed due to formula supplementation,[13] a practice that became even more prevalent when mothers are infected with SARS-CoV-2.[1] While our study did not include a dedicated infant follow-up, investigating the health outcomes of infants born to mothers with different SARS-CoV-2 serostatus in human milk remains a crucial area for future research.

We expect that our findings will be of great interest in our new era where most infants will be exposed to SARS-CoV-2 and will need special help to avoid severe infection.

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