This Week in Virology

With Vincent Racaniello, Ph.D.

Episode 85: Hepatitis C virus with Professor Michael Gale

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Vincent Racaniello: Today, we have a special TWiV. I'm speaking with Professor Michael Gale about Hepatitis C virus. Dr. Gale is a faculty member in the Department of Immunology at the University of Washington's School of Medicine. He received his PhD with Marilyn Parsons at the University of Washington working on the parasite, *Trypanosoma brucei*. He then moved to the laboratory of Michael Cates at the same institution where his focus turned to innate responses to RNA virus infection. He continued his work as a faculty member at the University of Texas' Southwestern Medical Center then moved back to the University of Washington in 2007.

Research in the Gale laboratory is focused on understanding innate immunity to virus infection. His research has defined the retinoic acid-inducible gene or RIG-I as the major pathogen recognition receptor that triggers immunity against hepatitis C virus and the variety of other pathogenic RNA viruses. During virus infection, RIG-I or a related protein called "MDA5," bind to viral RNA, a process that triggers the production of alpha/beta interferons and the expression of interferon-stimulated genes. These processes induce the innate immune response that serves to limit virus replication and spread. Work in the Gale laboratory has focused on how RNA virus infections are sensed by the innate immune system and how viral gene products regulate such responses.

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Vincent: What is the extent of the global hep C problem? How serious the pathogen is it?

Michael Gale: It's a big problem because the estimate is we're approaching 200 million people globally with chronic infection. The problem is chronic infection. It's a very successful virus meaning that if you get exposed to it, you have a high chance of getting the chronic lifelong infection.

Vincent: What's the fraction of that?

Michael: Well, I guess the estimates range from – the hard data suggests 75%, approximately, of people exposed to hep C who are going to a chronic infection. Let's say that will vary between 68% to maybe 80%.

Vincent: It's higher than hep B, right?

Michael: Oh, yes.

Vincent: What's the mechanism of transmission mainly? It used to be the blood supply, right?

Michael: Yes. Now, it's IV. It's still a lot – the viruses are alive and well in IV drug users. That's the big problem in developed countries, IV drug use. It's very efficiently transmitted by needle sharing and exposure to contaminated blood products. It is also possible to transmit it sexually although the efficiency by which that occurs is probably much lower, so sex and drugs and blood products.

Vincent: [Laughter] What is the global distribution? Is it a big problem in the US or mainly elsewhere?

Michael: Okay. It's a big problem. In the US, approximately 2% of the US population has chronic HCV infection. Even though that sounds low - 2% - that means it's a big problem. That's what - 4 million people? The global problem parallels the US or is higher frequency. For example, in Egypt, 15% of the entire population is likely chronically infected with hep C. We don't know the numbers from Asia, from China, or the former Soviet Union. China and Russia, we don't exactly know what those numbers are but they're probably at least as high as they are in the US.

Vincent: How about southeast Asia?

Michael: In southeast Asia, hepatitis C virus is a big problem because IV drug use has increased dramatically there and then similarly in Japan.

Vincent: When we found it in the blood supply, what year was that, '89?

Michael: '89.

Vincent: Did initially the incidence decrease?

Michael: It decreased dramatically after Chiron came out with their first-generation serologic test. Blood supply got cleaned up maybe 50% to 60%. It wasn't really until the second and third generation test came on board by 1992 did the United States' blood supply get cleaned up, free of hepatitis C.

Vincent: We've talked about hep C just – we had Matt Evans on last year. We talked mainly about trying to make a mouse model and what you needed to get in but we haven't talked about the chronic infection. Why is having a chronic hep C infection a problem?

Michael: The problem is that over time, the hepatitis C virus will direct, simply put, the destruction of the liver. We only have one liver. When our liver stops working, we have to have a liver transplant or we die. Over time, the chronic infection associates with liver inflammation, it's what we call "hepatitis." The liver inflammation, and probably also viral direct processes work together to damage the liver to the point that it can no longer function properly.

Vincent: How many years does it take to do this?

Michael: Well, it varies. Say, 10 to 30 years.

Vincent: Everyone who is chronically infected eventually within 10 to 30 years will have liver failure?
Michael:

Michael: Not everybody. What we know is that the frequency who goes on to liver failure, what we call "end-stage liver disease," will be relatively low. I don't know, 2% to 10%, let's say. It depends on your lifestyle also. The frequency of people who go on to develop liver problems is quite high. Liver problems meaning elevation of liver enzymes that indicates that your liver is stressed out and you start to lose some level of liver function. That could be from 20% to 80%. There's a smaller percentage of people who just live a normal healthy life and may not even know they have hepatitis C virus.

Vincent: Yet they have infection though?

Michael: Yes.

Vincent: Is there also an association with hepatocellular carcinoma?

Michael: Yes. Hepatitis C maybe is the number one epidemiologically-associated infection with hepatocellular carcinoma in the United States.

Vincent: It's probably a consequence of the inflammation and liver damage. There's no oncogene involved with that?

Michael: There's no oncogene. It's thought to be a result of the chronic liver inflammation. The liver has this remarkable capacity to regenerate. For example, you can chop off half of the person's liver and it will grow back fully. As the liver damage takes place, liver cells die and the liver works to regenerate those cells. Think about that happening over and over and over. That combined with environmental insults to the genome [is] thought to drive the outgrowth of liver cancer.

Vincent: You have cell division where normally you wouldn't have it?

Michael: Correct.

Vincent: We know that's a recipe for accumulating mutations eventually. It's the same thing the transforming viruses do. They push the cell cycle and mutations accumulate and you eventually have a transformed cell and then an oncogene activated or something. I assume it's the same for hepatitis B, similar liver destruction and regeneration.

Michael: That's right. With hepatitis B and hepatitis C, there's a lot of thought in the field that the virus may encode protein products that have oncogenic potential so they could contribute to the dysregulation of cell growth. There's plenty of evidence for that in in vitro studies. But no smoking gun in terms of a viral gene as an oncogene has been identified for either but they have been suggested.

Vincent: The fact that there are so many chronically infected people means that a good therapy hasn't been developed yet, right?

Michael: Yes. That's correct. The bottom line is that's true. The drug companies that sell interferon, which is the current standard therapy today, would argue that. In reality only half of all HCV-infected patients who go on therapy will respond to the therapy. By that definition, that's not a good therapy.

Vincent: If you're diagnosed with hep C, you're given interferon therapy and you have 50% chance of it working. Working means it will remove the infection?

Michael: Yes. We call that a "sustained virologic response" in 55% of the patients overall. The problem is there are six different HCV genotypes, so six different types of HCV. Genotype 1 which is predominant in the United States, Europe, Japan, is the most difficult to treat. Genotype 1 runs about a 50% sustained virologic response rate.

Vincent: When you have the sustained virological response, does that mean you're cleared of the virus?

Michael: You would interpret that as yes, you're cleared of the virus.

Vincent: You can stop taking the interferon at this point?

Michael: That's right. Then after a year, we check and the virus hasn't come back, so we would consider that patient a "virologic responder."

Vincent: The other 50%, they continue to produce virus so we obviously need some other antivirals to take care of them. I know there's a lot of activity in many companies.

Michael: Yes, those are in the pipeline. It's exciting. The new generation of drugs target enzymes encoded by the virus and they have the potential to be very effective without the side effects of interferon.

Vincent: So they're targeting the RNA polymerase of the virus?

Michael: Yes. There are at least two classes and more like three classes that will one day be in the clinic. One of them is the inhibitor of the RNA, dependent RNA polymerase. The most potent and best – I shouldn't say "most potent" - but the best described and characterized target is the heptatitis C protease. There are many protease inhibitors in the clinical pipeline. Finally, and I think this is pretty exciting, there's a NS5A inhibitor. NS5A being a non-enzymatic protein encoded by the virus. It seems to be a compound that can bind that molecule and walk whatever it does.

Vincent: We don't know what that molecule does, right?

Michael: We don't know exactly. It's likely involved in replication but I think that's something to figure out.

Vincent: Yes. That's something just came out recently from Bristol-Meyers. We're going to do that on – maybe this Friday's TWiV because it's really interesting story. It's one of the few non-enzymatic antivirals.

Michael: Right.

Vincent: The goal would be to have at least three, if not more, so you can do combinations like you do to treat AIDS, right?

Michael: Yes. Otherwise, it's certain the virus will find a way to resist, mutate around. It can certainly mutate around a monotherapy. That's been shown in the clinical trials for the protease inhibitors. If we've learned anything from HIV therapy it's that we need a whole cocktail of drugs to throw out the virus at the same time.

Vincent: Do we understand why half of people treated with interferon don't respond to therapy? I think that's a lot of what your work is aimed at.

Michael: Yes. That's a big question. The bottom line is we don't understand. We understand a little bit but there's a lot to be learned. One of the things about interferon is that it's a biologic. That means it's a molecule that we produce ourselves and were infected with the virus. That binds a receptor on any given cell and it induces the expression of hundreds of genes literally. Any of these hundreds of genes could have antiviral potential to limit hepatitis C infection. We don't know what those genes are. We know the identity of the genes but we don't know which of the hundreds could have antiviral activity. That's an area that the field is starting to work on. We know that the virus can undergo genetic adaptation constantly suggesting that one or more viral proteins may adapt or mutate so that it can block interferon responses. We also know that there are genetic distinctions from person to person that could influence the response to interferon, so it's very complicated. The latter, the genetic distinctions from person to person, at least one of those what we call "polymorphisms" has been identified in the interleukin-28 gene which is an interferon-responsive gene and actually encodes a type of interferon that has antiviral potential. There's human genetic, viral genetic, all kinds of factors that are involved in the interferon response. Understanding any one of those you have to consider all these other factors weighing in, so it's very complex.

Vincent: When you treat a person with interferon, who's already been infected with hep C, you're mimicking what would happen when the virus infects you. The virus comes in and the cells produce interferon in response. Why doesn't that contain the infection initially?

Michael: Yes. That doesn't contain the infection initially for at least two reasons. First of all, during an acute infection, the first hours of an infection after you've been exposed, there's a race against time for the infected cell to produce interferon and induce these hundreds of genes that may have antiviral activity compared to the virus making its own proteins. One of those proteins at least of which can block interferon induction. We've learned it in the first 12 hours of infection, there's this remarkable battle between virus and host. With hepatitis C, the virus most often wins. That's because it synthesizes a protein called "NS3/4A." It's a viral protease. That's important for processing the virus into its little components so it can replicate. NS3/4A also targets a host molecule and it chops it off so that the cell can no longer produce interferon. Once that happens, we think that that serves as a foundation for chronic infection.

Vincent: What's that molecule that's the target of NS3/4A?

Michael: It's called "IPS-1." It's a molecule that is essential for signaling by a protein in a cell called RIG-I. If RIG-I gets turned on it leads to the expression and production of interferon from the infected cell and that could shut down infection. When RIG-I gets turned on, it signals through an adaptor protein called "IPS-1" that's required for this response. What the virus does as soon as it infects the cell, it produces an enzyme that targets IPS-1 and cleaves it, chops it up so that RIG-I pathway is now dead, can't produce interferon now.

Vincent: What's the role of RIG-I in that pathway?

Michael: The RIG-I serves as a pathogen-recognition molecule. In other words, if we get infected with the virus, any given virus, the cell that's infected - we have to know that we're infected. This is a fundamental basis of an immune response. It's called "self versus non-self discrimination." Processes inside of our body are constantly at work surveying for non-self molecules that would tell the body, "I'm infected. I need to clear out this infection, do something about it." RIG-I is one of those molecules. RIG-I, as we know today, surveys the intracellular environment for viral nucleic acids, non-self RNA basically. When it encounters a non-self RNA molecule that it can bind to, it induces a series of events that leads to signaling through IPS-1 and turning on interferon production.

Vincent: How does it distinguish a viral RNA from the cell RNAs that are all floating around in the cytoplasm?

Michael: Yes. That's a big area of research, a very important question. What we've learned is that RIG-I can distinguish non-self from self by virtue of what we call "pathogen-associated molecular patterns" (PAMPs). These would be non-self-motifs – signatures if you will – embedded in a viral nucleic acid. RIG-I recognizes RNA molecules that have a free 5'-triphosphate on one end as non-self. Most cellular RNAs lack a free 5'-triphosphate either it's covered with something or it's not exposed. Many viruses have this free 5'-triphosphate exposed. That's one PAMP signature recognized by RIG-I. There are probably more like short stretches of double-stranded RNA structure in cooperation with polyuridine tracts. If you put all those together, we will call that a "PAMP-motif" that distinguishes self from non-self by virtue of RIG-I recognition.

Vincent: Is there a phase in the early part of infection where the viral RNA is in the cytoplasm so it can be detected by these molecules?

Michael: Yes. This hepatitis C virus, like most RNA viruses, replicates in the cytoplasm of the cell, albeit, probably in cooperation with intracellular membranes. The cytoplasmic replication program is very important for recognition by RIG-I and similar molecules; whereas, a different type of virus, a DNA virus like herpes virus, replicates in the nucleus. RIG-I wouldn't be expected to recognize herpes virus in that context. Then the RNA viruses, like hepatitis C, replicate in the cytoplasm in association with membranes. We think RIG-I is somehow trafficked along intracellular membranes for this purpose of pathogen recognition.

Vincent: It's interesting you mentioned the DNA viruses. We had a seminar not too long ago by someone trying to understand what is the RIG-I for a DNA virus? What's the sensor? Most of the work is done using DNA viruses that go right to the nucleus. Yet they find that cytoplasmic sensors seem to be able to sense those infections which doesn't make sense to me because the capsid for many of these big DNA viruses docks right on the nucleus. The DNA goes in. There's no opportunity for the DNA to be sensed in the cytoplasm. I think there must be some nuclear sensors.

Michael: That raises a couple of points. Yes, I would speculate. There's some biochemical evidence for this if there are nuclear sensors, DNA sensors, probably. In addition, those DNA viruses, when they initiate their replication program, they do have a cytoplasmic component to their lifecycle because they send their messenger RNA out into the cytoplasm to be translated and metabolized and so on. It's likely that they're pre-messages or their messenger RNA could serve as substrates for recognition by molecules like RIG-I.

Vincent: Sure. That's a good idea. I haven't thought about the RNA side. But they would have to be sufficiently different from cellular messages to be able to trigger that. Yes.

Michael: Yes. We could consider that the naked RNA or ribonucleoprotein – RNA that's bound to proteins – could serve as non-self-recognition.

Vincent: So getting back to hep C, the RNA comes in to the cytoplasm. It activates RIG-I. Then you said RIG-I interacts with a second protein, IPS-1. That's the protein that the virus protease cleaves to block this whole series of steps which eventually leads to interferon production. But in the case of hep C, because you're cleaving this IPS-1, it doesn't occur. In cells infected with hep C, you don't see a robust production of interferon?

Michael: That's correct.

Vincent: Okay. I know that IPS-1 is an interesting protein because it's in the mitochondrion, right?

Michael: Right.

Vincent: Do we understand why it's there?

Michael: Yes, actually we're starting to – so it's actually expressed on the outer – it's located on the outer mitochondrion membrane. You can think of it as having a small transmembrane domain that anchors it to that outer mitochondrion membrane. The rest of the molecule, which is some 500 amino acids, is sticking out exposing the cytoplasmic milieu. What we've learned is it is not as simple as IPS-1 being planted on the mitochondrion membrane but its mitochondria-associated membranes. These special membrane compartments, if you will, that mediates cross-talk between the endoplasmic reticulum and the mitochondria. You can think of it as an extension of the ER that communicates with the mitochondria. IPS-1, when it signals, seems to be distributed to this mitochondria-associated membrane compartment we call the "MAM." My interpretation is that the MAM serves as an innate immune signaling platform. It actually serves to form a synapse between the ER and the mitochondria and that synapse is important for signaling and thus a requirement for IPS-1 to have a mitochondria outer membrane component so the synapse can form and we have a source of IPS-1 that traffic to the MAM. What everybody agrees on I think is that IPS-1 has to be localized to intracellular membrane in order for the RIG-I pathway to signal. So this membrane substrate is very important because it's going to serve as a site to collect all the signaling molecules together that confer downstream signaling to the RIG-I pathway.

Vincent: So having it on a membrane is just more efficient than having it floating around in the cytoplasm?

Michael: Exactly.

Vincent: It's very much like the RNA polymerases of all these plus-stranded RNA viruses. They like to sit on membranes maybe to make the reactions more efficient.

Michael: Right.

Vincent: Is it correct to say that the protease of hep C cleaves IPS-1 so that it's just free of the mitochondrial membrane?

Michael: Yes. The way I describe it is that the protease cleaves IPS-1 and it floats off the membrane. It's still present in the cell but it's not anchored on the membrane.

Vincent: It doesn't work to make interferon in that case?

Michael: Correct.

Vincent: So can you redirect IPS-1 to another membrane within the cell and see if it works in that location?

Michael: Yes, we can. There are some conflicting reports that if you redirect IPS-1 to the ER, for example, your endoplasmic reticular membrane, it won't signal. It has to be on the outer mitochondrial membrane. There are other reports that say you can redirect it to a variety of different membranes and it'll still signal. The answer isn't clear but I can take all of those reports and sort of distilling down to this idea that if you put IPS-1 on an ER membrane or a mitochondria outer membrane, it still going to be able to traffic to the MAM which is the connector between the two. Therefore, you'll still get signaling.

Vincent: Okay. If you could replace our IPS-1 with a form that was not able to be cleaved by viral protease, would that help our resistance to hep C infection?

Michael: Yes. Indeed, I think so. We've been able to show that in vitro anyway. If we replace IPS-1 with a mutant, IPS-1 that can no longer be cleaved by the protease, the cells are resistant to hepatitis C infection. They get infected but they clear the infection.

Vincent: Yes. Would that ever be a viable therapy in people do you think?

Michael: That's a genetic therapy, I think it could certainly be viable but it's got all of the caveats that any genetic therapy imposes. If hepatitis C were like Ebola virus and were like kill most of the people it infects in a short amount of time, that kind of strategy might be a consideration. Because it's a chronic infection and many people can live out their life span with hep C infection under the right conditions, a genetic approach to therapy is probably low priority.

Vincent: Yes. Especially since we can make antivirals in multiple ones so that would probably be a lot simpler than altering us but in principle it's an interesting approach.

Michael: Yes, it is. For example, we can engineer a cell in culture, even a mouse, and show that that approach could be effective. My excitement really right now is in this new small molecule therapy, the protease inhibitor, NS5A polymerase inhibitor. They're going to be online soon.

Vincent: I read very recently that another kind of organelle that IPS is located in is a peroxisome. I don't know if you've seen that paper. I don't know much about peroxisomes but I know they're involved in lipid metabolism. Is that a particular significance to hep C which also requires membranes?

Michael: Yes. Actually, that paper is quite interesting because it suggests to me that the biology of IPS-1 maybe more extensive than we think. So the answer to your question: the peroxisome and lipid

biosynthesis is very important contributor to hepatitis C virus pathogenesis. People with chronic HCV infection often develop what's called "fatty liver." Fatty liver is the formation of these massive amounts of fat droplets in the liver that just compromise liver function. Why that happens, we don't know. IPS-1 involvement in the peroxisome and maybe part of the pathway of lipid biogenesis could be revealing somehow that pathogen recognition could intersect with lipid biogenesis in terms of viruses because of the linkage of RNA viruses in lipid membranes. So that's an area that could be thoroughly explored.

Vincent: Yes, I think there's probably a lot more to everything in the end than we think, right?

Michael: Absolutely.

Vincent: If you find IPS on the mitochondria, it doesn't mean that that's the only place. Often we're guilty of finding something and then just focusing on that and not looking. Then ten years later, "Oh, my gosh, it's here as well." And it explains so much.

Michael: Yes, it's funny how that works. I go back and look at our lab notes from eight years ago and they make sense only now.

Vincent: Sure. I know in hindsight things make a lot of sense. The IPS is a target of hep. Is there anything downstream? This is a little jargony here but there's a whole pathway of signaling from sensing hep C to making interferon and IPS is in that pathway. Is there anything else in that pathway that the virus interferes with?

Michael: Yes. There are several components. First of all, the pathway is certainly is not linear. It branches off at multiple points. Any of those points maybe antagonized or regulated somehow by the virus. The virus is recognized by RIG-I then initiates signaling downstream. The end-product would be the expression and function of genes that respond to interferon gene products. We call them "interferon-stimulated genes." We've learned that hepatitis C virus may encode proteins that can attenuate signaling through the interferon receptors. Once interferon is made and it engages its receptor to drive the expression of interferon-stimulated genes, that signaling is compromised, could be compromised. In addition, HCV infection may induce the expression of cellular inhibitors of the interferon signaling pathway called "suppressors of cytokine signaling." In addition to that specific interferon-stimulated genes, their protein products maybe targeted for regulation by hepatitis C protein products. That would suggest to me that those genes that are targeted are the important gene products for controlling hep C infection.

Vincent: As you said, there are many of these interferon-stimulated gene products, do we know of any of them that are particular importance for clearing hep C?

Michael: Well, for clearing, I don't really know. For having antiviral activity in a controlled experiment - yes, several of them identified IFITM1 – these are just the…

Vincent: Gene names.

Michael: Yes, the gene names. Okay. IFITM1, viperin, and so on, so there are a handful of them. For people who get exposed to hepatitis C and spontaneously clear the infection, the speculation would be that these handful of gene products are getting turned on and clearing out the infection. Which could be true but that has yet to be tested and observed in a patient.

Vincent: Could these have therapeutic value at some point? I know that the interferon treatment is not without side-effects. Maybe using just a few select interferon-stimulated gene products might remove those side products and more specifically target infection?

Michael: Yes, that's what we think. So there are a couple of ways you could approach that. If we were able to identify, let's say, ten interferon-stimulated gene products that we know are THE ten – and this is speculative, of course - the ten genes that actually drive down hepatitis C virus infection. We could approach that by modifying interferon therapy temporally to maximally induce that subset of genes, change this therapeutic regimen approach. Alternatively, one could consider developing therapeutic approaches that would either mimic the function of those gene products like small molecules or drive the expression of those genes individually.

Vincent: It's always a problem with the multi-component system, right? You have probably many interactions. As scientists, we try and simplify, have a reductionist approach. We take one ISG away. We add one. But there are probably so many networks going on. It's hard to do that. You have to simplify otherwise you don't get anywhere.

Michael: Right. You know the new contemporary approaches are the systems biology approach. I think there's an application there for something as complex as this but that brings in multiple approaches, multiple technologies and then bioinformatics to incorporate everything. Those are the kind of approaches we're going to have to take to fully understand virus-host interactions.

Vincent: So one thing I've never understood is where hepatitis C virus came from. For many human virus, especially contemporary ones we know, there is zoonoses essentially. Influenza is a zoonosis. How about hep C? Is there something in animals that looks like it?

Michael: Well, hepatitis C is a bit of an enigma. It seemed to emerge into the human population in a big way after World War II.

Vincent: So we can look back and see that it's clinically something that looks like hep C?

Michael: Right. Serologically it was known as "non-A, non-B hepatitis" by process of elimination serologically and then after it was cloned, molecularly identified in 1989, we called it "hepatitis C." Then there have been some retrospective epidemiologic studies that sort of show its emergence around the period of while the veterans were returning home from the war. There was a study that was published in *Science* maybe ten years ago now that tried to date the age of hepatitis C virus. It goes back actually – it's considered a fairly recent emergence into the human population. There is an age difference between, for example, genotype 1a and 1b, which were subtypes of the same genotype. So one may have emerged earlier as the parental and then diverged off and now into six different genotypes. The zoonosis question is of high interest: where did it come from? The only other animal that can sustain hepatitis C virus infection is the chimpanzee. The speculation is that there was a zoonotic event from non-human primate to human where it may have jumped from a chimpanzee population. I have heard scattered reports of some molecular epidemiology from wild chimpanzee cohorts, troops, but no solid evidence has been published to say the virus is resident in chimps and jumped to humans.

Vincent: Of course, Beatrice Hahn does this with HIV-1.

Michael: Yes. She's done a wonderful job tracking this zoonotic event. We've had discussions with Beatrice about examining…

Vincent: Her samples, right? Sure.

Michael: Her samples for HCV, I think. Other people probably talked to her as well.

Vincent: So she gets urine often I think.

Michael: Urine and stool.

Vincent: I guess you would find hep C in stool particularly, right?

Michael: Presumably, we would be able to detect in stool with molecular techniques as infected cells will be shed.

Vincent: Maybe not urine, right?

Michael: Maybe not urine.

Vincent: Yes. That will be very interesting because that's an enigma for that virus. For a lot of others, we can say, "Okay, here's an animal version," most beautifully for HIV-1 and 2. Years ago, measles virus we think came from Rinderpest of cows. There's enough sequence homology to be able to tell that. So that would be very interesting. If they don't just pop out of anywhere…

Michael: No. There's a reservoir and then there's some sort of transmission event.

Vincent: Well, chimpanzees wouldn't be unreasonable because we know that we get their viruses. The chimpanzee population is dwindling I understand.

Michael: It's dwindling and when people [come in] and directly examine blood of wild chimps, we may have recovered blood somehow the – there's not a predominance of hepatitis C. I don't think there's any direct evidence and has been presented except for these scattered reports that I've seen it.

Vincent: Interesting. So the idea would be that maybe it was acquired from a chimp. At some point, made its way to Europe and then with troop movements after World War II, that really again to globally spread.

Michael: So I think that could be a speculation. Yes.

Vincent: That's interesting. So how did you get interested in hep C?

Michael: Well, my true love I guess in my training background was intracellular signaling. Then I applied that as my training progressed to intracellular signaling events in viruses. I got some heavy-duty training in virology. At the time, I was greatly inspired by working – just came out of Jim Darnell's lab at Rockefeller describing these new molecules called the "Stats." At the same time, I was deeply inspired by [unintelligible] Taniguchi's work identifying the interferon regulatory factor, factors 1 and 2. Beautiful signaling paradigms there that related to host defense. I took those interests and sort of married those with virus infection. In my post-doc, in Michael Cate's lab, we studied the virus host interactions of interferon function. At that time, hepatitis C had just been cloned and there was some effort to treat hepatitis C virus with interferon. I got really interested in pursuing – trying to understand how interferon works. We know from Darnell's work, it signals through the JAK-STAT pathway; from Taniguchi's work, their interferon regulatory factors that induce the expression of interferon. How do those operate during a hepatitis C infection? The beauty of that interest is the conditions were just starting to treat hepatitis C infection with interferon injection. I thought, "What better way to study interferon than study it in a human setting with a chronic virus infection." We've been doing it ever since.

Vincent: Yes. Well, it's got applicability clinically because what you find can help people. As always, viruses are probes into these cellular pathways. Without them, it will be very hard to dissect this whole signaling pathway.

Michael: Yes. Viruses have uncovered really some major nuances of cell biology over time.

Vincent: We have actually not talked much about innate responses on TWiV. It's probably fair to say that hep C is not the only virus that interferes in the pathway, right?

Michael: Yes. I would say every pathogenic virus has a way of interfering with these pathways.

Vincent: Otherwise, they wouldn't exist.

Michael: Right.

Vincent: So we teach students that if you're infected with the virus, you mount an innate response. If that is not successful, then your adaptive response kicks in and takes care of business. In the case of hep C, do we understand why that doesn't happen?

Michael: Yes. There's a lot of focus in this area. Typically, the innate response will develop from the site of infection. So in the liver, the innate response would be induced for example through a RIG-I pathway in addition to other pathways from the hepatocytes at the site of infection. The products of that response like interferon and other primatory cytokines and chemokines play a major role in limiting virus replication and spread but they also play a major role in recruiting, informing, and driving the maturation of an adaptive immune response, the pathogen-specific adaptive immune response. With hepatitis C virus, as I said earlier, within the first 12 to 24 hours of infection, the virus produces an enzyme that knocks out the RIG-I pathway. So this important arm of the innate response is shut down. So the products that are normally made that would limit virus infection aren't made. So the virus replicates. It spreads to new cells. In addition, that innate response that is now missing can no longer inform and drive the maturation of the adaptive immune response. We know during chronic hepatitis C is the adaptive immune response – so let us look at the T-cell response, can be described almost as anergic. Meaning the response is present but it's severely attenuated. A speculation would be that because the innate response has not informed that adaptive T-cell response in the right way, those T-cells are anergic. Being that it's just a speculation, but it's testable. In terms of the humoral response, the antibodies that are made during the adaptive immune

response, there's plenty of antibody made. The virus undergoing genetic adaptation constantly just mutates largely around hypervariable regions of the viral genome that just mutate around the most important epitopes if you will for neutralizing the virus.

Vincent: Similar to HIV?

Michael: Exactly.

Vincent: Waves of mutants that are resistant to whatever antibodies you're making. By the time new antibodies arise, that can neutralize the variants. You now have new variants. So it doesn't neutralize. The antibody response is active in a hep C. It's just that the virus is getting around it.

Michael: It's active and diagnostic.

Vincent: Interesting. That's similar to the situation with the respiratory syncytial virus vaccine. I don't know if you remember this but in the '60s, a vaccine was made which failed. It was a formalin-inactivated vaccine. It didn't induce protective antibodies in kids. Now, last year, it was found that it's a poor ligand for the innate system. You get very poor inflammation after giving this vaccine and very, very low affinity antibody production as a consequence.

Michael: That's of high interest to me and I want to describe some observations we made recently. We deleted the IPS-1 gene from mice. I made a knockout mouse. When we infected those mice with virus, they had no resistance to the infection for one thing. They died very rapidly. One of the characteristics that we observe is that we had a complete loss of the innate response to the virus infection. In addition to that, the adaptive response was totally altered. In fact, the mice made antibodies but the antibodies completely lacked neutralization capacity. The mice made T-cells. The T-cells were dysfunctional. In fact, the key T regulatory cells that will normally control an inflammatory response failed to expand. So there all these downstream problems with the adaptive immune response that we attribute to the simple knockout of this IPS-1 gene which suggests to us that the innate response plays a major role in governing and driving the maturation of adaptive immunity - including humoral and cell-mediated immune…

Vincent: That's a really good point. It's not that the innate defense is just a wall that blocks – a low wall. It's talking to the next wall which is the adaptive system. It just goes to show that – that's why as you said we need systems biology to understand all these interactions.

Well, I have to stop and bring you to your seminar now. I want to tell you that I've always loved this work of yours on hep C interacting with the innate system. I've been working on polio for many years and about six or seven years ago, at a meeting in Italy, I heard you talked about RIG-I and hep C. I was looking for a change in what we were doing. When I came back here and I said, "We have to work on this protein." It turned out there was someone here who had discovered MDA-5, a fellow by the name of Paul Fisher. So I emailed him and he actually provided a post-doc and some antibodies and we began working on that which I'll tell you about over dinner.

I appreciate you talking with us today. I think our listeners really enjoyed hearing it and I'm sure they'll have some questions, which we always get on TWiV. If we do, I'll pass them along to you because you're the one to answer them.

Michael: Yes. Thank you very much.

Vincent: You're welcome.

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Transcribed by Raphael Fernandez of *The Learning Blog* (http://raphaelfernandez.com/).