In Reply We wish to thank Zhou et al for their insightful comments on our study.1 The authors make an excellent point that some of the neutralization activity of human breast milk against SARS-CoV-2 may be attributed to other nonimmunoglobulin factors in breast milk.

We agree it is interesting that 4 of 20 prevaccination samples of breast milk exhibited neutralization activity. We proposed as 1 possible explanation a previous SARS-CoV-2 exposure in these participants resulting in anti-SARS-CoV-2 immunoglobulin A (IgA) antibodies in breast milk. Lack of specific IgG in 3 of these 4 samples does not refute prior infection. In our study, we only assessed anti-receptor-binding domain (RBD) antibodies, but antibodies against other unmeasured SARS-CoV-2 epitopes may have contributed to neutralizing activity.2 As the authors suggest, it is also equally possible that other, nonimmunoglobulin components of breast milk contributed to the observed neutralizing activity. However, the source of this neutralization activity was not universal, given 80% of prevaccination samples exhibited no neutralization activity.

The authors suggested that the lack of association between IgA or IgG antibodies and microneutralization activity implies that IgA and IgG were not main contributors to the observed neutralization activity. We respectfully disagree. We would not necessarily expect such an association, given that variation in anti-RBD IgA or IgG epitopes and binding affinities can modify neutralization activity. Furthermore, in a previous study2 of 34 lactating parents with an active COVID-19 infection, we did detect a positive association between anti–receptor-binding domain (RBD) antibodies, but antibodies against other unmeasured SARS-CoV-2 epitopes may have contributed to neutralizing activity.3 As the authors suggest, it is also equally possible that other, nonimmunoglobulin components of breast milk contributed to the observed neutralizing activity. However, the source of this neutralization activity was not universal, given 80% of prevaccination samples exhibited no neutralization activity.

The data presented in Figure 4 demonstrated that purified IgA had distinct neutralizing capacity because this fraction is free of other proteins. We propose attribution of the neutralization activity of the non-IgA fraction to IgG but acknowledge that this fraction contains other components.

The authors refer to work that showed breast milk collected prior to the COVID-19 pandemic had pseudoneutralization activity against SARS-CoV-2.2,3 These results are relevant to this discussion, but caution is warranted when comparing results from pseudovirus microneutralization assays with wildtype microneutralization assays, as conflicting results have been reported for these assays studying microneutralization activity of breast milk from individuals thought to have COVID-19 against pseudotype and wildtype SARS-CoV-2.3

The authors also suggest that lactoferrin may be a source of some of the neutralization activity observed in the non-IgA fraction, as lactoferrin is known to have wide-reaching antimicrobial activity and was recently associated with inhibition of SARS-CoV-2 via multiple avenues in vitro.4 However, other studies have demonstrated that recombinant breast milk lactoferrin shows limited inhibition of SARS-CoV-2 compared with breast milk in vitro.5 If lactoferrin was a significant source of neutralization activity against SARS-CoV-2, we would expect to see consistently higher microneutralization activity in all prevaccine samples as lactoferrin is found in a large proportion of whey proteins.

We agree that previous research makes a compelling case that other nonspecific and nonimmunoglobulin components of breast milk may contribute to neutralization capacity against SARS-CoV-2. We also emphatically agree that future research detailing such components is a valuable endeavor to identify novel therapeutics and optimize recipient infant protection.

Bridget E. Young, PhD
Kirs M. Järvinen, MD, PhD
Antti Seppo, PhD

Author Affiliations: Department of Pediatrics, Allergy and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

Corresponding Author: Kirs M. Järvinen, MD, PhD, Department of Pediatrics, Allergy and Immunology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave, PO Box 777, Rochester, NY 14642-0001 (kirs.jarvinen-seppo@urmc.rochester.edu).


Conflict of Interest Disclosures: Dr Young reported grants from National Institutes of Health and the Gerber Foundation. No other disclosures were reported.