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LETTER TO THE EDITOR

WILEY

Potential protective effects of breast milk and amniotic fluid against novel coronavirus SARS-CoV-2 through decoy receptors

1 | OBJECTIVE

Infection of pregnant women during previous coronavirus-mediated pandemics, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), was associated with high rates of fetal and maternal demise. However, the characteristics of COVID-19 in pregnant and non-pregnant women are very similar, and while severe COVID-19 in pregnancy brings increased risk of preterm birth and intensive care admission,[1] most pregnant women, be they symptomatic or asymptomatic for SARS-CoV-2 infection, do not experience severe complications in pregnancy.[2] Similarly, cases of SARS-CoV-2 vertical transmission are rare and there is low risk of serious disease for the neonate.[3] Understanding the mechanisms of resilience against severe COVID-19 in pregnant women and the newborn is critical to ensure ongoing vigilance in care and to provide insight into disease pathogenesis and therapeutic opportunities.

Angiotensin-converting enzyme 2 (ACE2), CD26, CD147, and neuropilin-1 (NRP-1) are some of the key molecules identified as contributing to the entry of coronaviruses such as SARS-CoV-2 into human cells.[4] There is immense interest in these and viral entry facilitating proteases such as transmembrane serine protease 2 (TMPRSS2) as therapeutic targets for limiting infection. Soluble forms of viral entry receptors have been postulated to act as viral traps for SARS-CoV-2 by preventing interaction of the virus with membrane-bound forms.[5] We predict that elevation of these at the maternal-fetal interface contributes to the lack of vertical transmission and limits severity of disease from SARS-CoV-2 infection in the fetus and neonate.

1.1 | Hypothesis

Soluble (s) ACE2, sCD26, sCD147, and sNRP-1 are elevated at the maternal-fetal interface.

2 | STUDY DESIGN

Samples of amniotic fluid (AF) collected at >37 weeks of gestation (North of Scotland Research Ethics Committee: 06/S0801/77) and breastmilk (BM) collected at 2 (2W) and 6 weeks (6W) postpartum (Wales Research Ethics Committee: 2004/024) archived prior to the COVID-19 pandemic were available. All samples were rendered cell free by centrifugation and breastmilk defatted prior to archiving. ELISAs (DuoSet, R&D Systems) were performed to determine the concentration of sACE2 (catalogue number: DY933), sCD26 (DY1180), sCD147 (DY972) and sNRP-1 (DY3870) in AF and 2W and 6W BM (Figure 1A). Statistics were performed using a one-way ANOVA; a p value of $< .05$ was deemed significant. Immunoblotting analysis (ACE2 (15983), CD26 (67138), Cell Signaling Technologies; CD147 (AF972), Bio-Techne; NRP-1 (ab81321), Abcam) was used to confirm findings from the ELISAs and reveal different isoforms (Figure 1B). The blots were checked for non-specific binding by treating the membranes with secondary antibodies only; this revealed no non-specific bands.

3 | RESULTS

All samples were rich in the molecules of interest; BM had significantly higher sACE2 and sNRP-1 but lower sCD26 than AF (Figure 1A). Comparable levels of these soluble receptors in adult plasma are denoted by the dashed lines on the graphs. These average markers are from analysis of adult plasma concurrently using the same immunoassay. Different isoforms of sACE2, sCD26, and sCD147 existed in AF compared with BM (Figure 1B) and might provide information about the origins of soluble forms of these mediators[6] and dictate their biological function.[7] For example, the shorter isoform of ACE2 lacks SARS-CoV-2 spike high-affinity binding sites[7] so AF sACE2 might be less efficient in acting as a viral decoy. The lower molecular weight (MW) form of CD26 is typically associated with its membrane-bound form (which can be shed) compared with the higher MW of the naturally occurring soluble form,[6] suggesting that BM sCD26 originates by shedding from mammary epithelial cells whereas AF contains the naturally occurring soluble form. As these two isoforms have shared and distinct properties, there are likely different functional effects of sCD26 in AF and BM that might include the ability to bind SARS-CoV-2. CD147 evidently has various degrees of glycosylation, with BM exhibiting numerous

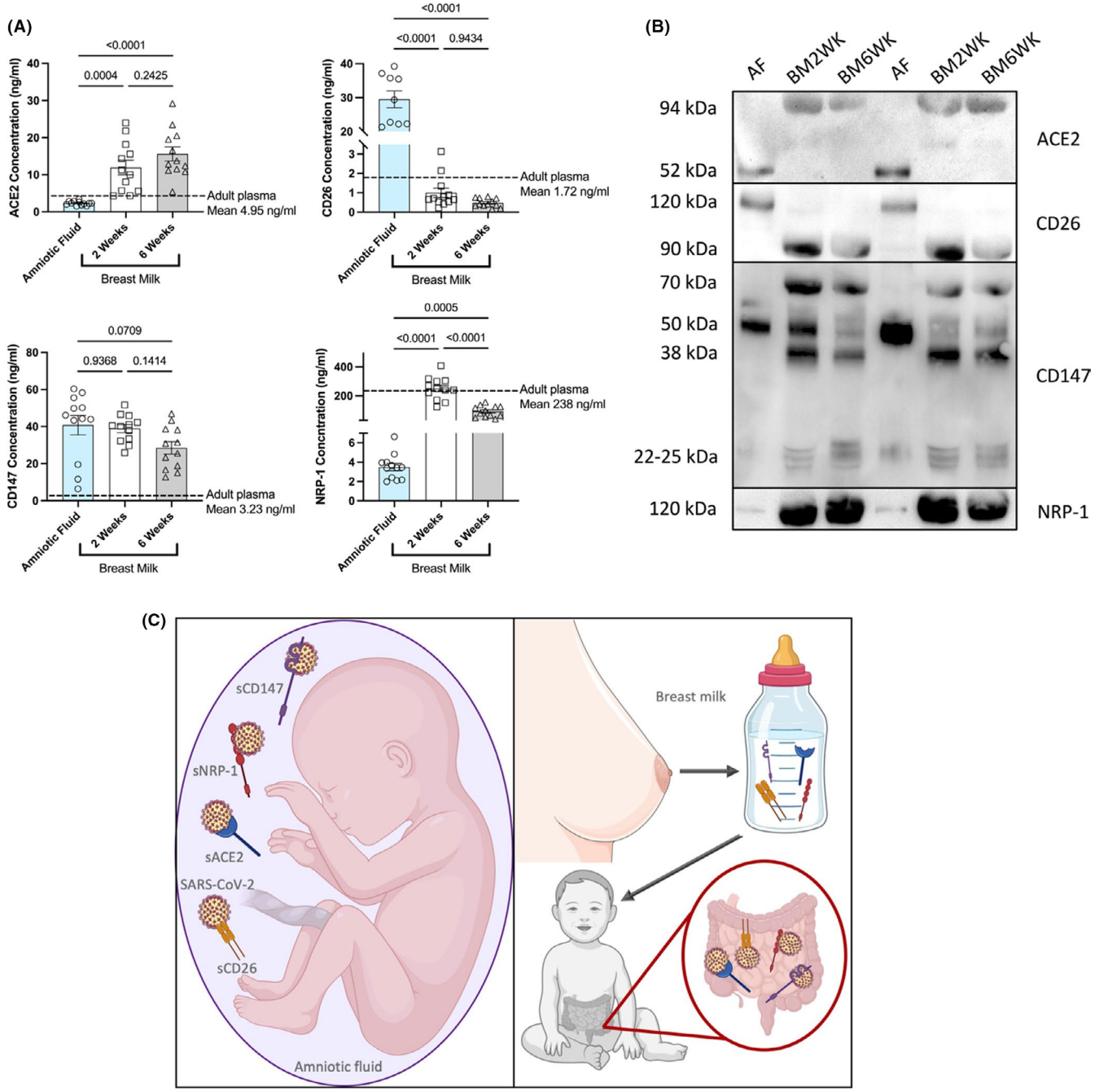

FIGURE 1 Presence of soluble SARS-CoV-2 host cell entry-associated molecules in amniotic fluid (AF) and breastmilk (BM) at 2 and 6 weeks postpartum. (A) Soluble molecules (ACE2, CD26, CD147, and NRP-1) were measured in AF and BM using ELISAs. (B) The presence of these soluble molecules was confirmed with Western blotting, and isoforms were identified. (C) The presence of these soluble molecules in AF and BM which the baby ingests and aspirates may confer resistance to SARS-CoV-2 infection and/or severe COVID-19 (the images in (C) were created with BioRender)

isoforms in comparison with the one isoform observed in AF. The level of glycosylation is correlated to CD147 function, with low glycosylation (LG) forms being unable to self-aggregate and stimulate matrix metalloproteinase (MMP) induction of which the high glycosylation (HG) forms are capable.[8] SARS-CoV-2 severity has been associated with an increase in MMP activity.[9,10] The presence of the LG forms in the BM and AF suggests a tighter regulation of MMP activity, potentially preventing severe disease.

4 | CONCLUSION

These results suggest that both AF and BM are rich in soluble forms of at least some of the molecules that regulate SARS-CoV-2 host cell entry and could help protect the fetus and neonate from infection by acting as decoy receptors and contributing to innate immune protection in the gastrointestinal and respiratory tracts (Figure 1C). Such a mechanism might also be relevant for other viral infections. Future

work should focus on evaluating if AF and BM inhibit viral entry into host cells and comparing the relative efficacy of different isoforms. This could reveal a novel protective mechanism for the baby against SARS-CoV-2 infection and/or severe disease and provide further evidence for a protective role for breastfeeding.

KEYWORDS

amniotic fluid, breastmilk, neonate, SARS-CoV-2

CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTION

April Rees: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (lead); Validation (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (equal). **Steve Turner:** Resources (equal); Writing-review & editing (equal). **Catherine A. Thornton:** Conceptualization (equal); Funding acquisition (lead); Project administration (supporting); Resources (lead); Supervision (lead); Writing-review & editing (lead).

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