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Culturing the First Synthetic Viruses

SYNTHETIC VIRIONS TO DETECT SARS ORIGINS

Every New Year, we look to the future and dream of what is possible. ReachMD radio is proud to present our special series focused on future medicine.

Understanding the mechanisms through which viruses and animals mutate and emerge as human pathogens help us to predict and restrict the impact of natural pandemics and intentionally borne virulent diseases. Towards this end, the cultivation of complex synthetic viruses looks promising in the area of research. Looking into the future, will these efforts allow us to subdue viruses and animals to be part of the emerging human population? You are listening to ReachMD, The Channel for Medical Professionals. Welcome to a special segment Focus on Future Medicine. I am your host, Dr. Mark Nolan Hill, professor of surgery and practicing general surgeon and our guest is Dr. Mark Denison, professor of pediatrics and associate professor of microbiology and immunology at Vanderbilt University School of Medicine. Dr. Denison is a leader of research published in the proceedings of the National Academy of Sciences and the creation of a synthetic SARS-like virus that is helping us understand the origin of the SARS outbreak.

DR. MARK NOLAN HILL:

Welcome, Dr. Denison.

DR. MARK DENISON:

Thank you.

DR. MARK NOLAN HILL:

Dr. Denison, just to begin with. How do you build a synthetic virus?

DR. MARK DENISON:

Well, the tools are quite readily available. The first thing we do of course is work with the NIH and the CDC and all of our Institutional Biosafety Committees to be sure that we are doing is being done under the safest and most secure conditions, but to answer question about the actual construction, we first identify from sequent databases, alignment of sequences and develop what we think is the sequence that we want to construct. Then we work with building it into small fragments or CDNA fragments and take it to commercial companies that are available for synthesizing DNA fragments. We asked them to synthesize the fragments, they then send those to us, and we then assemble them in the special biosafety level 3 laboratory and use those then to introduce or drive the whole assembled genome into cells and then out these particular viruses are capable of directing synthesis of virus directly from the genome itself.

DR. MARK NOLAN HILL:

Now, this is very carefully controlled from the government as such?

DR. MARK DENISON:

Well, we work in a special biosafety level 3 laboratory and every stage of our research is controlled from the level of course the research funding, but we have taken special initiatives to work in what I would sort of call an open source approach with between the institutions that are involved between regional policy ethics and law core and with interaction with the NIH to be very clear about what our goals are, of why we are doing the work, what the critical research questions are, and then why this particular approach is essential rather than standard approaches.

DR. MARK NOLAN HILL:

Dr. Denison, review with us just for a second. How do you determine the sequence?

DR. MARK DENISON:

Well, we did not in this case. This was done by investigators in China and elsewhere, but basically the strategy, which is one that all conditions would appreciate, its sampling of animals; in this case bats, but I am not sure what the unfortunate people who were who got to do this job, for taking that rectal swabs, taking the material from those swabs, isolating out the RNA genome, and then doing what's called reverse transcriptase polymerase chain reaction or RT-PCR in the sequencing facility to identify particular sequences from coronaviruses.

DR. MARK NOLAN HILL:

Now, the virus your team synthesized is characterized as the largest synthetic replicating organism ever made. Can you put in context for us the significance of this accomplishment?

DR. MARK DENISON:

Well, I think the significance is that its incremental, it's evolutionary, and perhaps a little revolutionary just in terms of the approach. There are many efforts to make synthetic genome in organisms, the largest of which are bacterial genomes, which have been the genomes themselves, the synthetic genomes have been published by the J. Craig Venter Institute, but they have not yet been used to

generate a replicating organism. So smaller viruses have been made synthetically such as polio virus, but those were from viruses that were already known to be capable of replication and they were simply reproduced synthetically.

DR. MARK NOLAN HILL:

Okay, so you have got the synthetic virus. Now what do you do with that and how does that help you really understand the disease?

DR. MARK DENISON:

The virus that we generated is in fact what we think of this a precursor to SARS, not SARS itself and so SARS if you think of it evolutionarily in animals and then in humans, so it may have distanced itself substantially from the original virus. So the potentially human antibodies made against SARS might not be effective at neutralizing or blocking the original virus that might have come out of animals. So one of the important components for us is that if we have the original animal virus, we can track how it became SARS in animals and we can also test reagents, antivirals, and vaccines against the original virus itself.

DR. MARK NOLAN HILL:

Now, is this really identical to the animal virus?

DR. MARK DENISON:

Yes, except for one important component that it was, you know, the original animal virus intact would not grow itself. What we had to do is take a small piece of the SARS spike protein and put it into the bad virus so that it could then enter into and replicate in the virucells, the same cells that are used to grow SARS.

DR. MARK NOLAN HILL:

Well, this is incredible. How much time does it take to actually synthesize a virus?

DR. MARK DENISON:

Well, it's like working in my workshop. The first table I build takes me a long time, but once I know how it doesn't take very long and in fact, it is as a sort of a gestimate I would say that if someone gave us a sequence now and everything went clearly and according to plan and everybody did what we needed probably within a week or 2 weeks, you could generate a synthetic virus from a sequence.

DR. MARK NOLAN HILL:

If you have just joined us, you are listening to a special segment Focus on Future Medicine on ReachMD, The Channel For Medical Professionals. I am your host, Dr. Mark Nolan Hill and our guest is Dr. Mark Denison, professor of pediatrics and associate professor of microbiology and immunology at Vanderbilt University School of Medicine. We are discussing the creation of synthetic viruses to

respond to emerging infections.

Dr. Denison, you mentioned that it might take a week or so to synthesize the virus; that seems remarkable in an incredibly short period of time. Years ago, when we first started doing this, how long did it take?

DR. MARK DENISON:

No one's been making synthetic viruses except in the last 3 to 5 years, so that standard approaches would have been to try to grow the virus in culture and then to try to understand its biology where the molecular biology and the details of its replication would have been limited and taken perhaps a months to years to one really understand the detail.

DR. MARK NOLAN HILL:

You expected years to come that the synthesis of the viruses will become actually more expedited?

DR. MARK DENISON:

I would expect so and one thing we are interested in is working with all the different systems that are emerging around the nation with sequencing in commercial CDNA synthesis and analysis of gene expression in different animal hosts to try to really integrate those into a plan, which could be implemented in case of a new epidemic.

DR. MARK NOLAN HILL:

Are certain viruses easier to synthesize than others?

DR. MARK DENISON:

That would be my speculation of course that the smaller the genome and the more that is known about it, easier it is for example, the polio virus genome as I mentioned is like 7,000 nucleotides, the coronavirus genome is about 30,000 nucleotides, but there are poxviruses and bacterial genomes that of course are much much larger and thus more complex, but the strategy, there's nothing potentially different or more complex about the strategy, just the implementation.

DR. MARK NOLAN HILL:

If you can culture a virus, is there any reason to synthesize that virus?

DR. MARK DENISON:

Now that's a good question and I think my answer is yes and I will give you one example. If I could culture a virus, lets say, a new bat

coronavirus, but I want to be able to introduce multiple mutations, so if I know across all coronaviruses, there are 30 different mutations I could introduce, which would replicably attenuate that virus or make it less virulent, potentially the vaccine, I could go to that original sequence and then I could synthesize that original growing virus with all of those 30 mutations at once, a strategy, which may take months or years to do if you did it by standard approaches one mutation at a time.

DR. MARK NOLAN HILL:

Now talking about mutations, we talk about these mutations and allow these viruses to jump from animals to humans; can we learn to fight these viruses before they jump?

DR. MARK DENISON:

That's an interesting question. I think the answer would be yes and in one way is knowledge of what they do and how they grow and how they jump, so for example, if we knew that a virus was coming out of particular animals reproducibly, we might be able to go back if we could grow to study a vaccine that potentially could be used in that animal source, that if it was a domestic animal source and block the replication of the virus in the animal host to reduce the risk to humans.

DR. MARK NOLAN HILL:

Now how far are we from actually doing something like that?

DR. MARK DENISON:

I don't know. I have to see how people respond to our work, (laughs). I think theoretically it can now be done, practically it would have to target that people felt was important enough to take that kind of approach. One example of vaccines were vaccines are being used of course in animals, would be like a rabies vaccine, like where there may be date that's laced with a rabies virus vaccine like attenuated virus and areas where there may be endemic rabies to try to reduce the load of rabies in an animal population.

DR. MARK NOLAN HILL:

We recently interviewed a colleague of yours, Dr. James Crowe about the reconstruction of the 1918 flu virus and we talked about the security under which they had laboratory most operated. That was similar to your work?

DR. MARK DENISON:

We operated under the levels of biosafety and security that are implemented for SARS. SARS is not considered to be a select agent or as higher risk as the AVN.

DR. MARK NOLAN HILL:

Why is that?

DR. MARK DENISON:

I think because of the limitations that it demonstrated in its spread, its transmission, and its maintenance in human populations whereas the influenza virus that Dr. Crowe was describing working with was the one that obviously killed millions of people in 1918 and 1919, and the mechanisms by which that occurred are still not completely known and so the potential risk to human populations are considered to be much higher.

DR. MARK NOLAN HILL:

Dr. Denison, several of us including myself, I am sure, are wondering these regulations and the control. Tell us specifically what is that like?

DR. MARK DENISON:

Its actually, I am also involved in biosafety regulation and policy on a regional level in the South East and at Vanderbilt. I think one of the things I can emphasize here most clearly is that communication among investigators and regulatory agencies and biosafety is really our best security, i.e. that understanding how to work safely with the virus and to prevent its transmission is really the safest way to do it, but we have a specific laboratory where we work under completely negative pressure environments so that nothing can escape. We have redundant systems, which can control all of that. So if one system fails, then basically we have 2 additional backups and this virus is not capable of spreading readily in the air and so our biosafety cabinets in all of the rooms and all of our equipment is meant to just keep it in a very small space.

DR. MARK NOLAN HILL:

Are the investigators like yourself tested frequently?

DR. MARK DENISON:

We have guidelines in place for being sure that we have pooled bank blood so we know what our circumstances are. The most important thing; however, let me reemphasize is the approaches and basic biosafety approaches to working with any virus are just as effective with SARS as they are with a non-human pathogen and so if we work with it correctly and we follow the guidelines and we are in communication with our biosafety committees and with people who regulate this work, then really its quite safe to work with.

DR. MARK NOLAN HILL:

How have you shared your information and your research with other physicians and researchers?

DR. MARK DENISON:

We have shared it in many ways, in many open for obviously biopublications in the proceedings in the National Academy Sciences and multiple open for including programs on emerging infection and biodefense, the American Society for Virology and another for that were scientists gather, so there's no component of this that we feel needs to be precluded or hidden from anybody.

DR. MARK NOLAN HILL:

Is there anyone in the country that is doing something similar?

DR. MARK DENISON:

There are many groups that are interested in the questions of synthetic biology like I said those who are working in the coronaviruses such as polio viruses and I am sure other people are considering these approaches to more rapidly be able to make attenuated vaccine strains of viruses by introducing multiple mutations.

DR. MARK NOLAN HILL:

So as clinicians, we are thinking your making synthetic viruses, were learning about these diseases, how far will it be from the synthetic virus to actually make any change or modification in our treatment regimens?

DR. MARK DENISON:

Well, of course, in our case we are more interested in response and prevention and we are working on the cutting edge of these particular viruses. If it was a virus that became an endemic human virus, then I think the application will be very immediate in terms of our ability to think of testing antivirals, testing vaccines, and thinking about how to apply that to blocking an epidemic.

DR. MARK NOLAN HILL:

Can the information that you obtain in your research be extrapolated to other viral diseases?

DR. MARK DENISON:

I think its possible to use these approaches to study really the origin of any epidemic or pandemic virus, it also might allow us to think about how other viruses, for example, transmit in domestic animals or transmit from animals to humans. Ultimately the approaches we take could be really used to understand bacterial infections as well.

DR. MARK NOLAN HILL:

I want to thank our guest, Dr. Mark Denison. We have been discussing the creation of synthetic virus to respond to emerging infections. I am Dr. Mark Nolan Hill and you have been listening to a special segment Focus on Future Medicine on ReachMD, The Channel for Medical Professionals. Be sure to visit our website at www.reachmd.com featuring on-demand podcasts of our entire library and thank

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