Immune

With Vincent Racaniello, Cindy Leifer, and Stephanie Langel

Episode 3: Two epitopes, four serotypes, and a partridge in a pear tree

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Transcribed by Kim Barker

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VINCENT: From Microbe TV, this is Immune, Episode number 3 recorded on December 12, 2017.

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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 NY, Cindy Leifer.

CINDY: Hi! Welcome everyone back.

VINCENT: And, from Wooster, Ohio, Stephanie Langel.

STEPH: Hey now, how's everyone's December lookin' like?

CINDY: Mine's a little snowy but not too bad from what I hear we have had less snow in Ithaca than in, for example, New York City, which is a little crazy.

STEPH: That is crazy.

VINCENT: So we had our first snow on Saturday, got 3 inches or so. And today it's kind of, it's raining today. It's now warmed up, all the snow has melted; it is raining and pretty dreary here in New York City.

STEPH: I'd kind of welcome the snow, I like the cold I don't know if it's, you know an Ohio Midwest thing, an Ohio thing, but I know Vincent you like hot hot weather, that is not my jam.

VINCENT: I do love hot weather, you know when I was younger I loved snow, I don't know, I'm less tolerant. The problem is, all the hot states are not the right politics for me.

STEPH: That does make it challenging.

VINCENT: Like...forget Florida, South Carolina, North Carolina, Virginia even, although Virginia seems to be coming around.

STEPH: What about Alabama- that seems to be an important state today?

VINCENT: Oh my gosh, that is such a key state. You know if a democrat could win the senate in Alabama, we would have a good chance of defeating the tax bill.

STEPH: That's true and I'm sure that that's where the opposition is campaigning against using those exact talking points. It will be interesting, I mean, I don't know...I, there's good signs being done in Alabama too, there's good signs there.

VINCENT: Ah yeah, sure, Birmingham for sure, but you know these are little...I heard a statistic today; 15% of the population of Alabama is college educated.

CINDY: That's it?

VINCENT: Yeah isn't that unbelievable?

CINDY: Wow...

VINCENT: And those are the core voters for the current administration.

CINDY: Do we know the numbers nationwide? I don't know that.

VINCENT: I don't know offhand, no. I heard that on Pod Save America, my latest podcast interest. Pod Save America is by a couple of former Obama and Clinton speechwriters and they started at the beginning of 2017 and they are probably one of the hottest

podcasts out there. They are, you know, kind of a liberal what's wrong with the current administration thing and people love them of course, they're doing really well. Hey listen to this; we're proud we got over 1000 downloads on the last couple episodes of Immune...

STEPH: Oh no, what'd they get?

VINCENT: Between 1 and 2 million per episode.

CINDY: No way, wow...

STEPH: That's our goal! That's our goal for Immune...1 million.

CINDY: So everyone listening out here, all you need to do is recruit about 1000 or 10,000 people each and we'll be good.

STEPH: Yes. and I will say something about Alabama, I think there is a sentiment, probably in scientific communities or just like maybe more liberal based groups is to kind of just disregard Alabamans or people who are in Alabama and I would say the opposite; I mean I'm for Alabama people because the science needs to get there, and if we say oh they're just, forget Alabama I'm not even gonna bother with them; well those people that need science the most are there. So, I kind of am doing the science for those people, that's kind of how I look at it.

VINCENT: You know, I'm of the persuasion where all people are are good, humans are an amazing species, we should help each other, and you know, science discoveries should go to everyone, everyone should benefit, but you know, there's a culture of hatred throughout the world and people hate each other. Ah, it's just terrible, it should not be that way. You know animals are good to each other except when they eat each other but...so we should follow their lead. Alright- we have two stories for you today...

STEPH: Yes.

VINCENT: ...and one of them we'll spend a little less time on, but it has to do with the dengue vaccine, which is called Dengvaxia, which some of you might have been reading about in the news, we actually covered it pretty extensively on TWiV, but I thought maybe we have some listeners who don't listen to TWiV, and you should know about this. And so…

CINDY: Absolutely

VINCENT: …so Dengvaxia is a vaccine produced by Sanofi, which is directed against dengue virus, which of course is a mosquito borne flavivirus vectored by *Aedes aegypti*. Primary infection; rash, fever, joint pain, conjunctivitis, but if you get re-infected with a different serotype, and there are four serotypes, you can get very very serious disease involving hemorrhage and shock and you can die of that. So that's serious dengue. And so this vaccine is supposed to prevent both primary dengue, which is not often life threatening although I think you can get serious disease in about 1 in 10,000 primary infections, but it's the secondary infections that are a problem. And we think that- and I think there's some good evidence for this- that the secondary enhanced disease is caused by antibodies. So the current model goes: you make, you get infected with dengue say type 1, you recover, and then sometime later in a different season when there's other serotypes circulating you get infected with a different serotype, you make a memory response to type 1 dengue, those antibodies will bind to the other dengue that you were infected with but it will not block infection, and it will allow them to get into cells that they don't normally infect, like cells with receptors for the Fc portion of the antibody.

CINDY: Right.

STEPH: We could talk a little bit more about antibody dependent enhancement now if you'd like?

VINCENT: Yeah, you can, go ahead.

STEPH: Sure. I know I think Cindy had brought up the different types of effector, effector responses for antibodies. We know that they're produced by B cells; they can either be in a membrane bound form on B cells or they're in a secretory form secreted from plasma cells. And what we're really talking about here is the secreted portion. They serve many functions, one of them being opsonization. And opsonization is essentially where the antibodies are gonna flag a pathogen by binding to it, and then it can be uptaken by a macrophage and phagocytosed. But what Vincent is referring to is essentially a primary infection you have the ability to do that amongst other immune responses- maybe there's some T cell work there but essentially in a secondary infection, that antibody is I'm assuming weakly bound to dengue and so it's not able to be flagged. It does not become phagocytosed, and then it essentially can bind to the Fc receptor as Vincent mentioned, and it's binding by the constant portion of the antibody and uptaken and then that virus can just keep replicating inside monocytes. And so it's really, it's I think was maybe first discovered in dengue virus. Scott Halstead in the 60s, and I know HIV it's been proven as well. Now there's some other viruses *in vitro* it has been shown, like Ebola, other types of viruses, but I think really proven, dengue and HIV seem to be the two viruses.

VINCENT: What do you think Cindy? Do you have any thoughts on ADE?

CINDY: I guess the only thing I was gonna add is the really interesting aspect of this is you mentioned the serotypes for dengue virus, and I think it's really important because when there's four serotypes, which you may wanna explain what a serotype is a little bit more, but it's just a variant that's slightly different. And so what happens is these antibodies are designed to be exquisitely specific for recognizing a particular pathogen. And when that pathogen varies slightly, they don't do as good of a job. But the interesting thing about this is that if you got exposed to for example, dengue 1, and you made a good strong response against dengue 1, and then get infected with dengue 2, what it does is it trips up the immune response. And so the immune system sort of thinks it's still seeing dengue 1, and so it generates this memory response. And so it responds rapidly with the same armamentarium that it did against dengue 1 the first time, but it doesn't work as well against dengue 2. And so the primary immune response against dengue 2 is not developed properly, and so you employ this not as good an immune response, which would be excellent against dengue 1 but when it's applied against dengue 2 it doesn't work that well. And what happens is it enhances the ability of dengue 2 to infect cells it normally wouldn't. And so instead of acting to protect, it causes worse disease.

VINCENT: So the, it, what's interesting is now as you're saying that, it doesn't happen with every virus right?

CINDY: It doesn't it doesn't...it really requires...

VINCENT: I'm thinking of polio, where I spent half my life thinking about polio, and three serotypes, you never get this. You know.

STEPH: And I wonder if that's because the serotypes don't differ that much in the epitope...

VINCENT: Maybe.

STEPH: …you know, I, it seems to be that antibody dependent enhancement it really is the change in the epitope that causes whatever the other serotype is to trip up the antibody's ability to bind to it, that could be why. I think in one of the links that you have in the show notes it did say if you get infected with the first serotype and then get the second or the third it's worse than if you got infected with the second or the third and then subsequently got the first. So there seems to be...you know...

VINCENT: Yeah, there's a sequence of events.

STEPH: Yeah yeah, which is interesting.

VINCENT: With polio, if you are infected with type 1, you can get infected with types 2 or 3. The antibodies do not cross-neutralize. But I don't know of I don't know of many human multiple infections like that. It may be that there's enough protection that…you know. But the vaccine has all three serotypes in it.

CINDY: And that's that's really important, because I read that that's one of the only ways you can try and get around this problem is to immunize with all of the potential things that you could come in contact with simultaneously...

VINCENT: That was the idea.

CINDY: ...which is problematic, right.

STEPH: Yeah.

CINDY: So they were able to do that for dengue but the other virus that's really close to that that we're going to talk about in a little while is Zika, so it's another flavivirus that's similar, and it, they think that dengue and Zika have this same thing so if you get infected with one, this can induce this antibody dependent enhancement of the other disease as well. And so being close in evolution or however you wanna call it, they can enhance each other. And so if we think about immunizing against flaviviruses, another one is yellow fever virus, right, so if we tried to immunize against all the dengues and Zika and yellow fever all together, what about all the ones we haven't seen yet? And so you could still have this happen to new emerging diseases that we haven't seen yet.

VINCENT: Now what happened with Dengvaxia is that it was licensed after a phase three trial, big trial, and many many thousands of people in different sites in Latin America and Asia. And after it was a three dose regiment, they had control and placebo groups, you know like 10,000 kids in each group, ages 9 through 16 I think, and a year after the phase three trial, they had data which showed that it protected against primary dengue and it, they didn't see any enhanced disease. So it was licensed. And it's been licensed in Brazil, Philippines and Mexico. But of course it's been now five years, and they've been collecting data, and now it looks like there is some serious disease. And there's a wonderful article by Scott Halstead in Vaccine, and we'll link to that, and he says there is disease in this trial, it's a 1.4%, so it's just, you know, between one and two hundred kids but that's too much. It's in two groups: serious disease is in kids who were never infected with dengue…

CINDY: That's correct.

STEPH: Right.

CINDY: Right.

VINCENT: …and then got the vaccine, and that was like having a primary dengue infection. So then, when they went out into the world, and got dengue out in the wild, then they got serious disease.

STEPH: And essentially it sensitized them…

VINCENT: Yeah, right.

STEPH: …to whatever they, yeah, if they saw it in the wild, and I was curious I mean, did they not, I mean they knew that antibody dependent enhancement was a thing. This is something that people have been publishing on, so when they initially licensed it and it had only been a couple years, I'm just surprised, I don't know I would have thought they would've waited that five years so that they would know.

VINCENT: Ah well, that's a good question, but remember, they had invested almost 2 billion dollars into this vaccine.

STEPH: Whooo whoo…

CINDY: Yeah.

VINCENT: I think they wanted to start earning something back.

CINDY: Yeah.

STEPH: Sure, sure.

VINCENT: And how long would you wait? You know, they figured alright a year we haven't seen it- here's the thing though, and I learned this from the Halstead article; you can't depend every year on having the same dengue seasons, the serotypes differ...

CINDY: Yeah, right.

VINCENT: …and you know sequences as you said previously of serotype infections can make a difference, so they didn't see it that first year, and then later they did.

STEPH: Well and I mean in these populations, you're gonna have a large majority, I don't know what, 70-80% that already has seen dengue, so you have that variability, and I'm just, I mean now they're dealing with a really large PR issue, you Google Dengvaxia, and...

CINDY: Oh yeah.

VINCENT: Yeah, yeah that's a problem.

STEPH: …Duterte, that dictator on the Philippines, he's all riled up and talking about how he's gonna come after them and so, and I'm also curious you know, your guys' opinion, I I mean I think this move to try to make a vaccine for a disease that pretty much dominates in poor populations is a good thing, I mean we, I like to think that more pharmaceutical companies would do this.

VINCENT: Yeah, yeah.

STEPH: But, you know, is this as a ripple effect going to make other companies, if they're even considering doing something like this…

VINCENT: Good point.

STEPH: …where they wouldn't make their money back maybe anyways, I just worry about those effects too.

VINCENT: Good point, I don't know the answer.

CINDY: Yeah, I don't either. Yeah it's definitely something to worry about.

VINCENT: The other population that experienced serious disease were kids who- the placebo group, right? Where they didn't get the vaccine. And of course, if those, if some of them had been already infected then they went out in the world and got dengue infection and got ADE and serious disease as well. So two different groups. Now here's the cool thing or the interesting aspect of this; this vaccine was made by taking the yellow fever virus vaccine, which has been used for many many years, made a DNA copy of it and they substituted the glycoprotein out from dengue all four serotypes into that backbone. So the idea was that's a proven vaccine…

CINDY: Right.

VINCENT: …safe, so let's just put dengue glycoprotein into it and that was licensed and used. And there's some idea now that protection against severe dengue requires a T cell response. Right? A CD8 positive T cell response. There's some data to suggest

that. And the epitopes that are recognized by those T cells are non-structural proteins that are not dengue in Dengvaxia, they're yellow fever; and that's not good enough apparently. Isn't that interesting?

CINDY: That is very interesting.

STEPH: Definitely.

VINCENT: I don't think it's as simple as that, because here's a paper and what's shown is antibody dependent enhancement of severe dengue in humans: a study in Nicaragua, where they showed that the antibody levels determined right if you had a good level of antibody you were protected against serious disease, and lower levels were not protective. But I think probably it's a combination of that and the T cell.

CINDY: Yeah, we're gonna have to cover that in a little bit more detail, cause for those who don't know a lot about this; so T cells and B cells see very different things. So B cell antibody, B cell produced antibody see things in their native structure, so would be a virus in its normal state with all its structural components the way they're organized, and that would be the normally infectious form of the virus. Whereas T cells, because of the way that they recognize what they recognize, they can see things that are inside the virus. And they have to get processed and then presented in a way that activates a T cell. So they can see fundamentally different things, that can complement each other. And for viruses, antibodies can prevent the virus from infecting a cell, and we'll also talk about other ways that neutralization can occur, I think in the paper we're gonna talk about. But the T cells, what they do is they they recognize virally infected cells and try to eliminate them from the body. So there's really these very different mechanisms that T and B cells use during infection, and so having both is great but most vaccines are not able to induce a T cell response. The ones that are closest able to induce a response has both T cells and B cells as a component are usually these attenuated viruses, and they have their own, er attenuated pathogens, it can be a bacterium too, they have their own safety issues and things. So the closer you are to a natural infection, the more you are likely to induce all of the types of immunity that are required to protect. And the further away you get from the natural infection, the harder it is to induce those, the more you'll induce antibodies but maybe not T cell responses and that might not be as protective.

STEPH: And another concept that's you know as immunologists we think about a lot, especially vaccinologists, is correlates of protection, or correlates of immunity.

CINDY: Yes.

STEPH: And what type of response is going to predict an individual's ability to fight the infection or to raise an anamnestic or a memory response. You know, I think for vaccines we think about, ok antibodies is what's important, but as we discussed, maybe it's a T cell response, maybe it's both, maybe it's CD8 or CD4 T cells, and we don't, I mean it's kinda…there's only a few pathogens that we actually know the correlates of protection. I know in the virus that I work with that's definitely something we would love to know; if I could draw the blood of a pig in a herd and I could tell the farmer, you know, OK this animal is going to be able to respond very well, this one is not, and it's just, without knowing that you don't really know the target of what you're trying to achieve.

VINCENT: I think what's interesting is that there is another dengue vaccine in the works. It's being developed at the NIH here in the US, and that vaccine's got the wonderful name TV003.

STEPH: Catchy.

VINCENT: I should say double-0 3 right?

CINDY: Double 0 7.

VINCENT: Yeah. And that is all dengue. It's an infectious attenuated vaccine like Dengvaxia, except TV003 is all dengue. What they did was delete I think 20 or 30 bases from the 3 prime noncoding region of the viral RNA, and that attenuates the virus so it doesn't cause disease. And in one trial they gave it to volunteers, and then they challenged them with a dengue vaccine strain, which didn't cause disease, and all of the volunteers were protected, none of them developed serious disease, and they developed what's called sterilizing immunity. In other words, when they were challenged, that challenge dengue virus never replicated in them. So that would probably be what you need to prevent, you know, serious dengue disease.

CINDY: Now in that one did they have all four serotypes represented?

VINCENT: They did, yes.

CINDY: Mmhmm.

VINCENT: It's all four. So, that's going on in other phase trials, we'll see what happens with that, that's a number of years off before it would be licensed, but to me that seems to be the one that should have been developed, but of course hindsight is always 20/20 right?

STEPH: Right, right.

VINCENT: So, who knows. But now what do you do? So Philippines has said no more, we're not using this vaccine. I don't know what Mexico has decided to do, but I heard that Brazil is just going to immunize kids 15 years and over, and the logic there is by the time you're 15 you probably have had at least one infection of dengue. Right?

STEPH: Right.

CINDY: Right.

VINCENT: Now of course the alternative would be to do a serology test on everyone before you immunize them, but that's kind of logistically complicated I guess.

CINDY: Yeah, and I'd imagine...

STEPH: And the places...it's expense right- like the places where dengue might be the highest might be areas where they don't have good mosquito control maybe it's poor areas and serology tests just, you know, wouldn't be feasible. But...I guess though if you vaccinate 15 and older and they see most of those children...it's just hard to consider the fact that these are kids- I always think back to that, it's like ugh...you know.

VINCENT: Yeah, yeah. Well the thing is that there have been some very cool rapid antibody detection tests developed in the last five, ten years. And many of them involve cards, just papers saturated with reagents and you can put a drop of blood on it and get a result anywhere almost you know you...

STEPH: Right.

VINCENT: ...you don't have to be in a physician's office so.

CINDY: But I guess what the studies show, including the Science paper that you mentioned, testing the children in Nicaragua, it's not just presence of antibodies, but it's the amount of antibody you have…

STEPH: Yep…

CINDY: …so you're measuring a titer, so a titer is just the dilution at which you don't detect it anymore, so the more you have to dilute something the higher the titer, the more antibody that was there to begin with. So if you have high titers it's very protective but if you have low titers, you have this potential for this enhancement. And so just looking at detection is not enough, we would need to do serotyping for the titers in order to be able to determine whether those children should or should not be vaccinated.

VINCENT: Mm yeah, that would be hard to have that degree of granularity right? I think you could just say yes or no and then make a decision; alright this person has had dengue, we're not gonna vaccinate them, cause I think what you're saying, and probably that's the way to do it, it would be too costly.

STEPH: Mmhmm.

CINDY: Yeah.

VINCENT: To do the actual titer. Anyway, it's an interesting problem. It's not a nice one, because the way I look at it, it just fuels the anti-vaccine crowd, right?

CINDY: I know, that's what I'm really worried about. And so, at least we're discussing this here and explaining that you know this happens all the time this is a natural phenomenon, this is not a vaccine specific effect.

STEPH: Right right.

CINDY: It's really important to emphasize that. But it is I mean it is a public relations problem now, because this is all over the news and people think oh, vaccines are dangerous. In this case you know maybe it is but it's not any more dangerous than having been infected before and having this potential. And so it's just understanding the disease better, that's why we need to study these things that's why we need the money in science to be able to understand these processes better and predict this before it happens.

STEPH: Mmhmm.

VINCENT: This, we should point out that there have not been any cases of severe dengue associated with the vaccine in any of these countries, it's just they have based this decision on the clinical trial results, and so they have avoided problems right by acting quickly...

CINDY: That's correct.

VINCENT: …so that's good. So we'll figure out how to get around it I'm sure, cause you don't wanna not immunize against this disease right? That's...

CINDY: Right.

STEPH: Right.

VINCENT: ...kind of not a good solution, although it seems, that seems to be what they wanna do in the Philippines, but I suspect that they're gonna figure some other thing out.

STEPH: Cause it definitely, I think it's also more than just the initial you know, they call it break bone fever, and people have problems even after. Maybe I had a friend who she went abroad, she came back and she's had issues ever since then. So it definitely leaves people incapacitated.

VINCENT: For sure, for sure.

CINDY: Mmhmm.

VINCENT: So we had a guest on TWiV, Nina Martin, who's a virology PhD student at Hopkins, and she told us her story of getting dengue-she was working as a volunteer somewhere. And she says to this day she has recurring health issues as a consequence right? So even though we say you know, primary dengue is usually mild, it can have long lasting effects.

STEPH: Right.

CINDY: Do do we know why that it would have long lasting effects? Is there some sort of mimicry with a self-antigen that would cause long lasting autoimmune type of reaction?

VINCENT: That's a good question, I don't know.

CINDY: I don't know either. Maybe our listeners know.

STEPH: Yeah maybe they can write in. Do you know does Nina do you remember what she said her recurring, I mean did it have to do with her you know, being sore? Her bones or muscles?

VINCENT: Don't remember, but we can put the episode in the show notes so people can listen.

CINDY: Yeah for sure.

VINCENT: It was a very interesting story, you know she said she was working and usually she used mosquito repellent. But one day she was out for a long time and she could feel that it had worn off and she started to get bitten, and it was after that she developed a fever, rash, conjunctivitis, bone aches. She recovered but she said periodically, oh I think one of the things was she gets blurry vision you know.

CINDY: Wow.

VINCENT: Just out of nowhere and other, maybe also bone aches or something, I don't know. But you know similar things happen with chikungunya…

STEPH: Yep, mmhmm.

VINCENT: Where you have these, many people for the rest of their lives have recurrent arthritis, bone aches, so Cindy I guess when you see arthritis you think about some autoreactive process going on right?

CINDY: I do, I tend to think that, yeah. It could just be damage that was happening during the disease that has now lowered a threshold for inflammatory sequelae.

VINCENT: Really interesting. Ugh it's just, immunology, infectious diseases, just fascinating.

STEPH: Fascinating.

VINCENT: That's why we're talking about it right?

CINDY: Right and and one interesting thing out of this end part of the discussion here is it's not just infection right? We often think about the immune system, and vaccines, and infectious disease, and influenza and everything, but it also has a lot of other dimensions to it with autoimmune disease, cancer, cardiovascular disease, they are all underlying components of the immune system.

STEPH: And I know we'll love to talk to some people maybe in our future allergy, cancer, there's a lot of different things that I think we're gonna tackle in the next couple months to bring a non-infectious disease perspective to immunology.

VINCENT: For sure, although mine is always gonna be infectious, but I'm only one out of the three so that's good.

CINDY: We'll pull you away!

VINCENT: Maybe, maybe. I like, I think cancer's really interesting, I think rheumatoid diseases are fascinating, and I'm looking forward to learning about them as well. Now we have a paper, which I think is is related in many ways. And is really interesting both from a technical and a theoretical viewpoint. And it was published in Cell, it's called "A Human Bispecific Antibody against Zika Virus with High Therapeutic Potential". And this is an article with many many authors. It's a big collaboration. First author is Jiaqi Wang, and the last author is Davide Corti. And it's from Duke-National University of Singapore Medical School, Universita della Svizzera italiana, University of California Berkeley, a company called Humabs BioMed SA in Switzerland, San Raffaele Scientific Institute, the CNR-Institute of Neuroscience in Milan, National Infection Service, which is in the UK, University of Zurich, and the first two authors, let's see, wow number 11, that's the first two authors, they contributed equally, and then number 12 also the last two authors...

STEPH: Last three!

VINCENT: Last three!

CINDY: Last three, that's right!

VINCENT: It's so hard! Oh my gosh. So there are a lot of names on this that I know and you probably know as well. I know of course Eva Harris, I know Antonio Lanzavecchia, a famous immunologist right?

CINDY: Yes, yes, he is. And Federica also, Sallusto.

VINCENT: Sallusto. And so this is cool because, alright Zika of course was in the news in past years, it's kind of calmed down now, because there are fewer infections, but of course people were concerned. This is a mosquito-transmitted flavivirus. A flavivirus just like dengue virus, which emerged in 2015 and was associated with birth defects in initially Brazil and then many other countries. And so if a pregnant woman is infected, first or second trimester, likely to give birth to a child- the most well-known defect was microcephaly, a reduced brain size and reduced skull, but there are other congenital defects arising as well. So there's a lot of interest, lot of vaccines being developed, and this paper talks about a different kind of vaccine. So, we talk about vaccines, we mostly talk about active vaccines, where you give the recipient a modified form of the pathogen, a protein from the pathogen, or an attenuated virus or an inactivated virus: you develop your own immune response. But sometimes you need to use what we call passive vaccines, where you give the products of the immune response. And that is typically antibodies.

CINDY: Another name for it is passive immunotherapy.

VINCENT: And you get it when you're in utero, right? From your mother.

STEPH: Yes.

CINDY: Yes.

VINCENT: You get your mother's antibodies and when you're born, cause you can't make your own yet. Now the famous ones that I know of; rabies, if you get bitten by a rabid animal, you will immediately start to get vaccinated but you'll also get some rabies antibodies injected at the bite site and that helps to get rid of virus, and those are antibodies made in volunteers, they're humans who are immunized with the vaccine and then they take serum from them. And you're basically getting a mixture of all their antibodies that's in their serum. Another one that I know of well because it played out here at Columbia back in the 70s; Lassa fever virus emerged in Africa, and a number of nurses who were working there started dying, it turned out this was a new virus that no one had seen before, and one of the patients, a nurse called Penny Pinneo, she was flown here to Columbia University Medical Center. And she eventually recovered, and they stored some of her serum. Sometime later, I don't know exactly how long, Jordi Casals was a virologist working at Yale, and he got this virus and was working on it and got infected. Now turned out he lived here in the neighborhood of Columbia Medical Center so he was admitted to Columbia Presbyterian. They found out he had Lassa fever virus infection, and they gave him some of the nurse's serum. And they saved his life. I love that story.

CINDY: There's another one where Ebola…

VINCENT: Ebola right.

CINDY: …in 2014 Ian Crozier was infected while he was working as a doctor there in Africa, and they gave him also immunotherapy.

VINCENT: That's right. So now we're moving to instead of giving people whole serum from people who are immunized, of course with Zika you can't give people, I'm sorry with Ebola you can't give people Ebola virus, so we're making monoclonal antibodies. So let's talk just a bit about what is a monoclonal, is that, does that seem like a good thing?

STEPH: Sure.

CINDY: Sure.

VINCENT: Cindy, you could probably do that better than I could, why don't you tell us?

CINDY: Sure I can take that. So when we develop antibodies, each one of our B cells makes one and only one way of recognizing. So they make one, what we call, a specificity. So it can recognize one thing. And so if you take those B cells out of an animal and you clone them and allow each one to make many many copies of itself, you can have one well or one group of B cells that all make the same antibody. So that was how it was originally done, and what would happen is they would take these B cells and they would fuse them with basically a tumor cell. And so the tumor cell would enable that B cell to live forever, because if you keep B cells in culture they'll just die out of the body they'll only live for a few days or a week. And so they keep these and they fuse them, so they make the two cells go together into one, and so that, what's called a myeloma, will then make the antibodies of the specificity you want. So you could take for example if you're immunize a mouse, you could take the mouse's spleen out and isolate all the B cells and fuse them to a whole bunch of different tumor cells, and they'll all be making a different specificity of antibody. And then you can sort them into individual wells and figure out which well has the antibody that you want. And so what basically you've done is have one B cell making one specificity of antibody, and then you can isolate that antibody, and that's a monoclonal antibody. Now things have gotten a lot more sophisticated now and we can actually clone out a sequence, the sequence that the B cell is making, the antibody that it's' making and we can clone that *in vitro* and then produce that in massive amounts. And so, that's important because if you wanna start changing the activity or the functionality of the antibody by changing the constant region where you wanna alter the specificity slightly, you can do that just by some *in vitro* molecular technologies.

VINCENT: What I think is really cool is that, and we have to do a paper on this at some point- you can clone from people say who have been infected with influenza, individual B cells, clone out the antibodies…

CINDY: Yep.

VINCENT: …and identify individual specificities and find broadly neutralizing antibodies, is that what…it's so cool.

CINDY: Yes, you can yep.

STEPH: It's very cool.

VINCENT: It's just amazing. So that's the basis of the Ebola monoclonal therapy that you mentioned. I think the preparation called ZMapp was a mixture of three different monoclonal antibodies. They had immunized mice with virus like particles, so they were not infectious so they could do it you know in a regular old lab, and then they as you said took all these individual antibodies and just found those that reacted with virus.

CINDY: Now there's one big problem with this. And so if you make these antibodies in a mouse, those antibodies look a lot like mouse antibody to a human cell or a human if you inject them in that. And so a human will make an immune response against those mouse antibodies.

VINCENT: Right.

CINDY: And that can cause a problem. And so what we moved to in this ability to clone these specificities and put them into new things is what you can do is take the specificity, just that part of the molecule, and clone that into a now entirely human antibody. And so what you've done is you've taken that specificity and put it into the human context. And so now those antibodies when you inject them in a human look just like all the other antibodies in that human, and they're not generating an immune response and they're not rejected.

VINCENT: So we call that humanizing the monoclonal right?

CINDY: Yes.

VINCENT: That's a term we'll probably use so just so people know that when we say humanizing we make it look like a human except for that combining site.

CINDY: Right.

VINCENT: And that's important. Turns out you can do other things to the antibody to make it last longer in the blood when you give it to people right?

CINDY: Right.

VINCENT: You can modify the...so antibodies look like Y molecules, they're 4 polypeptide chains, and the bottom of the Y, the stem, you can make changes in that, That's called the Fc portion, to make it longer lived in the blood. And they appear to be important, because if you give someone antibody you don't want it to be gone in two weeks, you would like it to last longer.

CINDY: Right.

VINCENT: So there are lots of cool things that you can do to make them last even longer. So, this paper's about making monoclonals to Zika virus.

CINDY: And there's one other thing I think we should mention at this point, and you sort of said it; they look like a Y molecule, these antibodies, and that's important because they have two times that they can bind something. So if you put your arms up in a Y and your legs are the constant region, each one of your hands has the ability to bind something. Now they bind exactly the same thing, and that's also important for their function, but they can be interchanged. And so that's what they're doing in this paper, we'll come to that, is they can change and make one side have one specificity and one side have a different specificity. So it's this ability to do this cloning and molecular mumbo jumbo here. But it's really cool because it allows you then to bind two different things, and that never happens in nature.

VINCENT: Now this...people are making monoclonals against Zika because- we don't have vaccines yet and it's gonna be awhile- and a monoclonal you can you can actually make the monoclonal relatively quickly and put it through some clinical trials and get it licensed and then say, if a pregnant woman got infected you could treat her in theory with the monoclonal and prevent invasion of the virus into the fetus say, or if you had a pregnant woman in an endemic area for Zika you could give her therapy to prevent infection, we call that prophylaxis. So there are multiple applications, and of course, the longer the antibodies last in your blood the better off you will be. So many groups are doing this- I chose this paper because it's got a really cool twist to what they're doing. So basically what they do here is to use the technology that Cindy mentioned to make monoclonal antibodies against Zika virus and then they focus on three different ones here- the main- they give them numbers, and one of them is called 190, I think another is 195 and the other is 200 something. And they do, one of them, 190, is very interesting because it apparently strongly blocks infection with many different isolates. We should say there's just one serotype of Zika virus. Now let me just pause and talk a little bit about serotype.

CINDY: Please do because for those of us who are immunologists I don't know if I fully understand that.

VINCENT: It's kind of an archaic term. So, in the old days, pre- cloning and sequencing I would say, you had a virus, let's say you had two virus isolates and you knew they were polioviruses. You could inject either one of them into rabbits or both of them into rabbits, raise antibodies against them, and then you'd say, do the antibodies against this one virus, can they block infection with the other one as well? And that's how serotype was defined. And so we had a hundred different rhinoviruses, we made antibodies against them all and saw which ones could neutralize the other, and that's how we would say ah, there's 100 serotypes of rhinovirus right or 3 serotypes of poliovirus. There's only one serotype of measles, there's one serotype of mumps virus, etc. So it's an antibody neutralization based definition. Well along comes sequencing, and now instead of doing a neutralization test you can just sequence the genome of the virus and look at the structural proteins and say ah, this is a type 1 polio or a type 2 polio or a type 3. So now, we use genotyping as a substitute for serotyping. We can tell whether a given virus is gonna react with antibodies to a related one simply by looking at the sequence of its structural proteins.

CINDY: Now do always the serotype and the genetics match? Or is there sometimes incongruency in that?

VINCENT: Well, the more we sequence the more isolate, the more genotypes we see, but as far as I know, you know no one's tested all the genotype/serotype correlations, nobody makes antibodies to every virus anymore, so. I think that we're confident that the sequence can tell us where to place the virus so now we make phylogenetic trees and you do that with all the Zika isolates and there's one serotype. And then I think Michael Diamond has looked actually at neutralization and has concluded that there's one serotype. So we can confidently look at the sequence and say you know, what an antibody's gonna do against it. So here we have Zika virus isolates we've, and these individuals made monoclonal antibodies. They found one called 190 that can neutralize many strains, and they also have introduced a change in the Fc region of this monoclonal antibody.

STEPH: I like the name of that.

VINCENT: LALA.

CINDY: La laaaa.

STEPH: LALA.

VINCENT: Isn't there a movie or something like that?

CINDY: La La Land.

STEPH: La La Land, I haven't seen it but I heard it's good.

VINCENT: This abolishes binding to Fc gamma receptor and complement, so in terms of antibody dependent enhancement, that would interfere with that right?

CINDY: Yeah it completely blocks it right.

VINCENT: So we were worried when Zika emerged whether there would be antibody dependent enhancement because it's a flavivirus like dengue virus. So there's been some indication at least in cell culture that you can get antibody dependent enhancement. But so here they just remove the sequence in the Fc portion that would lead to that and, so if you treat someone with this antibody you won't have to worry about ADE.

CINDY: And they showed that pretty clearly too.

VINCENT: Yeah the assay for that is interesting; you take a cell, I think it's K562 right?

CINDY: Right, non-permissive.

VINCENT: Which normally you can't infect with Zika virus.

CINDY: Yeah.

VINCENT: But if you add an antibody to the virus, it will then get into these cells, which have Fc receptors.

CINDY: Yep.

VINCENT: And it will infect them. But as far as I understand in people no one has really shown ADE in Zika virus infected people, at least not yet, there have been a couple of small studies. Alright, what can you do with this monoclonal antibody? Well they do a lot of other stuff, which is kind of de rigueur, and it's less interesting to me, but they can determine exactly where this antibody binds. And let's introduce a term that we'll use over and over: epitope.

STEPH: Yes.

VINCENT: So Cindy, what would you say is an epitope?

CINDY: So, we start with an antigen; an antigen is a part of any kind of protein or anything that can be recognized by in this case we're talking about an antibody. Now the part that actually interacts with the antibody is called the epitope. So it is the primary sequence or the secondary structure if it's a folded protein that's directly interacting with the antibody.

VINCENT: And it's typically short for a linear epitope right?

CINDY: Yeah, it can be quite short.

VINCENT: Like what, 8, 10 amino acids? Something like that?

CINDY: For a T cell we talk about epitope definitely being 8 to maybe 11 amino acids. I think it depends on the antibody structure and how big, how interactive the binding is between the antibody and the antigen for the epitope.

VINCENT: OK. Now, just to be clear, antigens are not only proteins right?

CINDY: That's...well...in the case of a B cell, the antibodies can bind to things that are not proteins. For T cells, primarily for CD4 and CD8 T cells it's gonna be a protein, mhmm.

VINCENT: OK, but here of course for Zika virus we're talking about an epitope on the virus particle, which is a protein.

CINDY: It is.

VINCENT: And what they do is they say when you make monoclonals one of the first things you wanna do is say where is this binding on the virus particle?

CINDY: Right.

VINCENT: And back in the old days when monoclonals were first developed, the only way you could really do that was to select viruses that are resistant to neutralization with the monoclonal antibody. And what you'd do is you grow the virus in the presence of

a monoclonal, and there will be some altered viruses with mutations in their genomes and all you need is one amino acid change in that epitope, and that could in theory block the antibody from binding.

CINDY: Yep.

VINCENT: And then you could sequence the virus and say ah, look here's...we've done 20 different monoclonal antibody resistant viruses and they're all within this short area, that must be the epitope.

CINDY: And didn't they also map? So by competition with different antibodies, so if they knew where one bound if another one competed for it then they would know they bound either the same or overlapping sites?

VINCENT: That's right yep. You could do that as well, exactly. And then you could say ohp, that was actually done for polio and we knew there were three or four you know, major regions against which mouse monoclonal antibodies were directed. And then, if you wanna know what happens in people that's a different study right? Because it's not always the same.

CINDY: Right. Yeah. And it's more sophisticated now right?

VINCENT: Oh so now, in this paper, they do it, they solve the cryo-EM structure of the virus complexed with the antibody okay? So the cryo-EM structure's the atomic structure basically, you know, here they do it at pretty low resolution, 22 angstroms but you can get a very high resolution structure by cryo EM and you can do it really quickly! So you just add high amounts of the antibody to the virus, you put it on a electron microscope grid, you take some pictures, and you can solve the three-dimensional structure, see exactly where the monoclonal is binding. That's what's amazing here is that they used that technology whereas 10 years ago it was really hard to get a virus structure. Now, we wanna know where the antibody is binding sure- let's solve the structure.

STEPH: Yeah and it's very it's very easy and clear to see where they bind at the different vertexes on the complexed version of the monoclonal and the Zika virus. That whole panel is amazing.

VINCENT: Yeah, it's gorgeous, there are a lot of beautify pictures here. So they've solved the structure of the 190 antibody complexed with the virus particle and you can see where it's binding. And they do some experiments that talk about how this antibody will prevent infection. Cause if you think about it, so the antibody is binding to the exterior of the virus particle of course, it's binding to the viral glycoprotein, and it could block attachment of the virus to cells, it could block entry from the endosome, it could block fusion, it could even aggregate the virus before it can even attach to the cell, and that would prevent attachment. So all these mechanisms are known mechanisms of antibodies blocking virus infection. And they do some experiments which suggest that aggregation is one but this is a...what you can do with antibodies that's great; you can remove the two antibody, the two specificity areas up on the two Ys from the Fc, and that will now- you can separate the two arms right? The two Fabs we call them, and now they can't cross link virus particles, so they can't aggregate it anymore but it still neutralizes, so there must be more than one mechanism...

CINDY: Right.

VINCENT: ...of neutralization. Now the cool thing here is that if you wanna use this antibody for therapeutic purposes, you have first you have to show that it works in an animal, so they used mice that they infect with Zika virus, and they treat them either before or after infection with Zika virus with this monoclonal antibodies. And they show that you can get protection, you can actually get protection up to four days after infection. But you're always gonna get monoclonal escape mutants. And in fact, they show you, they got one in mice infected with Zika and treated with this 190 monoclonal. It's really easy to do. In fact, I don't know of any any monoclonals that neutralize viruses for which you don't get escape mutants. So that's not good, because if you treat people if an escape mutant arises within a few days that will nullify the treatment with the antibody. And they look at a couple of different antibodies here, and they show with all of them you can get escape from neutralization. So then they said, let's combine two of these monoclonals together; and this is the cool part of the paper.

STEPH: Very cool.

CINDY: Definitely.

VINCENT: Which is why it makes it to Immune. They said OK we have monoclonal 190 and 185; they appear to recognize different epitopes on the virus particle, which you can tell by selecting monoclonal resistant variants or just looking at the binding site by your structural information. And then, they construct what they call a bispecific antibody.

CINDY: I have to give a shout out to my post-doc advisor, David Segal. He listened to our first podcast, I'm not sure about the second one, but he was one of the original people who worked on bispecific antibodies.

VINCENT: Cool! Oh, that's great.

STEPH: That's very cool.

CINDY: Yeah.

VINCENT: So basically you have, you know, at the tips of the Ys you have the combining sites for the epitopes and all they do is put two in tandem right?

STEPH: Yup.

VINCENT: But you can manipulate the DNA so you have the combining site for 190 you have the combining site for 185, and now you can make an antibody which has two combining sites. And I guess they have a little linker in between to space them out is that right Cindy?

CINDY: Yeah.

VINCENT: Yeah.

STEPH: Now for the FIT though-so this bispecific format they use, they call it FIT Ig, which is somewhat neat, but it stands for, it's the antibody fragment in tandem like we mentioned. And now I looked up, it said that there wasn't a linker necessary, but maybe I missed that in this paper.

VINCENT: Oh that, it could be.

STEPH: Maybe they include one- I think that was kind of the benefit of this FIT format, and if you kind of picture you know we described a Y antibody, if you just hold up your hand as you're listening it's kind of this Y, just kind of stick another little molecule on the sides of the 2 Ys, and that's what it's gonna look like and it's gonna be able to be tetravalent or be able to bind those different sites. So it's a really neat format and I like the name too, FIT.

VINCENT: So instead of an antibody now binding two of the same epitopes, this can now bind two each of two different epitopes, you know. One on each...cause each arm of the Y has two different combining sites. And so, these antibodies bind the virus with higher affinity than the individual monoclonals and they neutralize infectivity. And the cool thing is they neutralize all of the monoclonal resistant variants that they generated against the three monoclonals that they work with in this paper: 185, 190 and 230.

STEPH: And Vincent I had a question about that, maybe you'd be best to answer. They, to be able to determine if this was going to develop an escape mutant they passaged it nine, I'm sorry eight times.

VINCENT: Yeah.

STEPH: Is that sufficient? I just don't know like what would be considered a sufficient amount of passaging? And for those who don't know, when we try to determine if a mutation is going to result in an epitope changing we're gonna just passage that virus in the cells, I think you said K562, for many different passages, whether that's every 2 days you passage or whatever the cell type requires, but I just didn't know, is 8 a lot? Should they have kept going?

VINCENT: Well, you could probably argue that you should keep going until you get a variant right? Because they say with the regular monospecific antibodies it takes three or four passages to get resistance. And they did 8, so it's obviously better but the question is, can you go 20 or 30 or 40? If I were them I'd keep doing it right?

STEPH: Yeah it just would be interesting to know, because I would imagine it yes, of course with binding two different epitopes it's gonna be harder to develop escape mutants for both of those.

VINCENT: Right.

STEPH: But likely, it will happen eventually and so it would be nice to know at what point. And you know some, a virus like HIV, which you know, mutates at a high rate- I don't know necessarily what Zika's is in comparison to other viruses, you just have to wait longer. So you know it was just a point I thought I'd bring up.

VINCENT: Well I think that if you do the math with the antivirals for HIV, you know it's easy to get resistance to one. Two is harder but doable and three is really really hard. Three different drugs. So here, it's like having two drugs, two monoclonal antibodies. So I think eventually you would get resistance. The question is whether it's therapeutically relevant right?

CINDY: Right.

STEPH: Right.

VINCENT: Cause Zika infection is is short, it's not like HIV, which goes on for you entire life right?

STEPH: Sure.

VINCENT: So there's a finite length of infection, and so as long as you're ok within that time, whether it's a month or two, whatever, then I think you'll be alright with this. So obviously they have to continue to look at that but, it's improved clearly. And I just think it's so cool. And the question is- Cindy can you put three specificities in there?

CINDY: I have no idea.

VINCENT: That would be cool right? And finally they put FIT 1 in mice and it protects them from Zika virus infection even if you give it 3 days post-infection. So that's a pretty exciting development for Zika, and I think it's it's...they're gonna they say we're gonna go ahead and look at rhesus macaques, a non-human primate model, and see if it blocks fetal infection right? Cause you give it to the pregnant mom and infect her and see if it'll block fetal infection.

STEPH: Yes.

VINCENT: They also talk about how modifying the Fc to make it to extend its half-life and finally maybe deliver the antibody via a vector instead of giving people the protein, you could give them a virus vector that makes the antibody, and that would be interesting. And I know that for HIV that's being done. They have cloned a broadly neutralizing monoclonal antibody gene into an adenovirus associated, adeno associated virus vector. And that gives you lifetime expression or at least long-term expression of the protein, and that's going into clinical trials for HIV, and that will be interesting here as well. Anyway, thought it was technically cool and also gave us a chance to talk about monoclonals and epitopes.

STEPH: And viruses!

VINCENT: Viruses, Fc. Yeah I must admit I really love viruses so...I'm sorry I'm not gonna apologize for picking it but it is immunology too right?

STEPH: Oh for sure! And it definitely satisfies, I think last month we had somebody who wrote in and said she wanted to hear about viruses in immunity so, we got to check that off. And yeah I enjoyed the paper as well and it really gives you an insight into how these new technologies...because really it would have been difficult to engineer an antibody like this, you know, 20 even maybe 10 years ago, so the technology has definitely got us to a point where we can develop some pretty cool and effective things for for the things that ail us.

VINCENT: I have to mention too that the Cindy you mentioned your former post-doc advisor or thesis advisor that developed this technology?

CINDY: Yeah he was one of the first ones, yeah.

VINCENT: So I had a colleague here years ago, Sheri Morrison, she's now at UCLA, but she developed the humanizing technology.

CINDY: Oh wow!

VINCENT: And she has a patent for that. Which you can imagine is quite lucrative.

STEPH: Yeah I can imagine.

CINDY: Yeah Dave was at the NIH and they don't let you...

VINCENT: Nope they don't.

CINDY: ...make money from those things.

VINCENT: Alright. We do have e-mails again; thanks everybody. And let's go through some of those. Cindy can you take that first one?

CINDY: I sure can. So Anthony writes, *Mice Infected with Low-Virulence Strains of Toxoplasma gondii Lose Their Innate Aversion to Cat Urine, Even after Extensive Parasite Clearance.* And so he says, this paper was covered on TWiP 60. *And he says, to split hairs, the infection in mice does not remove the fear of cats. It removes the hard-wired aversion to cat urine.* And so this is in response to one of the comments that I made last time about the immune response changing cat behavior then I mentioned Toxoplasma infection in the brain and how that changes cat behavior and he is correct, it removes the response, the aversion response of the mouse to the cat urine smell. Not specifically fear of cats.

VINCENT: Yeah we have to be grateful to our listeners who correct us at every turn right.

STEPH: Yeah yes.

CINDY: Yeah.

STEPH: I should actually listen to that TWiP I don't think I got to that one.

CINDY: Yeah I have to say, you know, one of the things that's really hard about doing this science communication thing is being willing to get out there and say things that might not be 100% correct and allowing people to correct you and being OK with that. Because it's not so easy for those of us who have trained in science...

VINCENT: Yeah for sure.

CINDY: …and trained to be you don't say it until you're 100% sure.

VINCENT: You know a lot of people have noticed that we are very ready to admit when we make a mistake on our, on all the podcasts that we do. And they like that. They say that they didn't think scientists would do that. And it's fun for them to learn that and I think it's an important thing that we do- we make mistakes and we, at least I do I admit it you know, because I think that's how you make science go forward.

STEPH: Absolutely.

CINDY: Yeah.

VINCENT: We had Dixon a couple of TWiPs ago say something and then later in the episode I pointed out that it was wrong, what he had said. Here's what he said; he said a serine protease cleaves at serines and I said no-it has serine at the active site. He said oh, my mistake. And I said do you want me to take it out and he said no you can leave it in. And so a listener wrote in and said that was so encouraging to me because I'm so afraid to ask questions or say things and now, if Dickson does it it's good for me you know so.

STEPH: Oh my gosh...

CINDY: So we make people feel good too.

VINCENT: Yep.

STEPH: Yeah and as a grad student I know that's something we just have to get used to of course. We're having candidacy and we do thesis defenses and you really, I think that you know doing a podcast like this of course for any grad student might be outside their comfort zone but it's good training and I would encourage any other students to you know take that step, that's how you're gonna learn and it's gonna build you good you know, thick skin. So, and it's good for science, in the end.

VINCENT: Will you take the next one, Cin- um Steph?

STEPH: Yeah sure.

Steve writes:

Hi Vincent, Cindy & Steph,

Thank you all for what is sounding like a well-honed team effort on 'Immune' already, which I'm sure is going to give TWiP a run for its money as my most eagerly awaited podcast from the microbe.tv stable! Not sure what the (y) is. I'm assuming maybe that was a smiley face.

VINCENT: Probably yeah.

STEPH: Probably.

Only once a month, for such a many-sided subject seems like it's going to take a long time to build an overall picture of the components and functions of the immune system though. So I wondered if you could do something to bring together the Audiommunity collection with the new Immune archive, so that listeners can easily go back and pick up some of the subjects already covered–such as MHC/grafts, T-cells, B-cells/germinal centres.

The Audio-and he...I think it's audio immunity...but maybe it's audiommunity...

CINDY: I think it's Audiommunity.

STEPH: OK. I...well; Vincent can talk about how he doesn't like that name either because nobody knows what the name is.

VINCENT: If you have to think about pronouncing it…

STEPH: It's kind of a problem.

CINDY: Immune seems easier.

STEPH: Yeah.

VINCENT: I love Immune.

STEPH: Love immune. OK Steve continues: *The Audiommunity podcast followed a bit of a random pattern, but, it would, nevertheless, enable picking up more background on the subject between the Immune installments. You do include some of the podcasts in your guests listings, but the earlier episodes are missing, and the order is a bit confusing. (Even on their (Audiommunity's) own site, they just list dates for the early ones, with no indication of what they cover until the links are opened–however, some are very good.)*

I'm going back through them now, but I think I'll need to go over them many times before the different components begin to sink in and be properly remembered (I think I've had a book on 'Complement' in the 'to read' pile by my bed for about 30 years!). It would also...it would be easier if the initials were explained when first mentioned. That's definitely something I need to work on as well. *I for example, knew what the 'Major Histocompatibility Complex' was, because I was very impressed by a Scientific American article I read on it in the 70's; and I knew that 'CD' meant 'community of differentiation'.* However I will say, I give you credit.

CINDY: Cluster of differentiation.

STEPH: It's cluster of differentiation. You tried, I guess. I mean it was from the 70s.

CINDY: It's ok, he has good memory.

STEPH: So… cluster of differentiation…*but I didn't know that the 'm' of 'IGm' was for 'membrane', and had forgotten that the 'T' in 'T-cell' was for 'thymus'. These terms are generally talked about by their abbreviations, but it's hard to take in, if one can't pin them down to a place or function by knowing what the name means. A program giving an overview of the key pieces of the whole immunity jigsaw–and how they were discovered–would be appreciated.*

Another thing that would be useful: I particularly like your coverage of the 'current affairs' affecting science–in all your podcasts–and I would like to be able to share them with friends and people who could help 'the cause', without having to post a link to a long programme and expect them not to be put off in finding the relevant parts. This week's discussion about the graduate students' stipends would be a good one to share widely, for example. The running time points help, but it would be best if, as well as the audio or video of the whole programme, clips of the individual topic discussions could be included as well, so that they could be easily referenced and shared. (I would also offer to copy and edit the individual pieces for you, but I'm long term sick and could not do it very reliably.) Oh, I'm sorry to hear that.

Anyhow, it's great that you've managed to come up with yet another podcast- applause to Vincent- that's a first choice when seeking something intelligent to 'chill' to…Should we say instead of "Netflix and chill" should it be "Immune and chill"? *And all the more so for not just being jammed with background music and noisy, unrelated, adverts, as happens with a lot of YT presentations by others.*

Many thanks,

Steve

In Luton, Bedfordshire, England.

Thank you Steve, for the long note. And I'll kind of just start. Audiommunity- yes I would say just go ahead and start listening to that podcast, I think it would be difficult to link...I mean I haven't really listened to all of their podcasts to even know what the content was, so I think it'd be difficult to link. But surely it would be a good supplement if you listened to that as well.

CINDY: But the good news in response to this is, we are in the works in planning Immune 101.

STEPH: Woohoo!

CINDY: So we're gonna cover topics, I don't know what format it's gonna be in quite yet, might be some videos, with some nice cartoons to explain topics, seems like we might start with complement because everyone seems to really like complement.

STEPH: Which is weird.

CINDY: It's very complicated. But yes so we have the works in the plan and we're gonna do that, and that will probably help a lot of individuals.

STEPH: Definitely

CINDY: Understand we're talking about.

VINCENT: Are you teaching next semester Cindy or are you teaching now?

CINDY: I am...so I teach now, I'm teaching, the final exam was yesterday and today for the veterinary students, but in the spring I teach our advanced immunology course. And we're gonna basically be going through Janeway's Immunobiology in one semester, with some extra things sprinkled in on more current topics as well. So it'll be pretty intense, but I'll be reviewing, brushing up on my T and B cells, which are not my forte. So I don't teach the entire class, but I teach some of it and, but we'll be able to sprinkle that in and see what we can do. I'd like to talk to Vincent more about the format of how we should present that. So we'll work on that offline and you guys'll be looking for that after the new year.

STEPH: Yeah.

VINCENT: Alright. Peter writes:

Greetings Immune Team.

I am much enjoying the new podcast, a great addition to microbe.tv. My own immune system has not been well this autumn: I caught influenza before I had my annual flu shot (the timing would suggest that I caught the virus on a flight or at an airport) and then developed secondary bacterial pneumonia, with pleurisy that required a few days in hospital. After a couple of months I am starting to feel better but still have a persistent nasal drip which my doctor has so far failed to find an effective treatment for.

Enough about me.

You should go to Tahiti for a month, maybe that'll get rid of it. Not that I've been there, I don't know.

I have some immunology questions for the team.

If our cells are infected with viruses, are there any intracellular defences that can eliminate the virus from the cells or is just a matter of triggering apoptosis to try to kill the cell before viral replication, or if the virus blocks apoptosis pathways, sending cytokine signals to instruct NK cells to come and destroy the infected cell?

CINDY: Yes, yes and yes.

VINCENT: Yes. And you will hear about it here! Right?

CINDY: I'm sure we'll do a paper on it...the intracellular defense mechanisms against viruses are really fascinating.

STEPH: Yeah there's a lot of questions there, I think we could use that for fodder for future episodes.

VINCENT: Yeah for sure.

STEPH: But yes all around.

VINCENT: Yep. And: *What happens with viruses that become latent? I presume that the immune stops virus replication but is unable to eliminate the virus from the cells, since viruses can break latency the blocking of viral replication is clearly an active process by the immune system.* So we're gonna get into that too.

CINDY: Yeah.

VINCENT: There's definitely...there yeah there are definitely antagonists encoded in these latent viral genomes. And of course some of them go completely silent; they don't make any proteins, so they are silent, invisible to the immune system. But we'll talk about those.

*On the subject of latent infections, in the UK the chickenpox vaccine is not on the vaccination schedule, but the National Health Service offers a free shingles vaccination (Zostavax) at age 70, which I understand is the same as the chickenpox vaccine, but a higher dose. The higher dose being needed because it is intended for the elderly who have a poorer immune response. It seems to me that having a single shot of varicella vaccine at a younger age would act as a booster helping to repopulate the immune system with the appropriate immune memory cells and thus maintain the zoster virus in a latent state. Do you think that giving someone...*it doesn't actually maintain it in the latent state, it's just if there's a reactivation it will take care of that, right?

CINDY: Right.

VINCENT: *Do you think that giving someone in their mid-50s a single varicella vaccination would boost immunity and have the same effect as Zostavax at seventy in preventing shingles? Maybe this idea is rubbish, but I would welcome your opinions on this.*

Alright. Saturday Night Live what do you have to say?

STEPH: Well, I just did a simple, I Google searched and the CDC, Zostavax, it's approved by the FDA for people aged 50 or older but it's not recommended for people ages 50-59. I'm assuming that it's because if you were to get a booster at, let's say, age 50 that that

immunity will maybe peak in your 50s but maybe it will wane by the time you get to 60 or 70 when you're more likely to contract the virus because your immune system is waning.

CINDY: Or reactivate.

STEPH: Or reactivate sorry rather. So I think that's why it's recommended for that age group but I think Cindy might have an update on that?

CINDY: So I think you're right, and remember that this is in the US too.

STEPH: Right.

CINDY: But there's the new vaccine at least in the US that was just approved recently, and that's Shingrix, which I think I'm saying that properly, and that one is recommended at 50. And the reason why is because that one seems to provide longer lasting protection. So the Zostavax wanes within four to five years, or protection wanes a lot, whereas the Shingrix vaccine seems to remain protective for a lot longer, so you can immunize earlier and maintain that protection.

VINCENT: Yeah that's quite an interesting one, Shingrix, it's a, I think it's a glycoprotein-based vaccine.

CINDY: I don't remember the details.

VINCENT: And we did cover that on TWiV, and it's really quite protective. It had really good numbers.

CINDY: It's 90…

VINCENT: Something.

CINDY: …97% efficacy I think it has? It was very high.

VINCENT: It's remarkable. Yeah.

CINDY: Now the one thing that we didn't mention yet is this idea that in the UK the Chicken Pox vaccine is not on the childhood vaccination schedule. I guess I didn't realize that!

STEPH: I didn't know that either.

CINDY: And that's an interesting thing.

VINCENT: Should we stop there or go on some more e-mails? It's up to you guys, we're at an hour fifteen, so.

STEPH: Well it's funny my husband he listened to this, and the first thing he said to me was hey you went over an hour, and I said well ok thanks for being our time keeper.

VINCENT: Should we stop?

STEPH: I mean...

CINDY: Sure.

STEPH: And we'll still do our picks is that true?

VINCENT: Yeah let's do some picks sure. That's right. Steph what do you have this week?

STEPH: OK so there's been some conversation on TWiV, Dr. Jon Yewdell had discussed about maybe needing to work 80 hours a week to get a faculty position. Now there's been a lot of conversation around this, and you know I'm not really gonna give my opinion on that but I think what's missing is maybe advice for people who feel like they could just improve their work efficiency for whatever position they wanna get in science. So one thing that I'm reading is it's called "Deep Work" and it's by oh, Cal, I'm gonna pull this up so I know the last...Cal Newport. Now he basically advocates for having long stretches of time where you are deeply focused in one thing. He feels that people who are creative, which, as scientists he feels as well as we all do we are creative, we have to come up with experiments and think about what we're gonna write, instead of having shorter very distracted bouts of deep work. And this is something that I struggle with; I kind of thought multitasking was the best way to work, I enjoyed the feeling of getting a lot done, but this advocates that that's not the best way. And I just wanna warn people- if you- probably you're listening to this podcast or on social media, this book will probably offend you because he does not use any social media and advocates for the complete like giving off of all social media, because he feels it takes away from dep work. Now I don't agree with that but this book does have some good you know, tips and tricks of how to kind of get into that deepness and maybe be more efficient during those hours at work. So you can have a life outside of the lab. So I recommend that. And then, I just wanted to give a brief update on the

grad tax situation, which I think you know many people were interested in. I don't think it was, the senate did take that provision out of the GOP bill that got passed. But the House of course was the one that proposed it, so this week they're gonna, over the next couple weeks decide on what's in and what's out. And in the show notes there was about 31, and I'm pretty sure all republican but I don't know exactly, they wrote a letter about how that is gonna be so damaging to science and to young people. So there are some people speaking out in Congress, and I would say you know keep calling your people but that that's kind of the update. The GOP tax plan has passed, but in terms of this grad student tax I don't, we're not sure yet.

VINCENT: Well remember it has to be reconciled between the House and Senate and so they will have to vote at least two more times.

STEPH: Right.

VINCENT: So this is a great letter, where they recognize that grad students are important for the future of this country, which is nice to hear. But you should still call your senators and representatives, because there's plenty of time to convince them that this shouldn't be part of the tax bill. In fact, that the tax bill shouldn't be passed at all in my view, because it's gonna kill us as well who are working, but the student part is ridiculous.

CINDY: Yeah.

VINCENT: Cindy what do you have?

CINDY: So speaking of being distracted on social media, the other day someone posted a video, and I literally had tears going down my eyes, I'm willing to fully will admit that. And many of you have probably seen this. There's a heartbreaking video of a starving polar bear. And this was recorded by a National Geographic video recording team. And it's gone completely viral I mean this video if you haven't seen it you have to watch it, but be prepared, it's gut-wrenching. But there's been really mixed response to this video and some people you know they're using this to argue that this is what we can expect with climate change, which I think is very real, but other people were harshly critical of the film crew by saying why didn't they help, and National Geographic put out a little note about the video providing more background information about how it was recorded and some responses to some of these criticisms including why didn't the film crew help this poor bear. And you know, it's a wild polar bear that's starving for food and you are two people in the middle of nowhere not close to a city or town and with no weapons or anything to protect yourself- that would have been ludicrous for them to help this bear, but nonetheless they felt really compelled to take this video so that people could see this, what was happening. So I'm linking to the National Geographic response so you can see the information that they're providing about this video. But if you haven't seen the video you should see it.

VINCENT: Nature's pretty tough.

STEPH: It is, it is tough.

CINDY: It is.

VINCENT: Alright I have a little more uplifting pick. Mine is a video which is in a blog called Colossal, which is all about visual and graphic art, it's really an interesting blog. But this is a fellow named Tatsuo Horiuchi. He's a 77 year old artist. He retired from another job, he decided he wanted to paint during his retirement, but he didn't wanna buy paint and didn't wanna buy a drawing program for his computer, so he used Excel, which he had from work, and he figured out how to draw these incredible drawings of you know, forests, mountains, cherry blossoms, fish.

STEPH: He's amazing.

VINCENT: And they're gorgeous!

STEPH: It's amazing.

VINCENT: And he prints them out. And I'm just amazed he used Excel. It's a very short video, he's a neat old guy and he said maybe in 10 years I'll be able to sell some of these. But I'd buy one because they are really pretty, you know.

CINDY: They are really pretty.

STEPH: Definitely.

VINCENT: Really. And just to use Excel, which I use and I kind of grit my teeth when I use it.

CINDY: I know!

VINCENT: To paint…and they have a video of him painting and stuff so it's pretty cool. And listeners if you have picks you can send them in-we'd love to hear yours as well. And that's Immune number three. You can find it at apple podcasts, microbe.tv/immune,

and of course your favorite pod player program on your phone or tablet will get it, just subscribe so you get all the episodes. And send your questions and comments to immune@microbe.tv. And we thank all our supporters for helping us financially. If you wanna know about that and contribute, go to microbe.tv/contribute. Cindy Leifer is at Cornell University.

CINDY: Thank you.

VINCENT: Thank you Cindy. And you can find Cindy on Twitter at cindyleifer. Steph Langel is at Ohio State University, thanks Steph!

STEPH: Thank you!

VINCENT: Steph is at stephanielangel on Twitter as well. And I'm Vincent Racaniello. You can find me at virology.ws. Music on Immune is by Steve Neal: stevenealpercussion.com. Thanks for listening to Immune, the podcast that's infectious. See you next month.