Expert Interviews

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Cellular Immune Responses to Syphilis: New Research

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Season 5, Episode 5

Dr. Tara Reid from the University of Washington Division of Allergy & Infectious Diseases and Dr. Meena Ramchandani discuss Dr. Reid's recent study to identify key *T. pallidum* proteins which stimulate CD4 T cell response to syphilis.

Topics:

- Syphilis
- CD4
- CD8
- antigens

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Disclosures

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Transcript

Read along with the audio or jump to a particular chapter.

In this episode:

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introduction**[00:00] Introduction**

Hello everyone. My name is Meena Ramchandani. I'm an infectious disease physician at the University of Washington in Seattle. This podcast is dedicated to an STI [sexually transmitted infection] review for health care professionals who are interested in remaining up to date on the diagnosis, management, and prevention of STIs.

In this episode, we welcome back Dr. Tara Reid from the University of Washington. Dr. Reid's research focuses on bacterial pathogenesis, and T cell immunology, specifically looking at the immune responses to syphilis. Welcome, Tara. It's great to have you on this episode again.

Dr. Reid

Thank you.

study-overview**[00:40] Study Overview**

Reid TB, Godornes C, Campbell VL, et al. *Treponema pallidum* periplasmic and membrane proteins are recognized by circulating and skin CD4+ T cells. *J Infect Dis*. 2024 Aug 16;230(2):281-292. [**PubMed Abstract**]

Dr. Ramchandani

Let's talk about an article that you published with colleagues in *Journal of Infectious Diseases* in August of 2024. For our audience, it's titled “*Treponema pallidum* periplasmic and membrane proteins are recognized by circulating and skin CD4+ T cells.” Tell us a little bit about why you did this study, and what was your hypothesis?

Dr. Reid

So, there's a lot of data, a lot of immunohistochemistry data, and microscopy that shows that there's a CD4 infiltrate in early syphilis lesions. We know this. We also know that people who are infected with HIV who have really low CD4 counts can have really severe presentations of syphilis infection. And so, we knew that CD4 T cells were important. We also know from the rabbits that splenocyte responses, including CD4 T cells, are important during infection. But what we didn't know was what antigens the CD4 T cells are recognizing, what bacterial antigens they're recognizing to stimulate their effector functions. So, we sought to identify those

antigens.

Dr. Ramchandani

And you enrolled four cohorts in the study. Tell us a little bit about the different cohorts, and why did you choose these different participants?

Dr. Reid

So, this is really important. We initially started out by enrolling people with secondary syphilis. So, these are people that have symptomatic syphilis. We can see their syphilis. We also looked at people with latent syphilis, including early latent and late latent. So, these are people that clinically, we think are infected with *Treponema pallidum*, but for whatever reason, they don't have outward signs of the infection. They don't have lesions.

Thirdly, we enrolled people that don't have syphilis at all right now, but they've had syphilis in the past, so they've been treated for syphilis in the past. This is a nice group to study, because whatever immune responses we detect in them against *Treponema pallidum*, those are more durable responses, because they don't actually have an infection right now. Maybe they were treated five, ten years ago.

And then lastly, as with most of these kinds of studies, we like to enroll people and study folks that have never been exposed to syphilis, and they have negative RPRs [rapid plasma reagins], negative treponemal tests, as well. I'll add, though, that we did not include people with primary syphilis initially because we wanted to test for adaptive immune responses, and typically, those take maybe a few weeks to develop. I think that we are currently of the mindset that we might go back and do these studies also in people with primary syphilis to see if we're able to identify CD4 T cell responses in that group, as well.

cohorts-rprs-cd4-counts**[03:40] Cohorts' RPRs and CD4 Counts**

Dr. Ramchandani

What was the average RPR in the cohorts that you studied?

Dr. Reid

So, amongst the cohort with secondary syphilis, we measured RPRs ranging from 1:16 up to 1:512. So, the average was maybe 1:128. In our group of participants with latent syphilis, again, that includes early latent, and late latent, we had RPRs range from nonreactive, up to 1:256. So, we measured, or kind of guesstimated the average there to be like 1:16. And I'll note that the participants with a nonreactive RPR actually had a positive treponemal test.

Dr. Ramchandani

Did you study persons with HIV? And what was their average CD4 count?

Dr. Reid

We did include people living with HIV. I think there were eight in total in this study, and their average CD4 count across all groups was about 686. I'll note though, that the minimum CD4 count that we measured in our participants living with HIV was, I think, 312. But we've had people that had a CD4 count greater than 1,000. So, in general, I would say that in this cohort of people, they weren't terribly immunosuppressed at all.

Dr. Ramchandani

But there was quite a range. And it sounds like everybody was on antiretroviral [ARV] therapy, right?

Dr. Reid

Everybody was on ARVs. Correct.

study-protocols**[05:15] Study Protocols**

Dr. Ramchandani

Tell us a little bit about how you identified *T. pallidum* specific T cells.

Dr. Reid

So, I was lucky, in that I had a great example of tools to use to find pathogen-specific T cells. So, David Cole has used these pathways to study and identify T cell responses to different viral proteins during herpes infections. We used those same experimental protocols, and applied them to syphilis. In summary, I'll say that from PBMCs, which is the first tissue type that we studied, we take the entirety of the PBMCs from a blood sample, stimulate them with whole *Treponema pallidum* antigen. So, we take the bacteria, smash it up, and use that to stimulate these cells in a petri dish, outside of the body, and then measure the activated CD4 T cells. So, these are the T cells that get stimulated by the treponemal proteins. We can then sort those activated CD4 T cells, so, you're separating those from the ones that aren't activated. So, these *T. pallidum*-specific CD4 T cells, we can sort, and then grow them in culture. So, now we have millions, and millions, and millions of these cells that we can use for downstream studies.

Dr. Ramchandani

And so, these are finding these CD4 T cells from the blood, correct?

Dr. Reid

That’s correct.

Dr. Ramchandani

And for our audience, what does PBMC stand for?

Dr. Reid

PBMCs are the peripheral blood mononuclear cells. So, these are your immune cells that are circulating in the blood. We can take the blood, and separate it into different fractions. So, you have your red blood cells, you have your plasma, and then there's a small fraction that are these white blood cells that we can separate from the rest of the blood.

interferon-gamma-response**[07:20] Interferon Gamma Response**

Dr. Ramchandani

So, tell us a little bit about the interferon gamma response and why you chose this measure.

Dr. Reid

The T cell response that we see in tissues is TH1-polarized. So, what that means is there's a CD4 T cell subtype that produces interferon gamma in response to stimulation through its antigen receptor. So, we used

interferon gamma as a measure of activation for these CD4 T cells. We also, use IL-2 (or interleukin 2). This is another effector molecule produced by these TH1 CD4 T cells. In addition to those two cytokines, in vitro, in these assays, we measured proliferation as a measurement of how activated these cells were, or if they were proliferating in response to different *Treponema pallidum* antigens.

Dr. Ramchandani

That's helpful. So, you would have the CD4 T cells that you sorted out, and then enriched, and then you would put them together with the *Treponema pallidum*-specific proteins, the different proteins, let them cuddle together, and then measure the interferon gamma response and the IL-2 response, and proliferation.

Dr. Reid

Exactly. I like cuddling.

why-expand-t-cells**[08:40] Why Expand T Cells?**

Dr. Ramchandani

And tell us a little bit more about enriching and expanding the T cells. Why was this done?

Dr. Reid

Yeah, that's a great question. So, in your peripheral blood mononuclear cells, there's T cells, and other immune cells that are responsive to so many different antigens that we encounter day to day, even antigens and pathogens that we've encountered throughout our lifetime. So, in this complex milieu of different cellular T cells and B cells, we wanted to find just the ones that were responsive to *Treponema pallidum*.

When we measure those using the assays that I mentioned before, when we measure those, those cells can be quite rare. So, amongst all of the PBMCs, all the CD4 T cells, they could be as low as less than 0.01%. That's incredibly rare, but we start off with millions and millions of cells. Even if we sort and find 100 or 1,000 cells, we can then use those cells to answer these questions. But it's such a small number, right? Only 100 cells. How do you do experiments with such a few number? So, that's why we expand them. So, we just grow them out in culture, so then we have millions to play with, and ask these questions.

Dr. Ramchandani

Did you find that people who had, let's say, secondary syphilis had more T cells than those who had, let's say, latent syphilis? Or was there not a difference?

Dr. Reid

Not necessarily. And it's hard to make that direct comparison based on how much blood volume you take from a person, how efficient your isolating of PBMCs was, and then additionally how many cells you start with as you're stimulating with *T. pallidum* and then getting the *Treponema pallidum*-specific CD4 T cells out. So, we didn't measure that specifically. We were more interested in how many we could get expanded.

I'll also add that when we take these *Treponema pallidum*-specific T cells out of the PBMCs, or out of the blood, and then expand them, it's a polyclonal expansion. Right? So, there's a lot of different types of cells there that could be responding to different *Treponema pallidum* antigens, but it's important to think about, because it's not just one CD4 T cell that's responding to one antigen, or maybe even a few. It could be hundreds of different T cells that are responsive to a hundred antigens. It could also be a hundred different CD4 T cells that are very closely related, and they're sisters, and they're all clones of each other, or near clones, and they're all responding to the same antigen. That's another scenario. So, by measuring that

absolute number, it doesn't necessarily give us any more detail about the breadth of the CD4 immune response for an individual.

Dr. Ramchandani

There's a lot of variables.

tissue-resident-memory-cells**[11:50] Tissue Resident Memory Cells**

Dr. Ramchandani

And were you looking at also, tissue resident memory cells? And what were your findings? So, you got some from the PBMCs and the blood, but what about the tissue?

Dr. Reid

We were excited to investigate what was going on, again, at that interface between the bacteria and the immune system. Right? So, by looking in tissues, we're able to do that. We use a different protocol to answer these questions. So, I mentioned that these *Treponema pallidum* specific CD4 T cells can be quite rare in the entire blood volume. Right? It could be very, very rare, so we have to expand them. We have to separate them and expand them.

It's a different story in tissue though. So, within skin biopsies, there's bacteria present, there's treponemes present. We can stain them. We can see them. So, there's antigen there. Because there's antigen there, we think that there's influx of antigen-specific immune cells. So, our hypothesis was, within these skin lesions, you're naturally increasing the number of pathogen-specific cells. So when we take that biopsy from that little piece of flesh, again, there's bacteria present, and these T cells, all we do is grow those T cells out from that piece of skin tissue, and then expand them. So we grow them out and just get as many as we can. And then that, again, polyclonal CD4 T cell line is what we used further downstream to test for specificity against different *Treponema pallidum* antigens.

Dr. Ramchandani

That makes sense, because it's much more concentrated, especially if you have an active infection going on in the skin.

Dr. Reid

Correct.

Dr. Ramchandani

And I can see why you'd want to go back, and take a look at those with a primary lesion, as well, because I think that would be really interesting findings.

identifying**[13:56] Identifying *T. pallidum* Antigens**

Dr. Ramchandani

How did you find or identify the *T. pallidum* antigens?

Dr. Reid

That's a really great question. So, I will preface this by saying that *Treponema pallidum* has a relatively small

genome for a bacterium, but it does have over 1,000 predicted proteins. So, there's over 1,000. We expressed each of these proteins individually, like one at a time, but it would've taken us a long time to do all 1,039 proteins. So, we started off by targeting our expression of these different proteins to known outer membrane proteins.

Dr. Ramchandani

Because they're protective.

Dr. Reid

Because they're protective, potentially. We also targeted things that we knew were highly expressed, either during in vitro culture, or during active in vivo infection. And then we also included proteins that were known to be B cell antigens, or targets of antibodies during rabbit experimental infection, and then also human infections. So, this comprised our list of goal proteins to express. We didn't express everything, either because of different experimental challenges, but at the end of the day, we ended up with 89 proteins that we could use in this library of proteins to test, and look for CD4 T cell responses. At the end of the day, it was less than 10% of the entire proteome, but we tried to use a rational approach to get started.

expressing**[15:34] Expressing *T. pallidum* Proteins**

Dr. Ramchandani

And how did you express these proteins?

Dr. Reid

So, we use a cell-free *E. coli* expression system. So, it's nice because without all of the entirety of *E. coli*, we're able to express these proteins without LPS, which is important for these in vitro T cell cultures.

Dr. Ramchandani

And is it because LPS can potentially activate the T cells?

Dr. Reid

Potentially, but also, for PBMCs, because PBMCs are such a complex mix of different cell types, the LPS could stimulate different immune responses in that setting, as well.

Dr. Ramchandani

And for our audience, LPS stands for lipopolysaccharide.

Dr. Reid

Correct.

how-many**[16:15] How Many *T. pallidum* Proteins?**

Dr. Ramchandani

And so, what did you find when you did these experiments? Which *T. pallidum* proteins were recognized by the CD4 T cells? And you can separate it out in terms of those from the blood, and those from the tissue, if they're different.

Dr. Reid

So, in sum, we included eight patients in this initial study, and found CD4 T cell responses to 14 different *Treponema pallidum* proteins.

Dr. Ramchandani

Out of the 89 that you had started with.

Dr. Reid

Correct. Those included outer membrane proteins, because we made an effort to include those in this study. So, things like our lipid transporters that are in the outer membrane, so, presumably essential to the treponemes, also, responses to a group of proteins called *Treponema pallidum* repeat proteins, which have a lot of different implications. We also, found CD4 T cell responses to a highly reported-on protein called BamA [β-barrel assembly machinery A], that's also known to be a target to a target for antibody responses. In addition to these things, so, these are kind of proteins on the outer membrane, including a fibronectin protein (fibronectin binding protein), we found CD4 T cell responses to proteins that are under the outer membrane, so, things that would not be exposed to the host immune system, from an intact, alive spirochete. So, those include some of our diagnostic proteins, so some of those proteins that are used in our treponemal tests. And then we did detect a response to a single cytoplasmic protein.

differing-strains-proteins**[18:05] Differing Strains and Proteins**

Dr. Ramchandani

So, we know that there's different strains of *T. pallidum*. How different are the strains in terms of the proteins that you're talking about? And do we have to think about that variable when you're thinking about the immune response?

Dr. Reid

Yes. So, that's a really juicy question. So, there are strain differences, and there are differences amongst strains in some of the proteins that we included in our library. So, one would say, "Well, geez, Tara, did you include multiple types of each protein to represent all these different strains?" We did not do that initially. We included proteins that represented the nickel strain. So, this is our laboratory strain that we've had circulating, and propagated in rabbits, or in culture for, I think it's over 130 years now, maybe 120 years. But we started there. My rationale for doing that was that it was shown in other highly variable proteins, TprK as an example, that the variable regions of the protein consisted of B cell epitopes, whereas the conserved regions of their protein contained the T cell epitopes. So, extrapolating from that, we presumed that the T cell epitopes, the things that we're after, would be located in the conserved portions of these proteins, compared to the B cell epitopes.

Dr. Ramchandani

And did you find any difference in the recognized *T. pallidum* proteins, for example, in those who had a history of syphilis, or for example, those who were asymptomatic?

Dr. Reid

So, yeah, that's a really great question. I really want to answer that during my career, but we had a limited number of patients enroll in this initial study.

Dr. Ramchandani

Eight patients, right?

Dr. Reid

Eight patients, right. So, it's a really small number, and I’m not powered to answer that question.

Dr. Ramchandani

So, maybe a future question.

Dr. Reid

Yeah, in the future. So, the other complicating fact is when we diagnose an asymptomatic infection, or symptomatic, sometimes that's not entirely clear. If somebody is diagnosed as having early latent syphilis, is it that they truly have no lesions in their body, or that we just didn't look hard enough to find that primary chancre?

Dr. Ramchandani

That's a very good point. And they have found that a lot of people diagnosed with early latent syphilis might actually have had primary or secondary that got missed on exam.

Dr. Reid

Yes. And then another level there is, if I'm saying that somebody has early latent syphilis, versus late latent syphilis, is late latent truly late latent, or is it syphilis of unknown duration, and we just don't have the timely serologic data to distinguish these two things?

Dr. Ramchandani

Yeah, that's a great point.

immune-response-durability**[21:08] Immune Response Durability**

Dr. Ramchandani

And do you think the immune response is durable and/or protective? I know you only had eight patients. Are you planning on following these patients over time?

Dr. Reid

To answer the first portion of your question, do I think it's durable? Yes. I think that these immune responses are durable, and I think that because we were able to enroll some patients that had previously treated syphilis. So, when we took their PBMCs, when we took their blood, they did not have syphilis. They were treated three years ago, five years ago, one person 10 years prior to coming to our study, and we were still able to find these circulating CD4 T cell responses. In addition to that, one of our participants who had secondary syphilis, we were able to detect skin CD4 T cell responses, and then even after treatment, six months afterwards, we're still able to find these *Treponema pallidum*-specific CD4 T cells in their healed, normal skin. These things together make me feel confident in saying that, yes, these are indeed durable responses.

Your next portion to the question was, how protective is this? Again, we don't know yet. I will say that some of our participants do come back to this study, either because they were reinfected, or to be enrolled in a different cohort. And just very, very anecdotally, we were able to identify at least one case of somebody who

was initially enrolled with symptomatic syphilis, was treated, living their life, happy, and then re-enrolled with early latent syphilis, and we were able to detect differences in their CD4 responses between those two episodes of syphilis. So, again, this is an *N* of one, very anecdotal, but interesting.

Dr. Ramchandani

It is very interesting that you did have a patient who was reinfected with syphilis during your study, and you were able to see a difference in immune responses. And was this a difference in terms of the number, or type of proteins the person had CD4 T cell responses to?

Dr. Reid

Yeah, so, in this situation, the person did have *Treponemal pallidum*-specific CD4 T cell responses. But with our admittedly limited library of proteins, we weren't able to identify CD4 T cell responses to any particular antigen. So, none of those 89 that we included. But, on re-enrollment, we were able to detect within that panel of 89 proteins, CD4 T cell responses against particular antigens. So, there's a difference there. We weren't able to detect anything, again, with our 89-protein panel, but later, after reinfection, we were. So, what does that mean? We're not certain yet, but there was a difference.

immune-response-hiv**[24:10] Immune Response and HIV**

Dr. Ramchandani

Do you think that the immune response would change in someone with HIV? For example, someone who had a low CD4 T cell count, or who was not on antiretroviral therapy?

Dr. Reid

Yes. I really do think that there would be a difference. It's hard to say if there's just a difference in the robustness of the CD4 T cell response, because there may be fewer overall CD4 T cells in the body, versus a different breadth of response. By that, I mean, it's unclear to us currently whether active HIV infection with a low CD4 count means that people aren't responding to as many antigens, or if their responses are just muted across the board, because the total number of CD4 T cells is low. So, it kind of gets at the question of whether it's a sheer numbers game, or if there's just a general CD4 T cell dysfunction that might impair the response against *Treponema pallidum*.

Clinically, we know that this is something that happens, that people living with HIV, that have uncontrolled viral replication, they've got low CD4 counts, they're at increased risk of developing neurosyphilis. They're more likely to have atypical presentations of their syphilis. And importantly, this is the thing that I think about the most, we're much, much, much more likely to present with malignant syphilis (or lues maligna), which is a really, really striking presentation of *Treponema pallidum* infection.

Dr. Ramchandani

And it probably depends on also, where their CD4 count is. For example, if their CD4 count is 10, versus their CD4 count is 190, that might have a different response. And whether or not they're on antiretroviral therapy can also influence that as well.

hla-class-restriction**[26:02] HLA Class Restriction**

Dr. Ramchandani

Tell us a little bit about HLA [human leukocyte antigen] class restriction, and how did you determine this?

Dr. Reid

So, we were focused on CD4 T cell responses. These CD4 T cell responses are dictated by the CD4 T cell receptor (so the TCR receptor) and how that T cell receptor interacts not only with an antigen, but also, with the MHC class II molecule on antigen-presenting cells.

Dr. Ramchandani

Immunology 101! This is great!

Dr. Reid

So, MHC class II—that is our major histocompatibility complex II—which also we discuss as our HLA type. So, if we're reliant on our HLA type to bind specific antigens, and interact with the TCR-R

Dr. Ramchandani

The T cell receptor.

Dr. Reid

The T cell receptor, it is important to understand the T cell class restriction, to understand the antigens that we are detecting. Another way to say that is, somebody with one particular HLA type might respond to *T. pallidum* differently than another individual with a different HLA type. So, although we might have detected responses to these 14 proteins in this particular group of individuals, another group of individuals with a different HLA type might respond totally differently, and maybe we won't detect those same 14.

Dr. Ramchandani

So, it's very important, thinking about the immune response.

Dr. Reid

Exactly. Also, super important if we're trying to generalize the CD4 T cell responses to bigger populations. So, everybody in the United States, everybody in the world, because if we intentionally include a CD4 T cell antigen into a vaccine, we want that antigen to be recognized by the majority of people.

cd8-t-cells**[28:05] CD8 T Cells**

Dr. Ramchandani

What are some next steps in your research in this area? And what are you planning on doing?

Dr. Reid

Oh, so, exciting!

Dr. Ramchandani

The CD8s?

Dr. Reid

Yes. Well, first, I'll say, for next steps, just as you were asking about this difference in asymptomatic, versus

symptomatic, reinfection, all of these things, we're hoping to continue enrolling, to get our *N* up, and be able to have enough people to say whether there's a difference in the CD4 T cell response between people that have a symptomatic reinfection versus an asymptomatic reinfection, such as early latent syphilis. So, that's one exciting thing that I want to get at. Another exciting avenue for this research to branch out into is understanding not only the CD4 T cell response, but also the CD8 T cell response, which is a huge black box. We really know almost nothing. And so, I'm excited to get into that CD8 realm and better define that.

Dr. Ramchandani

And are you able to do some of the similar laboratory techniques to sort on CD8 T cells, to do the same types of experiments?

Dr. Reid

So, yes, we're able to isolate CD8 T cells that we think are *T. pallidum*-specific, but to test for reactivity to particular antigens, it's a little bit trickier than it is for CD4 T cells. So, CD8 T cells, as you might remember, tend to respond to intracellular antigens. So, these are antigens that are presented to the immune system by very specialized antigen-presenting cells, and then also, other cells in the body, in the context of MHC class I.

So, to do that experimentally in the lab is a little bit different, and so we're working on understanding those pathways for antigen presentation, because it is possible that *T. pallidum*, one, has some portion of its life cycle within cells, maybe less likely, but we don't know. Or two, there is a cross-presentation mechanism that's important for presentation of *T. pallidum* antigens in the context of MHC class I.

Another interesting thing to think about for these CD8 T cells is whether or not they are indeed antigen-specific, or if they're more on the innate-like spectrum, meaning that maybe they're responding to more generalizable bacterial antigens versus *T. pallidum*-specific proteins.

Dr. Ramchandani

This is very exciting. I look forward to hearing more and seeing what results you end up having as your experiment continues over time.

Dr. Reid

Well, thank you. I'm excited to get into it.

most-common-question**[31:00] Most Common Question**

Dr. Ramchandani

What is the most common question you get on this topic, in terms of the immune response to syphilis, or syphilis vaccines?

Dr. Reid

It depends on who I'm talking to. I would say that the most common question I get from people that aren't used to thinking about syphilis, or syphilis immunology, is why don't we have a vaccine yet? So, that's a very common thing, and so, it's multifactorial, right? But we're working on it. I think that there's more and more people here in the U.S. and abroad that are actively working on these questions, and it's actually a very, very exciting time to be a syphilis immunologist. Amongst my friends who love to talk and think about syphilis, I think the question about the CD8 T cells is really common.

closing**[31:50] Closing**

Dr. Ramchandani

We appreciate you being on this episode, Tara. It was fantastic, and I learned so much from you. Thank you for being here, and for your time, and discussing the immune response to syphilis. We really appreciate you.

Dr. Reid

Thank you for all the work that you do.

credits**[32:00] Credits**

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