Expert Interviews

National STD Curriculum Podcast

New Chlamydia Pathophysiology Research

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Professors and Drs. Daniel Rockey and Scott Grieshaber discuss their recent research on *Chlamydia* pathogenesis, a unique chlamydial persistence pathway, and impact of their work on future research with National STD Curriculum Podcast Editor Dr. Meena Ramchandani in the second of two episodes.

Topics:

- Chlamydia
- pathophysiology
- STI
- STD

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Transcript

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introduction**[00:00] Introduction**

Dr. Ramchandani

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 STD [sexually transmitted disease] literature review for health care professionals who are interested in remaining up-to-date on the diagnosis, management, and prevention of STDs.

Hi, everyone. Welcome back to Part 2 of this episode where we have Dr. Daniel Rockey and Dr. Scott Grieshaber join us for this episode. Dr. Rockey is a Professor in Bacteriology in the Department of Biomedical Sciences in the Carlson College of Veterinary Medicine at Oregon State University and Dr. Scott Grieshaber is a Professor in the Department of Biological Sciences at the University of Idaho. And both of their research focuses on *Chlamydia* pathogenesis and the interactions between *Chlamydia* and the mammalian host, and this is a second episode focusing on an article titled "Metabolic dormancy in *Chlamydia trachomatis* treated with different antibiotics," which was published in *Infection and Immunity* in February of 2024. Welcome, Scott and Dan. We're so happy to have you on this episode today.

Dr. Rockey

Thank you very much.

Dr. Grieshaber

Yeah, Thank you.

background**[01:16] Background**

Dr. Ramchandani

So, I guess what I want to turn to now is the article that you wrote on "Metabolic dormancy in *Chlamydia trachomatis*treated with different antibiotics." Can you describe for our audience why you did this study? What led up to the findings to start doing this study?

Dr. Rockey

This all began, as a lot of research projects begin, with sort of an incidental observation when an

undergraduate student of mine, Addison De Boer, was conducting work to examine how *Chlamydia trachomatis* reacted when exposed to drugs for 24 hours, and then having the drugs removed, and what happens to the *Chlamydia* inside those cells.

And we used three drugs. We used ofloxacin, which is a quinolone, we used tetracycline (TET), and we used chloramphenicol. These are different drugs, they target different pathways within cells, and we were just trying this out. And, very quickly, we determined that when you look at ofloxacin-treated cells at 1 or 10 micrograms per milliliter, which we called low or high doses, treat it for 24 hours, remove drug for 48 hours, you saw a lot better growth of the organism in the cells treated with high levels of drug. And this was surprising. And you always say, "What did we goof up? We probably goofed something up." And so you do it again and it turns out the same. And so this began this line of investigation, what is going on in these cells treated with ofloxacin?

I will add, first of all, that treatment with tetracycline, chloramphenicol, and other antibiotic we tested leads to what you would consider more typical results, where those treated at low concentrations survived better than those treated at higher concentrations. But this anomaly that we identified with ofloxacin was how the work got started. And then we worked through the process, tried to understand the timing, and then, fortuitously, I was able to talk to Scott about this, and he has a great technique that he can tell you about that allowed us to expand on these results and validate them in our analysis.

research-methods**[03:39] Research Methods**

Dr. Ramchandani

Scott, can you describe briefly the methods that were done?

Dr. Grieshaber

So, we were actually studying something similar. We were looking at penicillin and some other stressors, and then we talked to Dan, and Dan pointed out this ofloxacin, but it was kind of funny. He didn't actually tell us what the result was. He said, "Try this." And so we try it, we're like, this is weird. This is the exact opposite of what we would've expected. Called him back and "you know this is totally bizarre." He said, "Yeah, you got the same result!" So, this is kind of where this collaboration started with this ofloxacin observation.

So, basically, we developed these *Chlamydia* that have these promoter reporters. So they express a green fluorescent protein (GFP) in the RB [reticulate body] state, so from an RB promoter, and they express a red fluorescent protein that's active in the last step to making an EB [elementary body]. So we call these an EB promoter, and these are two different proteins that we know are involved in development. We can grow *Chlamydia* in culture and we can use microscopy and video microscopy, we can record them over time and watch the developmental cycle. They express green and then they grow. So, you get this increase in green fluorescence and that's growth, and then eventually as they transition to the EB form, you get this increase in red fluorescence. We have this great kinetic readout that can show us the developmental cycle. Green growth switches over to red growth. We used this system. We tried the ofloxacin, like Dan said, we tried low and high concentration of ofloxacin. So, after Dan talked to us, we tried his system, we infected cells, we treated them with low ofloxacin or high ofloxacin, and we did our video microscopy where we watched *Chlamydia* grow every 30 minutes over 48 hours.

So, for this experiment, we treat them with ofloxacin at infection and we wash it out 24 hours later. So the idea was to see how these cells recover from this antibiotic assault. And, like we said, we saw the same thing Dan said. We get much more recovery the higher ofloxacin used than lower concentrations.

But the other thing we observed was you see aberrant forms at low ofloxacin concentration. So you see these larger RB-producing, RB-promoter active cell forms inside the cell, and then you wash it out and you see inclusions re-form. But the interesting thing is the aberrant ones weren't the inclusions that came back to the

developmental cycle. Those cells never changed. They never died. They were still expressing some of that GFP promoter. They stayed bright green, the inclusion stayed intact. They stayed dormant inside the cell even after removal of ofloxacin. However, there are other *Chlamydia* inside other cells that actually started the developmental cycle and they were unseen. And then if you looked at the high ofloxacin, none of them were aberrant looking, and those are the ones that recovered. So, basically, if they weren't expressing from the RB promoter, they weren't green, they recovered. If they were green, they didn't recover. And that was sort of the beginning of this observation.

Dr. Rockey

And what was remarkable about that, and what Scott's work really added here, is that the forms that recovered were absolutely invisible at the beginning, in his experiments and in our experiments. And they appeared to be truly dormant forms that were frozen at some very early point in development before these microbes could express any of the proteins that we were looking at. And so that's the novel part here, is that we have this dormancy associated with a form that doesn't look like an aberrant form. And, as he said, the aberrant forms tend to not mature to typical chlamydial inclusion.

intracellular-forms**[07:24] Intracellular Forms**

Dr. Ramchandani

And you found that with the ofloxacin, the other antibiotics, you saw the aberrant form at different concentrations of the drug. Is that correct or am I saying that wrong?

Dr. Rockey

So, these are antibiotics that we don't associate with the formation of aberrant forms. Okay. I've done experiments with tetracycline, and I can show that in a very narrow window you can make aberrant forms with tetracycline, but it's not physiologically relevant. These antibiotics tend to shut the microbe down.

So, aberrancy is not associated with what we're looking at here. Yes, in the tetracycline, in the low drug, take the drug off and forms that were not visible turned into normal developmental forms. So, this is a dormancy or persistence that appears to be separate from what people say with aberrancy, and that's what's probably the novel aspect of the work that we are describing.

Dr. Grieshaber

Exactly. So with chloramphenicol concentration dependent, as Dan said, higher concentration, the less recovered, but there were always these non-expressing cell forms that weren't expressing GFP from the promoter. And Dan looked at other genes as well. And tetracycline was a little more sensitive, probably kinetically, it's a little less reversible. So there was a delay, as you'll see that in the paper, there was a delay in recovery with tetracycline, but I think, in the end, these are considered static antibiotics, and so they're holding *Chlamydia* in this static form that then could recover. But again, these were antibiotic concentration-dependent versus the ofloxacin was a reverse. The more ofloxacin, the better they did.

Dr. Ramchandani

So, the *Chlamydia* is existing in a completely different form that was not recognized until now.

Dr. Rockey

I think, yes. The question is whether or not these forms are elementary bodies or if they are in some state that's between an elementary body and a reticulate body. But they don't appear to be expressing detectable protein, in our assays, prior to the period of dormancy or during the period of dormancy, and then they

become normal after the fact.

Initially, back in the fifties, aberrancy was identified via electron microscopic analysis of cells treated with penicillin, and it was then shown that any beta-lactam can generate aberrant forms during culture. As people continued to look at this, they determined that many different stressors, including amino acid starvation, interferon gamma treatment, iron starvation, and, interestingly, coinfection with HSV-2 [herpes simplex virus type 2] can all lead to aberrant forms in *Chlamydia*-infected cells. This seems to be a default stress response in *Chlamydia* as they infect a cell. In most cases, if you take the stress away, the microbe can revert back to the normal developmental cycle using an approach that's not totally elucidated as of yet.

Dr. Ramchandani

So, it's possible that we think that viable and culturable *Chlamydia trachomatis* was found after the drug was removed, because the *Chlamydia trachomatis* had gone into this new state. So, tell us about what you think about what this new state is.

Dr. Grieshaber

So, if you go back to the basics of the chlamydial developmental cycle, right? So, we'll start at the beginning with the EB cell. The EB is this metabolically active cell. It's using as many resources it can find to make ATP [adenosine triphosphate] to keep itself alive and happy, but it's not replicating. But it does have, at the end of the cycle or before, it preloaded itself with a whole bunch of effector proteins. So these are proteins that inject inside the host cell, reprogram the host to make the host cell more amenable to chlamydial replication. So, they have these preloaded effectors and they have this type III secretion apparatus, which is essentially molecular syringe on the outside of the EBs that they can use to inject these protein effectors to then reprogram the host cell. So when the EB attaches to the next host cell, it is primed and ready to reprogram the cell. It has these injectosomes, these needles, they inject these proteins, and that leads to the EB getting inside the host cell. So that's all before *Chlamydia* restarts the developmental cycle. This is all like basically a wound spring. The EB is programmed to do this, it touches the next cell, it then reprograms the cell to gain entry, and then this is where the cycle starts again. This is where we are with this process, we don't understand what happens here with response to these antibiotics. What happens during this process for that EB that's just entered the cell and before it gets to this replicating RB form?

So somewhere in that process, it seems to be trapped and recalcitrant to ofloxacin at higher concentrations and to some extent recalcitrant to TET and chloramphenicol. But those make sense, right? The more you give it, the harder it is to recover, but still, they're trapped. But the ofloxacin conundrum is really the fact that, that these EBs that have just entered don't fully form RBs yet, but we don't know what they're doing.

persistence-vs-resistance**[12:50] Persistence vs Resistance**

Dr. Ramchandani

And do you think this antibiotic stress is the same thing as resistance? My guess would be no, because as soon as the drug is taken away, then you have viable and cultureable *Chlamydia trachomatis* that pop out.

Dr. Rockey

So that's a really interesting question and addresses a really interesting issue and this idea of resistance, and persistence, and tolerance. And it's important to think about the differences here.

In clinical settings, *Chlamydia trachomatis* has never been shown to have an antibiotic resistance phenotype. There hasn't been identified any genetic resistance in *Chlamydia trachomatis*, which has been very good. I would argue that we have to keep our eyes open to the possibility that could happen in the future, but as of now, no. But persistence occurs all the time, as we talked about.

And the difference between persistence and resistance: First of all, resistance is defined as the process when a microbe acquires a genetic change that leads to a population emerging that is, phenotypically, a hundred percent resistant to the drug. So it's a genetic change.

Persistence discusses the organisms that are genetically identical, but a fraction of which resist either the drug, or the immune attack, or any other stress in the cell, and once the stress is removed, that organism grows out. But if you take that organism and look at it, it is not resistant to the stress, whether it's a drug or an immune-mediated process. The population that grows out will express the exact same phenotype as the input microbes.

So, resistance is when the population changes genetically and becomes a collection of organisms that is uniformly genetically and phenotypically different. Where persistence, there's no genetic change, it's just a fraction of the organism survive better, and then they go on to perpetuate the infection. And individuals that work in this area will also split persistence and tolerance into two different groups. And there's a lot of biology that separates them. But what we think about mainly is persistence versus resistance.

Dr. Ramchandani

And that's great.

pathophysiology**[15:22] Pathophysiology**

Dr. Ramchandani

And so, what do you think the findings of this paper tells us about the pathophysiology of *Chlamydia trachomatis*?

Dr. Grieshaber

That’s a great question. I think, in the end, what this is telling us is that our models of aberrancy are maybe not complete, and that's sort of impacting our ability to understand persistence in a clinical sense. So, the disconnect between understanding persistence in a natural infection and persistence in the lab. This is just one more avenue that *Chlamydia* can use to persist in patients.

And so what's not clear to us, and we're in the process of trying to develop this as a bigger idea, is that how these other kinds of tolerance/persistence play a role in clinical persistence. So, in addition to aberrancy, are there other aspects of chlamydial biology that lead to clinical persistence? And that's where this paper and our studies are kind of leading.

Dr. Ramchandani

Which is so interesting because when we give patients a course of antibiotics, let's say doxycycline, or sometimes in some cases a fluoroquinolone, they're able to treat and cure their infection. However, in this setting, at least in vitro, with this paradoxical increase in productive growth when the cultures were treated with higher concentrations of the fluoroquinolone. So, I'm curious to hear your thoughts on this and what you thought of this paradoxical increase. It was quite a surprise.

Dr. Rockey

Yes. And, as always, once you discover something that's kind of interesting, you try to dive back into the literature and understand what is known and not known. And it turns out that this quinolone paradox had been pursued in other systems, free-living microbes, beginning in the seventies, and they showed similar types of results with *E. coli*, etc. And the logic that developed out of those studies, which continue to this day, is that there's probably two different metabolic processes that are affected by quinolone antibiotics. One is

the classical thing we think about with gyrase, etc., where you're messing with DNA packaging and replication. But secondly, it's thought that these quinolones might be affecting reactive oxygen species abundance in the bacteria. And the balance between those two is what might be leading to this unusual quinolone paradox that we've observed in *Chlamydia*.

Dr. Grieshaber

And just to get back to your question about clinical significance, our studies are really showing that higher concentrations of the fluoroquinolones are shifting fluoroquinolones from lytic to static, right? So, basically, we're just forcing more organisms to go into this static role rather than lytic role. So, it's acting more like a static antibiotic. So, there's still the immune system, there's still other stressors that *Chlamydia* has to dodge from being attacked by a static antibiotic, it's just not lytic. And I think that's probably one of the issues with *Chlamydia* treatment in general is that almost all antibiotics at some level are static and not lytic because of *Chlamydia*'s unique developmental cycle.

Dr. Ramchandani

That's really helpful. I wonder if, in the clinical sense of it, it affects how long we treat with an antibiotic, the different concentrations of the antibiotic, or even what antibiotics in the setting of *Chlamydia trachomatis* treatment that can cure a patient.

Dr. Rockey

The message that I always want to get out to the public, if I'm talking to anyone, for me as a non-clinician, get tested if you think you might be positive. Testing and treatment are quite effective overall, even though we're identifying some of these interesting quirks about the microbe in the presence of some of these antibiotics, testing and treatment are very effective in helping individuals prevent *Chlamydia* disease in the community.

why-is-this-important**[19:18] Why is This Important?**

Dr. Ramchandani

Why do you think it's important for us to understand the molecular aspects of dormancy? And has this been explored before in the setting of *Chlamydia trachomatis* or other *Chlamydia*?

Dr. Rockey

I like to think that we're working on defining the true nature of persistently infected chlamydial cells, which hopefully will reflect the true nature of *Chlamydia*-infected patients.

Persistence is a key part of the natural history of almost all *Chlamydia* during infection and disease, I would say certainly in humans. We'd like to think we're defining the nature of this process, and if we as a field can elucidate the molecular steps in chlamydial persistence in vitro, we may be able to identify ways to help eliminate *Chlamydia* persistence in the patient.

Dr. Grieshaber

I'd just like to add even a different level. Antibiotics are well studied, they've been studied for a long time, but generally they're studied on free-living bacteria that grow in culture. And so, our understanding of lytic versus static antibiotics, treatments, times, and the way antibiotics are used are based a lot on obviously empiric data, but also studies of free-living organisms. And so, our studies are actually showing that you also have to think about the biology of the organism itself. Antibiotics are going to act differently on organisms that are replicating in different fashions, or going through different life cycles during infection. I think that's also an important part of this project.

Dr. Ramchandani

That's really helpful. And I can imagine any aspect of the microbiology of *Chlamydia* and the pathogenesis in the human host is really important in us thinking about in the future for a *Chlamydia* vaccine, and just understanding about how *Chlamydia* enters and lives in the human host cell, and the implications of that long-term.

next-steps**[21:06] Next Steps**

Dr. Ramchandani

What are some next steps after learning the information that you found out from this study? Are you going to continue this project? Where are you going to go from here?

Dr. Grieshaber

My interests are firmly set in the basic science of the chlamydial developmental cycle. And so we're really interested in where in the developmental cycle are these organs trapped, and how they recover. What this means for, in particular, this antibiotic and how this antibiotic works on *Chlamydia*, and how it might explain the fluoroquinolone paradox in other organisms.

We're focused on this early side now of *Chlamydia*, how it goes from entering the cell to the first replication. And so now we have some tools and some interesting areas to look at to say, "What is the biology of *Chlamydia* re-entering the replication cycle?" Because that's an important aspect of *Chlamydia* becoming sensitive to antibiotics. It's not replicating, it's not sensitive to beta-lactams. It's not replicating, it's not sensitive to fluoroquinolone. So, how does *Chlamydia* know when to start replicating? It's one of our big questions.

Dr. Rockey

My primary interests here involve trying to identify the state of this dormant form. Is it truly a unique form within an infected cell, does it exist persistently in that cell, and can we relate that to what might be happening in patients? Because I'd like to believe that if we can eliminate this dormant form inside cells that might lead to applications that would lead us to eliminate the dormant form inside patients.

Dr. Ramchandani

That's perfect.

Dr. Ramchandani

Are there any questions that people tend to ask you about *Chlamydia* pathogenesis, or from this paper that you find would be of interest to our audience you'd like to answer?

Dr. Grieshaber

Chlamydia is usually a conversation stopper.

Dr. Rockey

Yep. Well, I think people are interested in it. I'll run into people and they're uniformly interested in what's going on, and they hear the word *Chlamydia*, right! It's a stopper! But their brains are going when you're talking to them.

I was in a line at a Dairy Queen. I had a T-shirt on that had on the pocket of the T-shirt, it said, "Dan, Dan, the *Chlamydia* man." And one of my students had given me this shirt quite a long while ago, and I was wearing it. I went to the DQ, and a young lady at the counter looked at me and said, "What does your shirt mean?" I didn't know what I had. I looked at my pocket and I saw it. I laughed, and I said, "Well, this is what I do for a living. I work on *Chlamydia* and try to understand it." And she said, "You know, I thought you had it and you were owning it."

Dr. Ramchandani

That was funny.

Dr. Rockey

I think the public is very interested in the disease. I think they don't quite understand a lot about it, and I guess we don’t necessarily understand a lot about it either, and I hope that our work can help close the gap in what we don't understand and what we do understand.

Dr. Ramchandani

That's great.

vaccine**[23:55] Vaccine?**

Dr. Ramchandani

What about colleagues in the field? What areas of interest that you guys are working on that you think will be of great importance?

Dr. Rockey

Certainly, I think persistence and molecular pathogenesis are very important. How do we get rid of the persistent form and really can we define the persistent form?

But, certainly vaccination is a very challenging subject in this organism. You have this microbe that has existed with invertebrates, and then vertebrates, and now mammals and humans for eons. It's pretty good at surviving and persisting in these animals, and it's probably dealt with every immune response that these animals have thrown at it. So, to design a vaccine is going to require some very creative work. Those approaches and those studies are being done by many people in the field, and it’s a challenging battle.

Dr. Grieshaber

Our interest in my lab is we really need to understand the developmental cycle. How *Chlamydia* lives inside the cells and goes through this developmental cycle. And not just the simplest, but on a broad scale. If you want a vaccine, you pretty much want to make antibodies to it, right? So you want antibodies, but they're on the outside of the cell, so what are you going to target? So there's been a lot of interest in targeting the EB cell form and try to block them from entry, and that's probably one of the best avenues, but it's been really hard.

But now we're discovering that it's very, very easy for *Chlamydia* to either become an aberrant form, or this alternative aberrant form where they can hang out protected from an antibody response inside the cell before even germinating. So this is another aspect of the chlamydial biology that's protecting it from that outside immune system. So, then the shift becomes, well, we need to target as an intracellular pathogen with CD8, T cells, things like that, and I think that becomes more challenging from a vaccine standpoint. So I think that's why our interest is what's happening inside the cell, because this is where they're hiding from the immune

system is inside the cell.

Dr. Ramchandani

That's great. Well, I thank you so much for being on this episode today. I really look forward to seeing what you guys publish next and all your research in this area. It's an important area of work. And for our audience, I definitely encourage you, if you want to learn more, to read this article that was published in *Infection and Immunity* in February of 2024, and also see some of the amazing pictures of *Chlamydia trachomatis* in the cell. It's kind of in the cell with the microfluorescence. It's really awesome images.

So, thank you very much. I learned a lot. It's been a very informative session, and I really appreciate both of you being with us here today.

Dr. Rockey

Thank you very much for allowing us to talk about our favorite bug.

Dr. Grieshaber

Thank you.

credits**[26:40] Credits**

This podcast is brought to you by the National STD Curriculum, the University of Washington STD Prevention Training Center, and is funded by the Centers for Disease Control and Prevention.

Transcripts and references for this podcast series can be found on our website, the National STD Curriculum, at www.std.uw.edu. Thank you everyone, and have a wonderful day.