© National STD Curriculum

Expert Interviews

National STD Curriculum Podcast

Chlamydia: Microbiology, Persistence, and Aberrancy

May 7, 2024

Season 4, Episode 10

Professors and Drs. Daniel Rockey and Scott Grieshaber, national experts on *Chlamydia* pathogenesis, discuss foundational *chlamydia trachomatis* concepts with Dr. Meena Ramchandani in the first of two episodes on their February 2024 article about metabolic dormancy and different antibiotic treatments.

Topics:

- Chlamydia
- trachomatis
- STI
- STD
- metabolic dormancy

Daniel D. Rockey, PhD
Professor, Department of Biomedical Sciences
Carlson College of Veterinary Medicine
Oregon State University

Disclosures

Disclosures for Daniel D. Rockey, PhD

Scott S. Grieshaber, PhD
Professor, Department of Biological Sciences
University of Idaho

Disclosures
Disclosures for Scott S. Grieshaber, PhD

Meena S. Ramchandani, MD, MPH
Associate Professor of Medicine
Division of Allergy and Infectious Diseases
University of Washington

Disclosures
Disclosures for Meena S. Ramchandani, MD
None

Transcript

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

In this episode:

---

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 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.

Dr. Ramchandani

Hi, everyone. Welcome. We are lucky to 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. Dr. Scott Grieshaber is a Professor in the Department of Biological Sciences at the University of Idaho. Both of their research focuses on *Chlamydia* pathogenesis and the interactions between *Chlamydia* and the mammalian host. This episode will focus 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, both of you. We're so excited to have you on the show.

Dr. Grieshaber

Thanks so much for the invite.

Dr. Rockey

Yes, good morning. Good to talk to you.

chlamydia-microbiology**[01:14] *Chlamydia* Microbiology**

Dr. Ramchandani

Now this will be more of a basic science session. To orient our audience, can you talk about some of the aspects of the microbiology of *Chlamydia* and how it lives or replicates in the human host? And let's focus on *Chlamydia trachomatis*for this episode, although I understand we might digress to other *Chlamydia* as well.

Dr. Grieshaber

Yeah! *Chlamydia* is an obligate intracellular bacteria, that is, it has to live inside a eukaryotic host cell, and this is true of a long evolutionary line of bacteria in the *Chlamydia* family, goes back roughly, I think, over a billion years. There was a good paper that described that a few years ago from Matthias Horne's lab. But, essentially, they grow as parasites. They have to attach, enter a eukaryotic cell, and they set up a replication niche where they go through a developmental cycle where they change phenotypic cell forms. They enter as this small cell form that's infectious, it's called the elementary body [EB]. It's very small. It has this cross-linked outer membrane and its highly condensed nucleoid in its DNA structure. And that makes it a very, very small cell form. This cell form enters the cell and starts the process of infection. It needs to go through this germination process where it becomes this replicating cell, the RB. We call it the reticulate body. The reticulate body expands the infection so you get more organisms, and then those expansions of those organisms then produce more EB cells to then, you know, disseminate the infection. So *Chlamydia* separates expansion from dissemination, and after *Chlamydia* replicates the high numbers of creating these infectious forms, they then can lyse a cell, or egress from the cell. It's not quite clear exactly what percentage of the time they do each. That's something that's hotly debated.

But in the end, these EB forms exit the cell and then go find new hosts, new eukaryotic cells to host. So, basically, *Chlamydia* goes through this cycle where they find new hosts, enter, expand, and then go find new hosts again. And to do this, they go through this, like I said, this cell form, developmental cycle producing this replicating form, the RB, and the infection form, the EB. I'm sure I left something out, but I'll let Dan jump in.

Dr. Ramchandani

Thank you, Scott. Yeah, Dan, do you have anything to add?

Dr. Rockey

Well, I think it's interesting to think about the idea that there are *Chlamydia* in almost every animal out there, and they're different. As Scott said, the lineage goes back for at least many millions of years. And remarkably, when individuals went out in the *Alvin* submarine, and went down into the depths of a trench near Iceland, and took a scoop of muck from the bottom of that trench, and just did metagenomic sequencing on that, the number of *Chlamydia*-like organisms in that was really quite surprising and filled up every lineage of what we call *Chlamydia* nowadays. So, it's pretty remarkable how long the lineage has been around and how successful it has been.

Dr. Grieshaber

Exactly. And they all share this developmental cycle.

Dr. Ramchandani

Oh, wow, that's fascinating. So, goes back millions of years potentially, or hundreds of thousands of years, and all share this kind of same pathogenesis where they have this cycle of these different bodies that it resides in the human host: the elementary body and the reticulate body. Correct?

Dr. Rockey

Correct. I think that certainly in the sediments it's not clear exactly how the life forms are perpetuated, but in every contemporary chlamydial species, the organism exists intracellularly in a vacuole and has a

developmental cycle. In some *Chlamydia* that occupy niches inside protozoans, I think they might be as more commensal as opposed to strict pathogens. I think that sometimes the host will grow better with the *Chlamydia* inside.

Dr. Grieshaber

And, like Dan said, not every host has been defined to some unknown, whether they have a host or what the host is.

chlamydia-persistence**[05:23] *Chlamydia* Persistence**

Dr. Ramchandani

Now how does that relate to *Chlamydia* persistence in terms of like the reticulate body, the elementary body? Can you describe *Chlamydia* persistence and does *Chlamydia trachomatis* demonstrate persistence?

Dr. Rockey

When I think of persistence, I always want to think in terms of clinical and basic biological terms. Certainly, in virtually every animal that has a *Chlamydia* infection, persistence is a major part of that natural history. In sheep, for example, *Chlamydia abortus* persists through the life of the animal. Once it acquires it, often as a very young animal, but only manifests disease when a first pregnant ewe is in the late stage of the pregnancy. Pathogen emerges from persistence, then grows aggressively in placenta, leading to death or morbidity of both the ewe and the fetus.

In humans, persistence certainly is a part of the life history and many individuals who are infected are infected asymptomatically. The pathogen exists as almost a commensal in the host - that the pathogen exists without causing disease in the host. And, in the laboratory, *Chlamydia* can be induced into persistence via treatment with any number of different stressors that affect very different aspects of growth. And one of the defining aspects of this persistence is the generation of viable, but not cultureable, unusual developmental form that are called aberrant forms. As Scott mentioned, elementary bodies, the infectious form are very tiny. You can fit hundreds of them inside a vacuole, inside of a cell. Now aberrant forms tend to be much larger than that. They often approach half the size of the nucleus. These are organisms, live *Chlamydia*, that will make DNA, make protein, establish their niche, but they never, in the presence of the stress, they don't mature to a active, infectious elementary body. Take the stress away, and through mechanisms that are really unclear, this persistently infected cell will then generate new developmental forms, new elementary bodies or reticulate bodies, that can process through the rest of the developmental cycle. So, *Chlamydia* can be seen as persistent pathogens in patients, and we can demonstrate persistence in laboratory cultures.

Dr. Ramchandani

That's perfect, thank you. It's almost stress, the organism goes into this aberrant form to kind of tolerate that stress, and once that stress is removed, then it can go back into the replicative life cycle. Is that correct?

Dr. Grieshaber

I would just like to stress that what Dan said is really important, that persistence in infections is absolutely clear, right? We do know they form these persistent infections, and we're trying to model that in vitro as best we can. And so the best model we have is this aberrancy effect, but we really don't know a lot about how this works, and how this works in relation to this persistence during infection. And so, if you look at other bacteria, persister cells are these forms that are usually low metabolism, not replicating, and so therefore they're not great targets for antibiotics. They survive an antibiotic onslaught because they're not doing anything. And so *Chlamydia* persistence has always been on the other end of that and that's just an aberrancy. This idea that there's these cell forms that are staying sort of stuck in a part of the cell cycle that's active, right? They're

making proteins, they're synthesizing DNA, but they're not actually forming the infectious progeny.

And so that's our current model and it's called aberrancy, and it's the best model we have. But I think there are other aspects tof chlamydial biology that lead to persistence. This idea that some forms of *Chlamydia* are true persister cells. Like, the EB has really low metabolic activity. It makes energy, so it makes ATP [adenosine triphosphate] but it doesn't really transcribe or make a lot of proteins and it doesn't divide, so it's already resistant to antibodies that target cell division. So there's kind of this dual aspect of chlamydial persistence and we know we have this clinical persistence and we have these models and culture that we're trying to understand how those relate to clinical persistence. And one of these is aberrancy. And the other side of that is the fact that *Chlamydia* itself has these persister cell-like phenotypes.

model-aberrancy**[09:54] Model of Aberrancy**

Dr. Ramchandani

What kind of stresses instigate the aberrant form or *Chlamydia* persistence?

Dr. Rockey

So, it's kind of surprising how many ways you can make *Chlamydia* go aberrant. And certainly, we talk about antibiotics, but certainly, this is a means for survival inside cells that has evolved over the course of, certainly, the pre-antibiotic era. Stressors such as low amino acid availability, immune mediators such as interferon-gamma, and starving cells for molecules such as iron, can all drive *Chlamydia* into persistence. In fact, I think if you can stress the *Chlamydia* in any way that's non-lethal, they will tend to go into this default aberrancy pathway. And it's not clear exactly what's going on here, because what you have is this microbe, that was so small to start with, now is really quite large and occupies quite a bit of space, a single microbe within the cell. And this is documented in vivo in patients' tissues, from hysterectomy patients, they can see aberrant forms in vivo. But the function of it is not super clear.

Why does it need to be so large? That's the great question, I think, in aberrancy, and what we'll talk about. Scott has generated a lot of very nice video microscopy images that don't necessarily add clarity to that, and then try to say, "What is really going on in this aberrant form inside cells, and what is its role during the infectious process?" One very interesting inducer of aberrancy is coinfection with herpesviruses, in which a single herpesvirus protein has been shown to facilitate the development of aberrancy inside cells. So the interaction between the herpes and *Chlamydia*, which probably goes back a long, long ways as well, will lead to aberrancy within infected cells. So now I don't know what happens in vivo, but there's solid in vitro data on that one.

Dr. Ramchandani

Has there been any other association with other organisms like that?

Dr. Rockey

So, people have tried to co-infect cells with *Chlamydia* and a lot of other things, and remarkably, to me at least, they always seem to ignore each other. If you put *Coxiella burnetii*, which is another obligate intracellular bacterium, into a cell and *Chlamydia* in the same cell, they will form individual vacuoles that never seem to talk with each other. The *Toxoplasma gondii* has been infected with *Chlamydia.* The *Toxo* vacuole is morphologically, perhaps, as similar to the *Chlamydia* vacuole as anything, and they don't interact at all. And that's a eukaryote with a prokaryote. Even within the genus *Chlamydia*, if you put *Chlamydia pneumoniae* into a cell with *Chlamydia trachomatis*, they appear to not interact directly at all. They form individual vacuoles and have their own timescale. So, they seem to ignore each other inside cells.

dormancy-latency-or-persistence**[12:54] Dormancy, Latency, or Persistence?**

Dr. Ramchandani

So, Scott, I want to ask you a question. A term has come up, “metabolic dormancy.” And so, what is metabolic dormancy in the setting of *Chlamydia trachomatis* or other bacteria? And is this the same thing as latency or persistence? How do these terms kind of interact, or are they different, or the same?

Dr. Grieshaber

So latency would be like the classical herpes, even HIV, right, that basically the virus hides out completely and makes maybe a few transcripts, but very little effect on the host. And, in the bacterial world, we don't really think much about latency. We think more about, like you said, persistence or this metabolic dormancy, the fact that the cells themselves maintain their structure, they maintain at least some level of protein synthesis to maintain their energy levels, but at the same time don't cause disease. So they're ignored by the host because they're not actively destroying tissue, they're not doing this. So that, it’s sort of equivalent to the latency in the viral world.

For *Chlamydia*, this is just part of the issue. We know we have this persistence clinically, and we know we have these aberrant cells, but then we also have, like I said, the EB form, which is, in my mind, less like a spore and more like the dissemination form. So it's a cell form that's only job is to go find a new host. So, I don't know how much you know about microbiology, but there's an organism called *Caulobacter crescentus*, which is sort of a model system for swarmer cells. So cells that undergo a developmental form and then go find a new place to live. And I would say that EB is this metabolically active cell form that's maintaining its ability to infect cells, but it's waiting to find a new host cell. It doesn't divide, but it needs energy. So we showed a few years ago that it needs ATP, or glucose-6-phosphate, or amino acids, something it can use for its TCA [tricarboxylic acid] cycle so it can generate its own ATP, because it needs that energy to infect cells. So *Chlamydia* isn't quite a spore, but it's more of this disseminator cell that can keep itself alive for some timescale—I think we measured it on a time of like 48 hours—with an energy source that can then find a new host to infect. So, the role of aberrant cells versus these EBs versus this other form, which we sort of maybe talk about in this paper: *Chlamydia* who have entered cells but then not fully germinated as these other forms of low-energy cell that doesn't seem to be doing much, but maintaining its energy pools, maintaining its ability to eventually grow out again.

Dr. Ramchandani

That's helpful. And do we find, or is there any evidence, that all the *Chlamydia* change into aberrant form or, for example, can they exist, some in the aberrant form and some in an infectious form?

Dr. Grieshaber

The developmental cycle is complex. When the EB touches a host cell, it enters through this type III secretion system-mediated endocytosis, so it drives its own endocytosis by directly reprogramming the cell, enters the cell, and the model system we use just *Chlamydia* L-II; this takes 15, 10 minutes to get inside. After that, they go through this germination program and it takes about 10 hours before they start replicating, and then after replication, they start producing these intermediate forms, which then transition to EBs. So, as soon as 18 hours inside the cell, you have a mixed infection. You have both RB cell forms; you have IB [intermediate body] cell forms that are going to become EBs, so those intermediate forms, and you have a few EBs. So, depending on when you treat them or when they get the stress, those inclusions will have all the cell forms. So, you'll have aberrant cells, you'll also have EBs and E-cells, those infectious forms.

You have mixed environments that then last hours. I'm not sure we actually tested how long the aberrant form and the EB can coexist in the sub. We did some experiments where EB that forms, say at 18 hours post-infection, can still be there 30 hours later and still infectious. That EB still collects inside this inclusion, and if you give them a stressor, those EBs aren't affected by the stressor because they're low-energy metabolism. I hate the word dormancy, but they're these persister-like forms, right? They maintain their energy levels, but

that's it. They don't replicate. They don't synthesize a lot of protein. They're just maintaining themselves for release. They're basically just waiting until they can get out. So if you treat those cells with a stressor, you get aberrance out of the RBs, but you also still have the EB forms present. So you always have this mixed environment as soon as, say, 16 hours post-infection. So, to answer your question, yes, you'll get aberrants, but you'll also have EBs present most of the time.

Dr. Rockey

I will say that in vivo in patients, there's probably all forms present at the same time, certainly in the patient, and probably within some single cells. If you grow *Chlamydia* in optimal conditions in a laboratory and you look at a bunch of cells, you will find aberrant forms. They're present in normal infections as well, and that's probably because there's some little twitch of stress in those cells and it's driven them to aberrancy. So yes, you can find each of the developmental forms, certainly, in every patient, probably, and I would wager almost in many different individual cells.

closing**[18:27] Closing**

Dr. Ramchandani

Thank you. This was a really helpful overview to give a background for our audience. Thank you so much, Scott and Dan, for this really informative episode. This has been such a great session, and I learned a lot from speaking with you both.

Dr. Grieshaber

Thanks so much for the invite.

Dr. Rockey

Thank you very much. Good to talk with you.

credits**[18:48] 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.

© National STD Curriculum
PDF created March 10, 2025, 9:44 am

The most up to date version of this content may be obtained from:
https://www.std.uw.edu/podcast/episode/expert-interviews/chlamydia-microbiology-persistence-aberrancy