Michael Sikora | Sequencing Clonally Expanded T Cells in ME/CFS

https://www.youtube.com/watch?v=_Alla0IT59E&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK&index=13

Raeka Aiyar: Alright, so our next speaker is Michael Sikora who is here at Stanford University. He works in Lars Steinmetz's group in the Department of Genetics and they have been collaborating with Ron's group here for about a year now. Recently were awarded an RO1 from the NIH to work on this project, exploring the role of T Cells in ME/CFS.

Ronald Davis: Thank you. I invited Michael here to give you a presentation. Michael is a graduate student. Why I'm doing that is for you to see the quality of students that come to Stanford. These are the people who make a difference in the research.

To help him in his career path I just said, "You're in charge."

(Audience laughs)

You have heard these three faculty members, Mark Davis and Lars Steinmetz.

Ronald: Okay.

Ronald: And me and tell us what we have to do. He's taken on the challenge and I think this is really good for his career.

This is just an opportunity to see what a student is like and not have to hear everything from the the people who actually don't do the experiments.

(All laugh)

Thank you Michael.

(Applause)

Michael Sikora: Yeah, thank you Ron. I hope now that you've put me in charge I can stop doing the experiments, actually.

(All laughs)

But really it's an honor to be up here amongst so many really established researchers.

Today I'm going to be talking about a project that we have developed in the lab, that we are now applying in the context of ME/CFS. I tried to tone down the science to make it approachable for everyone. It may still be a little bit dense, but I will alert you guys to the important points that I think, at least for preliminary results, are promising.

The project that I'm going to be talking about today is about the immune system and, of course, there's been a lot of talk that the immune system may be heavily involved in ME/CFS.

As you may know there are many different pathogens that can infect humans. These are from viruses, from bacteria. Ron also mentioned fungal. For example, the flu and herpes are all viral infections and then you can get tuberculosis or pneumonia from bacteria. What you can see from this slide at least, is ... I mean this is only fraction of all the organisms that can infect people, but there are a lot of them and they can happen all throughout the body.

The immune system is tasked with actually detecting these infections and neutralizing them. This is just a figure showing a pathway that the immune system kind of travels through in order to detect all of these potential invading organisms.

Today I'm going to be talking about T cells specifically. This is a particular cell type within the immune system. The immune system is very complex. This is somewhat narrow, but just to let you know how these T cells work. They go throughout the body through this lymphatic system. They're also in the blood. The T cells go all throughout the body and search for all of your cells. These are heart cells, brain cells, skin cells, et cetera. They have this T cell receptor here. It's kind of like an arm on the T cell that feels around and looks at the cells, like indicated here, which may be infected or may not. Based on a very specific interaction of this T cell receptor with this cell, it will be able to determine whether the cell is infected and then if it decides that it is infected it will then kill this infected cell.

As I mentioned, there are many potential pathogens. Whatever bit in red here is presented to the T cell, that is very variable, so the immune system has to create a bunch of different T cell receptors.

I won't go into how that is done, but basically you have many, many T cells throughout the body and each one has its own T cell receptor. That way when you have any different type of infection each T cell with its own T cell receptor can then recognize such an infection and remove whatever invades the body.

You start with many T cells, each one with its own T cell receptor which you can see as indicated by colors. Each one has its own specificity. Upon infection, some of these T

cells will recognize an infected cell and it will undergo a process known as clonal expansion. This is a critical point in this talk. This clonal expansion, when this T cell recognizes the infection, it will duplicate itself, maintaining that same T cell receptor. In that way now the immune system has a bunch of these blue T cells that all recognize these infected cells and so you can create a stronger response to the infection.

A very key point here is this interaction with the T cell and the infected cell, which I mentioned before. It has to be very specific. This clonal expansion won't occur just randomly. It has to have something that it recognizes in order to clonally expand.

That may have been a bit of background, so I'll just summarize what I've said so far. The immune system is in charge of searching the entire body for infections. The immune system, and in this case T cells, will kill these infected cells. Each of these T cells have T cell receptors that are very specific for a particular infection. If it is specific and recognizes the infection it will clonally expand. It's rare that any one T cell will recognize a particular infection.

This is the first data slide that I'll present. I believe Mark Davis talked here last year and showed this slide. This is kind of the crux of the project that we are gonna do, which I'll talk about in the second part of my talk. Each circle is a pie chart and this represents the diversity of T cell receptors in a individual. On the top, you can see, are the healthy controls. Most of this pie chart is white, indicating the T cells are all ... they all have a unique T cell receptor, which means that there's been very little clonal expansion in these healthy controls.

However, if you look at ... here is Lyme patients and at the bottom is MS patients, which have been mentioned, multiple sclerosis. These are well known immunological diseases. There is a T cell response to these diseases with a known infection. Also, you can see here are the ME/CFS patients and they have a similar pattern. This suggests that there is at least an immune response in ME/CFS patients which you do not see in healthy controls and you do see in other diseases that are known to have activated immune responses.

Just to go back to the cartoon, in ME/CFS patients and these other diseases you have a lot of clonal expansion. The healthy controls just have these unique T cells.

That is the background and why we are starting our project looking at these clonally expanded T cells. Before I get into those results I will give you some more background.

This is the fundamental slide ... background. It is known as the central dogma of biology. Basically, I think most people are aware that we have DNA in our cells. These have genes, which and in your genes they make RNA which go on to make protein and these proteins are functional and they go out and do things like kill infected cells. We

use a technology known as RNA-seq. Basically what we're doing is reading the RNA in cells in our body. I'll walk you through that as well.

Here if you think of this line as your DNA. There are genes all throughout your DNA, indicated in red. These genes from time to time will be expressed and so they will make RNA and this RNA will go on to make protein. The basis for our project is the fact that RNA recapitulates the protein, which is what is going out and enforcing its function.

You can imagine these different genes have different functions. If you take heart cells and do RNA-seq the genes that are important for heart function will be expressed. Likewise, if you do it in kidney the kidney genes will be expressed and these other genes will not be expressed.

We wanted to apply this to these clonally expanded T cells. As I mentioned, this is rare and it's only if it's very specific for infection. We don't want to just sequence all of these T cells. You can see there are a bunch of blue ones, but there is still the orange and the red, et cetera. But the orange and the red aren't necessarily what we're interested in. We're interested in these blue clonally expanded T cells.

We are applying single-cell RNA sequencing, which is a new technology that is really gaining in frequency and has been helping in characterizing a lot of different diseases and development, et cetera. This is quite a straightforward concept. Single-cell is going to be more informative than averages.

Here is a smoothie and here is some fruits.

(Audience laughs)

If you take the smoothie you don't know is this piece, is it a blueberry or raspberry or blackberry? Rather, if you go in and have just the bowl of fruits you can pick one up and figure out oh, this is a blueberry, et cetera. To take this back to the T cells then, you can imagine if we take all of these T cells and look at the gene expression maybe we have these two genes expressed. But what if it is that the blue ones are expressing this gene on the right, these other ones are expressing the gene on the left, and so, in fact, the average is that both are expressed. So we apply this single cell RNA sequencing.

These next two slides are more data. If you don't understand it, it's not much lost. I'll tell you the main points.

This is a principal component analysis where we have sequenced T cells from healthy controls as well as ME/CFS patients. Each point represents a single T cell. They are placed on this principal component analysis plot based on their similarity. If you have two T cells that are exhibiting very, very similar function, they will be placed very, very closely on this plot. I think, maybe you don't understand the statistics, but if you look in

red are ME/CFS T cells, or T cells from a ME/CFS patient. In blue are T cells from a healthy control. You can see that there is a pretty clear difference between the ME/CFS patient and the healthy control.

I don't show this plot, but we can actually go in and look at the genes that are contributing to these differences in the ME/CFS and healthy control. However, the main point is that we do indeed see a difference in the ME/CFS and healthy control.

I've been talking a lot about these clonally expanded cells. This plot doesn't represent that. This is just a bunch of different T cells. This next plot that I'll show you is a heat map showing the expression of different genes in a bunch of different cells. On the the rows here are different genes. These are genes. They have expressed RNA. They have different function. The columns are different cells.

We sequenced about 200 cells. This is all from a ME/CFS patient and roughly we've ordered these cells from left to right based on the extent that the cells have clonally expanded. Immunologists can go in and look at these genes and characterize what are these cells doing. There are a bunch of interesting genes and they are basically as expected, actually. You see a bunch of granzyme B, granulysin, et cetera. These are genes that are actually responsible for killing the infected T cells.

To summarize this point. I mentioned we did the single-cell RNA sequencing. We were wondering maybe these clonally expanded T cells have a different expression pattern as these unique T cells and indeed we do find that is the case. These expanded T cells are expressing the gene on the right, for example, and these other ones are not. Basically, what we find is that this gene on the right is responsible or ones that are known to be responsible for removing infected cells. What we hypothesized then is that these, in the ME/CFS patients, we see an activated immune system and this activated immune system is likely actually killing some cells.

The summary of the overall talk, I think there're two main points. The first is that the immune system is activated in ME/CFS and that this happens only in the event of a specific recognition of an infection. When you look at those cells that are activated they do express the genes that you would expect them to express, which are genes that are responsible for killing infected cells. I think these are preliminary results, but it does present a lot of, I think, hope and some things to follow up on. There are a lot of things that you can correlate with ME/CFS. We don't know is it causing something, is this a secondary effect.

One very powerful thing with immunology is there is something that is activating the T cells and then they are going out and killing infected cells. There is work going on in the Davis lab to actually characterize which of this interaction with the infected cell and the T cell what is stimulating these T cells.

I have been talking this whole time as in the context of an infection, but of course there's a lot of hypotheses that it could in fact be from yourself, so there could be an autoimmune disease. Right now we have solid evidence that there is indeed activation; however, we don't know is this response to an infection or is this response to yourself? This is exciting, however we don't know do you want to activate the immune system or do you want to inhibit it. These are things that we are looking into towards the future.

The last slide to thank especially the OMF for funding and then all of the people who have done the work and given feedback. Thank you.

(Applause)

Thank you to our wonderful team of volunteer transcribers for transcribing the Symposium.