COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Cassian Yee (MD Anderson Cancer Center), October 26, 2021

Title: T Cell Immunity Of COVID19: Developing Biomarker And Therapeutic Strategies

NIH Project #: 3R01CA237672-02S1

YouTube Recording with Slides

October 2021 CIC Webinar Information

Transcript Editor: Macy Moujabber

Transcript:

Lauren Close:

Slide 1

I'd like to next welcome Cassian who's joining us today from the University of Texas at the Anderson Cancer Center. Cassian if you'd like to go ahead and get us started we'd love to hear your presentation.

Cassian Yee:

Slide 2

Can you guys all see the screen by the way? And you can hear me? Okay sorry I had to put the ears on. There's a little bit of ambient noise and I know the titles change a little bit, but this is a slide that describes topic of this particular discussion, which is really looking at another aspect of COVID immunity. Most people have been fixated on neutralizing antibodies and serologic response, and I think as a testament to the flexibility and agility of NIH, that's probably an oxymoron, but I think in this particular case it turned out that we were able to repurpose our lab and the supplemental funding provided by the NIH to shift from our pancreatic cancer work to COVID-19 work.

Slide 3

This is a busy slide but I think I only want to point out two things here. One is that the coronavirus family includes non-pathogenic viruses. Some of you recognize these. This nomenclature and then also the pathogenic coronaviruses. The SARS, the MERS and then the SARS-CoV-2 which we're discussing here.

Slide 4

Just a brief biology so that you can understand which structures we're talking about when we talk about T-cell responses, and the Spike Glycoprotein is what binds to the ace receptor and delivers the virus. [Sorry about that I don't know if I can go back one slide. Kinda messed up there. There we go.] But then the coronavirus obviously has RNA nucleocapsid envelope and so what I'm gonna talk about structural versus non-structural proteins and most of the antibody responses direct against structural proteins like the spike glycoprotein and S-protein or S-response which most IgM, IgA, IgG serologic assays are directed against.

Slide 5

So, this is again- I'm just going to actually focus on three things here. One is the antigen load in purple. Hopefully this is a successful response which the antigen load increases induces both antibody and T cell responses and then antigen load decreases. Now, obviously, in long COVID or in COVID that doesn't respond to this [oops sorry I did it again]- and you're gonna see the antigen will persist and that correlates to transmissibility. What I'm focused on is T cell response in the light blue here. This is a little bit misleading because this does not represent what you see here necessarily protective response. It's just a structural measure of the antibody and what I'm going to focus on here is let's dissect the T cell response more closely.

Slide 6

And the question is why are we interested in finding T cell epitopes to SARS-CoV-2? [And now it's just going on its own.] Ok, and the reason is because we want to understand the natural history of T cell immunity. It's much harder to measure T cell immunity than to measure serological response, and then plus if you measure these responses properly, maybe we'll predict whether you're protected or not or how bad it can go for a particular individual and also the effect of a certain intervention.

Slide 7

So, this is a paper, actually, just one year's work. One and a half years work. I have to really commend Dr. Ke Pan and Dr. Yulun Chiu in the lab who really drove this research in a very difficult time and it's coming on plays very closely.

Slide 8

Sorry I need a time check. How much time do I have? Because I forgot. Do I have five minutes or ten minutes?

Lauren:

You have 10 minutes and you're about three minutes in so you have plenty of time to expand.

Cassian:

Slide 9

Yeah, I'll try to not give you too much of a pressured speech here but I do want to point out one very important aspect here which is that when you're looking at the T cell response, you're looking at a bit of protein that's brought onto the surface of the cell, which is a nine amino acid sequence, a nine length peptide, or a 14 [inaudible] if it's a class 2 CD4 response. I'm just going to focus on the Class I-restricted T cell response. So CD8 T cell, through its T-cell receptor recognizes a nine amino acid peptide sequence brought to the surface which is bits and pieces of a protein. And that protein can come from anywhere.

It can come from the surface, come from- non-structural proteins come from transcription factors, what have you. So the T cell response is much broader than a potential antibody response and the problem is that you can't predict what this is just by doing in silico analysis, and I think that is the whole point of this discussion in the paper is that if you want to find out what that peptide, you need to go where the money is you elute off that peptide from the MHC of SARS-CoV-2 infected cells or expressing cells and then you run i through a tandem mass spec, there's a bunch of algorithms that we then use to sort of prioritize the peptides that we have. Now this is supposed to be a T cell with a T cell receptor and what I'm showing here is that not only we pull out the peptide but we validate its immunogenicity, meaning that that peptide is not only processed and presented on the surface of a virus infected cell, but that you can induce a T cell response and that T cell response comes from normal human peripheral blood mononuclear cells. That T cell response is sufficient to recognize an infected cell. So, it has sufficient affinity to recognize that target and that peptide is present with sufficient density that this interaction occurred leads to killing, and I'll talk about the TCRT in just a minute.

Slide 10

But I think this is the structure we're talking about. This is the SARS-CoV-2 genome ORF1a and 1b. There's a spike protein everyone knows about sticking on the surface. And then a whole bunch of accessory genes that are associated with the structure, remembering protein MGP nuclear capsid, this one we're going to talk about just a minute. Now remember, there's a whole huge part of the gene dedicated just to survival and function of the SARS-CoV-2 virus.

The spike protein, as it turns out, people have gone and looked at T cell responses, just looking at this part of the spike protein because, oh well, you know, it's accessible, let's go for it. And they come across

a number of peptides so you can see these are lists of peptides some nine or some longer shown along here and a number of different individuals have published on this and have made a big deal the fact that there are immune-dominant responses that they're dominant T cell major responses. Not only are people infected with SARS-CoV-2, but apparently in healthy donors as well, and so if you look just visually these tall bars here represent what the other scientists have called immune-dominant responses. And it turns out, oh my goodness, they're also found in healthy individuals who've never been exposed to SARS-CoV-2 as they know, so they say oh wow, there's some cross reactivity and so on and this makes it like a great story. And I'm not saying it's not true, but I'm saying it's flawed. And the reason it's flawed is that people are taking a whole bunch of different peptide epitopes, and you can see here the whole list coming from different parts of the protein and they've looked at responses in patients and they see variable responses and that's great and in fact if you look in the metabolic archive there are 2,000 class 1 epitopes and 1,400 class 2 epitopes so these are all predicted. Now just so you understand even more deeply into why I'm a little bit passionate about this is that you can take any peptide that binds the MHC very well, that's predicted to bind. So, you just map along the sequence you throw that into PBMC, you're going to get a T cell response. Now whether that peptide is actually presented with that T cell response is relevant or not, for the most part and you know this is controversial statement, probably, is that all these studies ignore that fact. They just want to know this reactivity and some of it may be real, some of it may not.

Slide 11

But we decided okay let's go for it. Let's see for these immunodominant epitopes, people are predicted okay they're all predicted. Do they actually generate a T cell that recognizes target? And unfortunately, we did all this work, Ke did actually, generate T cells we pull them out we expand we sorted them you can see it's nice cluster meaning they're all and tetramer-specific for this peptide. They do not recognize the SARS-CoV-2 expressing targets so zero flat completely flat you can see that right here, so not relevant, as far as we're concerned from an immunogenic standpoint. So, these are predicted but they don't elicit responses from a SARS-CoV2-recognizing T cell.

Slide 12

What do we do instead? We went for the money. We went after the peptide. We did the mass spec analysis. We looked at all these genes. And in order to make this as effective as possible, we engineered antigen-presenting cells to express these different parts of the SARS-CoV-2 and with different HLA alleles to be as broad as possible on a coverage. You can see we highlighted here non-structural protein membrane glycoprotein.

Slide 13

We did the analysis. We ran it against the database to make sure that these genes actually are expressed, and so this is how we substitute it what- we used to use tumors now but now we use the SARS-CoV-2

engineered cell and then we ran through this protocol as I explained to you earlier. We generated T cells against these peptide epitopes and then tested that these T cells do in fact kill the engineered cell.

Slide 14

And this is an example of a mass spec and you can deconvolute. You get a peptide sequence.

Slide 15

And this is what we got. Okay, we published five of these. There's actually 18 of these and you can see that four of them are found in a non-structural protein. One of them is found in membrane glycoprotein, and the top line is the sequence. We took the sequence from SARS-CoV-2. We compared it to all the sequences in the other coronavirus families. We see there's some identity and there's also some misidentity. It's possible that you know if you were previously exposed to one of these- I'm sorry one of these other viruses, you may still elicit a response to the SARS-CoV-2-specific T cell.

Slide 16

That's interesting. This is interesting, but what's more interesting is that turns out these targets and I've shown again the same five targets here in the non-structural protein region and also the memory glycoprotein region is that they're highly conserved among all the variants- alpha variant, and I don't show the delta variant, but the delta variant as well. These are the variations but you can see there's no variation. You know it's highly conserved partly because non-structural proteins may be responsible for helicase activity and so on.

Slide 17

So, we did this whole process that I did before for the predicted immunodominant. Found no responses.

Slide 18

But here we did it and we showed that, in summary, that these T cells- they do respond to the peptides flow targets but they also respond against the endogenously expressed membrane glycoprotein. Lots of killing here. Lots of killing here.

Slide 19

And I can show you the same thing for all four of them- five of them all together. The nice killing. So, these are- we believe in the multigraph and just to complete the multigraph, I've gone 30 seconds over time sorry. The last point I'm going to make here is that we're going to pull out the T cell receptor and

prove in fact that that T cell receptor can transfer specificity. So here we are cloning our T cell receptor into PBMCs and show that SARS-Cov2 targets are also recognized.

Slide 20

It's published just to prove from nuts to bolts that you know predicting does not lead to SARS-CoV-2 epitopes, but in fact, what you need to do is to elute the peptide. Some of them are predicted of course among the four thousands, but you have to go after the specific ones that are eluted and we actually have a whole bunch now, not just against spike and glycoprotein, but also NSP targets.

Slide 21-22

And I think that the question that we want to answer is you know is this assay with a more select group of peptide epitopes these T cell epitopes going to be relevant for looking at these T cell responses in more detail? You don't throw in those three or four thousand and then hope that you'll get some relevancy. And I think these are the questions that we're going to be asking as we move forward.

Slide 23

And then the last thing I want to make. Not everybody knows about Maurice Hilleman. He's a hero. He should be considered a superhero. He saved millions of lives literally because of vaccine strategies that he implemented for a lot of childhood diseases- rubella and so on. Mumps, measles, rubella. And also, Zhang Yongzhen who literally within 48 hours turned over the SARS-CoV-2 genome into the public domain for which Pfizer, and everybody else has made billions of dollars making these vaccines and saving lives and it was because of his work that really we get to where we are as quickly as we can.

Slide 24

And I just want to thank everyone here, and prove in fact that this was a supplement that came out of a pancreatic cancer grant and led to this sort of collaboration. So, I thank the Commons for allowing us to display some of this work. Thank you so much.