Immune

With Vincent Racaniello, Cindy Leifer, Stephanie Langel, and Brianne Barker

Episode 30: Immunology of COVID-19, Part 2

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Transcribed by Kim Barker

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VINCENT: From Microbe TV, this is Immune, Episode number 30 recorded on April 15, 2020.

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VINCENT: I'm Vincent Racaniello, and you're listening to the podcast about the body's defenders against disease. Joining me today from Ithaca, New York, Cindy Leifer.

CINDY: Hello again!

VINCENT: How are you? Are you well?

CINDY: I am good, I am well. Busy.

VINCENT: Good!

CINDY: But good, yeah.

VINCENT: No COVID-19, right?

CINDY: Well it's in Ithaca, it is not in my house, so that's...

VINCENT: Do you have a lot of cases? Do you know how many?

CINDY: We definitely have over 100. So, that's, it's, you know, that's a reasonable number for a pretty small town because we don't have any of the students in town. Both colleges have left town. So it's just just faculty and residents.

VINCENT: Any fatalities?

CINDY: We have had two. And they were imported from down where you are. As sad as that is, I mean it's really tragic but yeah our department of health actually has a report each day that they put out and they used to have a box that said total deaths and now they have two boxes that says total resident deaths and total imported deaths. So what happened was we took some very ill patients from New York City who were unable to get the care that they required and they brought them up here.

VINCENT: Got it.

CINDY: And unfortunately they weren't able to save them.

VINCENT: Wow. Also joining us from Durham, North Carolina, Steph Langel.

STEPH: Hey there. Good to be here, good to be back for our Immune part 2 of our COVID description of immunology. And things are going fine here. We're doing some COVID research, so I'm still coming to the lab, staggered of course. I'm not, you know, interacting with many people, we're doing temperature screenings and I wear a mask, so I feel very protected here.

VINCENT: Very good. And from Madison, New Jersey, Brianne Barker.

BRIANNE: Hi, it's great to join you guys again. I am hanging out in Madison and teaching lots of classes online. New Jersey has been hit pretty hard with COVID-19, but I'm doing okay here. I'm trying to make sure that all of my students are okay and help them get through the rest of the semester.

CINDY: How is your teaching going?

BRIANNE: It's going okay. I am teaching everything on Zoom, and I think it's working alright. I was pretty fortunate that my lab class was actually getting into a bioinformatics section right when this happened. So that was the most difficult class and we've been able to do a lot of data analysis. So it's goin okay but I think that both for the students and for us it's a big change and in some ways a lot more work.

STEPH: Yeah.

CINDY: Yeah I agree.

STEPH: Vincent, what about you? You're teaching right now?

VINCENT: Yeah my virology course is ongoing. We have two Zoom sessions a week, it's fine. But as you know I record all my lectures so I don't know half the class doesn't even show up cause they know it's gonna be on YouTube.

STEPH: Sure.

VINCENT: But it's fine. This morning I did a virology class in Knoxville, University of Tennessee Knoxville. The class invited me to spend an hour and we talked and it was great. There were 33 students there. And I'm supposed to go to Knoxville in September so I'm hoping that we start to travel again by then, cause it will be fun to see them all. One of the students she's got into a PhD program in New York and she said they said that you can come in July she said what do you think about that? I said I don't know, who knows?

CINDY: Yeah.

VINCENT: Who knows? It's a week by week thing.

STEPH: It is.

CINDY: Day by day sometimes, right?

STEPH: For sure.

VINCENT: So Brianne do you ever go outside or do you just stay inside all the...

BRIANNE: I do. You know, there's a lot of nice places around here where I can go out and walk, you know, sometimes I have to you know go into town to do different things. But I'm kept pretty busy with Zoom so I spend a fair amount of time indoors on my computer on Zoom.

VINCENT: Yeah, I do the same, I'm just here most of the day and I don't, I walk the dogs in the morning and that's it for me. I've been trying to go in once a week, usually the CO2 tanks or something I have to go on Friday to do that. But otherwise, let's see I go to the post office once a week, I wear a face mask. The post office is a funny place because they all wear gloves and face masks, which is good, but then they have this plastic sheet which is hanging down in front of the counter.

STEPH: Oh interesting.

BRIANNE: Yeah.

VINCENT: And it's funny.

STEPH: I wonder if that gets changed. Every day er...

VINCENT: I don't think so.

CINDY: Probably not.

BRIANNE: Yeah. Well I right before this was actually on a student senior honors thesis defense and she was talking about the importance of vitamin D in bone health and how sunshine and physical activity were so key, and I felt very worried about my bone health as I was thinking about my lack of sunshine and my lack of physical activity.

CINDY: I have been taking my vitamin D supplements.

STEPH: There you go.

CINDY: Because I live in Ithaca!

STEPH: I know.

CINDY: Everyone in Ithaca is vitamin D deficient anyway. It's actually snowing right now so yay...

STEPH: Oh my gosh, is it? Wow.

CINDY: Yeah, a little bit. It's flurrying. Not a lot. But there was a coating on the ground when I woke up this morning. I just want it to be done. I like snow, you guys know this.

STEPH: Oh yeah.

CINDY: But but once we get to like mid-April, I'm like I'm cold, please no more.

STEPH: Yeah.

CINDY: And especially being stuck inside I just wish it would get warmer so we could go hang out outside and do a little gardening or something, because kids stuck in the house all day, teenagers and parents stuck in the house all day, it's getting a little stir crazy.

STEPH: Do you wanna come down and visit me? It's a little bit warmer down here.

CINDY: It is, I know.

VINCENT: Alright so we're gonna pick up where we left off last time, and I'm gonna hand it over to Steph who did such a great job. And while you're starting I have a few e-mails from listeners I forgot to paste in so I'm gonna stick those in in case we get to them, okay?

CINDY: Yeah.

BRIANNE: Great!

STEPH: Yeah that sounds great. So I wanted to start off by kind of doing some dipping into TWiV this week and virology. Because Daniel Griffin, he's a M.D./Ph.D., he works at Columbia and Vincent you can correct me if I'm wrong, he's the Chief Medical Officer for...

VINCENT: So it's a physician patient network called Pro...

STEPH: Pro-Health.

VINCENT: ...Health

STEPH: Yes.

VINCENT: Which is New York, Connecticut, New Jersey, you know, one and a half million patients, 3000 doctors, he's the chief of infectious disease for that yeah.

STEPH: Right. So he's really in the thick of it right now in terms of the hotbed of cases and has been giving 15 to 30 minute updates at the beginning of those episodes. So I went through the last couple and I kind of compiled some of the things that Daniel was noticing in regards to patient outcomes and predictors or biomarkers of success or not success as to whether somebody would succumb to severe infection. And I thought we could go through these, because I think they're relevant and these things we are hearing about a lot in the news are kind of these hot button topics. We can work through those and then we'll get into part two, which we didn't get to, because it's just a vast topic and that would be herd immunity, who's gonna be immune, vaccines, vaccine development, there's a lot we're gonna go through with that. And then we'll kind of wrap up by talking about the future. Prevention, engineering controls, and what is it gonna look like gosh, it's hard to predict even weeks down the road but a year, I mean there's a by the history there's been a coronavirus outbreak every decade or so so what would SARS CoV-3 look like and how do we deal with that? So I'm gonna start by talking about steroids. And the reason I thought this was interesting was because Daniel was really observing that in the clinic there's a big question as to whether steroids should be given. Now steroids in general, they're called glucocorticoids. You make them yourself just by a natural enzymatic process when you're producing cholesterol and it's released by the body by the adrenal glands in a circadian way or a circadian rhythm. Interestingly we did do an episode on circadian rhythms.

CINDY: We did.

STEPH: Yes, I think it was early on.

CINDY: Yup.

STEPH: And talking about how release of certain factors can affect your ability to respond to vaccines and treatments. And so glucocorticoids are similar. They are released. But they're also released in times of stress and in response to stress. And they inhibit inflammation by dampening signaling pathways within the cell. So these would be pro-inflammatory signaling pathways downstream of what we call pattern recognition receptors, cytokine receptors and Fc receptors. All things that I won't go into but things that coat the surface of the cell. They're gonna react to its environment and then tell the cell downstream into the nucleus what proteins to produce. And so by programming these kind of, or dampening these pro-inflammatory effects, it's really the macrophages that are mostly affected because they have receptors for glucocorticoids, as well as our lymphocytes so macrophages of the innate system and lymphocytes of the adaptive. So steroids can really dampen the immune response across both arms of immunity, both innate and adaptive. And why we give them is because you likely have a pro-inflammatory reaction that you can't, your body is not tamping down on its own. And so it's treated for autoimmune disease, lupus, Crohn's disease, anything where you have this overreaction or even, I mean they'll give steroids like I had poison ivy pretty bad...

CINDY: I was just gonna say that I've had the same thing you know.

STEPH: Yes, if you have very strong dermatological issues then you would take steroids.

BRIANNE: And usually that's Prednisone...

CINDY: That's right.

STEPH: Yes.

BRIANNE: If we're thinking about what they may have received in the past.

CINDY: Right.

STEPH: Yes, yes, exactly. So these are given in response to an inflammatory reaction which we're seeing with COVID-19. But the problem is, if it's dampening your ability to recruit your adaptive immune cells, which would be the lymphocytes, so your CD4 T cells, your B cells, or even cells of the innate system that might help secrete things like interferon-gamma to reduce coronavirus, you don't really wanna give steroids while your body is trying to fight off an infection. And what Daniel was noticing is that if you're giving these pro-inflammatory dampening steroids in the first week, the patients were having poorer outcomes. However if you were able to give it in the second week, when patients were experiencing really severe immunological symptoms, they did better. And so if we kind of tease that out as to why that may be happening and, this is all, you guys can chime in but this is all hypothesis, because I think, you know, we're still waiting on published literature as to clinically what is best. But it does make sense to me that if during the first week if you have a time where the viral titers are high, and we are seeing that in patients that do worse, their viral titers are higher than those that are doing better. And so you actually you wanna recruit immune cells that can fight off infection and if you're dampening that response, that could be why you have poorer outcomes. However, if you get to the second week and your body, because you're older or you have co-morbidities, really is not good at recruiting its own immune cells and now you have this over-

inflammatory immune response, tamping it down at that point does make sense. And so I think, if I could hypothesize a mechanism that could be what's happening, you wouldn't wanna give steroids just as a blanket treatment and especially probably not at the beginning. But I didn't know if anyone had anything else to contribute but a lot of people have been asking me about steroids, and Brianne I think you were the one who brought up allergy medications.

BRIANNE: I did. And so the way you've just described things is sort of exactly how I've understood things as well. That much of what we're seeing in the first week might be sort of virus driven, and so there you want your immune system working kind of at full strength. But maybe later on some of what we might see might be immune system driven, and there perhaps tamping down that immune response is important. When I moved to New Jersey, I suddenly realized that it was named the Garden State for a reason. And not that my allergies were great when I lived in Durham, but I have a lot of seasonal allergies, and one of the allergy medicines that I take is, in fact, a steroid. And so one of the questions I asked Daniel was about whether people should worry about continuing to take their allergy medication, their nasal steroids, because of everything going on with COVID-19.

CINDY: What was his response to that?

BRIANNE: So his response was sort of we don't know.

CINDY: Yeah.

BRIANNE: It does seem like having them at the beginning would be problematic. And so he was a little worried about the idea of having them during the first week.

CINDY: Right.

BRIANNE: When I think about sort of my own allergies at least, I think having my allergies in check so I am not being confused with a COVID-19 patient and am staying away from my healthcare providers and am letting them do more important work might be a good thing to do. So I am continuing to take my nasal corticosteroids.

CINDY: Right. And so I think it's also true for people with asthma right?

BRIANNE: Yup.

CINDY: And that's even a little bit of a different category, because allergies are annoying to say the least, but asthma can be life threatening if you are not taking your steroids regularly and you begin to have more asthma attacks.

BRIANNE: Exactly.

CINDY: Because each time you have an asthma attack, you're causing more damage to the lung. And so I think that at least the Asthma and Allergy Foundation of America is making recommendations saying that you should continue to take your medications unless you talk to your healthcare provider and they say you shouldn't. So I think that the advice is to, especially for asthma, to continue to take your medications, because the potential of causing the lung damage or being in a state where your body is going into asthma attack or bronchoconstriction upon exposure to the disease might make it worse. So.

BRIANNE: Exactly. And I think that there also is a difference between some of the steroids that Daniel was talking about being used clinically that might be treated a little...might be used a little more systemically and suppressing the immune system more broadly than some of the steroids that we're using for asthma and for allergies that are much more locally delivered.

CINDY: Right. Of course it's locally delivered in the same place you're being infected...

BRIANNE: Yeahhhh.

CINDY: ...but, you know, I guess you know.

STEPH: And I think that's a part of, you know, what I'm learning a lot about science communication is it can be challenging for people to you know, choose us over maybe a cable news source or something, you know flashy on Facebook because ours it can be very nuanced. I mean I think what we're saying is that it could be that doing a nasal inhaler in the first week would suppress the ability of you to fight off the infection in the upper airways before things start to get worse, we just don't know. But I really I of course the official Association of Asthma and Allergy Foundation has said keep taking em. And I agree with Cindy. You don't wanna predispose

your particularly your lungs to then getting COVID or even another respiratory infection. So keeping with the asthma and allergy medications we all agree that would be, you should do that.

BRIANNE: Yeah I agree. And while we don't know a whole lot about what is happening maybe with that medication in the first week, we also don't know what's happening if you are in the midst of having asthma symptoms and COVID-19.

CINDY: That's right.

BRIANNE: All of those things are unknown. And we wanna prevent as much as we can.

VINCENT: I think Daniel's general approach is if you're on a prescription medicine, don't change it, talk to your doctor.

CINDY: That's right.

VINCENT: You know, you could be worse. Like people who are on ACE inhibitors for high blood pressure or...

CINDY: Oh yeah.

VINCENT: Should we...and Daniel says no no no there's no evidence that it's bad to take them so continue.

STEPH: Right. Right.

CINDY: I read an article on that and I think I got more confused. It's really really complicated. And in the end they said they might actually be helpful, because there's something to do with the expression level of ACE2 going down during infection, and the ACE inhibitors block ACE1 and that actually might promote the generation of the fragments that cause more hypertension. I don't know. It was very confusing but maybe a listener knows more about that and could enlighten us. But in the end yeah, I think the bottom line is we're not physicians and you should talk to your doctor. They hopefully are keeping up with what are the recommendations for them as physicians. And I would ask that question, because if they're not you might want to consult with a physician who is. Because I think it's changing so rapidly.

STEPH: Yeah. It would be a really great, and I know of a couple they don't know me, so I could approach them but, connected with our with my boss who works in lung pathology and asthma and allergy, it would be really neat to have an M.D. on to talk about what is it like dealing with okay it's allergy season, COVID-19, you're a lung specialist, that would be really neat.

CINDY: Even even without lung, the COVID-19 I think it's fascinating to talk about the types of immune responses that happen with allergy and asthma.

BRIANNE: Yeah my sister is doing a Ph.D. on some aspects of lung immunology.

STEPH: Oh, perfect timing for her.

BRIANNE: And I have recently asked her a couple of random questions and it it's been really interesting for me to learn how many things are known about lung immunology that I had no idea about and were never thought of...had never thought of.

STEPH: Yep.

BRIANNE: And how she considers the lower and upper lungs totally different organs- no one would think about both of them.

STEPH: Yes. So well then moving on, the next thing that Daniel had brought up was coagulation. That this was something that he was seeing potentially after trying to treat the cytokine storm, we brought that up last time, really driven by IL-6, and then you're dealing with the secondary issue of coagulation and micro clotting eventually leading to liver failure. Now I can tell you coagulation is like my least favorite. We all have our most favorite and least favorite. It's like complement and coagulation I can just like, you know, leave it, I'll let Cindy take this one.

CINDY: Well I have, we talked about complement.

STEPH: We have.

CINDY: And I teach it now but it was really hard to do that at first. But yeah coagulation is now becoming that for me. Because I think that the more we learn about it the more fascinating it is. And I think that for me I became interested in coagulation when I realized that macrophages could throw clots, and of course macrophages are my favorite cells, and that seems to be involved in some host defense. So then I started trying to figure out this whole coagulation cascade, which I can't say that I understand, but I have a collaborator that's quite good at it so I'll rely on her. But the bottom line is I think what we do know for sure is that there's something called a D-dimer, is a real biomarker for poor outcome. And so what is D-dimer? I won't go into the details and somebody so nicely put a picture into our show notes here...

STEPH: I was like I don't know if that's really gonna help.

CINDY: But basically but basically what happens is if you cut yourself you'll clot, right? We all know this, it's very simple. And what we don't think about it well that actually serves two purposes. One is you have to stop bleeding, otherwise if you don't stop bleeding you'll die and so that's number one. But clots also trap microorganisms that might have come in through those openings. And so in essence they're preventing the spread of microorganisms into the body. And this is really interesting because if you look at lower like invertebrates like flies, they have a process called melanization and so they basically create a wall or a barrier when there is a breach in the corpus or whatever you call it, the outside exoskeleton of the insect. When it's pierced and bacteria get inside, they wall them off. And that's a main mechanism. And so this clotting is actually a main mechanism to trap and block off bacteria and potentially viruses. So nonetheless, you know, this plays a role in host defense. But what we're seeing in the case of COVID-19 patients is that there is this cytokine storm, which is this massive influx of pro-inflammatory cytokines, and that's gonna promote changes in vasculature, vascular leak, there's going to be things on the wrong sides of blood vessels, and when that happens just like when you have a cut you're gonna get clotting. And there's gonna be deposition of something called fibrin, so that's a main part of a clot. And then what happens is when that clot as we know, starts to go away, it gets digested and eaten up, and it gets replaced by new extracellular matrix. And so that's when we have a scar or you know wound healing like that. But in the case of these micro clots what's happening is the clot forms and then it gets digested. And when it gets digested it releases these D-dimers. So what the presence of enhanced levels of D-dimer is telling you in the blood is that there are clots happening. And they're happening at a decent level, enough so that you're able to detect these fragments that are being digested from the clot, because it's trying to remove these clots. So now you have these clots and you're trying to remove these clots. The other interesting part about coagulation is there's two phases. So there's a hyper coagulation, so there's a formation of these clots, and then there's when you use up all of the clotting components, you end up hypo coagulate, and then you start to bleed out. And this is something that we see in sepsis. So when you have a high level of bacterial infection in the blood and you get sepsis, you get what's called disseminated intravascular coagulation or DIC, and this can happen in COVID-19 as well. And so you get these clots everywhere. But if you don't, if you can't rescue the patient at that point, it goes it kind of flips over and it's, it becomes hypo coagulant. And so the vessels start to get leaky, the blood pressure drops, and you start to go into shock. And so in the case of COVID-19 what's happening is you get this hyper coagulation at first with these little micro thrombi that can get stuck into vessels that feed into different organs and so the organs start to become compromised and shut down. But then also if it goes too far now you have this fluid that starts to leak into the lungs and leak into other tissues and then it becomes a real problem. So D-dimer is not, I would say not a great outcome if you're starting to see that you're in pretty bad shape and if they can't rescue you at that point it's not looking good. So I think we we do need to look at earlier biomarkers. And so I think the next thing that you had, unless you had a question about that or you wanted to talk more about that, I think the next thing is can we go earlier than that, you know, that moribund biomarker, you know, that's way closer to a poor outcome. Can we look earlier and see if there's something we can read out?

VINCENT: So why...this is the question, it's in the notes, why would SARS-CoV-2 infection cause coagulation and D-dimer increase? I don't understand the mechanism.

CINDY: So there's two things. One is just the massive amounts of pro-inflammatory cytokines will change the vasculature. But the other interesting thing is that virus infections in general, and I, it has not as far as I know been studied for SARS-CoV-2 at this point, but some viral infections, at least in macrophages for example, can cause changes in surface expression markers that will promote clotting. And so in the case of macrophages, what will happen is you can have flipping of what's called phosphatidylserine, which happens when cells undergo apoptosis as well. And that will activate a protein called tissue factor on the surface of cells and cause them to be pro coagulant. So there's potential that the infection itself is causing this, or it could be secondary to the cytokine storm that's happening as well.

VINCENT: You know what's weird is that this is just coming up now, you know, Daniel mentioned it for the first time last week, and you know patients have been cared for for quite a while, so I wonder why this is happening now or being detected now and we didn't see it before.

CINDY: Well I think when I looked at some studies that came out several weeks ago they were saying D-dimers were up in the patients who died.

VINCENT: Yeah I just go by Daniel and he never mentioned it before.

CINDY: Maybe they just started looking for it, I don't know.

STEPH: And you know what's interesting so this time what we're talking about, what Cindy's mentioning, it's late.

CINDY: Yes.

STEPH: And really there's likely very little detectable virus. And so earlier in infection is really gonna predispose your system as to whether you're gonna be able to have this severe of an event or are you gonna be able to recruit the immune cells to tamp down the inflammatory infection and inflammatory response rather and be able to balance it. And so that's seemingly the pathology. I do think it's immune, in terms of severe disease is immune-based.

VINCENT: Cindy what's the implication for blood oxygen supply if you have high D-dimer? Is there any?

CINDY: I don't know.

VINCENT: Cause you said if you have an embolus that can obviously block blood supply physically right?

CINDY: Correct.

VINCENT: But there's this other observation besides D-dimer that these patients, some of them do not have ARDS but rather have a high altitude oxygen problem, you know that mimics lack of oxygen at high altitudes and I don't know if it's connected to the clotting or not.

STEPH: That has been really fascinating to even really understand. And from reading and listening to what Daniel said, it's not every patient...

VINCENT: Yeah.

STEPH: ...but it's seemingly like maybe 30-40% where they're not experiencing...so with with ARDS I mean the body once it detects damage is gonna block off that area of the lung and that's why you need oxygen, because the body has blocked off that area and you can't get oxygen to it. So if you, so your blood oxygen is very low. But seemingly with this HAPE or high altitude...what does the P stand for...high altitude pulmonary edema, it isn't blocking off an area. So you actually have a lot of oxygen in your lungs or in your blood, you don't need additionally oxygen, but for some reason you still have lower pressure, and your lungs are still failing from the edema. So I don't know if that, I think that's very recent observation that they're gonna have to follow up on because seemingly that would be a different treatment that you wouldn't want to ventilate.

BRIANNE: Yeah that's what it sounded like from Daniel.

VINCENT: Yeah. He said they're not ventilating them they'll put you on your belly to...

STEPH: Prone you.

VINCENT: ...improve...

CINDY: Yeah I read that, yeah.

VINCENT: ...it's easier to breath there or something, yeah. We'll have to ask him that on Friday.

CINDY: Yeah!

STEPH: Yeah.

CINDY: Yeah.

VINCENT: You know I often don't wanna ask too many questions because he's got a half hour and I want him to get out and it's not like we have time to chat, because apparently all his physicians are listening in the first half hour to get information so.

STEPH: Sure sure.

BRIANNE: Right.

CINDY: Oh yeah, wow.

STEPH: They're like then we get the go.

VINCENT: Yeah.

STEPH: So yeah, I don't know Cindy we did have one more thing about other biomarkers. We could touch upon that.

CINDY: Yeah so going a little bit earlier when we're talking about the cytokine storm- I think it's interesting because everything was focusing in on a cytokine called interleukin-6 or IL-6. It's certainly not the only molecule that goes up when there's a massive inflammatory response. You have your procalcitonin, C-reactive protein is a acute phase reactive protein secreted by the liver. And so they definitely are correlated with poor outcome. But I also saw a recent paper where they started to do sequencing analysis of PBMCs and of BAL cells. And you know, IL-6 was not up there. So although we're seeing IL-6 I don't know who's making that? I would've thought it was the macrophages but I'm not really sure if it's the blood monocytes or tissues macrophages or alveolar macrophages, it might be from another source. But it's interesting that there certainly, IL-6 is not the only cytokine. Is it the most important one? I don't know, I think we'll find out, because people are trying to start to use anti-IL-6 therapy. So there's been, it got advanced, it's Tocilizumab I think?

BRIANNE: Yes.

CINDY: Is advanced now into like a phase 3 trial for this, because it was already being tested in phase 2/phase 3 trials. So they're, I guess, enrolling patients throughout the world it was crazy I saw something that they had treated like 2 or 300 patients in a 24 hour period because it's a global mobilization of a clinical trial, which is another fascinating aspect of this whole pandemic. But yeah so I guess we'll find out soon enough whether it will or will not be helpful. But the idea there is IL-6 is a major promotor of the fever and other tissue changes that occur in response to the cytokine storm. And so finding out whether that's a good target or not we'll know in a couple of weeks probably.

STEPH: Sure, sure. So I think our summary for that section is you do not want to get to the point where you have a cytokine storm and increased IL-6. It seemingly before that, finding ways to either through biomarkers or treatment options to make sure that you don't have this type of high pro-inflammatory response.

BRIANNE: And it seems like that response is happening maybe more in the second week than in the first week.

CINDY: Exactly, right, yeah. Which is an interesting thing because also, you know, where do we call it the first week versus the second week? Because now we're hearing a lot about asymptomatic, right?

STEPH: Yeah.

CINDY: So is that from someone when they first test positive or is it from when they have a first symptom or, I mean, because as far as the immune system is concerned, once you are exposed and the virus starts to replicate we would call that day 1, right?

BRIANNE: Yup.

CINDY: Because approximately five to seven days later is when you're going to have the real exponential phase of your T and B cell response. And so we would call, you know, after that seven days that would be week two, because we know the T cells and the B cells are becoming very active and then they start contributing to the pathology. And so...where do we call week one and week two?

BRIANNE: So this might really be week three or week four.

STEPH: Right.

CINDY: Yeah we don't really know, right?

STEPH: Yeah. So okay great. Well that section was great. I think we're looking at, we have about 50 minutes to get through the next section, which is gonna be a bit of a monster but I'm excited for it because of course it deals with adaptive immunity, which is opposite of coagulation, my favorite part of the immune system.

CINDY: Well you like your part I like my part.

BRIANNE: That's right! That's perfect.

STEPH: Exactly, no I know I tease. That's why we all, right, we all kind of find our little niches and what we love, so. So we're gonna talk about herd immunity first. It has been hotly talked about and, you know, what is it, what does it mean, and specifically for COVID. So herd immunity in general is when a population is immune to an infectious disease that...and this provides an indirect protection to those who are not immune to the disease. And there are three ways, two ways, one has like a part a and part b, but I'm gonna say three ways to develop herd immunity. The first one is quite draconian and I would not advise as a public health official who cares about the health and safety of society- and that would be let everyone get sick and let everyone recover and just see who ends up dying and see who ends up surviving, and the ones that survive are the ones that are immune, and you have likely herd immunity. Now that was something that there was a lot of press about England wanting to do that, and then I think they realized very quickly that the percentage of people that would die would be higher than what you would just expect for COVID, because the overwhelming of hospital systems means that people who have COPD from lung damage or other maladies would also die because they don't have enough ventilators or treatments, doctors, I mean the healthcare system, people in healthcare cannot all get infected, because then who takes care of those patients. So that's the first way. The second way...

CINDY: And I have to say that was really shocking to me, when it came out in the news that they were gonna do this. Because we had already been dealing with this for weeks and weeks and it was very clear, country after country, that that was not an approach that was gonna be a viable approach. And yet they said we're just gonna lock up our elderly people, and just let everyone else get sick, right? Which doesn't make sense, because you can't keep everybody in, and on top of that, it assumes that only the elderly are really susceptible and we know that's not true either.

BRIANNE: Right.

STEPH: Well that, exactly. There are young people dying. Of course it's a lower percentage, but we see it happening. So, so yeah that was shocking. And, you know, you hear people suggest that in like weird corners of the internet, but for a country to say that's their policy was very bizarre. But they did...

CINDY: The anti-vaxxers believe that too by the way.

STEPH: Yeah, well, I know.

CINDY: A natural infection is best, is their argument. Except for when you die.

BRIANNE: Unless you die.

STEPH: Or your family member of something, yes. So the second way then of course is a vaccine. If we have a vaccine that we can give, you know, a majority of people then that could prevent herd immunity, and then the third way is really what we're doing now, which is a very public health based approach where we are social distancing, we have cancelled much of our economy, I don't mean cancelled, we have shut down much of our economy, and that's the flattening of the curve, and out of these three things, when you don't have a vaccine you're left with let everyone get sick and have a lot of people die or social distance and have economic downstream effects, which are also going to be very bad. So I'm not here to say that what we've chosen doesn't have any consequences, but I do think out of the two that it's gonna be the best for society. And so...

CINDY: And this is an interesting thing to think about, because we often think when we talk about immunology what's going on in a cell, right? Like what happens when a cell is infected, how does it respond? Or an individual, you know, what happens if the individual is infected, how is their immune system mobilized. But this is thinking of it more in a community way, right? And so it is indirectly impacting the individual.

BRIANNE: Yeah. And population immunity is really interesting.

CINDY: Yeah and so then it becomes a an interesting argument because those individuals who feel like maybe they wouldn't be impacted by it but are impacted by societal changes that are made get very frustrated if they don't understand, you know, how their...what they do on an individual level in the community is helping others. So it's an altruistic type of community interaction in order to make this work.

BRIANNE: It is. And you can never, you know, you don't have the sort of controlled study to say oh you would've been affected if we didn't do this.

STEPH: Right.

BRIANNE: You sort of just have to be imagining that.

STEPH: Yeah we can't like section off a part of the population and say ok you guys don't do social distancing.

CINDY: Well, we've got those...

STEPH: Well I guess we, that's true...

CINDY: ...hey, unintentional studies happening, right?

STEPH: Between countries? Maybe between regions?

CINDY: Yeah yeah between states, between countries, yeah. It'll be interesting.

STEPH: Yeah. And you know, the number in terms of percent of people that need to have antibodies that protect them against a future infection, I think that's kind of debated, I know 70% is what a lot of epidemiologists say, but it can, it really depends on how long immunity can last, how infectious it is, so it can range from 70 to 90%. I think in measles you need a very high proportion?

BRIANNE: Yeah.

STEPH: Correct me if I'm wrong.

BRIANNE: About 95.

STEPH: Right. It is so infectious that any break in kind of our herd immunity can cause infections. So that leads us to we can break down exactly how vaccines work in a general way before we start talking about this big question of if you get infected will you be immune?

CINDY: Right.

STEPH: I'd be happy to do that or Cindy if you want to it doesn't matter. But I'll just kind of start in a general way and feel free to pitch in. You're gonna get vaccinated likely intramuscularly. And inside that vaccine is gonna be a piece of the virus. It can be a weakened part of the...er live virus or it can just be a subunit or a piece. And within that vaccine you likely also have something called an adjuvant. And those two things are gonna work in concert to stimulate something called a dendritic cell to be able to pick up that piece of antigen or that piece of virus called an antigen. And process it so it can present it to CD4 T cells in the lymph node. Now the adjuvant's very helpful because you think about a muscle, you don't usually have a huge amount of dendritic cells, but it's gonna activate cell types to secrete proteins to have the dendritic cells start to migrate there. Or it's going...the piece of virus is gonna travel through your lymph and go to the lymph node where there are a lot more dendritic cells. But it's...yeah go ahead Cindy.

CINDY: Oh I was just gonna say so for those who are not aficionados, you know, we often think of lymph nodes of those lumps in your neck when you're sick.

STEPH: Yes.

CINDY: And it's because all of those cells accumulate there to try and educate each other and learn and become activated and then go out and do their job but in wherever they can find the infection. And so the swelling is both the lymph and the number of cells that are there, because they make copies of themselves and more and more of them.

BRIANNE: Yeah.

STEPH: Right, and I'm only gonna talk about three cells just to keep it simple, flag the dendritic cell, that pick up the protein...cause there's a lot more goin on but dendritic cells that pick up the protein, they present it to T cells, and T cells are gonna help work with B cells to make antibodies. And antibodies really are what we think of what we're trying to develop memory for a future infection that a memory B cell and memory T cell can work in concert to then protect you in the future.

CINDY: And when you say memory can you just expand on that a little bit?

STEPH: Yes!

CINDY: Because I think that's the key point with vaccines, right?

STEPH: Sure sure. So the first time you see a virus or a pathogen, the first time you see anything in your immune system, you're gonna develop cells that are gonna remember that pathogen. And they start as naive cells. They don't know any better. But then they're gonna develop this memory and so they can in the future expand at a greater rate than when they first saw that pathogen. So the second time they see it, you don't have to wait 2 to 3 weeks, maybe you only wait one week. And that quicker response is what we define as memory. And not only the response rate but the ability to produce more antibodies that can bind the virus and neutralize it.

BRIANNE: Yeah exactly, it's sort of a qualitative and quantitative improvement that we see in those cells. And the important thing about the lymph node as Stephanie was mentioning is that we need all of those three cell types to work together to make all of this happen, and the lymph node is sort of the place where that can happen. And it's really nice to have a place to collect up all of those cells.

CINDY: So I sort of think of the dendritic cells as the detectives that go and find a piece of information, right? But then the memory cells sort of keep track of those fingerprints of the different microorganisms, so they can look for those fingerprints. And so if you're trying to solve a crime, if you have a fingerprint in the database you can solve the crime much faster, right? Than if it's a fingerprint you don't recognize and you have to try to figure out what happened. So if you have a new infection, it's a new fingerprint, they've never seen it before, and so you have to mount an entirely new response. But if you have a fingerprint in your database that you recognize, you can go after the criminal really fast. So the B cells and T cells can be mobilized really fast the second time around.

STEPH: Right. And so that leads us to then this big question is if you get infected, can you get re-infected? And there's a couple different things to think about. We can look at past studies, so we know that SARS was a pathogenic coronavirus, MERS is a pathogenic coronavirus, and both of those have shown that you can develop something called neutralizing antibodies or antibodies that block the ability of the virus to enter your cells. In some cases we had talked there's a link that they could wane after 3 to 6 years, but I know Lin-Fa Wang had looked at MERS and it lasted up to fourteen...er not I'm sorry, not MERS but SARS lasted up to fourteen years, and you could pull out those antibodies and they still neutralized. So pathogenic coronaviruses there's some evidence, but of course SARS really disappeared after 2004 and we didn't get to learn a lot about long-lasting immunity.

CINDY: Right.

STEPH: Now, there are human common cold coronaviruses, and what we know about their ability er our ability to develop immunity against them is it's not great. We get reinfected with common cold coronaviruses. It lasts maybe one to two years. But I wonder if it's the difference in the fact that they're a more mild disease, they possibly are presented to these important cells in the lymph node in a different way. So when we think about developing a vaccine, you want something that is going to powerfully present itself to the B cell and have this high affinity response to the pathogen. And we're still learning, the problem is of course we're early. There are some papers that show that you can get neutralizing antibodies to SARS-CoV-2, and if I had to put a bet on it I'm gonna bet on the adaptive immune system to be able to make those antibodies, but it's not gonna be what we call sterilizing immunity, is my guess. I think that...and sterilizing immunity is really you would see no trace of the virus. You wouldn't see replication, your body has been able to fully protect you and neutralize it. But I think with the vaccine we could get there. So that's kind of my thought on this is that we can develop neutralizing antibodies, but we need a vaccine for this longer-term protection.

BRIANNE: Right. And the idea with the vaccine is basically you are trying to show your immune system some part of that microbe, you know, and you know get the fingerprint in the database without having to have disease happening first. And so you are really trying to sort of mimic the first part of this in a safe way.

CINDY: Yeah it's like a fire drill, right?

BRIANNE: Yup.

CINDY: You pretend that there's an infection, you do everything you need to do so that as soon as there really is one, you can go quickly. So...

STEPH: Right. And a lot of the kind of onus on making sure this works is in the vaccine, what is gonna be the antigen that we present to ourselves and the antigen again being just the piece of the virus. There's something called the S protein, the spike, that certain...that's around the coronavirus. And previous studies have shown that that protein is very important to developing these neutralizing antibodies. And so a lot of the vaccine development is a vaccine that presents this spike protein to your immune cells and there are different parts of that spike protein so I think what people are trying to figure out right now is what part of that spike protein do we wanna drive the antibody response towards? And these are all things that they're gonna start testing in clinical trials. There are a lot of vaccines under development. I mean I'm very familiar with the mRNA vaccine, but I know I'm sure there are tons of others.

CINDY: Yeah.

STEPH: I know that the mRNA is in clinical trial right now, and that's just that you're injecting the more basic form of the protein, so the RNA that can be then translated into the protein of interest. The reason why they were able to come up with that so fast is really you're just plugging in the sequence, because that platform had been developed.

CINDY: Right. And I think that there's a nice thing to learn here too, is that we learned a lot from the original SARS, and we learned about the types of antibodies that are generated and whether or not they're neutralizing, and it was really important. And so some of what they've done is develop specific stabilized structures of the protein to which the antibodies are generated that you use in the vaccine. So that you're generating antibodies that are much more likely to be neutralizing. So they're called like the stabilized pre-fusion form, so that when you generate the antibodies to the vaccine it's generating the ones you want. And so yeah, the one that's the lead candidate right now is the mRNA vaccine and the nanoparticle. The other cool thing about that is that is the nanoparticle itself is adjuvanting. So it is itself the adjuvant. So there isn't anything else in that formulation except the nanoparticle and the mRNA.

BRIANNE: Yeah and this is a type of vaccine that is different than sort of the vaccines people have already received. It's a newer technology.

CINDY: Right.

BRIANNE: And so this is sort of the first big test for this technology. It's really exciting because we can make these vaccines so quickly. And so if this one works well, that's going to be generally really exciting as an idea that this is gonna be a way we can make vaccines quickly in the future.

CINDY: Yup.

STEPH: Right. So there had been a lot of press particularly when a lot of the genomes or the genetic information in these viruses is being published in this open source, it's called nextstrain.org, it's very cool out of University of Washington, we can put that in the show notes. But there was a lot picked up by the press that there are eight strains of coronavirus circulating, and it's based on mutations and can we develop immunity to all of these strains? But really, what I think is missed is the nuance is the difference between strain and just mutation. So for viruses they're mutating all the time, but it does not always confer something that matters, something that's functionally relevant. You have a buildup of mutations and in coronaviruses actually it takes a lot longer to develop these mutations because of its nice it has a way to fix those mutations. So there are not eight strains, there is one strain. Although we're seeing some evidence of maybe a deletion of a part of the virus called ORF8. It's a non-structural protein, and maybe that will matter, but remember ORF8 is not a driver of neutralizing antibodies, it's the spike. So likely as we've seen with other coronaviruses, the one that I worked on in my Ph.D. there was a different strain that emerged called an InDel, an insertion and deletion in the spike protein, but it made the virus less virulent. So that was one other strain that emerged and it was not more virulent. So I think that's something to keep in mind that it's unlikely that we would have to worry about these mutations in terms of immunity, because they're not functionally relevant.

VINCENT: Stanley Perlman mentioned that yesterday is this the pig virus that basically it ended up immunizing pigs this new isolate and it got rid of the original virus?

STEPH: Oh my gosh so that story is even more fascinating than the PEDV one.

VINCENT: Oh that's TGEV, right?

STEPH: That's TGEV. So Vincent, Cindy, Brianne, correct me if I'm wrong but there's not been another example of an enteric virus that mutates to get different tropism in the lung and then is able to...

VINCENT: Yeah, right.

STEPH: ...basically build immunity in the animal for both viruses.

VINCENT: Yeah.

STEPH: So porcine respiratory coronavirus was a mutation from transmissible gastroenteritis virus to different tropisms in the body. And they ended up kind of TG...the gut one ended up not being pathogenic because the respiratory one built immunity in the pig. It's an amazing story. I don't...

CINDY: Well there's the opposite that happens in the cat, right? There's an internal mutation that causes the FECV to be FIPV, feline infectious peritonitis...

STEPH: Infectious peritonitis virus?

CINDY: Yeah and so and that makes it worse.

STEPH: Right and that makes it worse. So this one made it I guess the opposite.

CINDY: Better. Yeah.

STEPH: Yeah.

CINDY: Fascinating.

STEPH: Fascinating.

BRIANNE: And one of many reasons we should continue to be looking in some of the different animal models, not just the standard ones.

STEPH: Yes! Of course, listen, stamp that on my forehead I just want everyone to know.

VINCENT: Well you know on TWiV before SARS-CoV-2 we used to talk about some of these other coronaviruses, I think we have to return.

STEPH: It's taken over, yes.

VINCENT: So the nextstrain.org is interesting. Nels Elde and I talked about it a few weeks ago. Between all the isolates from the beginning of the outbreak to the current there are just 20 nucleotide differences. That's how stable...

CINDY: Amazing.

VINCENT: ...this virus is. Only 20 and none of them as Steph said have any functional consequence.

CINDY: Wow.

VINCENT: They're just markers and Christian Drosten used one as a marker so he found in one patient a virus in the upper tract that was one base different from the virus in the lower tract. Just randomly, right? And they could tell that it was just the virus from the upper tract that was transmitted to other people using it as a marker, showing that that's where you transmit virus from the upper tract, which makes sense because it's hard to get stuff out of the bottom of your lungs to other people, right?

CINDY: Yeah! Wow.

STEPH: Yes. So you know, I think and there's been debate back and forth and I think different people can weigh in, different experts but in terms of generating a vaccine and then the question is are you going to drive evolution of the virus towards something. My opinion is just because of the way coronaviruses work that you would not and also I think the virus would either burn itself out or become so mild that there's no...the pressure is gone to change quote unquote and even if it did change it'd be less virulent but I'll let you guys comment if you have other opinions.

CINDY: I have no idea.

VINCENT: No we believe you we believe you!

BRIANNE: I agree.

STEPH: So okay so we're gonna make this vaccine. Likely everyone, I'm gonna say everyone but there's gonna be pockets of people either for access reasons or belief reasons are not gonna get it but we could say that this is gonna be a widely distributed vaccine. So it would be particularly bad if this, if antibodies against this vaccine made the disease worse if you got reinfected. And there are examples of this happening, and really one major one and that's dengue. And dengue virus you have an enhancement of disease because the antibodies from a different serotype that you previously were infected with are not neutralizing for a new serotype, but then these antibodies start to coat the surface of cells that...they just they don't act appropriately and you can actually have an increased amount of infection in the cells themselves. And so I think that's been a big question in the field. There's a couple studies and we can talk more about them, but generally coronaviruses haven't had this issue of antibody-dependent enhancement except for one, which is a cat, that feline infectious peritonitis virus, but that's because that virus infects macrophages.

CINDY: Right.

STEPH: And I think it has a lot to do with bringing antibodies in close contact with macrophages as their main tropism cell and then you start to...the Fc...if I'm holding up my hand in a Y shape the top, my two fingers are what binds the pathogen but the stalk, the long part is what can cause a lot of this damage from antibody-dependent enhancement. So I don't know, I don't really think we're gonna see that but if you guys have thoughts on that.

BRIANNE: Yeah so my understanding of the dengue situation is that the antibodies help the virus into macrophages and so it's sort of changing which cells get infected. And the- there's a paper that a lot of people are talking about about anti-spike antibodies and it seems like what's going on there is a little bit different. That instead of changing which cells can be infected, there are changes in what types of cytokines cells are making in response to the infection.

CINDY: Right I agree.

BRIANNE: So it's actually changing the macrophage from one type of macrophage to another. And so it's a bit of a different type of phenomenon. There was a recent TWiV episode where we talked to Stanley Perlman and he was really helpful in talking about this because he had done some studies on this.

STEPH: Right. Does he agree that it's probably not gonna be a thing?

BRIANNE: He he noted that the...while there were differences in the macrophages that were seen in macaques, there were not differences in the pathology in the disease seen in the macaques and so he didn't seem to think that it was particularly important for the disease. Although he said they didn't look long term at the macaques to see if there were differences in the lungs much longer after infection.

VINCENT: He also emphasized that it's different from ADE right? As Brianne has been saying yeah.

CINDY: And ADE is what we're mentioning here, this antibody-dependent enhancement, when the antibodies bind to the virus and actually promote it's internalization into a cell that it doesn't normally infect typically.

BRIANNE: That's the dengue thing.

CINDY: Right, exactly.

VINCENT: Is that a scientific term, the dengue thing?

STEPH: I like the dengue thing.

CINDY: The dengue thingy.

BRIANNE: I'm pretty sure, I'm pretty sure it is.

CINDY: But so I think we're all on the same page that it's probably not gonna happen with this. And I think that you know, what I was saying earlier where they're using this pre-fusion stabilized form of the antigen to promote more neutralizing antibodies versus non-neutralizing antibodies should also help with that as well.

STEPH: But I, I do, yes, I agree and I think, I do get the concern, particularly from vaccine companies because it would be really bad if it even was the potential.

BRIANNE: So bad.

STEPH: So I understand the concern.

CINDY: It would be really bad.

STEPH: Yes.

CINDY: And that's why we need to do safety studies, right?

STEPH: And that's why it takes so long!

CINDY: I mean, people want to rule these things out really really quickly, but I don't think that is entirely prudent. We need to be really careful.

STEPH: So Vincent had a question and maybe Cindy had an answer.

CINDY: So Vincent you were wondering that if you get passive antibodies...

VINCENT: Mmm right.

CINDY: ...would that block your own response to the virus? So we're talking about a major therapy that's being it's been all over the news, is this convalescent plasma. And so when you take the blood the liquid part of the blood from people who have recovered from the disease, it will have, presumably, antibodies that helped them survive the disease. And so if you take those then and give them to a very ill person, the theory is that it has antibodies in there, well that will then help them as well. Now the caveat to that as we talked about early versus late infection, whether you have a lot of virus or a little virus, so that's a question still. But the question is if you gave somebody these antibodies, this passive antibody therapy, would it block their own immune response? And I would say the answer is probably yes to some degree depending on when you give it. Because we know that and Steph can probably speak to this more than I can that when you have a baby for example, that's nursing and they're getting antibodies from the mom, those antibodies are protective and some cases, you know there's multiple reasons why but there's some cases where those babies will not generate an immune response to something they might be exposed to. And so the antibodies might actually bind the infectious agent before the baby's own immune system generates an immune response. Would you...I might not be 100% correct but I think that there is a possibility that it would block the response. What do you think Steph?

STEPH: Yeah I think depending on how high the concentration is, I think it's a little bit different because with the baby it's longer-lasting...

BRIANNE: Yeah.

STEPH: So you can't really give convalescent serum for every, you know, consistently for many months. And that's kind of the situation that you would have with either after placenta, the IgG wanes over like the first six months and then breast milk it's over a long time too. But, I think that probably what they're weighing is the FDA from what I understand with convalescent serum they approved it only for severe patients. And so likely your risk/reward is well, even if we slightly suppress the immune response they're likely older and they need protected more than we can worry about that. That's probably my take on that risk factor.

BRIANNE: Yeah I would completely agree. I know when you are making a memory B cell response, and you have antibodies that have already been made and that are already IgG antibodies that have already class-switched, those antibodies are able to actually suppress activation of naive B cells.

CINDY: Yup.

BRIANNE: Naive B cells have a receptor on their surface for IgG, so they sort of get suppressed while the memory cells are acting, because we don't want to have both naive cells and memory cells sort of using up resources.

CINDY: Yep.

BRIANNE: And so in that way I would expect that the passive antibody might block making a naive B cell response, but I completely agree with Stephanie that when we have patients who are extremely sick the first priority should be to help them feel better now, and more so than thinking about their later immune responses.

CINDY: Yeah I wouldn't worry so much about the later immune response. What I'm actually more worried about is if you don't know why those people are really sick, I'm not sure it's gonna make a difference. So if they...if they...

STEPH: Right. Like is the virus gone by that point?

CINDY: Yes, right.

BRIANNE: Right.

CINDY: If their own body is failing to mount a response for some reason, which we do see examples that people are saying that there are certain individuals that are not mounting a good antibody response, right? So in those cases, providing those antibodies to them in a passive way should help those individuals. But if somebody has already mounted their response, the virus is mostly under control and the pathology is being mediated by the immune response, I'm not sure it's gonna help.

VINCENT: Yeah I think these convalescent trials are gonna show that it doesn't help if you give it to a patient later in illness. Because even when this more serious lower tract illness begins the virus is already decreasing in the upper tract by then, so.

CINDY: Exactly, yeah.

VINCENT: It's not gonna help. It's helping earlier but who knows who's gonna be the seriously ill patients, that's a problem.

STEPH: Yeah.

CINDY: Yeah.

STEPH: Wow. Okay great! So we're kind of onto our next section here. I think we can talk about yeah, Cindy the testing the diagnostic tests and that might roll us right into serology testing.

CINDY: Yeah, so...

STEPH: I think that's about...

CINDY: So there's two main tests. And there's a whole lot in the news about tests and whether they don't work or they do work or whatever. And we could get into whether they quote unquote work or not or, I don't know. But the bottom line is there are two major classes of tests. One is testing whether or not there is virus present. And that's important to say it that way I think, because it doesn't necessarily mean it's infectious. And we all, those of us who work in this know that in order to demonstrate there's infectious virus you have to do a virus isolation and culture and show that it's actually live, whatever, you know I wouldn't say live, replication-competent virus, which means it could infect new people. So if they do a swab and stick a swab way up your nose into the back and they swab it or down your throat, they're going to do what's called a PCR test. And that's polymerase chain reaction. And I was reading about this it's fascinating because I did not know about the loop amplification mechanism. It's a PCR that they can run isothermally, so at room temperature er at least at one temperature I would say. And it can be done in 15 minutes, I was like whoa that's so cool! Anyway because you know we do real-time PCR and I think you know, that's great because you can detect it in a couple hours. But so the test is basically this is an RNA virus, but to do polymerase chain reaction you need DNA, so we turn the RNA into DNA and then you amplify it and basically the more copies the more fluorescence you get because there's a probe in there. And so you can tell if the person is infect...has the virus present or not. So they have a virus that you can pull out of them. Usually if you have symptoms along with that that would be considered a diagnostic positive. But there's issues where maybe a person has a lot of symptoms, it seems like, it looks like it and it smells like it but when you test it's not it. And that could be just a failure in the testing, so there's this whole thing of is a positive test a positive test? Probably yes, it's pretty accurate. But is a negative test a negative test? Maybe not. So that that causes a lot of confusion in people, you know trying to understand how all of this works. So that's the main test that they're using and to do that you do need pretty sophisticated equipment to run these PCR reactions to be able to test whether there's virus there or not. And the other thing is you need to get an you need to be approved to do this by the FDA and then you have to demonstrate that you can accurately detect positives and that you're not detecting positives where they're not positive and that they're negative when they're supposed to be negative. And so there's a lot of, you know, certification that you need to go through to be able to do that. Now, as I understand it they're sort of relaxing the rules on that and it's becoming the wild west and so it's not clear that every test that every company is putting out is as accurate as it should be and so that could be contributing to some of the negatives that we're getting that may not actually be negatives but they are in fact positives. So that's the first test, did you guys want to comment anything about that?

VINCENT: I just wanna say there are these reports coming out of Korea and some other countries that people are getting re-infected, quotes.

CINDY: Yep.

VINCENT: But I think those are failed negative PCRs, and these people are just shedding pieces of RNA not infectious virus by that point, and then they just test them a week or two later and they're, the test works and they're still shedding pieces of RNA and they say they're reinfected but I don't believe that they're actually reinfected, the original infection is just going on. And we know now that RNA virus infections can shed bits of RNA for a long time after the virus is actually gone.

BRIANNE: Yeah, exactly. I think that with this PCR, one thing that people should know about it is that the answer that you get in the lab is a number.

CINDY: Right.

BRIANNE: And then we take that number and sort of make a decision about whether that number means positive or negative. And in some of those patients there who went negative and then became positive they were really fluctuating right around the cut-off line. And so this might be more what cut-offs we're using for positive and negative as opposed to the patient actually really having zero virus and then having the virus get, come back or allow them to be re-infected.

CINDY: And it's the nature of this kind of amplification style of testing, because, you know if there's bits of anything in the air or on any surface that when you run these tests if you go long enough...

BRIANNE: Yup.

CINDY: ...pretty much everything will eventually come up positive. It usually is very rare that something stays negative for, you know, many many many many many cycles. And so we do this based on when a positive known control comes up and when a water or negative control comes up or doesn't come up. And so then, that's how you set the cut-offs, and so it's not an exact black/white.

BRIANNE: Yup.

CINDY: You know we think of things like a pregnancy test, you pee on the stick, it's positive or it's negative, right? And so sometimes there's a, is that really a line there or is it not, that can be a question. But some of these other tests we're talking about are not so black and white. Now, if you pee on the stick for a pregnancy test and it's black and white, you're actually looking for a protein in the blood, and so you can detect proteins or you can detect antibodies by a similar mechanism. And so there's what's called a serology test or an antibody test. Now the antibody test is getting a lotta news lately, because everybody wants to know whether they have been infected or not and whether they are now safe to go back to work or to go outside and to socialize. And the presumption is, if you have an antibody test come back positive, that your immune response has kicked in, kicked the virus, and you're good to go. And so what those tests are looking for are antibodies in the blood or in secretions, so you can use saliva sometimes or you know, even the nasal secretions. But there are several different ways that test can be done as well. The most accurate way is what's called an ELISA, where you look for those antibodies and you can actually quantify how much of them are there. And we're saying them because there's more than one class, and I'll let you talk about that a little bit maybe Steph in a minute. But the other test that's being developed is a rapid point-of-care test that probably is going to become the norm as we move forward, and that is you'll be able to take a sample, you'll put it on the end of the stick, and it will slide along and if it binds to something that's a control that will tell you if it's there or not and it's probably like gold or some other reagent that if there's an antibody there, it will get reacted and then it will come up as a line as a positive. And then there's a positive control and a negative control on that sample as well, and then you read whether there is or is not a line there and that'll tell you whether you have antibodies in that sample or not. And so yes in theory you would not have any antibodies if you have not been exposed and you would have antibodies if you were exposed. So that's theoretically what would happen. But there are nuances to that as well. So I don't know if you wanted to go into any more about that, Steph.

STEPH: Sure, yeah, wow. This serology testing. I mean it's, I would argue an even more challenging assay than the PCR assay in terms of creating something that's highly sensitive and by sensitive I mean the ability to correctly identify patients with the disease, and then also highly specific, which is the ability to test and ID patients without the disease. And that's kind of, scuse me, why there's been a lot of controversy because the FDA has relaxed regulations and so before when you were creating a serology test to look for an antibody, a company would have to submit data to the FDA to then be allowed to sell this as you know, a diagnostic. But now they're able to sell it and then send the data to the FDA and then the FDA can then decide, but they're already selling it so you might see in the news people who are receiving boxes of serology test kits that they're all defunct.

CINDY: Yup.

STEPH: They're not all sensitive or specific enough, and why is that the case? So there's a couple different reasons. For one, what the antibody binds to in the test has to be something that is highly recognizable by the antibody. And so we've talked a lot about the spike protein and so likely it is the spike protein that is being put in the test for the antibody to bind to. But it could, you might wanna double your chances and put another protein down in there, which could be the nucleocapsid protein because there's so much of...it's the most amount of protein in the virion. So how is that produced? How is that protein produced? What's the conformation? We make these things in cell culture-what was the type of cell you used? How did you purify it? And all of those different things are gonna vary from company to company. And if it's not validated extensively, you could have a station where it's,

you're getting either false positives or false negatives. And that relates to the fact that we have circulating common cold coronaviruses. And I think in the HKU, there's like four and they're all random names so I'm not gonna list them because it's, who cares about trying to remember those. But one of them has 50-60% cross-reactivity with the spike protein of SARS-CoV-2, which is not a lot but could be enough for something like IgM, or the first antibody that's produced after an infection, that is very sticky, might accidentally bind to that and then getting a positive test that maybe isn't really positive. And so all of these things that in terms of manufacturing of the test can lead to faultiness down the road. And then trying to determine based on is it IgM as I mentioned is this first, more innate-like antibody, where it's more quickly produced but it is kind of sticky, it's not very specific, provides some protection but you really wanna wait for that IgG with the nice high affinity to really show you that you have an antibody against it that's specific. So that's the reason why there's a reason why there's a lot of issues with this in terms of sensitivity and specificity. And you have to weigh those things. I think Daniel was talking about in the clinic they have, you know, you have to test that and weigh the different options and then they can decide okay this is the one we're gonna use. And if we're determining policy based on this, that makes me very nervous because of this reason. I mean we're gonna give somebody a test that says you're negative you can't go make money for your family but in fact, you know, it wasn't a sensitive enough test because if for whatever reason, that makes me very nervous. And so...and because the regulations are relaxed, which I understand in this circumstance but that's what we're dealing with right now, it's very complicated.

BRIANNE: That's only one of many reasons I find, this whole thing makes me very worried.

STEPH: Right.

VINCENT: I mean the real issue is that does this test actually tell you you are protected?

CINDY: No.

STEPH: Well that's a whole...right. That's a whole nother bag of worms with this idea of correlates of protection and protection is even kind of used loosely. Does this antibody to whatever protein they're putting in there, which is probably proprietary, they don't tell you. Does that mean you're protected? We don't know. And so if you're telling people you can go back to work but maybe that's not a protective level and people are different, so it's...yeah, Vincent that's key.

VINCENT: And I think it's very very questionable, I don't think they have a choice, I think they have to do this and they're gonna see if it's positive on this rapid test, are most people not gonna get reinfected? Because as you know you need to look at neutralizing antibodies...

CINDY: Yup.

VINCENT: ...to tell if you're protected. These tests don't distinguish.

CINDY: Nope.

VINCENT: And boy if you wanna make some money, figure out a 15 minute neutralization assay.

STEPH: Wow.

BRIANNE: Woah.

CINDY: That'd be amazing. There's a challenge for the listeners out there.

VINCENT: Although I guess, and you immunologists tell me, if you knew which epitopes generated neutralizing antibodies, couldn't you make an assay specific for those or would that be too hard?

BRIANNE: I don't think it's epitope to epitope specific.

CINDY: Yeah.

VINCENT: Well, yeah but we know on flu HA for example what epitopes give rise to neutralizing antibodies and others do not. So...

CINDY: So if we used the receptor binding domain, only the part of the spike protein that binds the receptor I suppose if you had an antibody that bound that it would tell you but it doesn't mean that...

VINCENT: That would be a subset, yeah.

CINDY: It doesn't mean that there aren't other antibodies that could also neutralize. So I think it would be...

VINCENT: So that's the problem here.

CINDY: Yeah.

VINCENT: If you have an epitope that is a neutralizing epitope, not every antibody against it, some of them will be slightly different and they won't neutralize it, right?

BRIANNE: Exactly. Yup.

VINCENT: Got it.

CINDY: That's right. And there might be other antibodies that bind to a different part that still might be neutralizing because of the way they induce a conformational change too I think.

VINCENT: Okay. Just trying to make money for us, that's all.

STEPH: Okay, we'll scheme after this, see what we come up with.

CINDY: It's not so simple.

STEPH: So we have about five minutes left, so Cindy am I assuming that 2 p.m.'s a hard deadline?

CINDY: I can hang out a little bit longer than that if you want.

STEPH: Okay, well I you know, that is really kind of the summary of serology. I'm gonna let you all pick out unless we wanna do a part three, I'm always up for talking about this stuff.

VINCENT: We should do a part 3, I don't think you should rush anything.

STEPH: Okay. That's fine. So we can either...masks are big...

BRIANNE: Yes they are.

STEPH: Yeah so if anyone feels passionate about any of these questions, or we can read a listener e-mail, I'll leave it up to you, whatever you want.

VINCENT: Steph, you wanted to say something about WHO., which I think is a fine organization.

STEPH: I think actually Cindy put that in there.

CINDY: I put that in there, yeah.

STEPH: Yes.

CINDY: I...yeah. I don't...I don't know, what do we say?

STEPH: Relevant...

CINDY: What do we say? So I'm distressed to say the least that I'm seeing that the president is withholding funding for the World Health Organization.

STEPH: In the middle of a pandemic.

CINDY: In the middle of a pandemic.

STEPH: Not sure that that's...

CINDY: And I feel like...I don't know what to say about it. It's irresponsible. It seems like a power play for not taking responsibility for what's going on here and trying to place the blame somewhere else where that doesn't make any sense. And so...I'm just, I'm fearful for what's gonna happen to us. Not that the World Health Organization is going to deny us stuff, I mean I think they understand that not everyone in the U.S. agrees with what our president is saying, but I think we need to...we need to worry about generally how continued, you know, over time and continuing even today this administration is viewing science and public health officials. And I think it's a part of the reason why we are where we are right now.

BRIANNE: Absolutely. I think that we mentioned earlier that this is sometimes making us think about immunology in terms of the population instead of as the individual, and I think that in a similar way we have to realize that we need to be thinking about what's happening in protecting us against viral infections like this in the world. It's not as if viruses are doing different things country by country. And the World Health Organization is really important in us thinking globally about what's going on with these infectious diseases. And we can't just worry about what's going on here. We need to think about what's going on elsewhere, because that will impact whether or not we see infections here.

STEPH: Yeah I mean I think any large organization is going to have its flaws. I think the Ebola outbreak in 2014 I think WHO. did get criticized for some of its responses but they had worked to, you know, make sure their responses were quicker and better. I think yes, there are points to criticize but overall in terms of a net benefit, this is an organization that is needed if we're talking about pandemic preparedness and allowing countries to be able to respond by sharing information and providing resources online that you know, maybe a lot of governments are not able...clearly...like ours potentially to respond quickly. And even governments of smaller countries who have even less structure. So the WHO. is something that can really serve as a source of structure to provide information and to have a consistency. And without it, despite its flaws, we are...we're worse off. I mean, we...that's the thing about viruses and infectious disease, they travel borders, it doesn't matter. We're not in, you know, 1820 where we're not traveling across the world in a second in an airplane. I mean, we're...we have to, the reality is it's 2020 and we can cross continents in a day. And we need a World Health Organization. So the timing of it, I mean it's just all, it's crazy. So I think we can say in general we're very pro WHO. and also holding them accountable for when they slip up. Just like we should hold ourselves and our government accountable for our responses.

BRIANNE: Absolutely.

STEPH: Yeah.

CINDY: Ending on a light note, you said that travel across the continent in a day- did you see this story of these people who took it was an Audi I think they got in it and because there was nobody on the road...now this is theoretically I think...but they said that this was anonymous. But they drove from New York City to California in like 28 hours.

BRIANNE: Wow.

CINDY: Isn't that insane?

VINCENT: Is that possible? Is that even possible?

CINDY: I don't know I did the calculation and for like 3000 miles it was like 115 miles per hour for 28 hours straight...that doesn't seem possible to me.

BRIANNE: Whoaaa.

STEPH: That's crazy.

CINDY: That seems a little crazy, because I know you can take Route 66 across, but it's not a straight shot and so, I can't imagine...

BRIANNE: No.

CINDY: ...that you could stay that speed constantly. And not get caught.

BRIANNE: It's hard to imagine there was absolutely zero people on the road.

CINDY: I know, not a single cop.

STEPH: Yeah cause listen, I drive around, I see...there's a lot going on in the RT, in the research triangle here at Durham.

CINDY: Yeah.

STEPH: There's a lot of people on the road.

CINDY: So, who knows if that's true but I thought that was pretty funny, so.

STEPH: Yeah, that's fun.

VINCENT: Alright. That's Immune number 30. We'll have part 3.

STEPH: Yeah part 3.

VINCENT: And probably 4 and 5 and 6 and so on...

CINDY: So send in your questions!

VINCENT: You know we're gonna have serology results, it'll be interesting to see and all sorts of things. Vaccine results, so...stay tuned. You can find this podcast on any podcast player. The show notes are at microbe.tv/immune and send us your questions and comments- immune@microbe.tv. If you like what we do, consider supporting us-microbe.tv/contribute. Cindy Leifer's at Cornell University. Over on Twitter, cindyleifer. Thanks Cindy!

CINDY: Thank-you!

VINCENT: Steph Langel's at Duke University. stephanielangel on Twitter. Thank you Steph.

STEPH: Yeah thanks this was great.

VINCENT: And Brianne Barker's at Drew University, bioprofbarker on Twitter. Thanks, Brianne.

BRIANNE: Thanks it was great to join you all.

VINCENT: I'm Vincent Racaniello. You can find me at virology.blog. The music on Immune is by Steve Neal. Thanks for listening to Immune, the podcast that's infectious. We'll be back very soon.

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