This Week in Microbiology

With Vincent Racaniello, Michael Schmidt, and Michele Swanson

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Episode 186: Crypto-metamorphosis

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Vincent: This Week in Microbiology is brought to you by the American Society for Microbiology at asm.org/twim.

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Vincent: Hi, This is TWIM, This Week in Microbiology, episode 186, recorded on Sept 20, 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello!

Vincent: How are you?

Michele: Not too bad.

Vincent: You've got football season started already there?

Michele: Oh yeah. We've had two home games that were both victorious and we've got a third home game on Saturday so lots of fun.

Vincent: I think one of them was Notre Dame, right?

Michele: Yes, I decline to mention that. We lost our home opener.

Vincent: Dickson was talking about that, he's an alum of that place.

Michele: Yeah.

Vincent: Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone.

Vincent: You're still there, you exist.

Michael: I exist. Charleston was fortunately blessed that hurricane Florence did not visit us here in Charleston. It unfortunately went up to the Wilmington area of North Carolina which is just across the border from South Carolina, and so as many of you will recognize, they have been

inundated by flooding because Florence brought mostly rain by the time it hit the coast it was down to a category one storm, and this morning I heard on NPR that the flooding rivers are releasing all sorts of all waste from the hog farm that are scattered across that are of North Carolina. And so people are very much concerned because you have millions of gallons of raw hog waste that are now being dumped into the local rivers that are in turn flooding people's homes. So they are out there warning people that should they get cut as they are taking out sheet rock or trying to clean up their homes, they should pay special attention to clean them up with soap and water and make certain that they get them looked at because it's not your normal river water. It's actually sewage.

Vincent: So can they use 70% ethanol handwash? (laughter)

Michael: That's a joke from the last TWIM! Yes indeed they can. But those poor folks up in North Carolina, we feel for them because for the grace of God it could have been us.

Vincent: So you didn't have to move out.

Michael: No, I was one of the designated jailees here at the institution, so I stayed behind and most of my neighborhood evacuated to Disney World. It was a hurrication, it's a new word, that the kids all had a great time, so.

Vincent: Good to hear it. I'm glad you didn't have to move and no damage was done either to your home or the university because that could be problematic.

Michael: Yes.

Vincent: Alright, before we start with science, I have a letter to read from Tom. And he starts by saying, a rant:

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Michele: Wow.

Vincent: So I thank you, Tom, and I thought I would read it up front because that's when most people are still listening. By the time you get to me at the end you're not listening anymore and I make a plea every episode to help us out. You can go to microbe.tv/contribute and you can do Patreon, give us a dollar a month, or other things as well. And Tom said feel free to read this rant on on all the other podcasts, and I will. I certainly will. (laughter) So Tom is in Austin and is a listener of several of our works, and we met Tom while we were recording TWIV 500 in Austin this past summer. So thank you, Tom.

Michele: Vincent, I think next time you can do it once more with feeling. (laughter) On your next podcast, to make it really a rant.

Vincent: Well, you know, rant, I reserve rants for science issues. I would like people to help us, but I don't know, I feel badly ranting for money. You know what I mean? It's not that we want to make money, we just want to pay our expenses, that's it.

Michele: And expand our reach for more face to face shows.

Vincent: Absolutely. I want more people to listen. We have a lot of listeners right now, which is great for science, but we want more. We want us to become a household name (laughs). Alright. Let's go on to our main paper, which Michele's gonna tell us all about.

Michele: Yes, this was published in August of 2018 in PNAS and it is entitled "Recombinant Listeria promotes tumor rejection by CD8+ T cell-dependent remodeling of the tumor microenvironment" and it is a work done primarily at Aduro Biotech at Berkeley in collaboration with a group at University of California Berkeley. The authors are Weiwen Deng, Victor Lira, Thomas Hudson, Edward Lemmens, William Hanson, Ruben Flores, Gonzalo Barajas, George Katibah, Anthony Desbien, Peter Lauer, Meredith Leong, Daniel Portnoy, and Tomas Dubensky.

So this is a remarkable application of what has been learned about a bacterial pathogen Listeria monocytogenes and it's I think just very exciting how microbiology can contribute to human health in many ways. So as background on let's talk about listeria, and this is a food borne pathogen and primarily affects the elderly, immunosuppressed, and pregnant women and their neonates, because this pathogen has the ability to survive in a white blood cell and also spread and invade in cells that normally are not phagocytic. So for example it can cause cross the placental barrier and get into the bloodstream stream of neonates and cause meningitis, or abortion and stillbirth. So we know that that you can get from unpasteurized dairy products, there have been outbreaks associated with ice creams, also deli meats, hot dogs, pates, things that aren't heated.

The other fascinating aspect of listeria is that classically it was used in really groundbreaking experiments to define what we now call cellular immunity. So in 1964, George McKinness, an Australian immunologist, published in Journal of Experimental Medicine that a number of intracellular parasites induce immunity in a mouse model that is not directed at the parasite itself, and it's not that that those immune factors are not in that blood serum, but instead that the defense can be transferred by white blood cells from one animal to another. So for example in that classic 1964 paper, they challenged or they infected mice with Brucella and then later challenge them with listeria, and found that the white blood cells were able to control the infection, the subsequent infection.

The reverse was also true. If they first infected mice with listeria and then subsequently challenge with Brucella or another intracellular parasite, they found that the mouse was

protected. So something about these intracellular parasites confers a broad protection against it and other intracellular parasites. So this then led to the birth of cell mediated immunity, and we now understand a whole lot more. In fact, we're gonna talk today about different types of macrophages, macrophages that are pro inflammatory and contribute to fighting infection of intracellular pathogens, but also cancers. And then the second type of macrophage that is responsible for, contributes to dampening the immune response, because of course once we get the defense systems have worked and cleared the infection, we now need to quiet the system so that we don't get collateral damage and tissue damage. So that's up will be what will be talking about today.

And what is really exciting, if we now fast forward to current day, is a number of scientists using various strategies are developing tools to stimulate our immune system to fight cancers, and we call this immunotherapy. So the challenge in making immunotherapy work is we first have to identify antigens, markers, that are unique to the tumor cells and are not present on our healthy tissues so it's a specific response. Identify which among those will stimulate a robust cell mediated immune response. But then another challenge is, as we understand what that antigen is, we've got to figure out a way to get it into the cytoplasm of the white blood cells, or the MHC1 pathway.

And that's where listeria comes into play. The vaccines also will need to be able, we need to be able to produce specific vaccines quickly, and by using the bacterium that we can genetically engineer and then grow in broth logarithmically, we can get a large quantity of the vaccine strain quickly. But another challenge is we have to overcome the what we call tolerance, so our immune system will see cancer cells as part of our self and they stimulate what we call self tolerance, and so if we are going to make immunotherapy work for cancer we'd also got to break that immunosuppressive blockade. So that's the challenge that this group has been working on, and their strategy is is to use this pathogen and ironically, listeria monocytogenes. So why is it a good candidate? We know from a lot of work done by a number of labs including the Portnoy lab that listeria, once it is ingested by a macrophage, it is able to break out of its vacuole and get into the cytoplasm, and of course that is where antigens can be accessed by the MCH2 pathway. We also know that listeria can then recruit cellular actin and make these fabulous actin tails that mediate movement of listeria within that host cell, but also it can push out of its primary host cell into neighboring cells and therefore spread from cell to cell, being hidden by the antibody response.

Michael: You've probably seen some of those videos on YouTube of the actin rockets propelling the listeria into its neighboring cell without ever leaving the cell, which is one of the great mysteries of how a listeria remains that facultative intracellular parasite. I did that, Michele, so you could actually take a drink. (laughter)

Michele: Those are spectacular movies. Julie Theriot has those on her website, so I encourage anybody who hasn't seen them to take a look. So in order to use Listeria as a safe, live, attenuated vaccine and take advantage of the fact that it naturally gets into the cytoplasm, the

scientists also then had to activate or inactivate the ability of this pathogen to get out of that macrophage and spread.

So they did that by mutating ActA which is a listeria protein that recruits the host actin to make these fabulous tails to spread. They also deleted a surface protein, an invasion factor named InternalinB, which experimentally has been shown to equip listeria to get into cells that normally do not eat bacteria, including liver cells, or hepatocytes. So in a previous paper in 2014 in PNAS, this group published a proof of principle experiment showing that listeria, when injected as a vaccine into a mouse with a tumor, could fight that tumor and not cause toxicity, not cause liver damage and not spread and do its normal pathogenesis. So they already knew that this listeria double mutant in ActA and internalinB could be a good platform for designing cell mediated immune vaccines directed at particular tumors.

So their experimental approach in this paper is to use a mouse model where they first inject the mice with tumor cells and allow a week to pass, so that the tumor cells can take hold and begin to develop tumors, and then they inject the mice with listeria that is not able to spread cell to cell but is now expressing an antigen which is known to be expressed on the tumor cells. So that's the vaccine strain. And what they find, remarkably, starting in figure 1, is that the tumor progression is blocked.

So I am looking for my notes, here we go. So what they did was measure the size of the tumor mass a week after treatment with this vaccine strain and showed in fact that the tumor growth was completely blocked, and that this required that the listeria expressed the tumor antigen and it required that the mouse also have the tumor cells. So they did not see an immune response as I'll describe in a moment unless we had both the mouse had the antigen and the listeria vector was expressing the antigen. So they wanted to understand how this miraculous protection against the tumor worked. So they began to look then whether CDAT cells were required.

So to ask if T cells were required, they used antibody against cytotoxic T cells to deplete that population and in that case now that listeria vaccine did not do any good, the tumor grew. So they also found that neither CD4 T cells or NK cells contributed. So now to ask, if these cytotoxic T cells were also sufficient to block growth of the tumor, they isolated this class of cytotoxic T cells from mice that had been vaccinated and had tumors, and they transferred them to a second mouse that had the same tumor. And again, they saw that these cytotoxic T cells were able to prevent growth of that tumor.

Michael: So if you will, they are fulfilling Koch's postulates with an immune cell. They're effectively extracting this magic immune cell that was activated by the listeria vaccine, the host manufactured these CD8 positive T cells. They extracted the T cell and transferred them to a new mouse that had not been vaccinated and then they asked the question, will it go out and

seek out the same tumor type and kill it? And as you just said, it indeed did. So it's really Koch's postulates without bacteria.

Michele: Yep, that's a great way to describe it to our microbiologists in the audience. That's a great analogy.

Michael: You have to have a score card to figure out immunology with all their abbreviations and the cytokines that are being activated and the multitude of expression paradigms that macrophages are able to accomplish, and I think if you just think about it as a simple microbiologist and Koch's postulates, you really appreciate the elegance and the fidelity of the work that these workers have contributed to the field.

Michele: It's really beautiful. And then they wanted to look in more detail at how to look in more detail at how this was working. So they, well, I'll add that they also wanted to make sure that it wasn't specific to the particular mouse model they were using, so they went through the effort of looking in a different mouse background and different types of tumor, and they found that the same miraculous protection was observed and the rules looked the same.

Vincent: Michele, could I ask you a question?

Michele: Yeah, sure.

Vincent: How were the mice inoculated and where do the bacteria go?

Michele: So they are inoculating the tumor cells subcutaneously, and the vaccine I believe is delivered IV.

Vincent: Do the bacteria remain systemic or do they home to the tumor cells?

Michele: We know that they will be ingested by macrophages and they can survive within the macrophages, but they do control experiments in the previous PNAS paper and show that they do not then spread into the liver cells as the wild type listeria would naturally do. So that's where the use of this live attenuated double mutant came in. So they next wanted to ask what are the immune correlates of protection? So what is the actual mechanism of the immune system being able to be recruited to fight these tumors?

They found that by isolating blood monocytes and then testing their production of interferon gamma, they found that one of the hallmarks of this defense was now a peripheral production of activated macrophages, and they show that in figure 1 B and G. They also found again that, and this is where they were able to show that it wasn't sufficient to put in the listeria strain expressing the tumor antigen, you also had to have the animal with the tumor cells continuously presenting antigen. So it was that combination that triggered the peripheral immune response to respond to this antigen and produce interferon gamma.

So they then went into a lot of detailed experiments to try to get insight to the mechanism. For example, they isolated cytotoxic T cells from a vaccinated mouse with tumors and analyzed

their gene expression using RNA seq, and they found hallmarks of T cell activation and cytotoxicity, and they also looked at the surface of these CD8 T cells using flow cytometry. So the idea here is basically just as when we look at people that we see in particular uniforms, we can deduce what their work is, what their job is.

So you can use flow cytometry to look at what surface markers are and then deduce what the specialized function of that particular white blood cell is. So in this case they found that these were CD4 positive but also contained an antigen called “Killer Cell Lectin-like Receptor” which the literature had already established is a marker for these short lived potent T cells which are amped up and ready to do their job killing infected or mutant cells like cancer cells. So they next moved on to a more sophisticated question. As I mentioned, one of the challenges with tumor biology is that tumors of course are cells of our self that are now replicating uncontrolled. Therefore, our immune system sees them as self and doesn’t mount a response. And there are particular cells in the vicinity of the tumor whose job it is to prevent inflammation and recognition of this tumor cell.

So these are the tumor associated macrophages that instead of expressing cytokines and functions that are in killer mode instead are suppressing the immune response. So they express IL10, for example. So they wanted to look then at the tumor microenvironment in animals who were successfully killing off the tumor cells and compare that to mice whose tumors were growing. In that way, they were able to gain clues to what cells were contributing and what molecules. So for example, they were able to look at the tumor vicinity and they found that there were fewer immunosuppresive cells called T-regs, there were much more, many more cytotoxic T cells. They also found more cells that are producing interferon gamma, producing nitric oxide which again is a toxic molecule that our immune cells use to kill microbes and other defective cells, and they also found more of these M1 type macrophages, macrophages whose job it is to suppress inflammation.

They also double checked and found that the listeria live attenuated vaccine itself was cleared from the animal as the tumor was cleared. So the vaccine strain did not hang around. From that set of experiments primarily shown in figure 4, they were able to conclude that the listeria live attenuated vaccine stimulated production of tumor specific cytotoxic T cells that leave the spleen and home to the vicinity of the tumor where they stimulate production of interferon gamma, nitric oxide, TNF alpha, that are all contributing to blocking tumor growth.

Michael: The bag of tricks that the human body uses to destroy things. They effectively turn them on.

Michele: Right. You might wonder, how is it that this live attenuated bacterial vaccine is able to break what is called cell tolerance? How is it that it is able to flip this microenvironment of the tumor from a suppressive one to a fighting one, and inflammatory one? They know that one of the things that microbes do, they have on their surface inside their cells molecules that are unique to microbes. We call those microbe associated molecular patterns. These can be

recognized by surveillance systems of the host, so we are equipped with receptors on the surface of our cells called Toll like receptors, and in the cytoplasm, Nod like receptors, to recognize minute quantities of these microbial products and trigger an inflammatory response. We now know that it is the capacity of listeria and other microbes to trigger this innate inflammatory response by stimulating NOD like or Toll like receptors. That is critical in shifting the immune response from an immunosuppessive response to an inflammatory response.

So the fact that listeria is not only delivering the tumor antigen but also triggering a local inflammatory response is kind of the one-two punch that is likely the explanation for why the listeria based vaccine is effective at triggering clearance of these tumors. So let me close with one more remarkable experiment, and this is shown in figure 5A, where they do the ultimate test which is once a mouse which had a tumor which was then cleared, if you will, by the live attenuated listeria, they allowed those mice to recover and then challenged them with more tumor cells. So this is much like if we get a vaccine and then years later we are reexposed to the same pathogen.

And what they found, remarkably, is the mice that had been previously vaccinated with the listeria vaccine were able to control that and prevent those tumor cells from forming a tumor. So they were able to use again flow cytometry to better understand that and found that in the animals that were protected, they had T cells that had the markers of memory T cells. So they were expressing not only these killer like and killer cell antigens but also a marker called T BET which is a marker of memory cells. So not only did the vaccine protect against the initial tumor, but also upon rechallenge there were memory T cells that coordinated a robust and more rapid response marked by increased numbers of CD8 T cells that were specific for the tumor antigen AH1 in the vicinity of the tumor and protected the mouse. So this I thought was a really amazing result that they were able to show that the vaccine has true, the live attenuated listeria strain did have the properties of a vaccine to mount a defense against tumors.

Michael: So if I might interject here about the first author, Dr. Weiwen Deng, this individual was born and raised in a small town in Southeastern China, where they earned a bachelors in biology from Xi'an Jiaotong University. That was then followed by PhD training in cell biology and immunology. So you can well understand how the elegance of this immunology comes through because it's they are effectively this individual's primary training. They did this under the mentorship of Bin Zhou, at Shanghai's Institutes of Biological Sciences and the Chinese Academy of Science where they investigated the regulation of the innate immune response including the Toll-like receptors that Michele just mentioned and the RIG1 signaling pathway.

After graduation from the Shanghai Institute of Biological Sciences, the newly minted post doc traveled to the US and then conducted post doctoral research in the lab of Dr. Dave Raulet in the Division of Immunology and Pathogenesis at UC Berkeley where the good doctor now shifted to tumor immunology and studied the role of NK cells in cancer immunosurveillance. So you can see how the background of this individual helped to shape, or how they were well set up to do this work. Weiwen then went off to Aduro Biotech which is also in Berkeley and

engaged in this early discovery in translational efforts related to the use of these live double attenuated deleted listeria strains which they referred to as LAD as they go through the paper. And the sting agonist in cancer immunology.

So I went out after Michele provided me the bio from Dr. Deng and I went and looked at Arduro Biotech's site, and like most pharma companies they have incredible graphics. And the paper we just discussed that was in PNAS, they have this wonderful graphic that summarizes the utility or how they anticipate that this basic science is actually gonna make its way into the clinic. And that's what Dr. Deng is presently doing, she's looking at the mechanism of how the LAD antigen efficacy is, she's looking to try or he or she is looking to try to understand what is going on. And the quote is, as a scientist I would like to know how LAD antigen works, and which antigen is the best choice for immunotherapy because those of you who are following the area of cancer immunology know that what the physicians are doing is they are bleeding individuals with cancer or they are trying to pull the antigens out of the cancer cell and then trying to make these vaccines using the antigens from the cancer cell. Fundamentally understanding which antigen will work best will be key to bringing this sort of technology/therapy into widespread use in the clinic.

So I was very excited when I read this paper. I sent it to my friends in our cancer immunology group and said this is a must-read because up to now, folks have been using lentiviral vectors to do some of this work. The lentiviral vectors I don't think have been as elegantly described as the work here, but that just may be because I'm not a virologist.

Michele: In fact, in figure 1B they compare their live attenuated listeria head to head with an adenoviral vector that is expressing the same antigen, and they don't see the same protection. I'm wondering if that is because they're not having the benefit of all of these microbial associated molecular patterns that are stimulating the Toll like receptors and triggering the cytokines constantly that is sufficient perhaps to push from this immunosuppressed environment around the tumor to the inflammatory one and then recruit the specific immune response.

Michael: It's all about that tumor microenvironment that I think is key, is that what you were going to say, Michele?

Michele: Yeah, exactly. And I'm with you, I think this is a really interesting challenge. This group is based at Arduro Biotech but they mention in the PNAS paper that many of the people are hold stock including Dan Portnoy who is a professor at Berkeley. This particular project was supported by both NIH grants to the Portnoy lab but also a grant from Arduro Biotech. So I think it shows a really promising partnership between the basic science that's happening in our universities and collaborating them with people in biotech who have the capacity to take it to the next step. Speaking of that, a listeria vaccine has been used successfully to treat pancreatic cancer. So in a paper published in 2015 in the journal of clinical oncology, they demonstrated the safe use of a listeria strain expressing an epitope called mesothelion which is common on

pancreatic cancer cells and were able to show in patients that they increased the survival time from 3.9 months to 6.1 months. So in that limited trial they were able to show safety and efficacy. So there's reason to think that we are not only saving mice with these strategies, but also we can really move this out into the trials, clinical trials.

Vincent: Was this listeria vaccine originally developed for these purposes or as a listeria vaccine?

Michele: It was designed I believe primarily for cancers.

Vincent: Yeah, because you don't have any vaccine for listeria infections themselves, right?

Michele: We know that listeria itself will stimulate a long lasting protective immune response, so you're infected once and then you're protected. I don't know, is there a market for a listeria vaccine?

Vincent: There might not be, I don't know. It's food borne.

Michael: I think it's too small. I know what they have been doing to treat listeria in the food industry is they have been using bacteriophage to effectively treat the surfaces, but that's as far as using a biologic to go after listeria that's not an antimicrobial.

Vincent: I remember Dan Portnoy said once, listeria won't kill you but you'll wish it did (laughter).

Michael: That sounds like Dan.

Vincent: So I'm looking at the scientific advisory board of Arduro on which we have Dan Portnoy and we also have John Mekalanos, another microbiologist. Drew Pardoll is a cancer clinician from Johns Hopkins, these are people I recognize. Frank McCormick is a guy I've known for many years who has worked on adenovirus oncolytic design using adenoviruses to kill tumors, and in fact years ago he had another company that was working on an ad and I went to a scientific advisory board meeting on that. And David Raulet, who is a very well known immunologist. They've got a couple of, quite a few clinical trials in progress.

Michele: It's exciting. I understand that the challenge now, now that we have a live attenuated vector, is identifying for personalized medicine of cancers, identifying quickly an antigen that is unique to the tumor, won't cross react with our normal tissues, and how do you do that? Evidently there are efforts underway to use computational biology to predict what kind of epitopes will stimulate a protective immune response.

Vincent: Right. We just actually started working on viral oncotherapy. There's a picornavirus that is an animal picornavirus that will only infect certain human tumors that have the right receptor for the virus. The cool thing is the receptor for that virus is only on certain types of tumors. So that's a prime example of that specificity. This idea of using bacteria as they say in the paper, it's gotten a lot of traction. Recently we had a seminar here just two weeks ago by

Jeff Hasty from UCSD, and he described the same thing. His lab is working on this, there is a strain of E. coli that you can purchase from Amazon as a probiotic that is not pathogenic and lots of people eat it and it turns out if you take this orally it will home to certain kinds of tumors, specifically. And he is putting tumor lysis genes in the E. coli to try and treat that. One of his post docs is now here at Columbia so at some point in the future I'm going to grab him and bring him on TWIM and have him talk more about that. Because this is growing now. And he actually said in his talk, Jeff Hasty, that this may outdo the viral oncotherapy. It may well be but not until I hope we get some funding for the viral oncotherapy. (laughter)

Michael: Ah, yes.

Vincent: You have to take care of your lab.

Michael: You have to keep the lights on.

Vincent: You gotta keep the lights on! I love that phrase.

Michael: You gotta keep the lights on.

Vincent: That was very cool, Michele.

Michele: Yeah, it's a great application of a lot of really beautiful cellular microbiology and cell mediated immunity studies that have been done with bacteria and now applying them to a problem that we are all familiar with, cancer.

Vincent: Alright. We now have a snippet for you. Or I should say, we have a snippet for you which was sent in by a listener, Justin. Now Justin sent in a link to a New York Times article. The NYT article is by Joanna Kline, it was published July 11th. The title is "This Snail Goes Through Metamorphosis, Then It Never Has to Eat Again." (laughs) It's great. I'll come back to the article, but Justin wrote, now that's a microbiome. So I saw the original paper, of course here on TWIM we do not rely on the New York Times for our journal information, we go to the original literature. And the paper describing this was published in June in the Proceedings B of the Royal Society. And the Royal Society has a number of journals and B is one of them. There is also A and C and others, presumably, but this is B, and you won't be able to get this because this is behind a paywall.

However, the name of the article is "Cryptic Niche Switching in a Chemosymbiotic Gastropod." And this, the authors are Chong Chen, Katrin Linse, Katsuyuki Uematsu, and Julia Sigwart. They come from the Japan Agency for Marine Earth Science and Technology, from the British Antarctic Survey, from Marine Works Japan Ltd, from Queens University, Belfast, and the Museum of Paleontology at University of California Berkeley. Another Berkeley paper. And if you have ever been to Berkeley, go to the library and right there in the lobby they have the skeleton of a T-rex and they have a skeleton of a flying dinosaur, what are those called, I'm forgetting the names. Anyway, hanging up in the atrium as well.

Michele: Cool.

Vincent: It's a cool place. Anyway, back to the paper. So this is about a gastropod. Does anyone know what a gastropod is?

Michele: No, what's a gastropod?

Vincent: It's a snail! (laughs)

Michele: Why didn't you say that!

Vincent: I want people to guess!

Michael: Pterodactyl is the flying dinosaur.

Vincent: Pterodactyls, that's right. Gastropods are snails and slugs, not just snails, but slugs, which don't have shells. Snails of course, you remember from your childhood, the lovely two things pointing up and slowly moving along the ground. That's a gastropod. This is about a gastropod. Before I tell you about this gastropod, which is very cool, we have to talk a little bit about metamorphosis. It's not something that humans do. We start out as a small human and we get bigger and bigger humans but we look more or less the same all the way and our organs are more or less the same. But in animals, metamorphosis means profound physical transformation in morphology.

Michele: I guess pregnancy would be the closest thing that humans do to metamorphosis, huh?

Vincent: I, well, we don't call it that, though--

Michael: That's called reproduction.

Michele: No we don't but.

Vincent: You would say an early embryo, a 16 cell embryo doesn't look much like (laughs)

Michele: I was just thinking about the changes undergo, that women undergo when they're pregnant.

Vincent: You could say that, Michele, yes.

Michael: We wouldn't dare, we couldn't possibly.

Vincent: Yeah, we wouldn't say that. But examples include insects and amphibians, tadpoles that become frogs, that's a metamorphosis. Lots of insects. These are called holometamorphic, they have very different body plans, they have different ecological niches in the larval and adult stages. They occupy what we call distinct trophic levels or food levels in the environment. There are also marine invertebrates that undergo metamorphosis, they have planktonic stages and they have a stage where they settle down and sit in one place as opposed to swimming around.

Michael: That'd be coral.

Vincent: Coral, that's right. And then there are, some of them are more dramatic than others, some can have multiple metamorphoses with different morphologies, and then there is a term called hyper metamorphosis which is used to describe those with many and drastically different phases. This often happens in insects and fish. Something common in these transitions is often the animals go through a transitional stage in which they don't feed. So insect pupa don't feed, the metamorphosing larva of fish, they don't feed, as well. So there are differences in the interactions of the organisms with their environments. Marine invertebrates, the larvae and the adults, are not only morphologically different, but they are spatially separated, they are in different places. In fresh water systems, some times, they are in the same place.

The key here is trying to reconstruct the food webs that these organisms occupy is harder in marine ecosystems because the food chains tend to be longer. On land, they tend to be shorter, but in the ocean they are longer. The exceptions are deep sea hydrothermal vent ecosystems. These are really amazingly productive ecosystems. They have a huge biomass, right. By the way, comparable to reefs, tropical reefs, in its density. But they have a pretty simple trophic complexity. So they say these are good models for understanding where metamorphosis can occur with respect to trophic ecology and energy flow. So that's the reason why they study this particular snail which they call a large bodied peltospirid gastropod. Its scientific name is Gigantopelta chessoia. It grows to 50 mm in length and it is endemic to hydrothermal vents in the southern ocean. It was only discovered in 2012, so you know, those deep dwelling life forms are hard to get at because you need equipment that can go down, a submarine.

Michael: A submarine, you need a big submarine to sample it and to prevent them from exploding when you bring them up to the surface.

Vincent: (laughs) That's right. So apparently this gastropod, Gigantopelta, undergoes quite a substantial transition from a grazing animal to total dependency on internal endosymbiotic bacteria, right? So in one stage of its life early on, it is moving around the vents and grazing, it's looking for stuff to eat down there. Then it transforms into something that doesn't eat anymore, and there it is dependent upon bacteria inside of it that produce the nutrients that it requires.

Michele: So it must be drinking. No eating, just drinking.

Vincent: Yeah, must be absorbing.

Michael: The hydrothermal vents are rich in sulfur compounds and reduced sulfur compounds, so it could be chemosynthetic.

Vincent: There are a number of animals down there at those vents that don't have any digestive systems, right? There are worms that are sealed basically and they contain bacteria that provide and the woman that the Max Planck in Germany whose name is just escaping me, she ran the ASM Microbe program last year, she was the program chair. Do you remember her name? Michele?

Michele: I don't.

Vincent: She was on TWIM years ago and talked about some of these sealed tube worms and their microbiomes. Very cool. This gastropod is unusual because it has a really big bacteria housing organ called a trophosome inside the body, which is derived from an esophogeal gland, so it starts out as an esophogeal gland and then differentiates during morphogenesis into this trophosome. Only 2 gastropod genera are known to have this organ. All the other mollusks that have these internal chemosymbionts put them in their gill tissues. Of course there, Michele, there would be lots of flow.

Michele: Flow, flow of water.

Vincent: So it's unusual to have them in this kind of organ, trophosome. So in the beginning when these gastropods are small, the Gigantopelta, they're not so gigantic, right, they start out small. The esophogeal gland is very small and it's not a trophosome. It doesn't have bacteria in it. And these authors, the hypothesis of the work here is that the metamorphosis and the enlargement of the trophosome is what is associated in the change of diet from grazing to dependency on internal symbionts. Somehow the symbionts get inoculated during this transition, we don't know how they get in there. There are some organisms where we understand this a little better, for example, the squid that Margaret McFull-Neigh studies, it has Vibrios in it that glow at night, so they're not eaten when they're feeding in the water, so they blend in with the moon, but in the day they go in the sand and those bacteria leave and then they come back in again. So that's pretty cool. In this case we don't know how the bacteria get in there, but somehow, they get in and the idea is that this trophosome as it is developing takes on chemosymbionts which are producing materials that the snail needs, building blocks to be able to live, and that becomes a trophosome and the snail sits down on the ground, doesn't move around, and spends the rest of its life that way.

Michele: Fat, dumb, and happy.

Vincent: Frankly, I'd rather move around, but (laughs) so what they did here is a very straightforward experiment. They did CAT scans of these snails. Incredible.

Michael: Micro CAT scans!

Vincent: Micro CAT scans! I have to tell you, on a recent TWIP we talked about micro CAT scans of ants.

Michele: Wow.

Vincent: They're smaller than this gastropod.

Michael: They're smaller than this because this is 50 mm.

Vincent: I gotta tell you about this TWIP, these ants, they eat a parasite which goes in their brain and makes them climb up onto a flower and stand there until they get eaten by a bird or

something else. So the parasite in the ant freezes it up, so they did micro CAT scans and they could see that there is a parasite in the brain of the ant clamped on to the neural tissue. So somehow, it is so freaky, right? This is in the brain of the ant which is behaving normally. And here's the cool thing, when the temperature rises, it is reversible and the ant goes back in the colony so it doesn't dry out. Then when the temperature drops it goes back. This is just amazing.

Michele: It's a very direct approach, to clamp on to the neurons.

Vincent: It's amazing. Alright, so here they do micro CAT scans. They go to the bottom of the ocean, chimney surfaces near these vents. They have a sampler that is on a sub called Isis, and it was during an expedition number JC80, 2,644 meters deep on segment E2 of the East Scotia ridge. You know, these are not cheap expeditions. So this probably happened a couple years ago and you have to book your time on it. We're lucky in microbiology, we just walk in the lab and do our experiments. Here you have to plan. So they got--

Michael: Unless it's tuberculosis. Then it takes a month of Sundays.

Vincent: To grow it, right (laughs) So they do micro CAT scanning on specimens of different sizes, from 2 mm to 23, and they create 3D images because that's what you do with a CAT scan. And they have a lovely figure, I'm sorry you can't see this because it's behind a paywall, but they have a 3D reconstruction of the digestive system and you can see how this esophogeal gland starts small and gets bigger and bigger and bigger. And eventually in these sessile adults, the ones that just sit there and don't feed, it's huge and it takes up a huge part of the snail and at the same time they looked by electron microscopy and you can see it fills with what are called bacteriocytes, which are cells containing the bacteria. These are endosymbionts, so they live within the cells of the host, and so it looks like the transition, the metamorphosis of this snail from a tiny snail that moves around and grazes to a sessile one that doesn't move is accompanied by an expansion in this trophosome and population with endosymbionts. So you can see that very nicely in these reconstructions. So you have a transformation in feeding ecology that is accompanied by this drastic alteration of internal anatomy. The point here that is very cool is you can't tell from the outside of the snail. They get bigger but you would never know that this is going on.

Michael: No, because a snail is a snail.

Vincent: Snail is a snail, right.

Michael: It looks the same on the outside.

Michele: But they provide it in Figure 1, really lovely colored schematics so you can see this amazing transformation, metamorphosis.

Vincent: Yeah, the trophosome is yellow and then other parts of the digestive system are blue, which don't matter anymore because see, those parts actually get smaller.

Michele: Are getting smaller over time, yeah.

Vincent: Because they don't need them any more, they don't eat anything.

Michael: And what you have to do is you have to follow the scale bars because otherwise the figure makes no sense. The one mm is this black bar, it's as big as a house in panel A and by the time you get down to panel F you're going my god, what happened here!

Vincent: Yeah, the line is getting smaller, it's just unusual because usually scale bars stay the same but right, it's changing with the size of this. And they call this cryptometamorphosis, for what is happening here. So this is a metamorphosis but you can't see from the outside, so it's crypto.

Michael: So it's like a Doctor Who, the Tardis, where it's bigger on the inside than on the outside.

Vincent: What they say is in the Gigantopelta there is no external evidence in either the shell or the body showing that the change has been completed, yet the internal reconfiguration changes the animal's ecology. Isn't that amazing? So that's basically the paper. I just want to add a few other bits of information which are interesting. They say this idea of having endosymbionts is a good idea because they say nutritional dependency on endosymbionts is often correlated with dramatic increases in the size and growth rate of marine lineages. So in other animals where this has been studied it is good to have an endosymbiont because you can get pretty big, and they go through a number of other examples of animals that, there are a bunch of other snails that live by these vents.

So these bacteria are chemosymbiotic, they are making chemicals, precursors that the snail needs, so that's chemosymbiotic. And then they are called holobionts because just like in the coral you have two different organisms. You have the snail and the bacteria and that's a holobiont. In the coral you have two organisms, as well. So this is a feature of vent ecosystems that you have a lot of symbioses and these animals can get pretty big. If you've seen pictures of the vents, there's a whole range of amazing organisms that live down there. Of course they are fed by the material that is coming out of the vent from within the Earth. There are also mussels that do things like this, they start out small and they get bigger. They say these could be another example of cryptometamorphosis. Tube worms is another one, giant vent tube worms, and I think those are the ones that we talked about a long time ago on TWIM.

Michele: But as the capacity of the microbes to efficiently extract the energy in the building blocks and provide them to their host that allow these animals to get as big as they do.

Vincent: That's right, yeah. And bigger than they would get if they had just been grazing down there, because there's not enough nutrients for you to graze and get really big. You could probably live but being a chemosymbiotic holobiont is the way to go down there, anyway.

Michele: Cool.

Vincent: So that's the paper, it's very cool, and the Times picked it up and so it is instructive to look at the Times, that's where the headline comes from, this is great. The snail goes through metamorphosis then it never has to eat again. That is a great science headline, right? It's gonna attract you. And the subheadline is the transformation of a deep sea mollusk is comparable to an average person growing as much as 60 feet tall with a giant sac of bacteria filling its guts. Isn't that cool?

Michael: It's very very cool.

Michele: Very descriptive.

Vincent: So then the article of course describes this, and as usual they interview the people that have done the work. In the ocean off the coast of Antarctica, a snail lives around scorching hydrothermal vents. Its name is Gigantopelta chessoia. From the outside it looks like any other shelled slug, but on the inside something strange is happening like no metamorphosis ever observed. And we call it cryptometamorphosis said Chong Chen, a deep sea biologist.

Michele: Fair enough.

Vincent: The article is very nice and it is accessible. You can find that on the New York Times. And so there you go, thank you Justin, and that's why Justin said, now that's a microbiome. Because it's allowing these snails to grow big, right. Isn't that cool?

Michele: It is.

Michael: Very cool.

Vincent: Alright, let's do a couple of emails, here. We can catch up because last time we didn't have time. Last time we had an argument and it went on and we didn't have time to do some emails.

Michael: (laughs) So we're falling behind.

Vincent: And so the first is from Adam:

Hi everyone,

In twim 182 it was stated that Maldi-tof id requires an isolated colony.

This is not technically true, there are special techniques that can id from blood culture without a colony, and databases are being developed to id multiple organisms if present.

Also interesting to note that there are antibiotic resistance assays being introduced that use maldi-tof. Some measure decrease of antibiotic overtime in the case of the presence of beta lactamases.

Thank you for the great show.

So as always, Michael, there's always an exception.

Michael: That's right and it was part of a snippet, so I always get in trouble when I go into too much detail. I knew about the blood culture and some of the advances in MALDI-TOF because of the power of that technology, and it is just how good your database is. I think as the databases get better, we will be able to use less pure samples to figure out who is there.

Vincent: Yep. Thank you, Adam. I appreciate it.

Michele: And those databases are a shared resource, correct, Michael? Or does each health center maintain their own?

Michael: They're unique to the company's instrument. Right now, there are two out there. There are two principal instruments that are dominating the market. I don't know if the databases are in the public domain because I don't have one of those instruments in the lab, but for the next TWIM, I'll find out.

Vincent: Alright, Michael can you take the next one?

Michael: Yes, Nicholas writes:

Hello TWiM team! How is everyone?

My name is Nicholas Gerbino and I'm reaching out to you from Rochester, New York, where I work at the University of Rochester Medical Center. I am currently a student in the Master of Public Health program at the London School of Hygiene and Tropical Medicine. I have only recently discovered TWiM but want to thank you all for the work you are doing.

My passion, above all else, has to do with poverty alleviation and securing health equity through the prevention of infectious diseases.… uh, the text is moving.

Vincent: Sorry Michael, I'm editing it and I made a mistake, go ahead.

Michael: That being said, I was quite pleased to see a paper published in The CDC's Journal of Emerging Infectious Diseases, for no other reason than the fact that it focused on the disease burden faced by some of the poorest among us. The title is "Poverty and Community-Acquired Antimicrobial Resistance with Extended-Spectrum -Lactamase-Producing Organisms, Hyderabad, India."

And as an aside, that's a free download for anyone who is interested, it's published by the CDC and it's in the public domain. So back to the letter.

This article serves as a reminder to all, that we either succeed together or fail together and must commit to serving the most vulnerable among us.

Thank you all again!

Nicholas

And he gives a reference. The article serves as a reminder to all that we either succeed together or all fail together and must commit to serving the most vulnerable among us. Thank you all again, Nicholas. And that's absolutely the case, I mean, the planet is a very small place and even though microbes are very very small, they seem to be able to move faster than Vincent travels (laughter)

Michele: In our food supply, in travelers, when we ship things.

Michael: Everything.

Michele: Long distances.

Vincent: Yeah, it's like he said. We either succeed together or fail together. Really bothers me when people in the US say, oh that Ebola is not our problem, we don't care about it. An infectious disease is a global problem always.

Michele: Yep.

Michael: I heard a fact on the Bill Maher talk show and it was by John Kerry, former Secretary of State, and he said that he was in the situation room when they were discussing the Ebola outbreak under President Obama's term and the predictions if you all recall were that we were gonna lose a million people in Africa due to Ebola. And President Obama told the group present, that's not gonna happen on my watch, and then he mobilized the French, the English, and the United States and we dispatched troops to that area of Africa and with the French and the help of the English we literally stopped that prediction from actually happening. And the models were pretty good, that we were gonna lose a million people. John Kerry volunteered that the President of the United States said not on my watch and made it happen.

Vincent: It's the way it should be, countries like the US should help other less able countries when they have such outbreaks. That's our job as human beings. I think that's great. It's interesting, I heard John Kerry on another podcast, he must be doing the circuit.

Michael: He's got a book.

Vincent: That's right, he has a book. And you know what, he sounded really good (laughs)

Michael: Yes!

Michele: He's a public servant.

Vincent: He was intelligent, he was kind, he didn't speak badly of anyone (laughter)

Michele: To whom are you comparing him?

Vincent: No one, in general, no, I would never do that as you know, Michele, but I'm just saying, it's too bad he lost because he probably would have been a very good president.

Michael: By 65,000 votes.

Vincent: Really, is that it?

Michael: That was it, one state, Ohio.

Vincent: Oh, boy. It's tough. Michele, would you be able to read Dallas's email?

Michele: I will. It's long. Shall I read all of it? And I have a reply.

Vincent: Okay.

Michele: So Dallas is writing:

Dear Twim team,

As usual an excellent podcast with the snippet being highly relevant to the future. You could use these rapid methods to obtain fast and low cost results for things other than antibiotics that impact growth rates like nutrition, nutritional history, phage resistance, etc. etc.

On the diatom paper, finding out how Fe is taken up by the diatom and the use of CO3 ion along with Fe was excellent (always wondered about that). Apparently most of the Fe in the oceans exist as particulate material such as Fe3O4 or Fe(OH)3 with a huge solubility difference between ferrous Fe++ and Ferric Fe+++ ions. Under aerobic conditions like the ocean surface or the water in your toilet bowl, the soluble ferrous is oxidized into insoluble Ferric materials, staining your toilet bowl in areas with anaerobic groundwater where ferrous ions are soluble (also driven by microbiology).

However, a statement was made that the increasing CO2 in the atmosphere will decrease the pH (true at constant alkalinity) and implied the pH determines the carbonate/bicarbonate ratio and decreases the carbonate concentration (true) even as to total of all carbonate species (free CO2, carbonic acid, bicarbonate and carbonate) increases (true). However the implication that this pH decrease would impact the Fe transport system may be wrong. The highly insoluble particulate ferric hydroxide Fe(OH)3 in seawater would increase in solubility with decreasing pH to the cube of the hydroxyl ion concentration effectively increasing the solubility much faster than the carbonate concentration decrease with increasing CO2. With iron concentration increasing and carbonate concentration decreasing, the lower concentrations of iron relative to carbonate combined with the iron solubility increasing much faster with increasing CO2 would say the Fe complexing system would preform better at high CO2. Back to the physical chemistry, mass transport theory I loved.

Another related part of the discussion regarding O2 production and O2 in the atmosphere seemed to be following the lines of Paul Ehrlich's faulty thinking back in the late 60's and continuing on. I keep hearing this thinking so that is why I am bring it up.

Back in the 60's, when I was a Ph.D. student in Edward Teller's Department of Applied Science at UC Davis, I encountered Ehrlich's intellectual impenetrability first hand. Ehrlich had been going on about how chemical X impacted algae growth in the oceans (true) and that the oceans

produce about 2/3 of the world's oxygen production (approximately true). However, Ehrlich had then extrapolated to claim that continued pollution of the ocean will impact the O2 on the planet and jeopardize our survival. This was where we parted company.

Years later, trying to stimulate the thinking of graduate level Environmental Engineers in a class I taught at USC, I turned Ehrlich's statement into an exam question. Ehrlich had continued to spout the same statement about which I had confronted him. He had ignored me then and continued to ignore the basic mass balance science of the situation. I had been a mere grad student, so what did I know.

The premises of Ehrlich's statement are true, but his conclusion is not. In the exam question my students were challenged to examine the case and then determine the actual impact of killing all the algae in the ocean with a permanent toxin.

Proving the conclusion is wrong was the easy part, because the premises' implicit assumption confused gross and net production of oxygen. Oxygen in the ocean undeniably produced by photosynthesis does not make a permanent contribution to the oxygen supply in the world, because it is mainly consumed by the metabolism of the organisms that eat the algae and the organisms that eat them, all the way down the food chain. This results in very little net O2 production. The actual inventory of oxygen in the ocean is small relative to the atmosphere.

The more difficult challenge is to estimate the amount of net production of O2 by the oceans when you know the gross photosynthesis. Many students tried working down the food chain, which is nearly impossible with available knowledge. However, a few thought outside the box, noting that net O2 from the ocean to the atmosphere is only created when carbon is buried in the sediments. One can look up the sedimentation rates and their carbon contents and calculate one mole of net O2 for every mole of buried carbon. Remember there are 2.1 tons of O2 in the atmosphere for every square meter of the earth's surface and few 10's of kg of O2/M2 of the open ocean (surface to bottom) and the open ocean sedimentation rates are very very slow.

If we used photosynthesis to remove and bury (sequester) all the CO2 in the atmosphere at 400 µ atm we would only add 400 µatm of O2 to an atmosphere containing 200,000 µ atm which would be near unmeasurable.

The world of science has expanded in all areas to the point where it is impossible to even know the boundaries of human knowledge making us even more interdependent on each other to achieve a realistic view of the real world and how it works. These podcasts help pass information to those of us who know little of biology, but know that it is important.

Dallas

Dallas E. Weaver, Ph.D. , P.E.

And he's referring to TWIM 182, where we discussed a carbonate sensitive phytotransferrin in diatoms that controls iron uptake.

Vincent: Wow.

Michele: So that's a lot for microbiologists to absorb (laughter)

Michael: But it's all about mass balance, he's absolutely correct, and those of us trained in the ancient times in microbial physiology, mass balance was a hallmark of what the beginnings of microbial phys were all about. We had to track where the carbon was going, we had to know how many moles of this and how many moles of that. And we had to be able to interconvert. It's a very important skill because you can dispatch people like Paul Ehrlich if you understand the mass balance. Clearly that was what Dr. Weaver was bringing up in his long narrative that I think is very very important.

Michele: Well, I shared, Dallas's ideas with Jeff McQuaid, who was one of the key authors on the paper that we discussed on episode 182, and he was gracious enough to provide a thoughtful reply, which I will now read. He writes:

Hello TWiM and Dallas-

This is a great question which really cuts to the heart of the iron cycle in the marine environment. In the ocean, dissolved iron concentrations are are far higher than predicted, and this is due to the fact that in the marine environment, most iron is complexed to an array of diverse (and largely uncharacterized) organic ligands – these can be anything from saccharides and hemes to humic-like molecules and siderophores. Ultimately, it is the kinetics between this soup of organic ligands and the dissociation constant of iron which determines the concentration of labile iron, and these 'gemisch kinetics' tend to dominate over inorganic Fe solubility. Understanding how changes in sea surface temperature and pH will affect the pool of naturally complexed iron is an active, and highly relevant, area of research — if this is interesting, please check out an excellent review which was published on iron in ocean biogeochemsitry in Nature in March of 2017.

In our work, we avoided the uncertainties of the ligand soup by buffered our concentration of labile iron with a large excess of the synthetic chelator EDTA. This allowed us to precisely calculate labile iron as the pH changed — we were extremely lucky in that much of this iron-EDTA chemistry in seawater was worked out by Susan Hunstman and Bill Sunda through a decade's worth of highly detailed experimentation. If you dig through our published datasets, you can see how it is these changes in the favorability of Fe-EDTA dissociation which drove the concentration of labile iron, swamping out changes in inorganic iron solubility.

Some of our next steps will include verifying this effect in other phytoplankton, and equal in importance will be moving away from simplified systems and synthetic chelators like EDTA to study how acidification-induced changes to iron and iron complexes will alter phytoplankton uptake rates, thus embracing the 'gemisch'.

Thanks again —- Jeff

Vincent: Nice. Thanks for that.

Michele: They are thinking hard about how experimentally they can look into more of the details of what is happening in the ocean milieu. (laughter)

Michael: The French. (laughter)

Vincent: Dallas, hopes that helps a little bit with your arguments. Alright, that's TWIM 186. You can find it at asm.org/twim wherever you listen to podcasts. If you do so on your phone or your tablet, you have an app and you can just search for This Week in Microbiology, please subscribe so you get every episode as we release it, we release 2 a month, and if you subscribe it gives us increasing numbers to show how we are doing and that is very important to us. So please subscribe, and as I mentioned earlier, you can go to microbe.tv/contribute for the different ways you can help us financially. And finally, questions and comments should go to twim@microbe.tv. Michele Swanson is at the University of Michigan in Ann Arbor, Michigan. Thank you, Michele.

Michele: Thank you.

Vincent: Michael Schmidt is at the Medical University of South Carolina, thank you Michael.

Michael: Thanks, everyone.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. Thanks to ASM for their support, Ray Ortega for his help, and Ronald Jenkees for his music. Thanks for listening everyone, see you next time on This Week in Microbiology.

(music)

Transcribed by Sarah Morgan