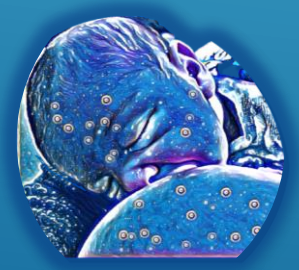
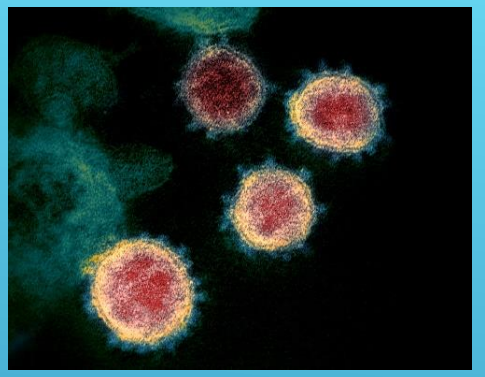
Immunology and safety of COVID-19 infection and vaccination during lactation

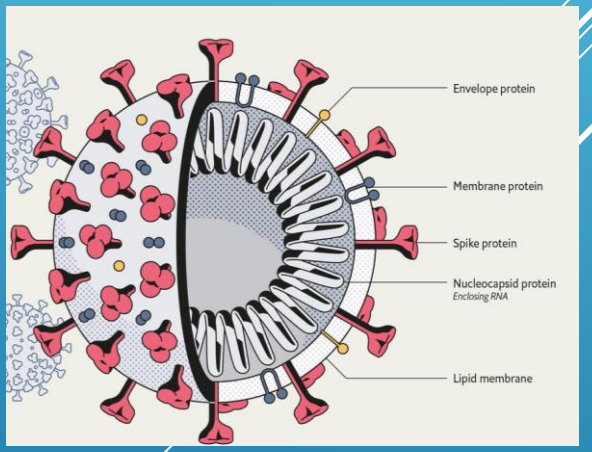
Rebecca Powell, PhD, CLC
Assistant Professor, Division of Infectious Diseases
Icahn School of Medicine at Mount Sinai
New York, NY, USA

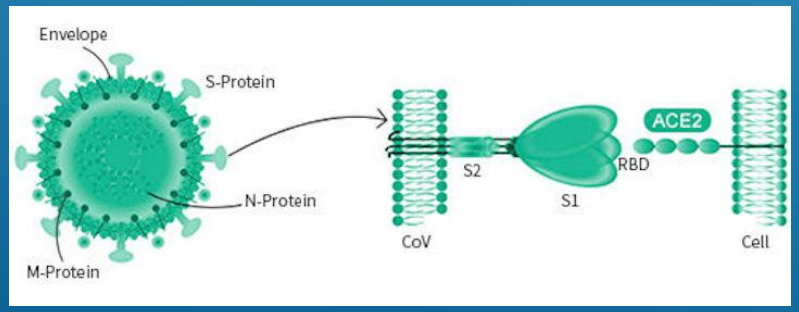


SARS-COV-2 (COVID-19)

- Like other coronaviruses, SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins
- The N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope
- The spike protein is the protein responsible for allowing the virus to attach to and fuse with the membrane of a host cell
- Spike has strong affinity for a protein on human cells called Angiotensin-converting enzyme 2 (ACE2)
- An enzyme attached to the cell membranes of cells in the lungs, arteries, heart, kidney, and intestines
- Spike is a highly important protein that is the main target for functional antibody binding







SARS-CoV-2 transmission

- The highly dominant transmission route for SARS-CoV-2 is via inhalation of respiratory droplets containing virus particles, some of which may be extremely small, forming what are considered airborne particles forming an aerosol
  - These droplets are formed from exhalation of any kind by an infected person, including breathing, talking, or even more so, from sneezing, coughing, or singing.
  - Though risk of contact with these aerosols are considerably higher when a social distance is not maintained, it has been demonstrated that transmission can occur over long distances indoors
  - Notably, infectious virus has been measured in the air for as long as 3 hours after particles were dispersed
- It is now well-documented that an infected person can transmit to another in as few as 3 days
  - Infected people are typically the most likely to transmit (i.e. transmitting the highest viral loads) very early in this transmission window, often before symptoms
    - Asymptomatic and/or presymptomatic transmission of SARS-CoV-2 is believed to account for nearly 60% of global transmission
- Though surface transmission is possible, substantial evidence indicates that this mode of transmission is of virtually no consequence

SARS-CoV-2 transmission

- Significant levels of viable SARS-CoV-2 is found in an infected person's saliva; however there is little evidence that oral transmission is a major route of infection, as the acidic stomach environment likely destroys the virus
- SARS-CoV-2 can replicate well in gut tissue and virus has been cultured from feces
  - There is evidence that a minority of cases globally may be due to fecal-oral transmission, particularly among children and/or where access to hygienic toileting is not available
- Viable virus has been detected in urine in certain cases, though this is not believed to be a source of significant global spread
- Determining the true risk of vertical transmission of SARS-CoV-2 from mother to infant in utero or during delivery via vaginal secretions is highly convoluted by respiratory exposure at birth, though placental infection has been documented using immunostaining of tissue
- **One major, important question from the outset of the pandemic has been whether a SARS-CoV-2-infected person's milk might be a vehicle for SARS-CoV-2 transmission**.
  - This question stirred considerable panic, and caused mothers and babies to be separated, particularly at birth, often with adverse consequences to establishment of the breastfeeding relationship, in some cases, irrevocably

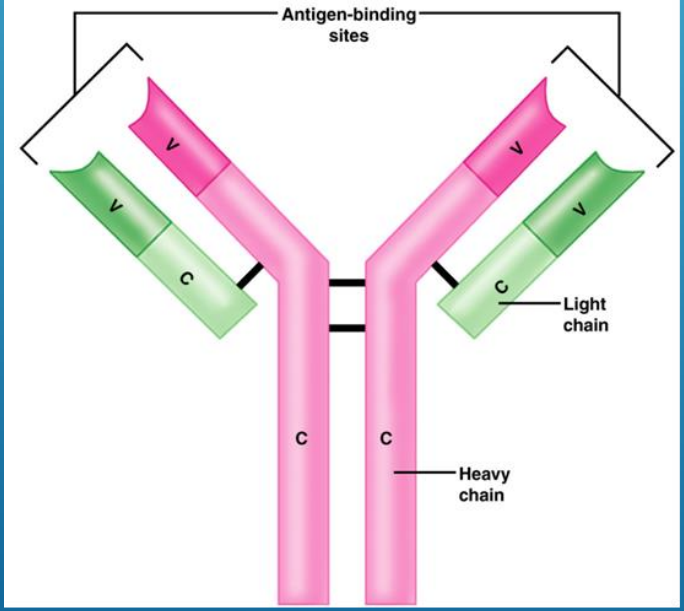
- **Now, more than 2 years into the pandemic, this question has been explored by several groups.**
  - There has been no evidence that SARS-CoV-2 transmits via human milk.
  - Numerous studies of colostrum and mature milk from women with acute SARS-CoV-2 infection have failed to find any viral RNA in milk samples.
    - This includes several early studies conducted in Wuhan, China that examined milk from infected mothers of newborns by RT-PCR
  - One early study from Italy and several of the Chinese reports documented both symptomatic and asymptomatic SARS-CoV-2-infected infants with probable mother-to-infant transmission (likely via respiratory droplets) or unknown transmission routes, still with no evidence of viral RNA in the mothers' milk samples
  - As cases of infected mothers continued to be analyzed, viral RNA was ultimately identified in a small minority of milk samples studied
  - In a single case, SARS-CoV-2 RNA was detected in milk at days 4, 5, and 7 after symptom onset, with subsequent samples found to be negative
  - In another study that included 18 women providing 64 milk samples, 1 milk sample had detectable SARS-CoV-2 RNA, on the day of symptom onset
    - Collection methods in these reports do not always include masking, cleaning of the breast, or even handwashing to avoid contamination of viral RNA from the donor's respiratory droplets.
      - viral RNA in such studies has been found on the breast skin
- **Even where viral RNA was detected in milk, infectious SARS-CoV-2 particles were not.**

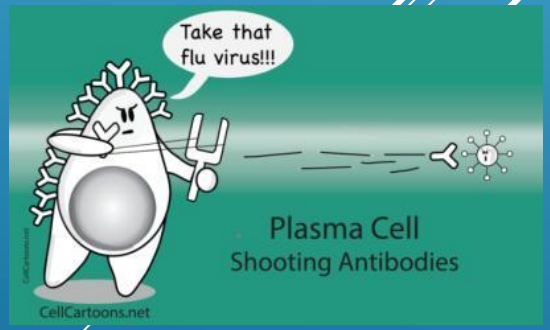
- Several meta-analyses to date have failed to find evidence of worsened health outcomes for infants of any age breastfed by infected mothers, or increased rates of infection for these infants compared to those who are formula-fed
  - In one prospective study of 19 women in New York City testing positive for SARS-CoV-2 infection at delivery, RT-PCR of colostrum samples found all but one to be negative for viral RNA, with no evidence of infection in the baby of the mother with the positive milk sample, nor in any other baby in the study
  - A larger NYC-based study of mothers with confirmed or suspected SARS-CoV-2 infection at delivery included 101 newborns monitored and tested while the mothers were still in hospital, with 55 followed for 10-25 days after birth
    - This study found only 2 infants to be SARS-CoV-2-positive (very low viral load) just after birth, with none of the infants in the study exhibiting any COVID-19 pathology, despite most newborns breastfeeding directly from the mother (with appropriate mask and hand hygiene encouraged) and rooming-in

Given the clear safety profile of milk from SARS-CoV-2-infected mothers, the WHO, CDC, and all major relevant associations recommend that infants not be separated from SARS-CoV-2-infected mothers and that breastfeeding should be established and not disrupted (depending on the mothers' desire to do so), in combination with masking and other hygiene measures

Antibodies

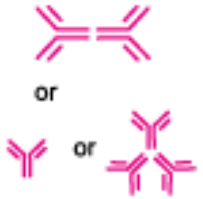
- Protective protein produced by the immune system in response to the presence of a foreign substance
- Antibodies recognize and latch onto the foreign substance in order to remove them from the body.
- When an alien substance enters the body, the immune system is able to recognize it as foreign because it's proteins differ from those found in the body.





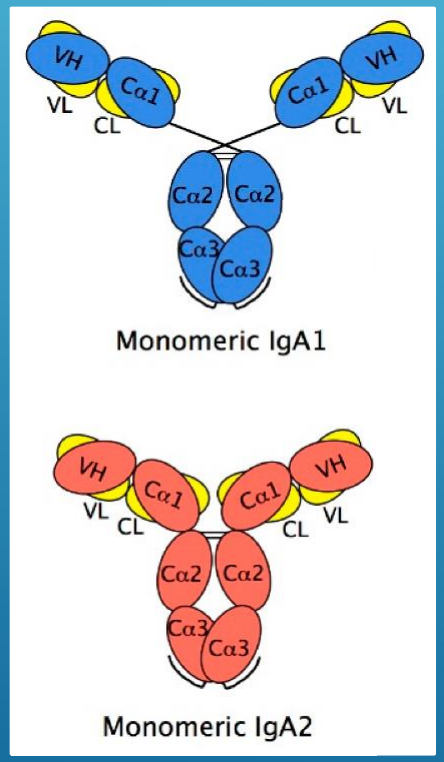
Not all antibodies are created equal

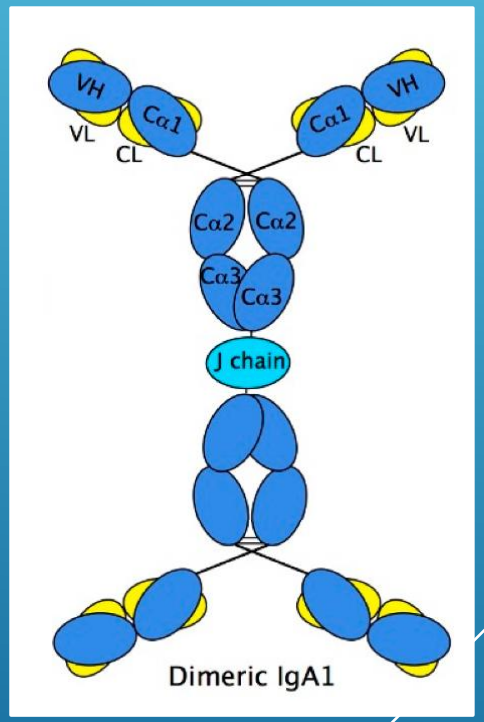
Types and characteristics of antibodies

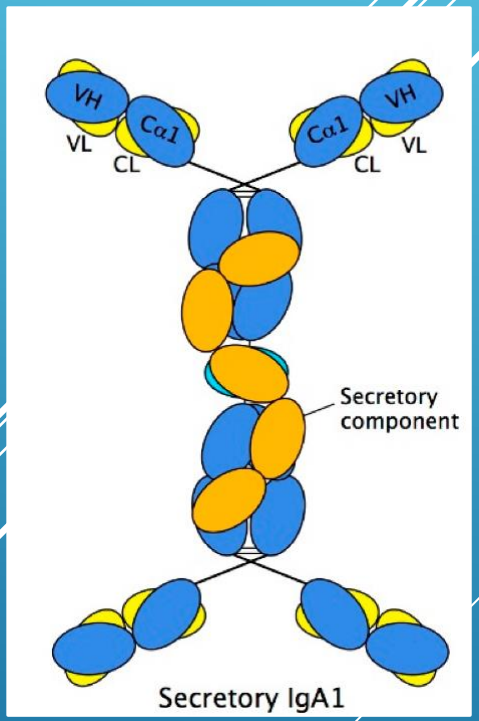
IgG		• Highest opsonization and neutralization activities. • Classified into four subclasses (IgG1, IgG2, IgG3, and IgG4).
IgM		• Produced first upon antigen invasion. Increases transiently.
IgA		• Expressed in mucosal tissues. Forms dimers after secretion. **~90% of milk antibody**
IgD		• Unknown function.
IgE		• Involved in allergy.

Antibodies in human milk

- Human milk antibody is ~90% IgA and 8% IgM, nearly all in secretory (s) form (sIgA/sIgM)
  - Polymeric antibodies complexed to j-chain and secretory component (SC) proteins





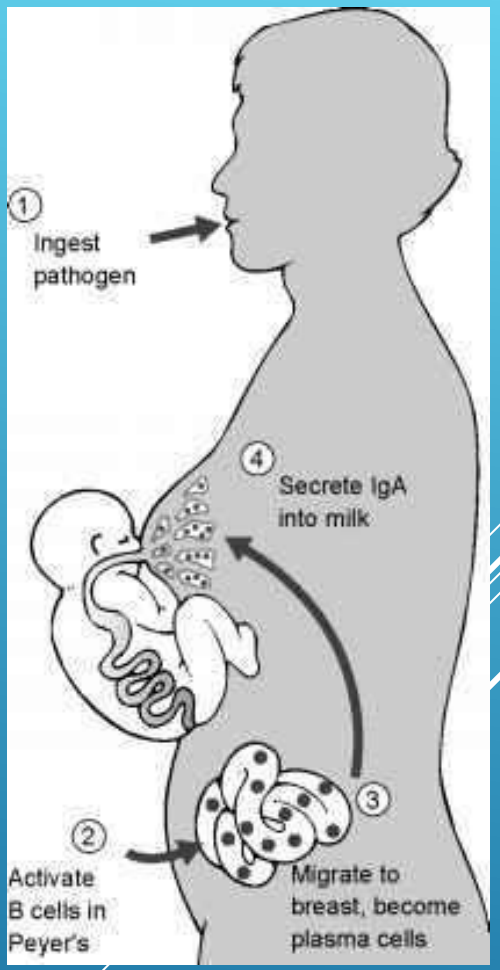


SC is critical for the protection of antibodies against degradation in harsh mucosal environments

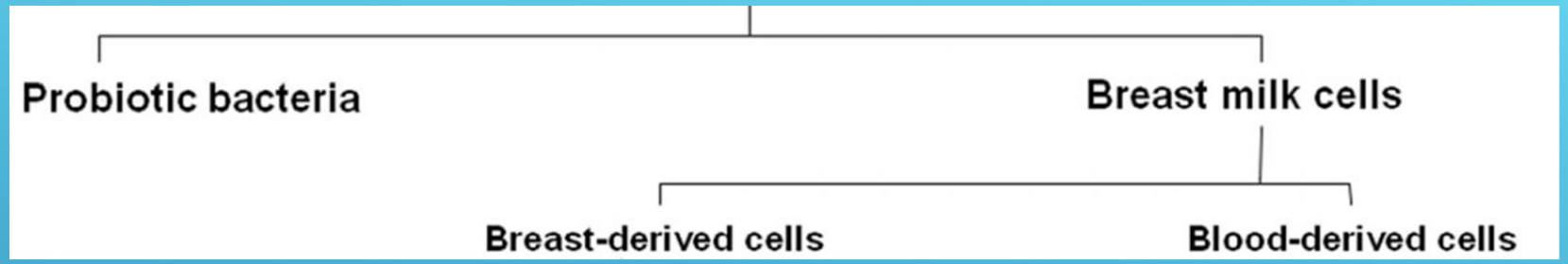
Antibodies in human milk

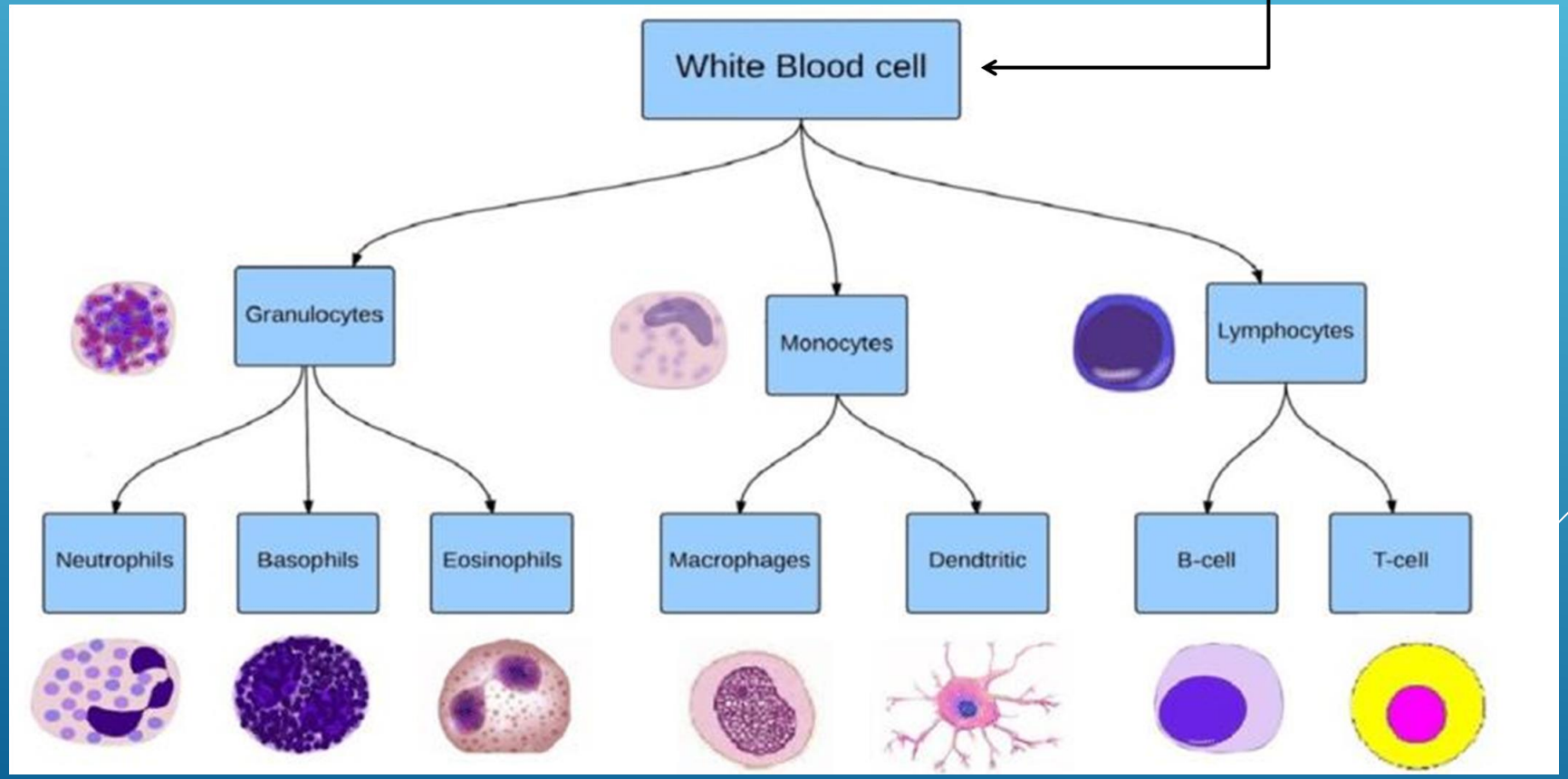
- In humans milk IgG originates mainly from serum
- B cells that produce milk sIgA/sIgM originate from the mucosa-associated lymphoid tissue (MALT)
  - Mainly originate in the gut (GALT) but some respiratory mucosa involvement as well

Known as the <u>entero-mammary link</u>

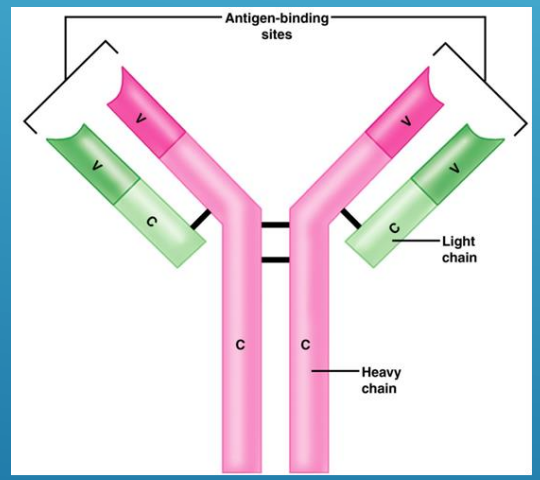


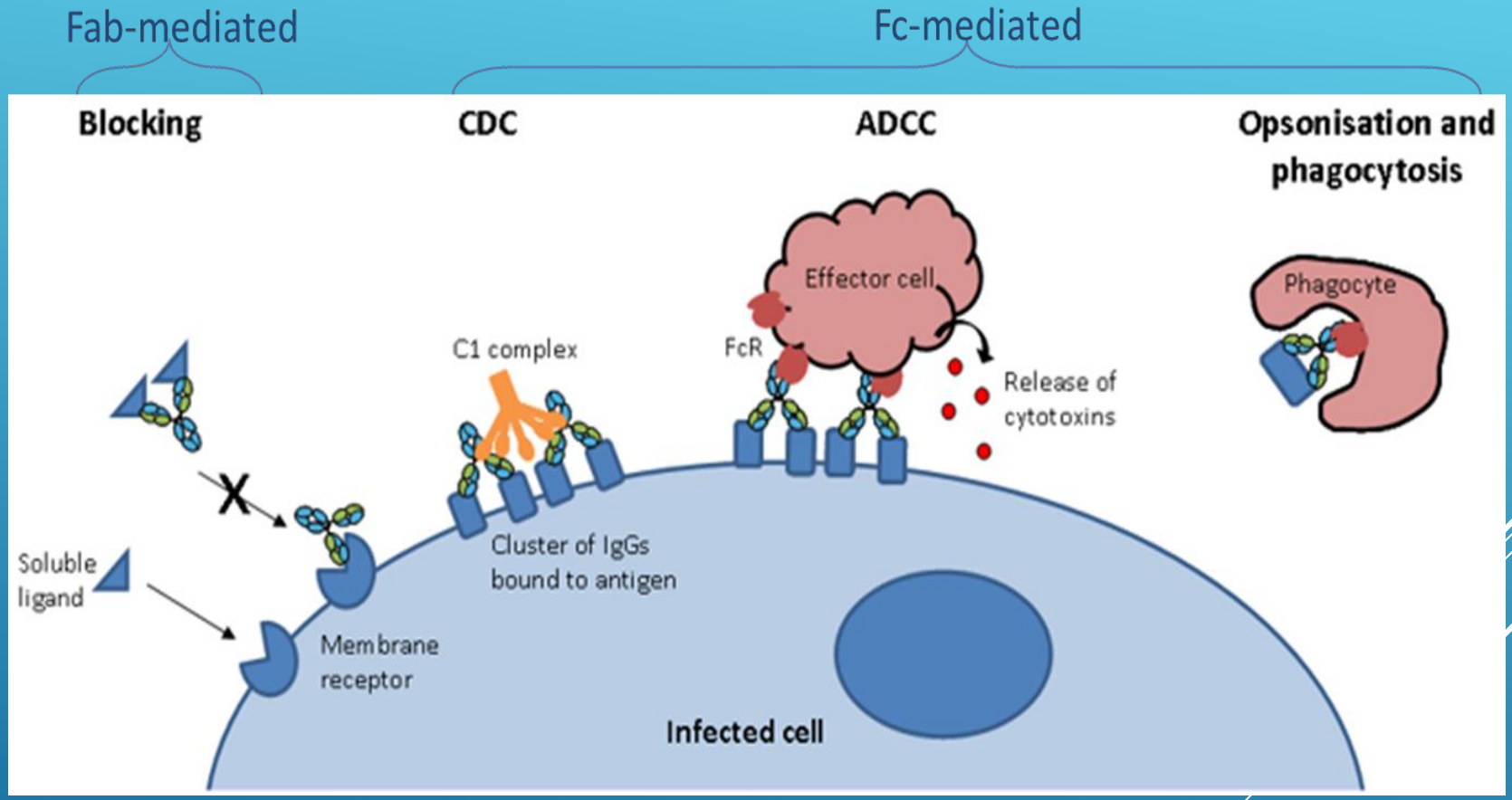
Cells in Human Milk





Antibody Function





Passive immunization by milk antibodies

- Passive protection works by coating the mucous membranes of the baby's mouth, upper respiratory area and digestive tract
  - Provides a layer of protection that may stop cells in those areas from being infected should the baby be exposed to virus, or potentially mitigate that infection, ie slow down viral replication.
  - Effect is temporary and the antibodies would be expected to be degraded or wash away within a few hours
    - Need to be replenished with every feed.
  - We expect the effect to be dose dependent, meaning the more milk the better and the best case scenario being an exclusively chest/breastfed baby who would get maximum milk exposure with no other fluids/foods.
  - Protective effect of the milk is also going to be affected by what else they are eating and drinking as that may more quickly wash away or degrade the antibodies.
  - When baby weans, the protection is gone. It does not last beyond a few hours after the most recent feed.
- Milk antibodies do not pass through the baby’s digestive system into the bloodstream
  - Would not expect them to show up in a blood test done on the baby.
  - This is different than antibodies passed via the placenta to a fetus in utero.
    - In this case, those antibodies do enter the baby's blood, they are born with these antibodies in their blood, and they last around 3 to 6 months.

Our COVID-19 studies

- SARS-CoV-2 infection study
  - Over 800 COVID-19-recovered participants enrolled
  - Today will outline data from 75 people

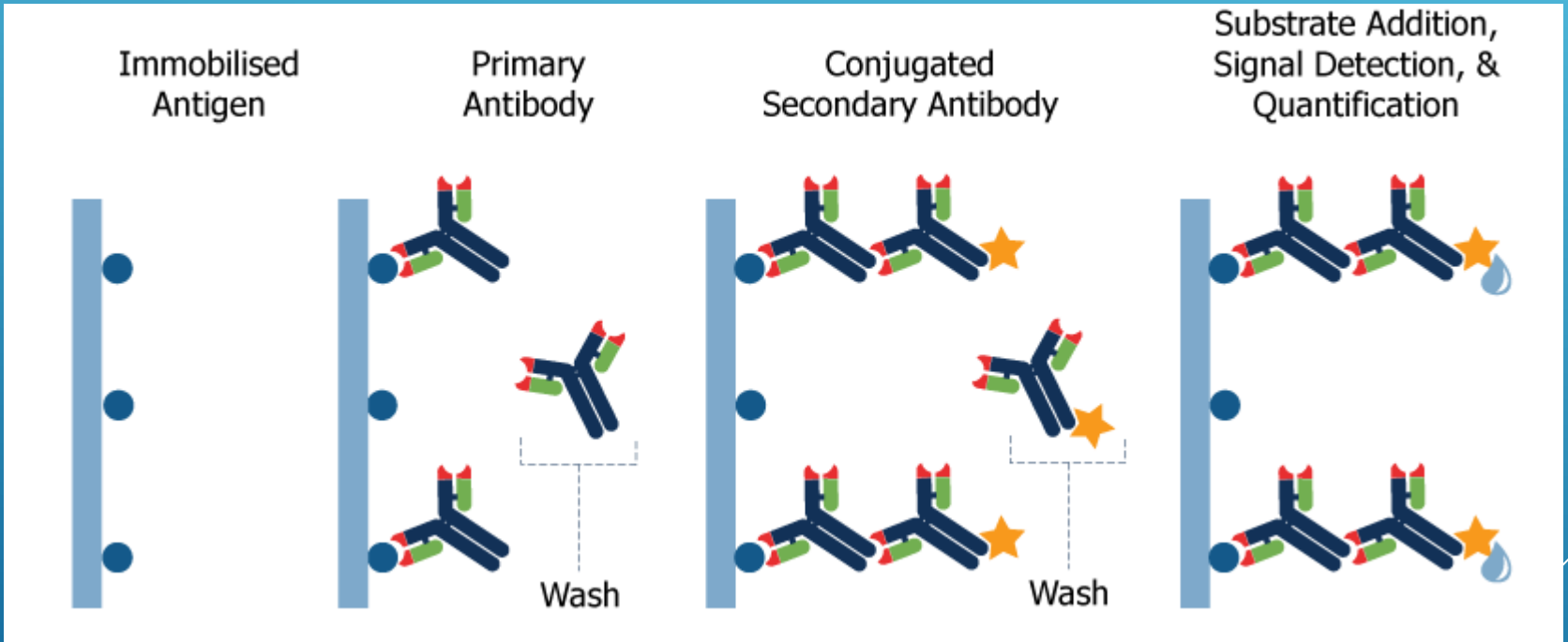
- COVID-19 vaccine study
  - Over 500 enrolled
  - Received Moderna/Pfizer/J&J/AstraZeneca
  - Pairs of samples analyzed: pre-vaccine and at peak antibody time point of 14-28 days (depending on vaccine type)
  - Today will outline data from 60 people

Participant/sample info

- ~30mL (1oz) of milk is obtained per sample from consented study participants using electronic or manual pumps.
- Participants had a (PCR) confirmed SARS-CoV-2 infection or had been vaccinated against COVID-19
- Milk was obtained ~4 weeks after infection or at various post-vaccine time points
- Participants continue to pump monthly samples
- Milk is pumped by the participants and frozen in their homes until sample pickup.

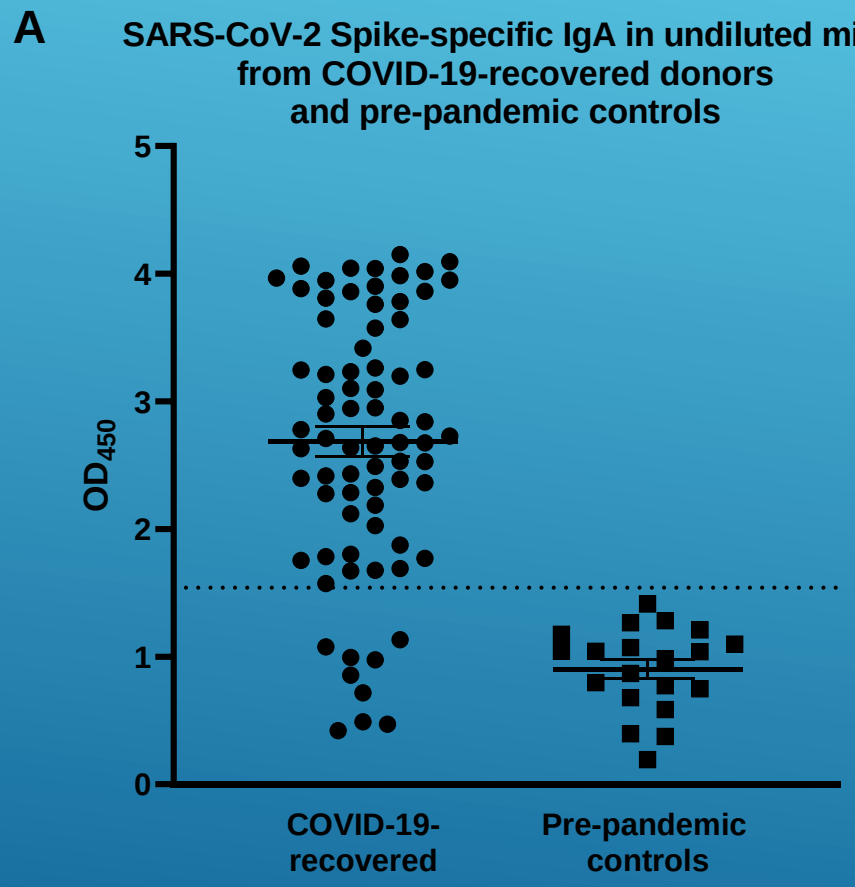
Testing milk for SARS-CoV-2 antibodies by *ELISA*

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying soluble substances such as the level of antibodies in milk specific for a viral protein



Results – COVID-19-recovered donors

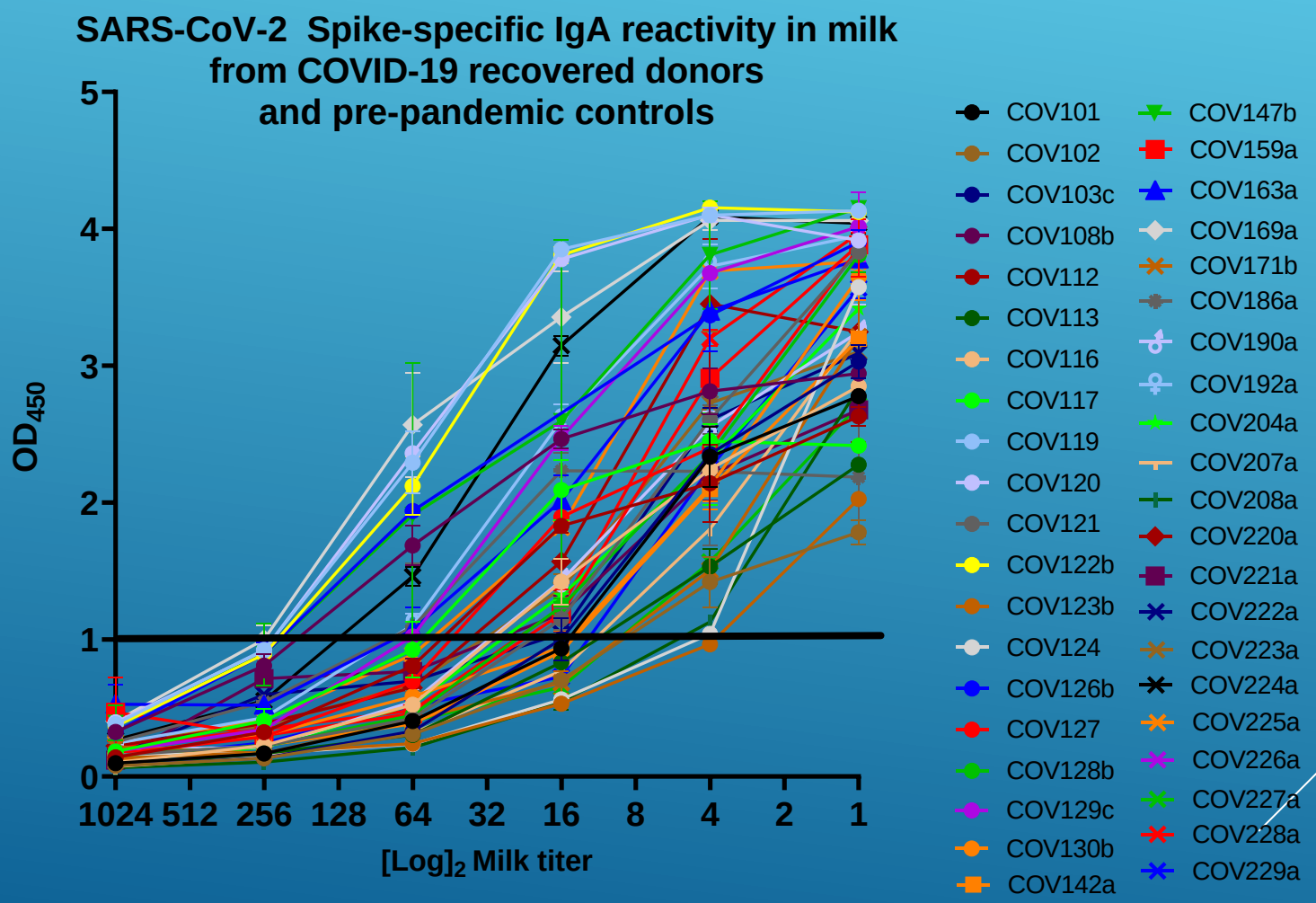
Initially, undiluted milk samples obtained 4-6 weeks post-infection from 75 COVID-19-recovered donors, and 20 pre-pandemic milk samples obtained prior to December 2019 were screened in our **IgA** ELISA against SARS-CoV-2 Spike



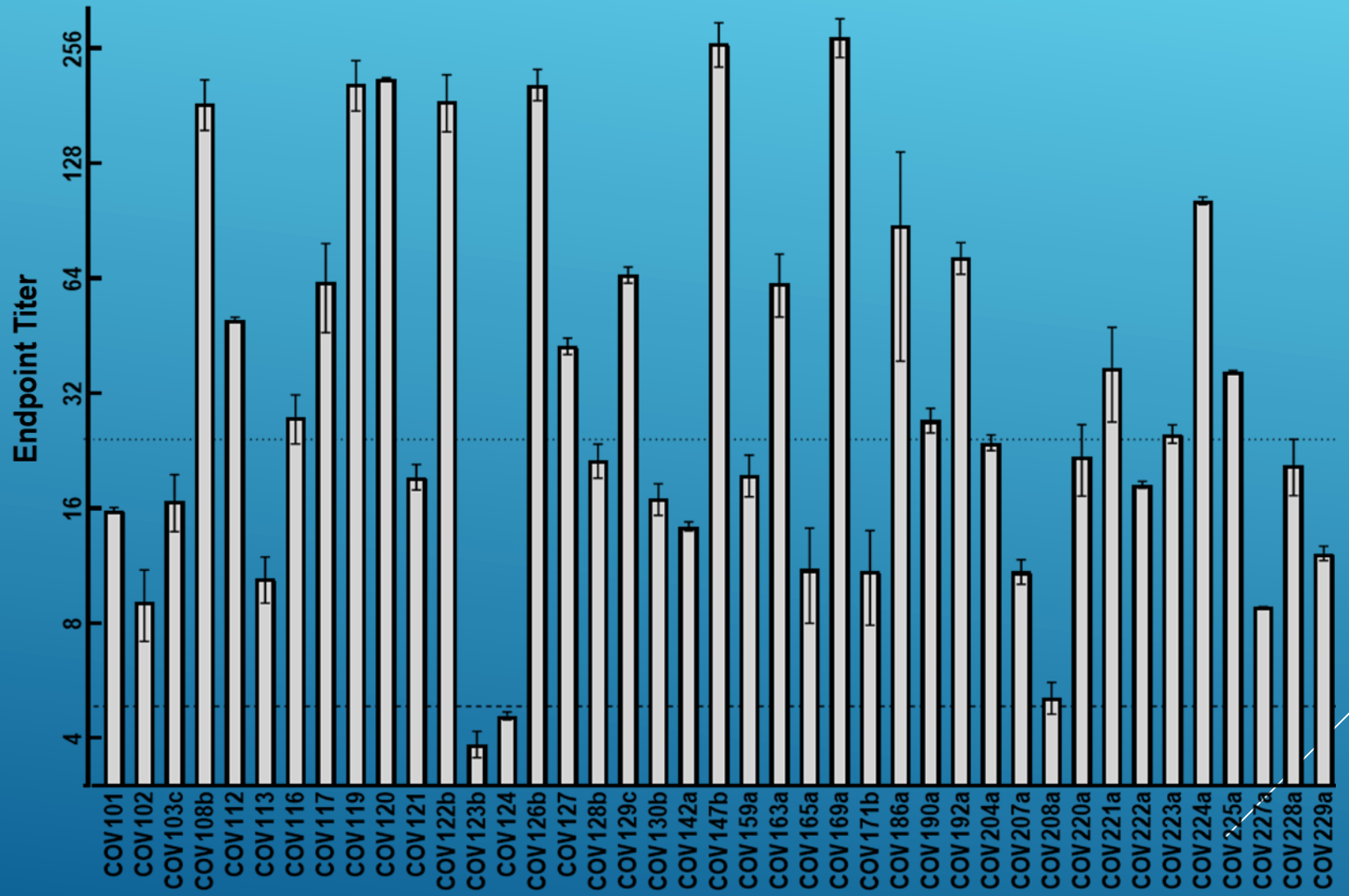
- 88% of milk samples obtained form COVID-19-recovered donors contain significant levels of SARS-CoV-2-specific IgA

Results

Following this initial screening, 40 of the Spike-positive samples were further titrated to determine binding endpoint titers as an assessment of Ab affinity and/or quantity

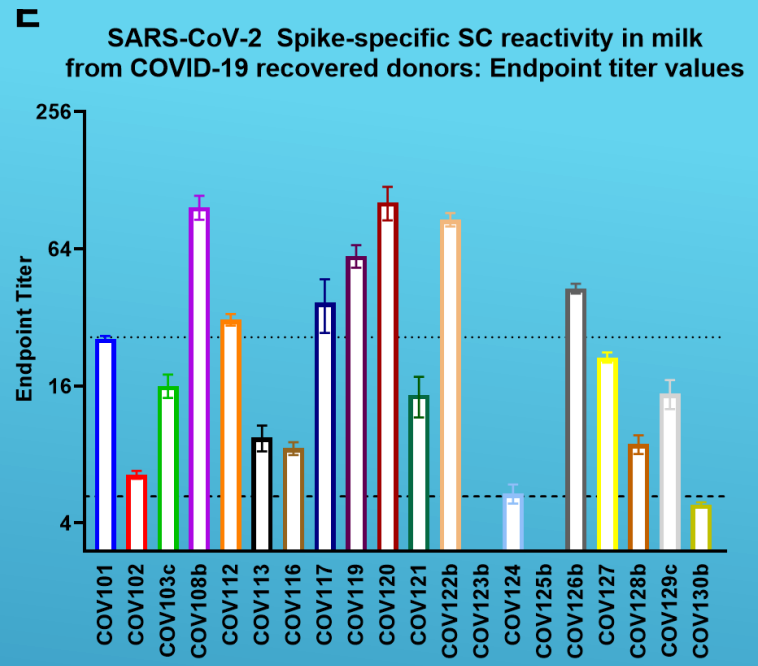


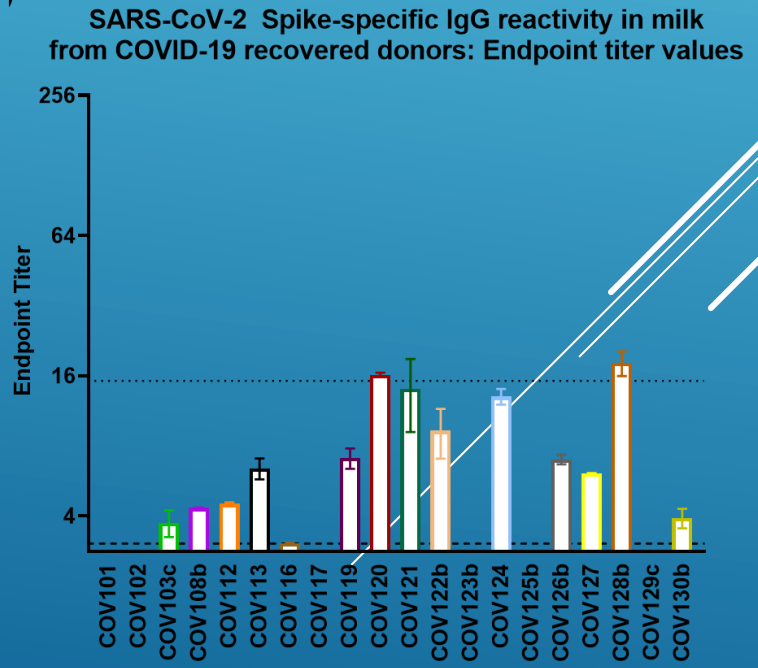
It was found that 38/40 (95%) of Spike-reactive samples exhibited positive IgA endpoint titers and 19 of these samples (50%) were ≥5x above the endpoint titer positive cutoff, therefore designated as ‘high-titer’



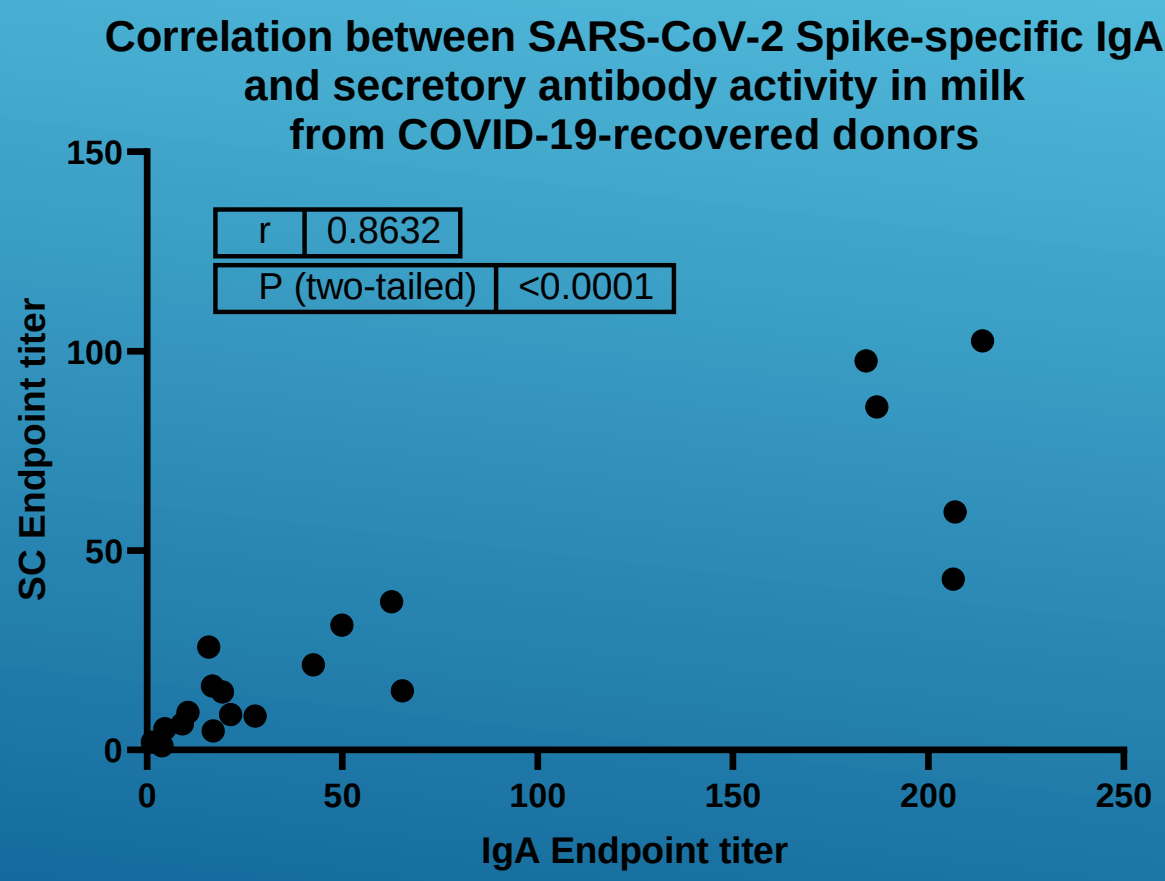
- Additionally, 20 samples were also assessed for Spike-specific secretory Ab and IgG.
  - (95%) exhibited Spike-specific secretory Ab binding activity
  - Of the samples found to be high-titer for Spike-specific IgA, 70% were also high-titer for specific secretory Ab

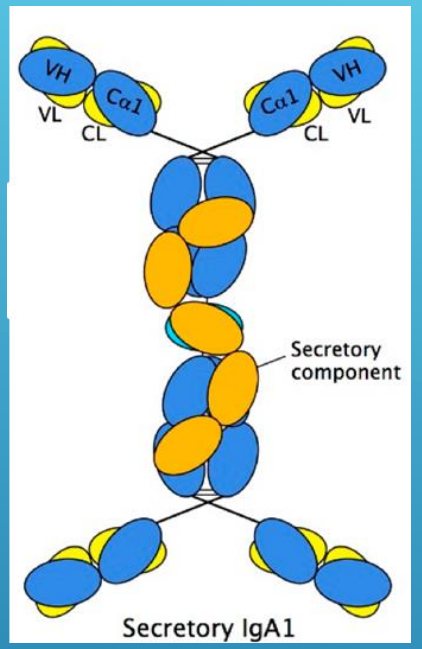
- 75% of samples from COVID-19-recovered donors were positive for Spike-specific IgG
  - 2/15 were designated as high titer (13%).



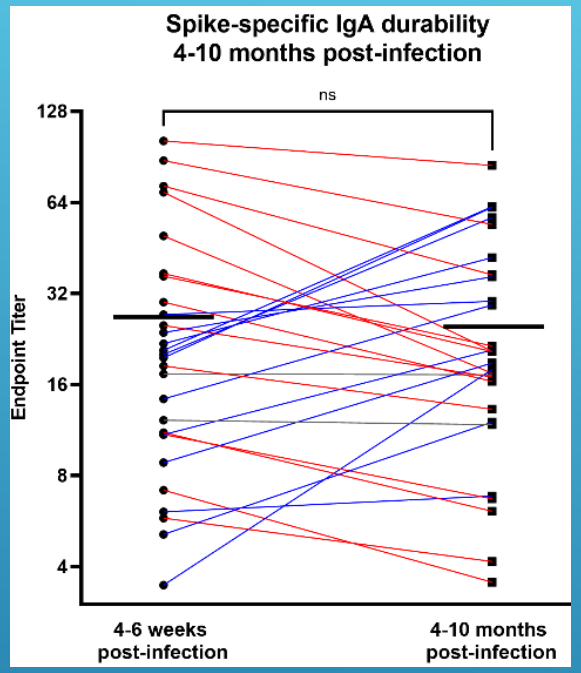


IgA and SC Ab binding was compared**. It was found that these values were highly correlated, suggesting that most IgA measured was in secretory form (sIgA)**, a highly durable form of antibody

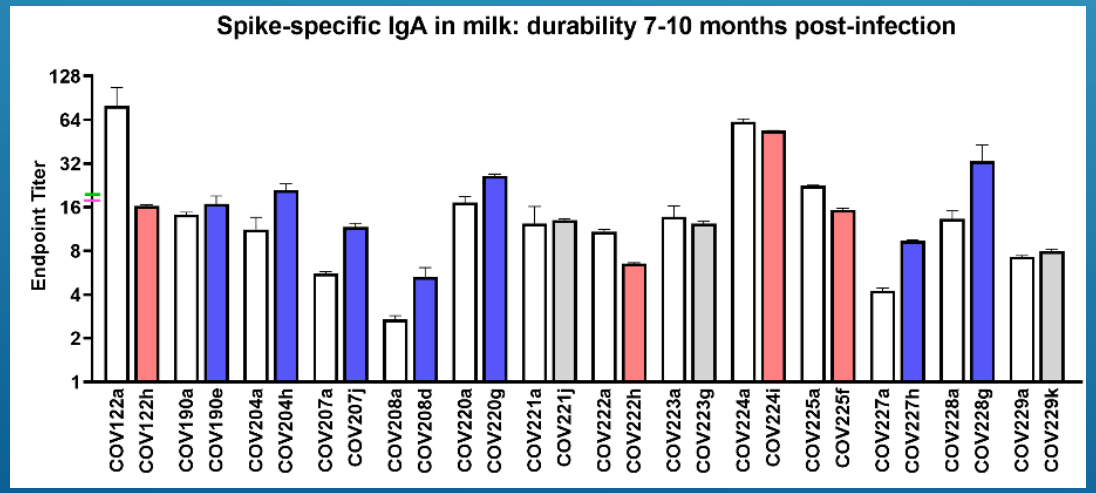




Durability of the milk IgA response up to 10 months after infection

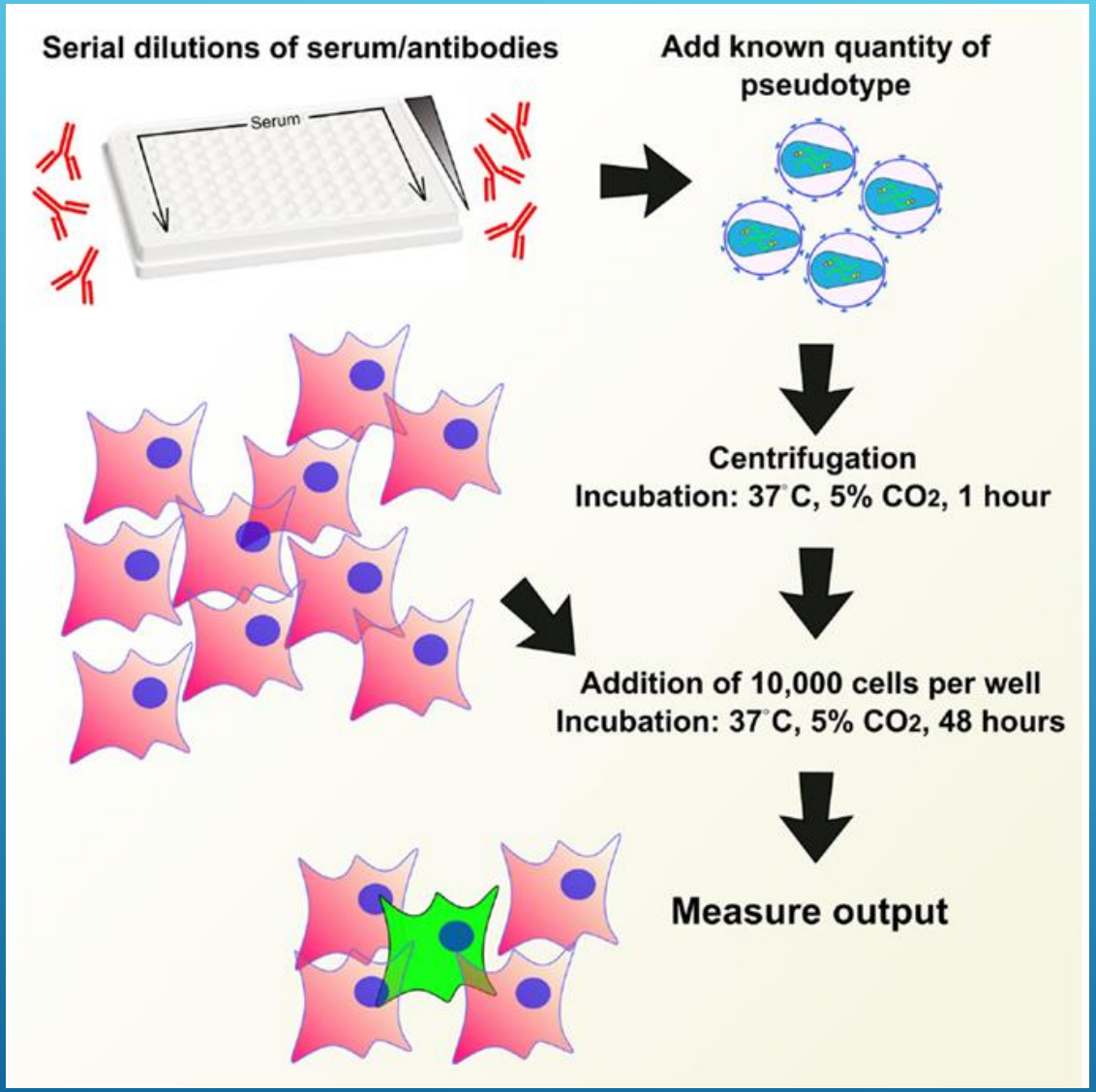


- 14 donors (50%) exhibited >10% decrease in IgA titer
- 12 donors (43%) exhibited >10% increase in IgA titer
- 2 donors (7%) exhibited no change in titer
- 2 donors (7%) exhibited >50% decrease in titer



- A subset of these samples with the longest follow-up, obtained 7-10 months after infection included:
  - 4 donors (29%) with >10% decrease in IgA titer
  - 7 donors (50%) with >10% increase in IgA titer
  - 3 donors (21%) with no change in titer
  - 1 donor (7%) exhibited >50% decrease in titer
  - All donors exhibited persistently significant titers

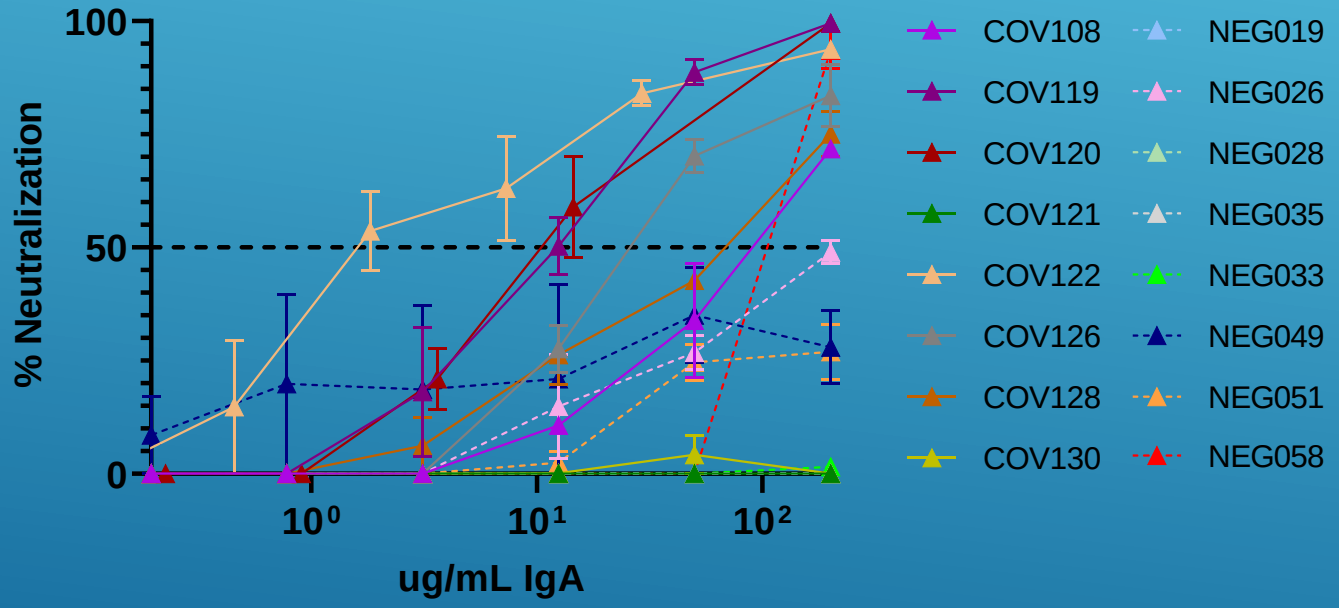
Measuring Neutralizing Activity



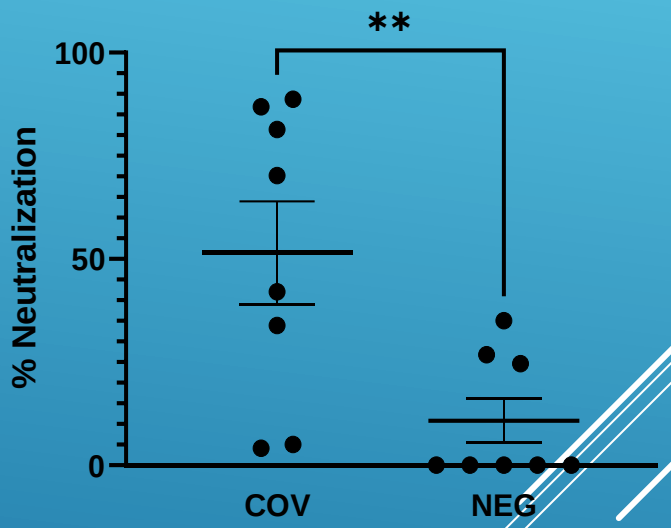
Total IgA was extracted from 8 COVID-19 and 8 control milk samples

All 8 COVID-19 samples had been shown to exhibit varying but positive Spike-specific IgA and secretory Ab

Neutralizating activity of extracted milk IgA against SARS-CoV-2 Spike-pseudotyped VSVΔG

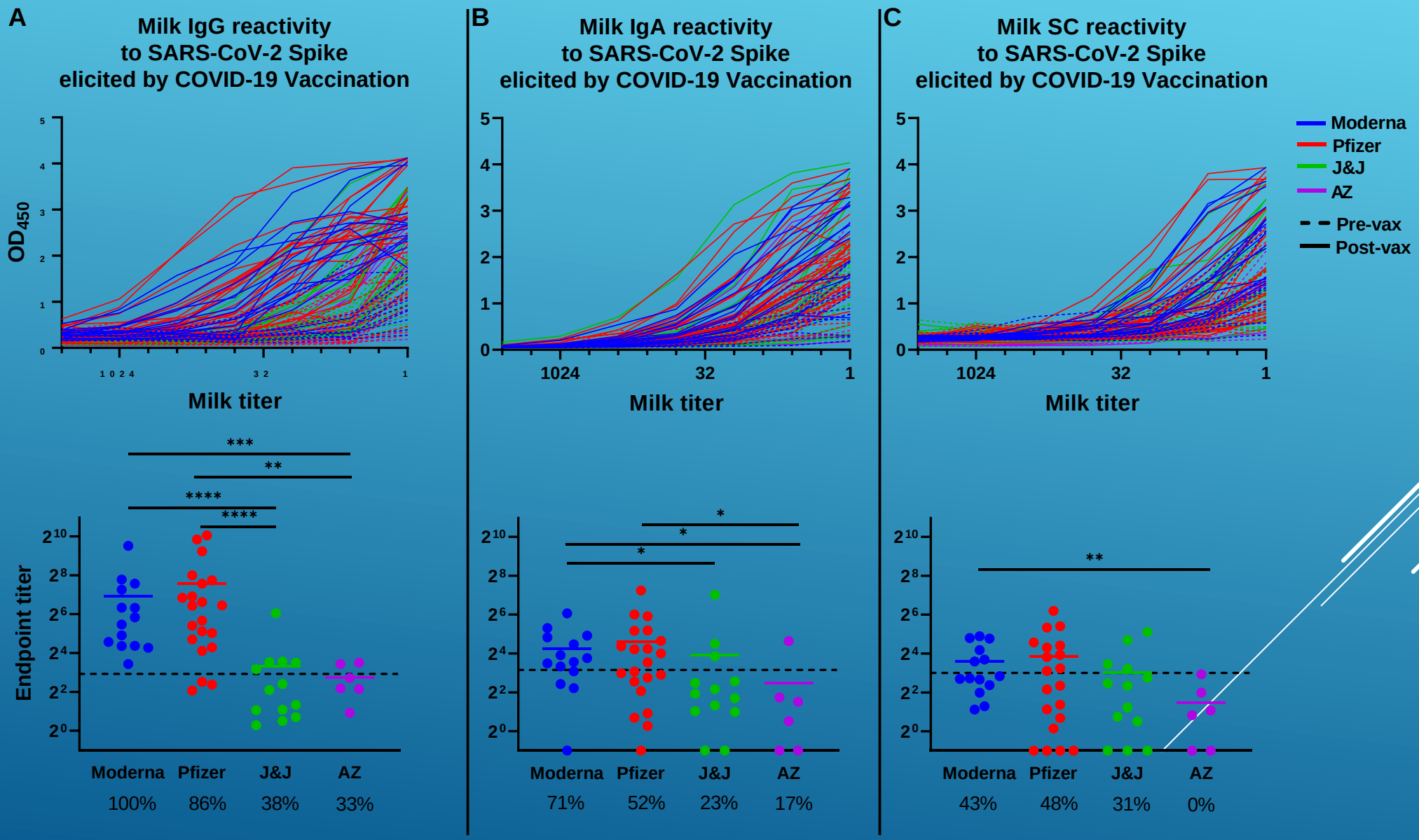


Neutralizing activity of 50ug/mL milk IgA

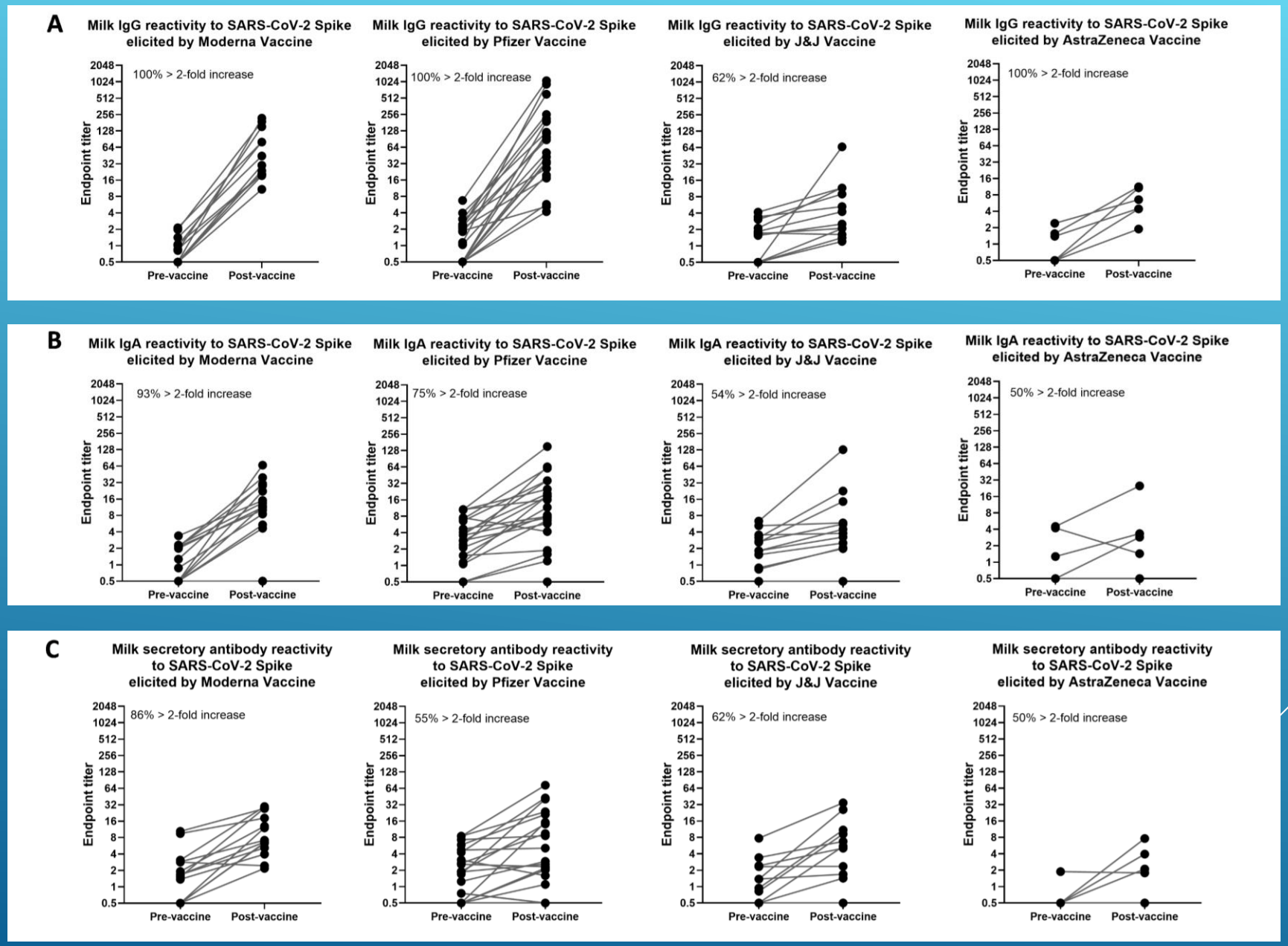


- 6/8 (75%) COVID-19 samples could neutralize the target virus
- The 2 non-neutralizing COVID-19 IgA samples also exhibited the lowest IgA endpoint titers
- **IgA binding and neutralization capacities were found to be highly correlated**

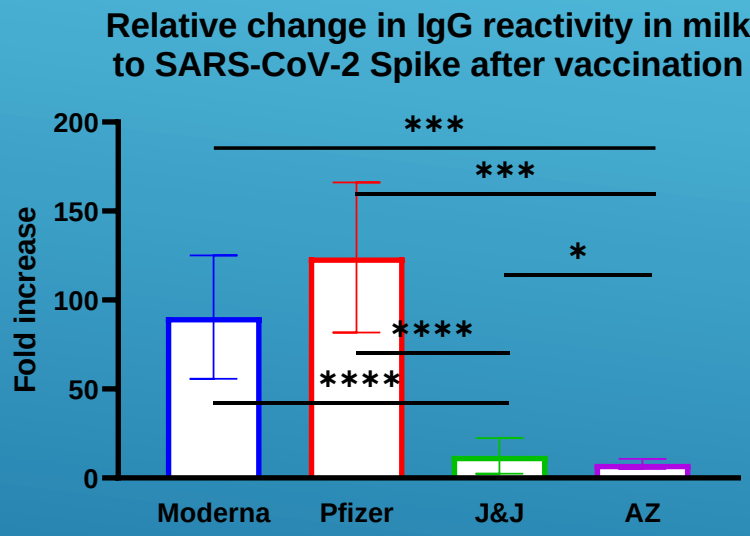
Results – COVID-19-vaccinated donors

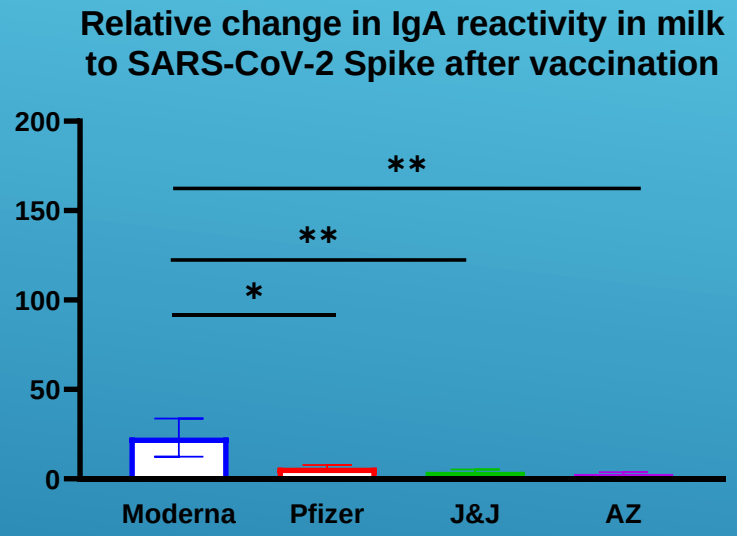


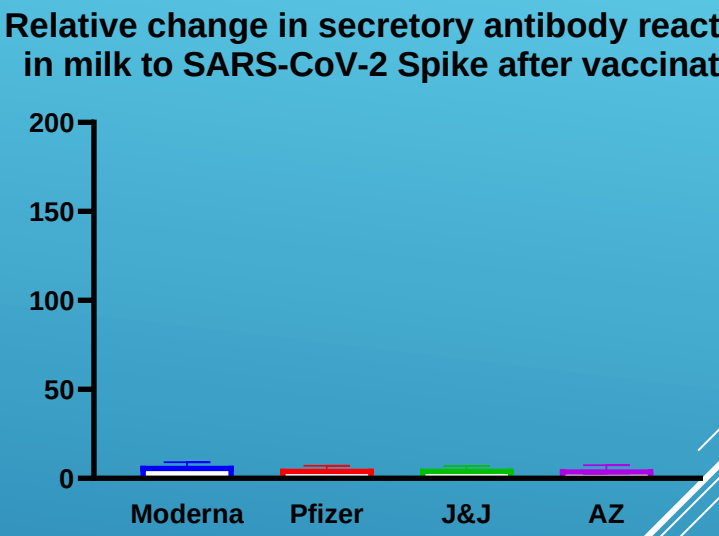
Relative antibody changes pre- to post-vaccine



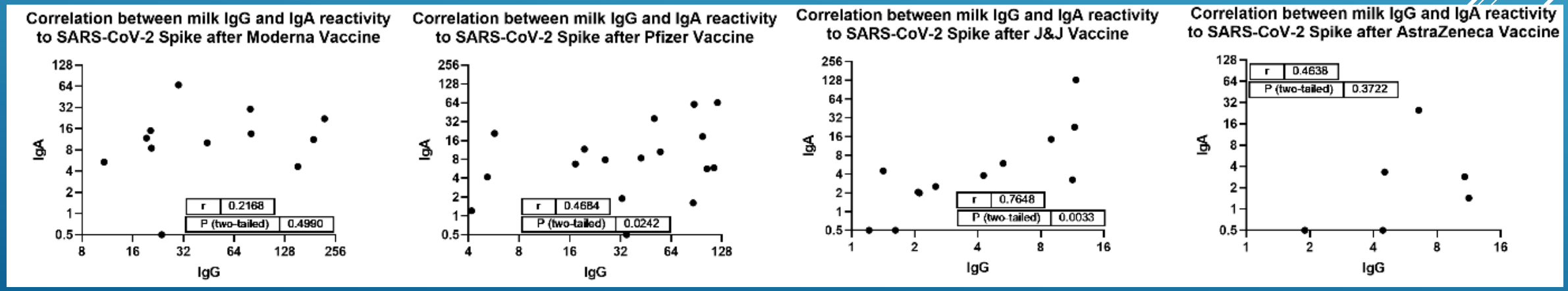
Relative antibody changes pre- to post-vaccine



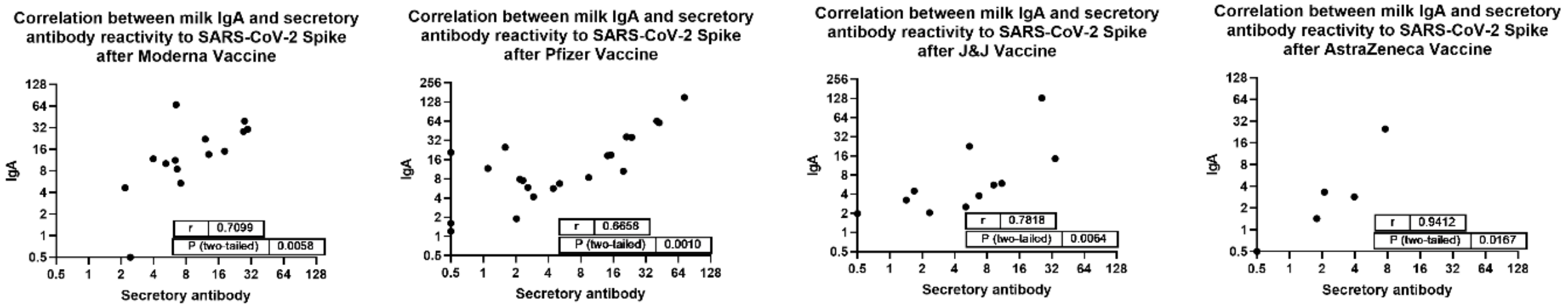




- IgG titers are significantly lower and less commonly detected for Ad-based vaccines compared to those induced by mRNA vaccines
    - These data likely mirror the serum Ab response.
        - Indeed, it has been demonstrated that serum IgG titer and neutralizing Ab elicited by Ad-based vaccines is significantly lower than those of mRNA-based vaccines
- Other groups have further noted that after mRNA-based vaccination, specific milk IgA responses are elicited by the priming dose of vaccine, but poorly boosted by the 2nd dose
    - Our correlation analysis also is consistent with this observation
        - IgG and IgA milk titers after 2-dose vaccination was only mildly related or unrelated
            - suggests the 2nd dose increased IgG but not IgA titers
        - J&J (single dose) exhibited a relatively strong correlation between IgG and IgA,



- Intriguingly, our correlation analysis of IgA and sAb suggested that relatively more of the IgA detected for AZ recipients may be sIgA, despite the low titers



Summary and Conclusions

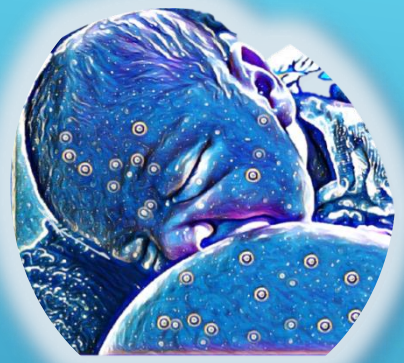
- Most milk samples obtained from COVID-19-recovered participants contains moderate-high levels of SARS-CoV-2 Spike-specific IgA and low-moderate IgG
- IgA was found to be predominantly in secretory form (sIgA) and very long-lasting
  - Beyond the potential for human milk-fed children, sIgA could be extracted for use as a highly targeted COVID-19 respiratory/oral therapeutic
    - sIgA is highly durable and resistant to degradation in mucosal tissue
    - With a strong, global recruitment campaign, it would not be difficult to source milk from COVID-19-recovered donors.
    - Our lab is testing the efficacy of this extracted sIgA in a small animal COVID-19 model system
    - Highly relevant for other infections and IgA deficiencies

Summary and Conclusions

- Unlike the post-infection response, the post-vaccine response is IgG dominant
- All mRNA-based vaccine recipient milk exhibited a strong IgG response, while milk from Adenovirus-based vaccine recipients exhibited a much weaker IgG response
- IgA response was moderate in mRNA vaccinees and much lower in Adenovirus vaccinees
  - AZ recipients exhibited lowest IgA.
- ~50% of milk of any group exhibited specific secretory Ab
  - Moderna appeared to elicit the best secretory Ab response of any vaccine
- These data indicate that mRNA vaccines are preferred for immunizing the lactating population (possibly Moderna is best).
  - Currently assessing:
    - Durability over time of these responses up to 1 year after vaccination
    - Effect of breakthrough infection and booster doses (matched and unmatched)

These data highlight the need to design vaccines with optimal protection of the breastfeeding infant in mind

Ideally, vaccines would be designed to elicit a potent, durable sIgA response for maximum efficacy in mucosal environments



ACKNOWLEDGEMENTS

Icahn School of Medicine

Alisa Fox

Claire DeCarlo

Xiaoqi Yang

Nicole Pineda

Krammer Lab (Spike protein)

Imperial College London

Dr. Natalie Shenker (AZ samples)

Medela

Support for milk shipping costs via Milk Stork

NIH National Institute of Allergy and Infectious Diseases

The milk donors!!