COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Rebecca Powell (Icahn School of Medicine, Mt. Sinai), January 31, 2023

Title: Comprehensive assessment of SARS-CoV-2-reactive antibodies in human milk to determine their potential as a COVID-19 therapeutic and as a means to prevent infection of breastfed babies

Rebecca Powell CIC Database Profile

NSF Award #: 5R01AI158214-02

YouTube Recording with Slides

January 2023 CIC Webinar Information

Transcript Editor: Lauren Close

Transcript

Slide 1

All right, so I'm a human milk immunologist at Mount Sinai in New York City. Today I'm going to talk to you about our work on the human milk immune response to SARS-CoV2 infection.

Slide 2

So just because not everyone's coming from the same background, I'm going to give a little bit of background on antibodies and other parts of the immune system in general. So antibodies are a protective protein produced by B cells in response to the presence of a foreign substance. They recognize and latch onto the foreign substance so that they can be removed from the body. When this foreign substance enters the body, the immune system recognizes it because its proteins differ from the self and any antibodies that might react with self are, at least in theory, not present and eliminated as part of the development of the immune system. So those proteins from a foreign element, like a virus, are very different from our own proteins. An antibody has two sides. It has the variable side where you see the Vs here. That's what contacts the pathogen like SARS-CoV2, COVID-19. Those are different for every antibody - it's a little bit different. It's variable region and that is how they recognize a foreign protein. Then this other side is the constant region or the FC region and that's what will mediate some other antiviral activities and bind to receptors on some of the cells of our immune system.

Slide 3

So there are different flavors of antibodies. I'm mainly going to be talking about IgA today because IgA is about 90% of antibody in milk. But in serum - so in our blood you mostly hear about IgG, so that's what's dominant in serum. Then there are other flavors I won't get into today, but just so you know there's many different types of antibodies and it's not so simple just to say antibodies and be done with it. There's many types.

Slide 4

In human milk, antibody, as I said is, about 90% IgA. Mostly, as a dimer, meaning two IgAs bound together. Most of that is going to be in secretory form. Secretory antibodies are polymeric so that means they have different types of proteins combined. In this case, they're a dimer which is mostly how you find IgA in milk. It is going to be bound together by another protein called j-chain that's made by the B cells and then wrapped up in secretory component.

Slide 5

I add this is a little cartoon of that. There are two kinds of IgA - I'm not going to get into that today - but they're structured a little bit different and IgA-1 is dominant. So here you see a dimer which is complex by j-chain which is made by the B cells and that's mostly what you find in milk in terms of the total composition. Then it gets wrapped up as part of its secretion into milk in this secretory component. So secretory component is critical for the protection against degradation and harsh mucosal environments like milk, like the babies' oral nasal cavity and especially the GI tract. So we evolve to have this highly protected form of antibody in our mucosal fluids because they are relatively harsh compared to serum. What's interesting is that one thing we're exploring in my lab is that secretory IgA derived from milk - because you can get milk in large quantities - as a therapeutic class of antibody against COVID or other pathogens may be highly efficient when you need those antibodies to be durable in those harsh mucosal environments like the respiratory tract or the GI tract.

Slide 6

Secretory antibodies - you don't have to get too wrapped up in this diagram, but as I said and for those that are interested, the way that they get their secretory component is that actually when the B cells transit to the mammary gland and they secrete that IgA dimer as it's being pumped into the milk, a piece of the receptor that pumps the IgA into the milk comes off and that's secretory chain. That is what wraps up the IgA. We also have IgG and other forms in milk. IgT is about 2% of the total and that's going to generally come from the serum, coming in at relatively passive manner. The B cells that ultimately produce milk secretory IgA actually originate mostly from the gut - from the gut associated lymphatic tissue. This is known as the entero-mammary link and you can imagine this is a very important evolved mechanism to help babies survive in an environment. As we were evolving or in low income settings today where modern medicine is not available and whatever the lactating person breathes in or ingests in the form of pathogen then an immune response is raised in the gut predominantly and then B cells from the gut transit through the lymphatics to the mammary gland and thus make antibodies that are highly durable

in the milk. This actually protect the baby from those very same pathogens and stop the baby from dying from those pathogens.

Slide 7

What is passive immunization? When a baby or child gets human milk they're not - it's not like they're getting vaccinated, it's not like they're getting infected themselves, but it's what we call passive immunization. This past protection works by coating the mucous membranes of the baby's mouth upper respiratory area and digestive tract. It provides a layer of protection that may stop cells in those area from being infected if the baby's exposed to virus. Or it may mitigate that infection, so slow down viral replication. This effect is temporary and the antibodies would be expected to be degraded or wash away within a few hours. This has to be replenished with every feed. Babies get their milk very frequently so this should not be a problem, but the effect is going to be dose dependent. So your exclusively breastfed baby who's not getting any other food or fluids is going to have the longest lasting coat of milk antibodies. Then a toddler who's also eating solids - it's not going to be that significant of an effect, relatively speaking. Now these milk antibodies do not pass through the baby's digestive system into the bloodstream. You would not detect them in the blood. They are a coating of the mucous membranes.

Slide 8

Our COVID-19 studies - we have two of them. I was NIH funded with an R01 for our infection study in 2020 as part of the emergency funding. We also have a vaccine study that I'm just going to mention at the end of my presentation. For our infection study, the participants had a PCR confirmed SARS-CoV2 infection. For our vaccine study they were vaccinated, but that's a separate study. We asked for about 30mls or 1oz of milk per sample using whatever pumps these participants had at home and were comfortable with.

Slide 9

The milk was pumped at home according to a schedule that we requested. It was labeled and frozen at home and then picked up, originally by me, during the shutdown and then later by courier or shipment by Milk Stork - a milk carrier company. Then this was designed as a longitudinal study so milk was obtained originally three to six weeks after infection and then we asked participants to continue to pump monthly samples for as long as they were able.

Slide 10

Initially, to show you the results from our COVID infection study, in a subset of samples we have many many samples we have hundreds and hundreds of participants. In this part of the study, we screened undiluted processed milk. So we just centrifuge the milk to remove the fat and the cells and we keep the skim milk fraction which is what we test. We looked at 75 COVID-19 recovered participants and also we used 20 pre-pandemic milk samples from other studies to form a positive cut off in terms of antibody against the COVID-19 spike. We use an ELISA which is a very tried and tested method to look at the response of antibodies in a solution of any fluid against any protein of interest. We get recombinant spike protein, we code it down to

a plastic assay plate, and we essentially just look at the response of the milk against that spike protein. We can quantitate how much antibody is there.

Looking at 75 people - this dotted line being our positive cut off which is based on our pre-pandemic control background levels in this ELISA - we found that just about 90% of milk samples obtained from these COVID-19 recovered donors had significant levels of SARS-CoV-2 specific IgA. This is an absolute positive cutoff level, so if you have samples from before an infection which we did not at the time for this subset and you could compare the relative increase for particular individuals, it's actually much more like a hundred percent of people have a response after infection. Our absolute cutoff is pretty stringent so in that sense it's about 90% past the absolute cutoff for positivity.

Slide 11

This initial screening 40 of the spike positive samples were further titrated, so serially diluted to find the binding endpoint titer, so this is an assessment of antibody affinity and or quantity. The way you do that as you is you basically look at what dilution of milk gives a optical density value of one so the amount of light in this ELISA assay. Then you can see each of these is the dilution curve. You can see every milk sample, although positive, looks very different because the quality of the response differs. So what we found is that of these 40 samples that were positive on the screen 95% of those had a positive endpoint titer so they were of high quality. About half of those were what we consider high titer, so a high quality or quantity of antibody in the sample, which is about five times the endpoint titer cutoff.

Slide 12

About 95% also had spike specific secretory antibody, which of course we're very interested in because that is the most durable class of antibody in milk. When we do our secretory antibody ELISA we look for antibodies. We look for secretory components so those could be on IgA, it could be on IgM, which also has a secretory form. It could be some other strange artifact like just free-floating secretary component but that's a lot less likely. But just because we measure IgA doesn't mean we know it's secretory IgA until we also measure secretory antibody and compare those data. What we also found was that about 75% of these samples did contain spike-specific IgG which is the dominant form and serum but can also be detected in milk. It was very low and as you can see only about really one past our high titer cut off and it was generally a low titer IgG response. But it's hard to detect IgG in milk because overall very little is in the milk.

Slide 13

It was very important was that we found that IgA and secretory antibody titers were highly correlated which strongly suggested that most or nearly all of the IgA was in secretory form. This is very important for potential protection of the baby. We also perform neutralization assays which looked at the ability of these antibodies to actually block SARS-CoV-2 infection. This was using a pseudovirus which is a harmless virus that's been made to have the SARS-CoV-2 spike attached to it and so we can measure how well the antibodies block infection. What we

found was that there was potent COVID neutralizing activity which strongly correlated with the amount of secretory IgA in the sample.

Slide 14

Then, finally, what we found more recently which is very important is that the milk secretory IgA infection response is very durable over time. When we have time points up to 12 months post-infection, what you can see is that there is hardly a significant change at all in these 20 or so people that we have looked at so far. You can see that when we do the statistical analysis there is no change between these groups at any time point in terms of the endpoint titer for the secretory IgA. This is a very very durable response over time after infection.

Slide 15

To summarize: the post-COVID-19 infection response is secretory IgA dominant which really is a classic mucosal infection response. The secretory IgA is neutralizing and it's very very long lasting over time. I didn't show you, but I'll just tell you that the post COVID-19 vaccine response is actually IgG dominant. It's a classic intramuscular injection response it does not follow this mucosal pathway at all. The adenobase vaccines like J&J and AstraZeneca actually had very poor responses in milk. Very low titers and very very low secretory antibody was actually found for any type of vaccine and it was very limited durability over time. So a strong contrast to the mucosal infection response the intramuscular injection response was really different and not very long lasting. So what this you know really tells us is that vaccines that elicit a potent secretory IgA response like your classic mucosal infection response - those vaccines are really needed. That's one of the overarching goals of my lab is - to design those vaccines with the lactating population in mind. We have some preliminary animal studies towards that goal right now.

Slide 16

I'd like to thank everyone in my lab the Kramer Lab for the spike protein Medela for initially supporting milk shipping costs by Milk Stork. I'm supported by the NIH and of course our milk donors. Thank you.