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

(music)

Vincent: This is TWIM, This Week in Microbiology, episode 171, recorded on February 15, 2018. Hi everybody, I am Vincent Racaniello, and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaechter.

Elio: Hello there, how are you?

Vincent: I’m well, how are you doing?

Elio: Okay, hanging in there.

Vincent: Everything good in California?

Elio: Yes sir.

Vincent: The world is all good, very good. Here in New York, it’s winter. (laughs)

Elio: Oh, what’s that?

Vincent: You used to know that in Boston, right?

Elio: That’s right, I forgot.

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

Michael: Hello everyone!

Vincent: Where it’s probably California like weather.

Michael: The trees are in bloom, I mean--

Vincent: Are you serious? In February?

Michael: When the Japanese magnolias begin to show their buds it is either a sure sign that there is a frost next week.

Vincent: Wow.

Michael: The flowering trees are flowering, it’s quite spectacular. The crab apples have blossoms on it.
Vincent: Nice.

Michael: It’s pretty.

Vincent: Very good. Maybe one day I’ll get down there to visit.

Michael: Maybe!

Vincent: It’d be nice. And that is our crew for today, Michele is busy doing important things but we will shoulder on and tell you some cool microbiology. We have two very good stories, as always. When you do four stories a month you can’t help but have four good stories because so much microbiology is published. We can figure out at least four good stories. We could even do more. But our first snippet comes to use from Hannah, who writes

Dear TWIM hosts, this new Nature paper about stingless bees demonstrates that they need a steroid from a mutualistic brood cell fungus to pupate. Thought it might make a good snippet.

And Hannah was indeed right. It will make a great snippet. It’s a really cool paper, and I think this is the kind of story that Elio loves.

Elio: I sure do. (laughs) I love tales of symbiosis, somehow seems to me that that tickles the mind more than a single genome going one at a time. When you put two genomes together, two organisms together, and they interact, it becomes interesting no matter what they do.

Vincent: It’s how the world works, always better when people collaborate, right?

Elio: Yeah.

Vincent: And instead of, you could do it yourself, but wow it is so much better when you collaborate. So this was published in Science Reports, a Nature publication, an open access publication of Nature, so you can find this online. The first author is Camila Paludo and the two last authors are Jon Clardy and Monica Pupo, and Roberto Kolter is on this paper, Elio.

Elio: So is Cameron Currie.

Vincent: Cameron Currie.

Elio: A big guy on the fungus ant relationships.

Vincent: So this comes out of the University of San Paulo, the Brazilian Agricultural Research corporation, Harvard Medical school, the University of Wisconsin, so it is a multi university and country work. It’s about stingless bees. Let me tell you a little about bees, because this is very interesting. Bees are believed to have originated about 110-130 million years ago. That’s about the time when angiosperms became the dominant flowering plants and then all of a sudden there was a lot to eat, so the ancestors of bees, instead of being predators, they became eaters of plants, phytophagous. So all of a sudden you have all these flowering plants and now you have a great emergence of bees from wasps and the bees diversify. Did you know there are 25,000 known species of bees on the planet? (laughs)

Michael: I thought there were only honeybees, but I’ll get a letter for that.
Vincent: No, there’s lots of bees, there are some that don’t sting, in fact. Which is the one we are going to talk about today. Now, as everyone probably knows, bees consume pollen and honey, but recently a Brazilian bee was found that also needs to eat fungal cells in order to survive. It is called the stingless bee Scaptotrigona depilis. It has to eat a specific brood fungus during its larval stage. When the eggs of this bee hatch, you can observe a white microbial growth between the cell wall and the surface of the food supply, and people thought oh this is a pathogenic fungus, must be bad, but it turned out that its a symbiotic fungus that is eaten by the larvae and is needed to complete development. And subsequently in a similar idea it is a fungus that is needed for development was found in other stingless bees, as well.

Now, I didn’t even know there were stingless bees, so this is all new to me. So what is the fungus doing? Well, symbioses are multi factorial, they can provide nutrition, they can supply defenses, they can communicate, and there are some other examples of fungal symbioses. The famous one is attine ants that cultivate fungi for food. They have fungal gardens and these gardens are protected from other parasitic fungi invading them by actinobacteria that produce small molecules that inhibit those other fungi, but not the fungi that the ants need. So it can be really interesting complicated arrangements here in the world.

So what do you need the fungus for? Well, in order to develop, undergo development, molt, the bees required ecdysteroids while are sterile derived hormones and those induce the transformation you need to go from an immature individual all the way to an adult. Insects can’t synthesize sterols, so they have to get it in their diet. They have to make ecdysteroids from dietary sterols. And that is what this paper is about. If you want the bottom line, they find that a certain species of fungus, Zygosaccharomyces, is the fungus that is eaten by these stingless bees and that provides ergosterol to the bees so they can make sterols, that they can make ecdysterols to aid in their development.

Elio: Its interesting, they historically had, there was a wrong turn because people thought that the fungus called Monascus does the job. In reality, what they thought was a pathogen I guess, but anyhow they found it.

Vincent: That’s right, that’s absolutely right, they thought it was Monascus. So what they do in here is they have, they collect this fungal growth that is accumulating inside the brood cells, and this is what the larvae are eating. They collect it, they tried to grow it and it wouldn’t grow but they thought maybe the brood cells are filled with high osmolarity food supply so they said let’s use high osmolarity medium.

Michael: Makes sense.

Vincent: They used 30% glucose, that’s a lot, right?

Michael: That’s a lot.

Elio: Syrupy.

Vincent: Syrupy. And it grows and they can isolate it from the food and they can sequence it, they can sequence the 18s and 26s RNA and it turns out to by Zygosaccharomyces. And it’s not, as Elio said before, Monascus. And so they can find some--

Elio: Which is another yeast.

Vincent: Another yeast.

Michael: Yes.
Vincent: In fact here they look for Monascus, they can’t find it. Instead they find Zygosaccharomyces. Zygosaccharomyces is the one that is eaten by the larvae of these bees. Very interesting. And they have some nice pictures of the mycelium of this fungus.

Elio: It grows in a very characteristic, sort of unusual way, doesn’t it?

Vincent: Yeah, you probably can tell us better than I can, why is this unusual?

Elio: It makes a pseudomycelium which is not known to happen in Zygosaccharomyces. Saccharomyces doesn’t typically make a mycelium or a pseudomycelium which is a short mycelium. And I don’t know if Zygosaccharomyces was known not to do it. So this is, they say it is unusual.

Vincent: Normally it would be dividing its cells, right?

Elio: Yep.

Vincent: So the first question they ask is can we feed Zygosaccharomyces to the larvae and will it substitute for whatever happens in nature, the real thing? So they grow up, they actually collect larvae and make sure there is no fungus associated with them, so they get eggs and they move them out of where they were laid so that there is no fungus associated with them. And they show there is no fungus there. And then they allow the eggs to hatch and so they have larvae, and then they give them Zygosaccharomyces. They inoculate them.

Elio: One microliter!

Vincent: It’s one microliter (laughs) I guess they injected it into the larvae and this is grown up of course in the lab.

Elio: They just add it to the 96 wells.

Vincent: That’s right, they put it right in the well along with the larvae, and then they say what percentage of larvae complete metamorphosis. So they get 71% with their Zygosaccharomyces and it’s 6% if you don’t inoculate them with Zygosaccharomyces. And then those 6% eventually they die anyway, they don’t live. And the others of course go on and develop into bees. So this Zygosaccharomyces gives them what they need to become, to mature, to go through their molt stages.

Elio: It really is a remarkable result, isn’t it?

Vincent: Yeah, I think it is very cool. In fact, when they got this, I bet they were really excited.

Elio: They better be, I mean, who thought of this?

Vincent: They were surprised, I mean, this is all new, right? So they were pretty surprised. So then they say, what is in the Zygosaccharomyces? They say it must be sterols of some kind because the bee larvae will take the sterols and make ecdysteroids which are needed for molting. So they extract sterols from fungus and they analyze it by mass spectrometry, which nowadays is pretty easy to do, and they find the major sterol is ergosterol. So then they take pure ergosterol and they add it to larvae and they get at two and a half micromolar 65% pupation. And that’s compared to 71% where they eat fungus. So that’s not bad, and it is 8% when you don’t give anything to the larvae, as you heard before. So ergosterol, or ergo-sterol, I guess you could say it, what’s the right way to say it?
Michael: Ergosterol.

Vincent: Ergosterol. It supports larval development, so presumably they eat the ergosterol which is produced by the fungus and they convert it to ecdysones which are then needed for the molting stages. And in fact, they find some of these in pupae collected from different colonies, they can find various sterols in them by mass spectrometry. And that’s it, really. So you need this and a probably a lot of other bees out there in the wild need fungus to undergo development. They have evolved over the millennia to need this. And what I found the most compelling is that they say farmers growing crops, they use antifungal pesticides.

Michael: Lots of them.

Vincent: Lots of them, and what is that doing to these poor bees, right?

Michael: And do we know if that could be part of the reason for colony collapse of just having fungicides out in the wild and you may be selecting for a fungus that no longer makes these important steroids that other types of bees may or may not need. And, you know, it’s a continuum.

Vincent: They say here that we should test the safety of antifungals mainly for wild bees. I don’t know if the honeybees need a fungus. Who knows, right? Maybe. We don’t know.

Michael: I think that the microbiome, this is another example of...

Vincent: A mycobiome, right.

Michael: A mycobiome. Well, it is! And it really illustrates the 2+2=12. And you know, you really see the codependence of higher organisms that do more complicated things like harvesting nectar and converting it to honey and fertilizing our plants, it really illustrates the importance of one member of a particular chain.

Elio: Yeah.

Vincent: Everything is interlinked, and this, yes, it’s a great story. Thank you, Hannah, for bringing it to our attention. It is a perfect snippet. I’m sure we will hear more about this story. Elio, you haven’t talked to Roberto at all about this, have you?

Elio: No, and he’s an author in this paper, which is totally surprising, but Roberto has a way of researching every corner of the microbial world.

Vincent: That’s nice. Well, if you can get support for it, that’s great.

Michael: That’s right.

Vincent: Now Michael, actually Michael also found this paper, so the same time you had sent it to me, Michael, Hannah did. And we also have the other one which I said, why don’t you do that one, Michael?

Michael: Yeah, and the one I’m picking up is, I traveled last week so I had the occasion to be contained in an aluminum tube for two hours and so I could read uninterrupted without email or anything else.

Vincent: You know, you can get WiFi on the plane.
Michael: I make sure it’s off. It’s the only peace and quiet you get these days. So this paper is from Science, and it was in the second of February issue and it is entitled “Patients with familiar adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria.” And it’s from a group at a Johns Hopkins University in Baltimore. The first author is Christine Dejea and we will have more to say about Dr. Dejea, if I’m not massacring her name, and she is in a large group led by Cynthia Sears. And it is a really fascinating story or study that shows the promise of translational science. It’s a story of colon cancer, which unfortunately is the second leading cause of cancer death in the United States. The last time they rolled up the statistics, the number of deaths per hundred thousand for colorectal cancer, colon cancer, was about 15 per 100,000 men and women. And the percentage of colorectal cancer deaths is highest amongst people age 75 to 84. And thinking about the background of this story and why it matters and why I gave you the age statistics will become apparent as we tease the paper apart, but on average, the lifetime risk of developing colon cancer is about 1 in 23 for men and women. So it’s at the same rate that we get health care acquired infections, about 4.5%.

So this is a pretty big deal. So the cancer rate however unlike healthcare acquired infection varies widely according to individual risk factors. And today, we are going to talk about the role that microbes play in this process in a genetically predisposed cohort or population of patients. And as we know, the colon contains trillions of bacteria that are isolated from the colonic epithelial layer where the colon cancer will develop by this dense layer of mucus and that there are bacteria out there like Akkermansia muciniphila that we talked about on TWIM 110 and we entitled that episode Exploring Unseen Life with Unpronounceable Words.

Vincent: (laughs) That was Elio’s.

Michael: That was Elio’s. And that used mucin as the sole source of carbon and nitrogen and used gastric mucin to colonize the GI tracts of a number of animals. So we know about bacteria that can eat mucin, but these trillions of bacteria that are in our colon can produce toxins and some of those toxins are mutagenic and or carcinogenic. And this magic mucus layer between the colon’s lumen and the epithelial layer where the cancer will develop promotes tolerance to foreign antigens by limiting this bacterial epithelial cell contact. And that is the gist of this story, is understanding this dynamic of how do bacteria that make a toxin that can cause a mutagenic event then develop into cancer? And you know of course there is inflammation involved and the colon is a nice mucosal surface and so you have mucosal inflammatory responses coming in.

So you begin to see it becomes a complicated story but this group has taken it apart nicely for us in this genetically limited group because we have the genes of the host, namely humans, interacting. And so I liken the mucus layer to the shield we are always hearing about in every space opera we have ever seen or heard, be it Star Wars, Star Trek, or even the newest crazy space opera out there Orville by Family Guy’s fame, Seth McFarland. And so there is always a need for a shield to protect the human inhabitants of the ship in the case of the space operas, or in the case of us, where our colon is no different than the space ship and this mucus layer is the answer or the shield. And so the authors tell us that we know that when bacteria breach this colonic mucus layer, within some cases biofilm formation, it then fosters chronic mucosal inflammation, which is bad. And in fact, this group has previously shown that biofilms on normal mucosa of sporadic colorectal cancer patients were associated with this pro, if you will, oncogenic state, suggesting that biofilm formation at this important epithelial layer is an important event in influencing the development of colorectal cancer.

To summarize the background, colorectal cancer individuals with sporadic so they are not genetically predisposed for it, frequently harbor abnormalities in the composition of their gut and inflammation is often associated with it. And the microbes making the toxins get past the shield and they result in the development of the cancer. So now here is where we get to today’s story and it is broken down into three parts.
Elio: Before you start, I don’t want to steal your thunder because you are probably going to get to say this, but
in a way, this is a remarkable paper because it is a very thorough examination that leads to really solid
conclusions in a field that didn’t always have that characteristic.

Michael: And that’s why I picked it, because of precisely why Elio brings this up. It’s because they lay this out,
they start with human observations, they go to not one but two mouse models, then they take it to tissue
culture, and then they spin it out at the end putting it all together for us in a straightforward model. And so that
is why I started out with the Star Wars metaphor talking about the mucus layer, and the mucus layer is the key.
So we are going to start with the observations that they give us in the beginning, because it really sets the tone.

So the observations of the human condition, they are going to test a hypothesis, and they really get to see what
happens. So to start, the microbiota associated with precancer lesions in heredity colorectal cancer remains
largely unknown. It only accounts for about 5% of the, I’m going to call colorectal cancer from now on CRC. So 5%
or about 1 in 20 of the CRC patients. And of these hereditary cancers, colon cancers, the familial adenomaous
olypopsis or FAP only accounts for a subset of that 5%, or it again is about 5%, so overall if you have 400
colorectal cancers, one of them will likely be an FAP causing cancer. So it’s not a really big cohort but this is
where this paper excels because they have access to these samples, and it doesn’t diminish the wonder and the
significance of the observations and the model that the authors are laying out for us.

So now recall that biofilm formation is an important epithelial event influencing CRC. So here to test the
hypothesis that the biofilm formation may be an early event in the progression of hereditary colon cancer, they
examine the mucosa of these FAP patients after a clinically indicated colectomy. So they have tissue samples
and they are going to simply ask the question, who is there? And so through the usual methods that we have
talked a lot about on TWIM, they are using 16S ribosomal RNA gene probes, they are adding a fluorescent tag
to it so it will light up under the microscope, and they start out with a probe that will light up many things, or a
pan bacterial 16s ribosomal RNA fluorescence probe using this NC2 hybridization. So each FAP patient exhibited
bacterial invasion through the mucus layer scattered along this colonic acid.

Vincent: That’s not right. That’s not normal, right?

Michael: No, that’s abnormal, and that is indicative that the shields have failed. And so why have the shields
failed? And I should point out that Michele--

Vincent: Causative or consequence, right?

Michael: It’s either the chicken or the egg, we don’t know which yet. But they are going to tell us. They are
actually going to tell us it was actually the chicken that is doing this. And it’s really fascinating how they get us
there. And I should point out that Michele was very intrigued by this, she contacted the first author and Dr.
Dejea is an expert in imaging in fish models and she has actually patented the process of examining these tissue
samples on the direct biofilm detection of tissue sections.

Vincent: You can imagine that that would be a clinical assay that would be used to look at risk for cancer, right?

Michael: Oh yeah, absolutely! Because every time those of us over 50 years of age have experienced the hose--

Vincent: None of us three, of course.

Michael: Ah no, I’ve actually experienced--

Vincent: I’m just kidding. I’ve had four. (laughs)
Michael: And so if you have a polyp, the beauty of the colonoscopy is they can take the polyp off and examine it. So this is where this technology can come into play. Now here is their first curious observation. Unlike the continuous mucosal biofilms in sporadic CRC patients, so in the CRC patient that is not genetically predisposed, they have this continuous biofilm that is associated with the colorectal cancer. So it goes along and it’s just not isolated. The tissues however, from the FAP patients, displayed patchy bacterial mucus invasion, and the average length was only 150 micrometers. On 70% of the surgically resected colon specimens collected from 4 of 6 hereditary tumor patients--

Vincent: Hey Michael, how do they know that cutting these out of the patients didn’t cause this?

Michael: I don’t know, that’s for the colorectal guys to talk about.

Vincent: Did they have some normal--

Elio: They snip it very fast.

Michael: Oh no, they had, they control it.

Vincent: They control it, abnormal tissue?

Michael: This is a well controlled study, they have if not more normal controls to go along with this.

Vincent: Alright.

Michael: The other substantial observation of this FAP cohort was that one of the FAP patients who received a course of prophylactic antibiotics the night before surgery, when they looked at his or hers, the biofilm was not detected in this mucus layer. So the antibiotics effectively quote unquote presumably could have cured this but they don’t touch on that. So that I thought was really interesting that a course of antibiotics could eliminate it. But I don’t think that is necessarily the case because biofilms are typically refractory to antibiotics, so I think that is just a curious observation. But as you can quite imagine, they next ask, what microbes were present in this novel mucus penetrating biofilm?

So again, instead of using a pan-bacterial 16s probe, they now use probes that are designed to recognize the major phyla associated with biofilms of the sporadic CRC, the quote normal cancer if you will. And the usual suspects they have the probes for bacteroides and prevotella, the proteobacter, the leptospiraceae and fusobacteria. And notably the FAP biofilms of these genetically predisposed individuals for colorectal cancer were composed predominantly of mucus invasive proteobacter. And those are things like E. coli and salmonella and vibrio. And they were responsible for about 60 to 70% of the community associated with these predominantly mucus invasive biofilms, and then the other 10 to 32% were bacteroides, which is very unusual. And the normal subjects that we consider to be causing problems like fusobacteria were not detected.

Elio: This is really a novel finding, isn’t it?

Michael: Yes!

Elio: This was not suspected before.

Michael: Wait until you hear who the players are, and I’m just going to jump to the players. The player was E. coli!
Vincent: Amazing.

Michael: E. coli! E. coli was the proteobacterium that was doing this! Good ol roly poly coli!

Vincent: (laughs)

Elio: It is also in feces is a minor component.

Michael: Absolutely!

Elio: Very little E. coli in them.

Michael: This was what was so remarkable associated with the stories. Now, one other more disturbing result was they saw invasion of this epithelial cell layer by biofilm community members was detected in all patients harboring the biofilms, which was similar to what was seen in these sporadic CRC patients. So now we have the human observations from the FAP patients, so some are writing this, screening FAP patients, they learned that E. coli and bacteroides--

Elio: At this point remind everybody what FAP stands for. You said it earlier, but might as well say it.

Michael: It stands for familiar adenoma--

Vincent: Adenomatous polyposis.

Michael: That’s it. And that’s why they call it FAP (laughs) And it’s basically adenocarcinoma. So E. coli and bacteroides have penetrated the mucus layer in these bugs. So they next ask, what’s up? Well, there is strong experimental evidence out there in the literature supporting the carcinogenic potential of molecular subtypes of both E. coli and B. frag. And we know that E. coli containing one of these genotoxic islands, and it was the one that contained a polyketide synthase, so in this genomic island there is a gene that encodes a synthesis for colibactin which is a genotoxin. So this induces DNA damage in vitro and in vivo along with tumorigenesis in an azoxymethane treated interleukin 10 deficient mouse. Suffice to say, E. coli makes a toxin that can cause cancer. The other thing is there was an enterotoxic B. frag which they abbreviate ETBF that also induces colon tumorigenesis in another mouse model.

So I know you can see the next set of experiments coming up because they are going to take us to these mouse models asking questions, but before we get there, there is still some human results that the authors think we need to learn about before we can go to the animal model. And again, this illustrates how carefully they assemble the story. So human epidemiological studies have associated both the B. frag that is this enterotoxogenic variant and this polyketide synthesizing island E. coli with inflammatory bowel disease, and then sporadic colorectal cancer. So they have this frozen bank of mucosal tissues from these 25 patients, and so they simply ask the question, can they culture out of this frozen bank of mucosal tissue the E. coli and the B. frag that have these toxins?

Well, as you might guess, since they are telling this about this, the mucosa of the FAP patients was significantly associated with the genotoxic producing E. coli to the tune of 68%. And the toxin producing bacteroides was present at 60% to compare to the mucosa of healthy subjects. And there was no preferential association of the enterotoxic B. frag or the PKS positive E. coli with polyp or normal mucosa from FAP patients and that this mucosal association occurred at a higher rate than what was expected to occur randomly. So they are building on this--
Elio: It’s really big news.

Michael: Oh, yeah! This is the microbiome’s potential realized.

Vincent: Well, adverse potential, right.

Michael: Right, excuse me, I’ll get letters for that, too.

Vincent: (laughs)

Elio: Is it like finding a new pathogen.

Michael: It’s defining new pathogenic potential for E. coli that we wouldn’t normally think about. So the author is in transition from their human observational studies to their animal models.

Vincent: Michael, can I ask you a question?

Michael: Sure.

Vincent: So this E. coli with the toxin, right? Do you and I have this? What’s the frequency in the population?

Michael: That I don’t know.

Vincent: I wonder, I mean as we will see it’s a problem to have this, but I just wonder.

Michael: And that’s how they end the paper! But I don’t know what the frequency is and that would be very good to effectively look at. The other issue is since this is on one of these if you will pathogenicity islands, what is the selective pressure for E. coli to maintain this extra luggage in its genome in this particular patch of the large intestine?

Elio: Good question.

Michael: So they didn’t get into that aspect as well, but that is for the molecular ecologists to begin to debate. So they now next take us into their beautiful mouse models. And the first is a well established model for cancer in mice, it’s azoxymethane without dextran sulfate, and then the other model is in the APC which stands for adenomatous polyposis coli (laughs)

Michael: That’s it. It gets mutated, I can’t keep all the cancer straight in my head. And so they then get to that and that is also got a deletion to test the hypothesis that the PKS genome containing E. coli that makes this genotoxic product as long as the enterotoxic B. frag cocolonization, they say they have to get together, enhances colon tumorigenesis. So they do the mix and match experiments, they have E. coli by itself, they have the enterotoxic B. frag by itself. They take us through that.

Vincent: They’re just feeding this to the mice, right?

Michael: That’s right. And they point out that the spontaneous rate of tumor formation using either model is very low, so that is good. So it’s just garden variety SPF specific pathogen free wild type mice were treated with
the cancer inducing compounds, azoxymethane, and then they add mono inoculated or co inoculated with these particular strains. They first report that the colonization was similar under mono or co colonization, the bacteria remained in the colon until tumor formation was assessed at 15 weeks. So you can put a lot of bacteria into model systems and they are quickly shed and gotten rid of. And the results were under the mono conditions, E. coli produced few to no tumors, but in contrast they saw pronounced tumor induction in the co colonized mice and that this tumorigenesis required the presence of both the bacteroides and the colibactin genotypes. So not only do we know the microbe responsible--

Elio: It’s the E. coli toxin that does the damage, right?

Michael: Right. So not only do we know the microbes responsible for triggering the event, we know we need two. We know the toxins responsible. So we know that’s the case because for when they do this next really cool experiment where they do an in frame deletion of the BFT gene from the bacteroides, and they delete the PKS virulence island, and they saw significantly reduced tumors. So, I mean--

Elio: They also have survival curves.

Michael: Oh yeah, the survival curves, since the audience doesn’t have access to the beautiful survival curves I didn’t want to torture their minds. They can probably see this paper, Science is probably in many of the libraries that folks have access to so they can probably see the survival curves. And they are the easiest things to interpret, because it’s all or none. And it is significant, not only to a p value 0.05, it’s significant out to p 0.0001. Talk about a smoking gun! So no discussion of cancer would be complete without introducing the immune system.

So they introduce us to IL17, which is an interleukin that is a pro inflammatory cytokine. So inflammation is bad, we know it exasperates cancer. IL17 is produced after it has been stimulated by IL23 and that is the extent of that aspect of the immunology. But they have this second model, the one involving the APC min delta 7 16. And they blamed the enterotoxic B. frag because the B. frag toxin was necessary for the synergistic tumor induction under the co colonization conditions. They point you back to the figure where they actually illustrate that elegantly. So now they then add the further spin of putting in this pro inflammatory cytokine, and as they reported when they measured IL17 expression by quantitative PCR they saw no significant difference. So PCR is only measuring gene expression, so they are looking for the expression level of IL17 mRNA. And they saw no significant difference in the overall mucosal IL17 mRNA levels between a 15 week ET, the enterotoxic B. frag monocolonized and the co colonized B. frag with the E. coli. But co colonization of IL17 deficient mice ablated tumorigenesis. So IL17 is somehow involved, but they didn’t see any changes in the amount of messenger RNA. And that is probably a consequence of the redundancy of our immune system being able to get to different spots by different routes.

So the immune story is not as nailed down as yet, but it’s still a component of what is actually going on. They then take us down further the immune system by looking at flow cytometry, asking the question, they tease this apart by looking for early colonic mucosal IL17 production by using a germ free system that were mono or co colonized. And they asked what is going on by looking at the innate and adaptive lymphocytes upset. And what they learn is that the germ free mice co colonized with these two bacteria displayed a trend towards an increase in total mucosal IL17 producing cells when compared to the mono colonized forms. So driven by both the adapter which is the T17 helper system and the innate which is a particular GDC17 cell. So while necessary for tumorigenesis, IL17 alone appears insufficient to explain this synergistic tumorigenesis seen these co colonized mice since robust IL17 induction in the animals receiving only the enterotoxic B. frag genes were only observed to have this meager colon tumorigenesis.

Elio: Is there a way you could sum up this part?
Michael: The immune system is---

Elio: Is there a take home lesson, is there a take home lesson here?

Michael: The immune system is involved in the formation of the tumors, inflammation is important, but we haven’t teased apart the complete story.

Elio: That’s clear.

Michael: And as they begin to take us through this, they then begin to talk about mucus and who is giving access to the endothelial layer or the epithelial layer of the colon, and they are asking the question, is it E. coli colonization alone? Does it have an impact on mucin depth, or do you need somebody else? And here is where they bring in the Akkermansia, which is the known human colonic mucin degrading bacteria. So what they concluded from this set of experiments is the order of addition for mucus degradation by the enterotoxigenic B. frag promoted this enhanced E. coli colonization in this niche. So if you will, the bacteroides is the founder, and E. coli comes in and you almost can take it back to the classic A B type toxins we talk about in bacteria, where one of them facilitates the binding of the toxin to the receptor and then there is the active site of if you will the toxin. And so the B. frag is the equivalent of binding to the colonic wall and then it facilitates E. coli coming in, supplying its genotoxin, or its colibactin, the DNA damaging toxin released by E. coli to the colonic epithelial layer.

Elio: Going back to the early discoveries of pathogens in the 19th century where you usually found in the majority of cases a single bug. Here you got two bugs they include in the pathology. And that is, there are other cases like it in the literature but this one is really very marked, it’s just a strong case for that.

Michael: Yeah, and so here is where they leave us. They begin to put it all together for us. Taken together all of their data collectively suggests that co colonization first with the enterotoxic B. frag and then this pathogenicity island containing E. coli that makes this genotoxin colibactin promotes enhanced carcinogenic through two distinct but complimentary steps. The first mucus degradation enabling increased E. coli adherence. But if you think about what E. coli normally does well as a pathogen, it sticks. It adheres, I mean, that is effectively how it causes UTIs, it adheres. And so the mucus degrading enables E. coli with this colibactin to adhere inducing an increased colonic epithelial cell damage by colibactin. And then we have the immune system. IL17 is induced, promoted if you will, primarily by the enterotoxigenic B. frag’s toxin with early augmentation by the colibactin E. coli containing strain. And so they propose that these mechanisms yield this cooperative tumor induction and as Elio said, this is a heretical concept. It sort of violates Koch’s postulates because you need to recover 2 organisms in order to get disease. And it is absolutely a fascinating story of how they take it through and I am going to do what Michele loves to do--

Vincent: Before you do that, let me just interject. So I asked you earlier who has this bacteria, they have this here, I forgot. ETBF and PKS commonly colonize young children worldwide, so a lot of young kids have it. So the idea is if you have these at a young age it could contribute to colorectal cancer you get later in life, you have got these all throughout your life. Now in this study they get the bacteria from patients with familial adenomatous polyposis, right? But they say this could even be an issue with people who develop sporadic colorectal cancer because in most of those people you have loss of this APC gene and they showed in this paper that mice lacking with a mutation in APC, they will get tumors caused by these 2 bacteria. So the possibility is that these two bacteria are involved in a vast majority of colon cancers which would mean we should screen for it. We should do a clinical trial, people with all kinds of colorectal cancers and see if they have these two particular bacteria, and if so, it would be a great screen early in life to look at your likelihood to have colon cancer.
Elio: It occurs to me that if you wanted to put this to use and prevent this kind of cancer in people who are susceptible to it by virtue of having the bacteria and the right immune system that you might want to think here of using bacteriophages.

Vincent: Exactly.

Michael: Exactly!

Elio: Antibiotics are going to clean out the gut and that’s not good.

Vincent: That’s right, use phage specific to this bacteria, that’s a great idea.

Michael: Or even better, you can use CRISPRs, CRISPR laden phage to come in and trim out the toxin out of the genome.

Elio: The phages are specific to a specific region instead of antibiotic.

Michael: Absolutely, I agree with that.

Elio: This paper may lead to something actually useful. It seems possible to me that knowing all this stuff you can do clinical trials in which you try to get rid of the specific bugs.


Michael: So as Michele--

Elio: Great paper.

Michael: As Michele left me with the first author’s bio, she wanted me to make mention that Dr. Dejea was born in the Bay area of California. She has always loved science, had a microscope even as a kid where she studied lots of insects. She got her bachelor in science degree at UC Davis and med micro was her major. She then went to earn her PhD at the Johns Hopkins University with Cindy Sears, where she switched to her lab in her third year seeking this translational research linkage, and I think she really got it. This science paper is only but one chapter of her thesis. She also has other papers in PNAS, Cell, Host Microbe, Mucosal Immunology, and as I mentioned earlier she pioneered the development of technology to detect biofilms on tissue sections that they have patented. And as to the most exciting day in the lab, its the day they saw the dramatic results from their first pilot with the mouse co cultivation experiment where these two organisms had to be present. And I think we can all appreciate that and then Michele writes in her note, she was especially proud and excited over the second figure where the significance factor was p 0.001 that I pointed out in the narrative.

The other thing that Christine points out to Michele in her conversation is she credits Dr. Sears with making connections with scientists with different expertises, and as you look at the author list of this paper you can see that this is truly team science. There is expertise from immunologists, there is expertise from cell biologists, surgeons are involved to get the samples, so this is really a collaborative translational event.

The other thing that Christine really wanted to highlight is to credit Payam Fathi, the second author, because this individual was talented and a devoted lab tech who would meet Christine at 5 AM every morning to begin their mouse dissections. This individual is now in a PhD program at Vanderbilt pursuing his interest in microbiology and when Michele asked her the famous question, what advice would you give to other students and trainees, Christine remarked, have courage and confidence to follow your own interests and at times you
have to follow your gut instincts about what observations are important and interesting. And she was especially most grateful for her mentor, Dr. Sears, who gave her the freedom to pursue the side projects. And this, folks, was a side project! So I mean, this is incredible.

Elio: This happens so often, doesn’t it?

Vincent: Yep.

Michael: Yeah. Right now, Christine has gone on, she presently is working for our Food and Drug Administration at the Center for Drug Evaluation and Research, and she is in the division of microbiology there. And she is responsible for evaluating processing for manufacturing biological therapeutics to ensure low risk of contamination with microbes or products like LPS. She is also the proud mother of two children ages 2 and 4 that were born as she was preparing to defend her thesis and during her first year of post doc with Dr. Sears. So again, this was a Science paper that I stumbled into on an airplane and it turned out to be one of the most remarkable reads I had on that particular trip, so.

Vincent: Really good job, thank you Christine. Congratulations and keep up the good work. Let me read a couple of emails from our faithful listeners. Harry writes:

Dear TWIMers, I hope my entry for the book is not too late—I’m sorry Harry, it is. I enjoyed Dickson on TWIM and I look forward to more cross pollination of the TWIX hosts on TWIM. I’ve been listening to the MicrobeTV podcasts since I took microbiology as an undergraduate in the SUNY system. The shows are so entertaining that I have continued to tune in as I completed a Masters in microbiology and immunology at Colorado State University. I did some work with the school’s biosafety department and have been interested in such matters ever since. I now work as a quality technician in medical devices where I can combine my micro education with engineering. Thank you all for your dedication to science communication.

Amir writes:

Hey, my name is Amir and I am a masters of science student of food science in the Hebrew University in Israel. I love your show, I listen while commuting to the lab and I am trying to develop a quick spectroscopy based method to detect bacteria in drinking water. I would really like to get this book about disinfection because I had a discussion with my PI about how exactly does chlorine ethanol and boiling kill bacteria? Do these treatments break the cells, break proteins, and other molecules and most importantly do they make the bacteria lose their optical fingerprints? I would like to hear your opinions on this question by the way. Thanks a lot for the podcast.

Michael, how does chlorine ethanol and boiling kill bacteria?

Michael: Each one is separate. Ethanol and boiling are a little bit different, but what you are effectively doing is changing the protein structure. We have all seen that television commercial, this is where they crack an egg into a frying pan pointing out that this is your brain on drugs, and the proteins congeal, well that is effectively how heat kills. It is of course coagulating and changing the tertiary structure.

Elio: I think alcohol works first by dissolving the cell membrane, it just breaks it up, so that’s the thing it does and when that happens after that it denatures the proteins. But the cells die the moment the alcohol hits them because the membrane is gone.

Michael: That’s right.

Elio: That’s how it sanitizers work all over the place, 66% alcohol.
Michael: Yes. And the other aspect is it will change the way they look because it depends on what you are actually looking at in your mass spec, and it’s time of flight and whatever else is going on. So you just have to have a good database of, you can boil for prescribed periods of time, you can add as Elio said the 66% ethanol and similarly the hypochlorite which will effectively add oxygen to everything. And you can see what it will look like.

Vincent: Do they destroy the optical footprints of the bacteria?

Elio: Yes.

Michael: If he is using mass spec he is probably--

Elio: I once wrote on that, a very small amount of hypochlorite will break up bacteria like there is no tomorrow. Minus five molar is enough to just break them up.

Vincent: Interesting, alright. One more from Oggy, Elio will like this one.

Dear TWIM team, at last I decided to drop an email to the cast of my favorite podcast. I started listening to TWIM following a friend’s recommendation some time last year and I chewed through the hundred and forty some episodes in just a few months. This podcast should be a mandatory material for graduate students for they could learn not only the science part but also the logic of analysis, succinct description of complex topics and the overall importance of communication in science. I wish you could produce an episode every week but I understand the reality of a busy PI. At least I can rely on the Immune podcast, also excellent and an occasional TWIV while waiting for the next TWIM. On a side note I would like to mention that I was lucky to see Elio’s Tales from a Blog talk this past December in Boston. How about that, Elio!

Elio: Well, that’s available!

Vincent: It was recorded? He heard you talk!

Elio: Sorry?

Vincent: Oggy heard you talk!

Elio: Oh, oh oh.

Michael: He’s at BU.

Vincent: Tufts.

Michael: Tufts. There at Tufts.

Vincent: He was lucky to hear your talk, Tales from a Blog. Needless to say it was fascinating even though I was familiar with most of the stories as I read In the Company of Microbes. I was hoping you could sign the book for me but unfortunately I forgot to bring it. By the way, in my lab, we have an Eppendorf rack used by Elio back in the day. I’m keeping it as a lucky charm.

Elio: (laughs)
Vincent: How about that, he’s got your old Eppendorf rack!

Elio: I can’t guarantee that, but (laughs) give it a try! Can’t hurt.

Vincent: Oggie has two papers that he suggests, a Microbiome paper going beyond your typical sequencing and show a caused consequence not only correlation. Elio should be pleased with this, and another paper, a fascinating tale of emergence of C. difficile with some help from a dietary supplement. I hope the whole TWIM team stays together for many years to come.

Oggie is a post doc in molecular biology and microbiology at Tufts University where I guess is where you were, Elio, right?

Elio: That’s right.

Vincent: Okay, thanks for your letters. We have more but we will save them for next time. This has been TWIM 171 which you can find at asm.org/TWIM and if you have a--

Elio: Boy, how time flies, huh?

Michael: Yeah!

Vincent: You bet. If you have a phone or a tablet and you listen to podcasts, just subscribe on your favorite player. Please subscribe, you get every episode. If you love us, consider supporting us financially. You can go to microbe.tv/contribute. There are a couple different ways you can do that. Finally, if you have questions or comments send them to TWIM@microbe.tv. We don’t have a book contest today, we will next time. We have plenty of books to give away. Elio Schaechter is at Small Things Considered, thank you Elio.

Elio: Thank You.

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

Michael: Thanks Vincent, thanks Elio!

Vincent: I am Vincent Racaniello, you can find me at virology.ws I thank ASM for their support of TWIM and Ray Ortega for his technical help and music by Ronald Jenkees. Ronaldjenkees.com. Thanks for listening everyone, see you next time on This Week in Microbiology.

(music)

Content on This Week in Microbiology (microbe.tv/twim) is licensed under a Creative Commons Attribution 3.0 License.

Transcribed by Sarah Morgan.