



## CONFERENCE TRANSCRIPT

### Welcome

Ellis Rubinstein, Chief Executive Officer,  
New York Academy of Sciences

### *An Academy Tradition of Convening Scientists*

Welcome to the New York Academy of Sciences. I think we have a packed room, so I think we should start. I am Ellis Rubinstein, the chief executive officer of the Academy, and it is a pleasure to welcome you all here.

As some of you may know, I've been CEO here for only six months. That's in contrast to the fact that this place has been here for 186 years and, some people might not know, is the third oldest, I think, scientific society that's been continuously running, and it's had an extremely wonderful history in many ways. Thomas Jefferson was a member, and Darwin was a member, so it's been global for a long time. I guess in my mind some of the best things it has done is to convene people across disciplines and barriers on important topics. Some people here may or may not know that the Academy was the first organization to run a major meeting on AIDS.

The meeting that we're going to hold here today is in keeping with a tradition that has gone back for some time of doing these kinds of important convening tasks. I would say, I think quite safely, that never in the history of the Academy could they have produced a meeting as rapidly as this one was done. It was only three weeks ago that I was talking to Dr. Ian Lipkin, who I am going to introduce in a bit, and we started speculating about whether there would be a need for a meeting that wasn't just a small meeting among coronavirus experts, or a big meeting like maybe the Geneva meeting, but something that would bring experts together across disciplines to talk about the interesting challenges that SARS is presenting. We were a little manic, we thought it would be a great idea, but we wondered whether we really could get an exceptional group together in such a short time, especially considering the pressure that people are under. Thanks to all of you here, we have done, I think, a remarkable job of bringing together a unique group, a unique mix of people.

### *Reengaging with Scientists*

Because of the importance of the meeting I am going to resist the temptation of telling you too much about the Academy, but I do want to mention a couple of things for you, because I think that, before starting things off, this meeting typifies what you will see from the Academy in the coming months, as we do a kind of a Renaissance or a reengagement of the involvement of real scientists in this Academy. To explain that and give you a couple of examples, I just wanted to mention to you that as we sit here, 4,000 graduate students and postdocs in the New York area have joined this Academy in one fell swoop, and it'll soon be six or seven thousand, thanks to the deans of all the hospitals, universities and the independent research labs in the greater New York area, as far north as Yale. This is our future.

A second thing that I wanted to mention is that in fall we'll be launching a series of seminars in the hottest fields of science: RNAi, tissue engineering, nanotech, imaging, and so forth. We'll be doing about a dozen of them, and the fact was that this meeting takes place because of the very conversation I was

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having originally with Ian about starting one in emerging infectious diseases, which we intend to do. For those of you interested in this topic, I hope that you've left your e-mail addresses, and if not please do, because we would be happy to let you know about this program when it starts in the fall.

### *Academy Meetings on the Web*

Another feature that's going to be quite novel: we have been convening in New York for some time; now we'll be convening on really exciting topics. But how do we reach out to the rest of the world? You'll see in this corner and in the back of the room some cameras. They're being operated by a very innovative team at Columbia University called Digital Knowledge Ventures. Digital Knowledge Ventures was trying to learn how to create really interesting distance-learning Web products out of professors' classroom experiences. We're ganging together to try to do this from our seminars and our conferences that we hold here, so that people sitting anywhere in the world can join the conversation in these hot fields, and not have to, by the way, watch a linear Webcast where you have to watch for thirty minutes a little, tiny head and a bunch of PowerPoint slides, but rather come to a screen and in five minutes see the key points that were made—one minute and twelve seconds on this, and two minutes and fourteen seconds of that, click and get a quick briefing. If you want to go further you'll get links to all of the things that were behind that.

This will be a perfect model of that, so all of you in this room will be able to come to our Web site at [www.nyas.org](http://www.nyas.org) and, starting on Tuesday, we'll have the raw talks as they exist with the questions and everything else up online for you. Then in the ensuing week or two the Digital Knowledge Ventures team will be enhancing that, so that there will be a really nice educational experience that will provide links to other exciting things related to the topics that we're discussing here. I recommend that if you yourselves don't, since you've participated in it, want to go to it you recommend it to others.

### *Meeting Cosponsors*

I need to thank very quickly our cosponsors who have made this meeting possible. Columbia University plays another role in this meeting; it's launching a brand new Center for Global Health and Economic Development. If you're interested in what that might mean, the dean of the Mailman School of Public Health, Dr. Allan Rosenfield will be here later, he's not quite here yet. Over lunchtime you can find out more about that.

Also, of course, I want to thank the NIAID, National Institute of Allergy and Infectious Diseases, and Tony Fauci and John La Montagne are helping with this. I am very pleased and thankful to our pharma friends, Pfizer and also Bristol-Myers Squibb, Merck Research Labs, and Novartis, and many of you folks here are from those companies. This is very exciting for us because it starts a partnership that is going to be evolving into the discussion groups that I was mentioning. We're very pleased to finally find ways to bring together industry scientists and academic scientists on a regular basis, here in New York and through the digital products. If any of you are interested in that, I'll be happy to tell you about it. We're in late-stage discussions with a lot of the heads of research in a lot of those companies, and we're quite excited about it.

### *Heroism in the Face of SARS*

To introduce this topic, I just want to mention, I have had a fairly long career as a science journalist, and so I have known a lot of scientists and a lot of journalists, and I have had a lot of high points in my career, not least when I was the editor of Science for the last decade. But I can't remember many occasions when I associated something with true heroism on the part of some scientists and some journalists. This has really been a kind of a unique circumstance, and I thought I would mention it, because I have



been thinking about it a lot for the last few weeks.

In Singapore and in China, some of our friends on the scientific and medical front and the journalists there have been at incredible risk to their own personal safety. One of the people, who will be presenting through Scott Hammer, who I am going to introduce in a minute, is Chen Zhu, a person we know very well, who's the vice president of the Chinese Academy of Sciences, assigned by the Chinese government to oversee the entire scientific effort there. He is not only risking his own personal safety—right now I think he's in the south of China—but he submitted, in the midst of unbelievable pressure, PowerPoints for this meeting, and you'll be seeing them. When one thinks about him and hopes for his health, and similarly the journalists here, some of their colleagues are on the ground in China and in Taiwan and in Hong Kong. One thinks of Libby Rosenthal of the Times who wanted to come here, who was the person who went to the epicenter of the disease in Guangdong and wrote that fantastic story on what is going on in the restaurants in Guangdong. She actually was here in New York a few days ago, she was dying to come, but she felt she had to fly back to China and report some more. So I think of her and I think of my former colleague at Science, Dennis Normile, the Tokyo correspondent who flew into Hong Kong to go to a meeting, knowing what he might be running into.

One more journalist I won't name, but I want to read you something I think you will find interesting. A journalist I have known for a long time in China, who is of course not only experiencing the same risk, but comes with a rather interesting point of view, I think to me and I think to you. She wrote me shortly after the outbreak the following little e-mail:

"I am among the people who feel rather grateful to SARS. Although it's a true disaster, it has helped solve so many of our problems. One, it knocked open the government information system and forced it to be more transparent. Two, it taught a good lesson to many government officials that they are public servants and they must be responsible for the people rather than their superiors. Three, it revealed the faults in our public-health system and made the government see them and mend the fence. Four, it redressed unhealthy habits of ours, like spitting at will. Five, it helped curb some social evils. Those who used to dine out at the public trough now don't dare to go out anymore but go home on time every day. Those who used to love to call meetings have to learn to do without them. Six, we can spend more time at home; people are caring more for each other."

I thought that was rather a remarkable e-mail, and then about three days later, after she heard about our meeting here, she wrote me a message. "Well, Ellis, it's a brilliant idea to call such a meeting of people from different disciplines talking about SARS at such a critical moment. I wish I could follow the event on the Web." Well, of course our Chinese journalist friend and all of the folks in China will be able to follow this event and your wisdom on the Web, as I mentioned.

### *On Ian Lipkin's Absence*

With that I want to introduce the person who probably was most responsible for starting this event, who I have mentioned before, and who is in my mind a heroic figure, Dr. Ian Lipkin. You don't see him here, even though he's on your program, because about three or four days ago Ian started to have a bit of a fever and a bit of a cough. The tests have shown up negative, and he doesn't have enough fever to qualify to be in the category where he would be quarantined for sure, but he's volunteered to stay home today not to put any of you at risk, and yet he's on the phone. Why did that happen? I haven't mentioned that. Because only days after we decided to run this meeting, and in the midst of all the other things that he had to do, he jumped on a plane and flew to Beijing to try to help the Chinese, as David Ho did, and spent three days there, and came back, and now he's got this little unfortunate fever. I think what we



want to do is hope that Ian is going to be very healthy in the next few days, but at least give him a kind of an ovation for having both put together this meeting and for having done all the other things that he was willing to do.

Ian, would you like to say something?

## **Introductory Remarks**

W. Ian Lipkin, Director of the Center for Immunopathogenesis and Infectious Diseases, Mailman School of Public Health, Columbia University, New York

### *A Personal Health Update*

I appreciate that, Ellis, that is very kind. First of all, good morning, thank you all for coming. It has been a real pleasure to pull together this meeting on very short notice with Scott Hammer. I have put together many symposia, and I can honestly say that the best person with whom I have ever shared such a responsibility has been Scott. He is really extraordinary and it has been a pleasure to serve with him.

I am very sorry to miss this symposium because it is stellar, and I think in part that is because I know so many of you, you are among the best. Many of you are among my closest friends. I am very sorry to miss this opportunity to meet with you, and I am very unhappy to miss a meal after this meeting.

First of all, I am certain I don't have SARS. Nonetheless, it is a very nasty bug and I have not been laid this low for many, many years. I was fine until Tuesday when I came back from NIH, where I was doing a presentation on MMR vaccine and autism. Yes, believe it or not this is still a very alive hypothesis, and I think it threatens many vaccine programs, and it's critical that it be addressed, so this is something that we're working on now. I very rapidly developed fever, about 38 degrees, and a cough. Although I didn't meet formal criteria for SARS, given that it was less than six days after coming back from Beijing, Scott and I felt it was prudent for me to go into seclusion at home. I still have a fever and a cough now, several days later, but I do feel better every day. For what it's worth, PCR analysis has shown no evidence of coronavirus.

The whole experience underscores for me, personally as well as professionally, the importance of finding sensitive specific tests to facilitate differential diagnosis.

### *Advising in China*

I want to thank specifically Larry for introductions to Alan, Hank, and Jayme in Beijing. I did my best to follow your advice and that given my John McKenzie, but it was difficult to wear a mask all the time, particularly at banquets. I have no regrets about going to Beijing. My understanding of the challenges that face the health-care workers there and the field officers is still poor, but much better than before. I was impressed by the dedication, depth, and breadth of talent in the Ministries of Science and Technology and Health. I had a call this morning from the minister of Science and Technology who tells me that the count is still dropping for SARS, probable and suspect.

The occasion of this visit gave me an opportunity to work very closely with Chen Zhu and to meet investigators supported by the Ministries. They were allowed, therefore, to have a meeting where they were able to find out for the first time what their various counterparts were doing in diagnostics, pathogenesis, therapeutics, and vaccine development. I am sorry I missed David Ho; we've actually never met, something that I really looked forward to doing today. Hopefully this will happen at some point in New York.



I was asked to assist as a special advisor by the Ministries, as an advisor for SARS, and I have tried to help over the past couple of weeks, and have interacted to some extent with Kathy Laughlin in making contact within the United States at institutions throughout the Northeast as well as at UAB and OHSU. It's the first time I have done anything like this; perhaps I am only fit for this sort of thing now. I can't say whether the catalytic event was switching coasts, joining the School of Public Health, coordinating a symposium, or simply turning fifty. Maybe it's a combination of all of the above.

Have a great meeting, and as Hilary Kopowski says, I am here on the phone but nonetheless shaking both your hands with profound admiration. To those of you who came because I twisted your arms, thank you in specific. I am going to listen in and participate as best I can, because we do need to write this meeting up. Scott and I have written some pithy provocative questions to keep things vibrant and fluid, and we do want the audience to interact with people up at the podium and in the panels. I look forward to seeing all of you in the very near future. Thanks very much for coming.

### *Opening the Program*

**Ellis Rubinstein:** With that, I am going to turn the meeting over to our other key player in this, Dr. Scott Hammer, who also had nothing but other things to do, and has spent unbelievable numbers of hours helping me put this together, I am sure you all know, since he's personally talked to many of you if not all of you. I won't introduce Scott because I'd like to meeting to get going. I think you all know in this room what a great scientist he is, and so Scott I'll turn the meeting to you.

**Scott Hammer:** Ellis, thank you very much, and special thanks to Ian Lipkin who was great to work with on this. We've known each other for a couple of years and he's a great addition to New York and Columbia, and I am glad to hear his voice over the phone here this morning, as we all are.

My role as part of this day is to actually substitute for Ian. I'll be a poor substitute, but I'll do my best and he can kibitz from afar. Just a couple of things. The way this meeting is organized, as you can see, is into four sessions starting with basic science, moving through clinical science, and then sort of the larger, bigger picture, if you will. We've asked the speakers to target their comments in ten- to at most fifteen-minute talks, and then to leave plenty of time for discussion. We'll stimulate those discussions from the podium here, but we also want this to be interactive through the scientific interchange from the audience. So think of your questions, and spontaneous additional comments are also welcome. We're going to try to leave plenty of time for that. The day will move very quickly, and I guess my major role is to keep everybody on time.

Without further ado, I'd like to essentially open the scientific program. To put us all on the same page as far as the up-to-the-minute data, the latest data from WHO and Promed report as of yesterday 7,739 SARS cases in 29 countries around the world, with 611 deaths, and still, obviously, by far the greatest proportion of cases being in China and Hong Kong and Taiwan and Singapore. We'll talk more a little bit about the epidemiology during the day.

The first session is "Coronavirus Biology and Pathogenesis." We have four terrific speakers. What we'll do is go through all the talks first, and then open it up to questions so we can be sure to stay on time.

## **Session I: Coronavirus Biology and Pathogenesis**

**Moderated by Scott Hammer**



## **Coronavirus Biology and Pathogenesis: Molecular Biology of Coronaviruses**

Paul Masters, Wadsworth Center, New York State Department of Health, Albany

**Scott Hammer:** The first speaker is Paul Masters, he's a professor of molecular genetics at the Wadsworth Center, New York State Department of Health. He's going to talk about the molecular biology of coronaviruses. Thank you.

### *Coronavirus Virion Characteristics*

**Paul Masters:** Thank you, Scott. I'd like to thank Ian and Ellis and Rashid and all the other organizers of the meeting for inviting me. I'd like to attempt to give you a rapid introduction to the basic molecular biology of coronaviruses to start things off.

Coronaviruses are enveloped, positive-strand RNA viruses. The virions have a punched-in spherical appearance, at least by negative stained electronmicroscopy, and a characteristic feature of members of this family are these surface projections, or spikes or blebs, that appear on the surface of the envelope. To the original electronmicroscopists this suggested the appearance of a crown, and hence the name "corona."

These are pleomorphic virions. They average somewhere between 80 and 120 nanometers in diameter; the spikes extend a further 20 nanometers from the surface. Here we see one that has burst and extruded its nucleocapsid into the surrounding medium. We see that it has a helically-symmetric nucleocapsid—this is unique among positive-strand viruses. All other positive-strand RNA viruses tend to have icosahedral nucleocapsids. This is one unique feature of this family.

### *Coronavirus Diseases*

Coronaviruses cause a variety of diseases in humans and animals, and I believe that Linda Saif, one of the next speakers, will tell us a lot more about that. These diseases are generally respiratory, enteric, neurologic, but there are also other types of pathogenesis. Coronaviruses are generally, but not always, highly species-specific. They've been grouped into three groups, originally on the basis of serological cross-reactivity, but more recently on the basis of genomic sequence homology, and by this particular criterion the SARS coronavirus appears to be the prototype member of a fourth group. Its sequence is sufficiently different from the other three groups to justify this classification, at least by present analysis, and I think Tom Ksiazek shortly will address this in his talk.

### *Spike (S) Protein*

Let me tell you a little bit about virion structure and other things encoded by the virus genome. The virus has four basic structural proteins. We're looking here at the prototype mouse coronavirus, mouse hepatitis virus, but all other coronaviruses are very similar, if not identical to this. There are three proteins in the membrane envelope, the spike S protein, the membrane protein, envelope protein E, and one protein component in the interior of the virus, the nucleocapsid protein N. I want to take a quick look at each of these.

The spike protein is a 150 kilodalton protein. It's a type I membrane protein, has a large amino-terminal ectodomain, a transmembrane domain, and a very short carboxy-terminal endodomain on the interior of the virion. The ectodomain is extensively glycosylated with N-linked glycosylation, and the protein oligomerizes into either dimers or trimers; this is still not well resolved, and these dimers or trimers



form the spikes that one sees on the surface of the coronavirus.

The functions of this the S protein are, first of all, to bind to species-specific host cell receptors. The next speaker, Kathryn Holmes, is the person who discovered the mouse, human, and feline coronavirus receptors, so I am not going to say anything more about that, because she's the person to talk to about that.

Binding of the S protein to receptor triggers a fusion event between the viral envelope and a cellular membrane, in some cases the plasma membrane, in other cases an endosomal membrane, and this results in internalization of the viral nucleocapsid into the cytoplasm. In some cases, but not all, the S protein also traverses all the way to the plasma membrane of infected cells, and so it can induce the fusion of adjacent cells to form syncytia. Finally the S protein is the principal viral antigen eliciting neutralizing antibody on the part of the host.

### *Membrane (M) Protein*

The other protein components of the viral envelope are a membrane protein M and an envelope protein E. The M protein is a 25-kilodalton protein. It's a triple-spanning membrane protein, has a short amino-terminal ectodomain, and this is either N-glycosylated or O-glycosylated, depending on which group of coronaviruses we're talking about, then makes three passes through the membrane, and it has a large carboxy-terminal tail, and this tail interacts with the viral nucleocapsid.

The M protein is the major component of the virion envelope, and it's the most abundant viral protein. The functions of the M protein are that it's the major determinant of virion morphogenesis, it is the thing that selects S protein for incorporation into virions during viral assembly. It also binds to the M protein during viral assembly, and there's some recent evidence that suggests it is M protein rather than nucleocapsid protein that specifically selects the genome amongst all the available RNAs in the cell for incorporation into the virion.

### *Envelope (E) Protein*

The other component of the virion envelope is a very minor component in size and amount; that's the envelope E protein. This is an 8&#8211;10-kilodalton protein; it's present in very tiny stoichiometric quantities in the virion, but it appears to play a very major role in interacting with M protein and bringing about viral budding. It is known that E protein determines the site of viral budding, and depending on which virus we're talking about that's either the ERGIC, the endoplasmic reticulum Golgi intermediate compartment, or the Golgi. So E, although not present in abundance, is playing a role, it is thought, either bringing about the curvature of the budding compartment, or perhaps tying off the neck of the budding virion.

It was Peter Rottier's group in University of Utrecht in the Netherlands who showed that expression of just M protein and E protein was sufficient to lead to formation of virus-like particles that have a morphology similar to virions and are exported from cells by a mechanism similar to that used by virions. This created a paradigm for assembly that's unique to the coronaviruses.

### *Nucleocapsid (N) Protein*

Finally, in the interior of the virus is the nucleocapsid protein. This is a highly basic protein, roughly 50 kilodaltons, and has an overall positive charge, but the carboxy-terminus of the N protein is acidic. It's the central region of the N protein that binds to RNA, the carboxy-terminus that binds to M protein during assembly. There's also some evidence that N protein may serve as a translational enhancer to lead to the



selective translation of virus messages during infection.

### *RNA Genome Characteristics*

What is the N protein bound to? It's bound to the positive-strand RNA genome. Because the genome of this virus is positive-strand RNA, like all positive-strand RNA viral genomes, it in isolation is infectious. If you isolate viral RNA and transfect it into the appropriate cells, an infection will ensue. The rest of the package is just for delivery of the genome to the host cell. This looks like a typical eukaryotic messenger RNA, it is a five-prime cap, it has a three-prime polyadenylate tail. There's two things that don't look like a typical eukaryotic messenger RNA. First of all, it's size. The genomes of coronaviruses range from 26 to 32 kilobases in length; that makes them amongst the largest mature RNAs known to biology.

Second, unlike a typical eukaryotic message, it contains multiple open reading frames. In fact, the genes that code for the structural proteins that I just talked about are found in the distal part of the genome.

### *Gene 1 Polyprotein*

The first two-thirds of the genome is occupied by this very large gene, some 20 to 22 kilobases, and this gene is translated into a very large 800-kilodalton polyprotein. It is translated via a mechanism called ribosomal frame shifting, and this polyprotein cotranslationally processes itself into the factories that synthesize viral RNA, that both replicate the genome and transcribe the genome. So there are, depending on which coronavirus we're talking about, two and in some cases three protease domains that are responsible for processing this polyprotein into some 15 or 16 polypeptides. There are membrane-bound domains that provide a scaffold, it is thought, for the assembly of this RNA synthesis factory, on some as yet to be characterized membranes within host cells that are at a distinct site from the site of budding.

### *Nonstructural Proteins*

Finally there's the business part of the molecule: the RNA-dependent RNA polymerase, an RNA helicase, and then many blank areas on the map, regions of unknown function. There's a great deal that's still not worked out about this non-structural protein. This is present in infected cells, but is not packaged into the virus.

There are also so-called group-specific nonstructural proteins. The genes for these, in yellow, are interspersed among the structural protein genes in the distal portion of coronavirus genomes. Their locations and numbers vary, depending on which of the three or four groups of coronaviruses we're talking about. For all of these group-specific nonstructural genes there's only one, a hemagglutinin esterase gene in the Group II coronaviruses, for which we actually have some idea of the function of the protein product. So this thing encodes a hemagglutinin esterase that provides an extra glycoprotein on the surface of some Group II coronaviruses, and very interestingly, this gene is known to have been acquired by horizontal transfer from some ancestor of the influenza C viruses. For most of the other group-specific nonstructural proteins, we know nothing about their function. Many of them appear to be nonessential, many are not expressed, and many have been looked at carefully and have no apparent role in pathogenesis.

### *Life Cycle*

Let me briefly run you through the coronavirus life cycle. As I mentioned before, the virus binds to specific receptors on target host cells, and binding and fusion results in the deposition of the nucleocapsid and ultimately the genome into the cytoplasm. The first thing that the genome wants to do is be translated by host ribosomes. This produces the RNA-dependent RNA polymerase complex, which then recognizes the three-prime end of the genome, and produces a full-length negative-strand copy of the genome. Also, by multiple steps the polymerase produces a series of subgenomic messenger RNAs.



These messenger RNAs form a so-called three-prime nested set, and each of them consists of a leader sequence of 70 to 100 nucleotides fused to some internal point on the genome. Each of these has a negative strand counterpart as well. The synthesis of the subgenomic RNAs is too involved to go into, but the consensus in the field is that the discontinuous step during transcription that joins the internal sequence to the leader actually occurs during negative-strand synthesis. Each of the resulting subgenomic RNAs then serves as a message for translation of one of the downstream genes.

Another remarkable feature about coronavirus RNA synthesis is the very high rate of RNA-RNA recombination that occurs in these viruses. If two strains or two mutants of a particular coronavirus end up infecting the same cell, something on the order of 25 percent of the progeny will turn out to be recombinants. This recombination occurs, unlike DNA recombination, not by a breaking and joining mechanism, but rather by a so-called template switching mechanism or a copy-choice mechanism, where the RNA polymerase will be copying one template, detach from that template, still hanging onto the nascent RNA strand, and then reattach to the homologous position of a different template and resume synthesis. That will result in a molecule that looks like this up to this point, and like the other strain up to here. That's how recombinants can be generated.

Resuming the life cycle scheme then, after synthesis of the subgenomic RNAs, which are another hallmark of the coronavirus family, these can then be translated into the viral structural proteins that I talked about. The membrane proteins S, E and M, initially go to the endoplasmic reticulum, and then begin to traverse through the default secretory pathway, ending up either in the ERGIC or the Golgi. The nucleocapsid assembles from the nucleocapsid protein, and from progeny genomes these meet up in the cytoplasm, and then bud into the budding compartment, resulting in virion formation. Viruses are then transported via smooth-walled vesicles out of the cell. So one virus can go in, a hundred to a thousand viruses can leave the cell.

How can we manipulate coronaviruses by reverse genetics? One way to do so is by the standard scheme that has been used for many other positive-strand RNA viruses. Because the genomic RNA by itself is infectious, if one can make a full-length cDNA copy of the genome, clone that into a transcription vector, plant a mutation of interest, and then transcribe from that infectious RNA containing the mutation of interest. This RNA can be transfected into the appropriate host cells and mutant viruses will be produced, assuming that the mutation one created was not lethal. This scheme for a very long time was not possible with the huge genomes of coronaviruses, but very recently full-length infectious clones of the porcine TGE virus, the 229E, MHV, and infectious bronchitis virus have all been made by various workers who are too numerous for me to list.

The disadvantages to this sort of system is that the resulting full-length clones are highly unstable and they're very difficult to manipulate, very difficult to work with. However, the major advantage of this system is this is the only means for doing reverse genetics on the huge gene 1 that occupies the first two-thirds of the genome.

### *Reverse Genetics by RNA Recombination*

For about a decade now my group and then other groups have been practicing a different method of performing reverse genetics on coronaviruses. We developed this method with MHV originally, using recombination, taking advantage of the high rate of RNA-RNA recombination, and using recombination with synthetic RNAs to construct site-directed mutations in the coronavirus genome that we could then recover by selecting against a thermolabile deletion mutant parent virus.



More recently, in collaboration with Peter Rottier's lab in the Netherlands, we constructed a virus that we call fMHV, because it is mostly MHV, all its components are MHV, except for the ectodomain of the spike protein, which comes from the feline coronavirus feline infectious peritonitis virus. This is a mouse virus now, but it can only grow in feline cells, and it can't grow in mouse cells. We take advantage of that characteristic then by forcing this virus to recombine with donor synthetic RNA that restores the ectodomain of the mouse S protein, and the synthetic RNA also contains some other mutation of interest to us, and we can recover mouse virus mutants on the basis of their having regained the ability to grow in mouse cells. This has proved to be a very versatile and robust means for performing reverse genetics on all of the structural protein genes of this coronavirus, and more recently other coronaviruses.

That's where I started, and that's my reminder to stop talking, so that the next speaker can speak.

### **Coronavirus Biology and Pathogenesis: Coronavirus Pathogenesis**

Kathryn V. Holmes, Department of Microbiology, University of Colorado Health Sciences Center, Denver

#### *Coronavirus Species-Specificity*

**Scott Hammer:** Thank you, Paul, that was a terrific introduction to the first session. I'd like to quickly move to Kathryn Holmes, who's from the University of Colorado Health Sciences Center, in the Department of Microbiology, and a world-famous coronavirus investigator who's going to talk to us about pathogenesis, including tropism and a few other characteristics. Kathryn.

**Kathryn Holmes:** I apologize for missing the left-half of all my slides, I think. What I am going to talk to you about today is the biology of coronaviruses as they interact with their [host] cells. There's a great deal of specificity in the interaction of coronaviruses with their host cells. The thing that has been very interesting to us over the years is the question of why each coronavirus, here human coronavirus 229E, has a particular host that it can infect and cause disease in, and other quite closely related viruses, such as the transmissible gastroenteritis virus of pigs, prefers to grow in pigs. This puzzled me for a long time. I was interested in mouse hepatitis virus and the human coronaviruses, and we have found that much of the species specificity of the initial infection depends upon specific receptor interactions. SARS coronavirus is the new one, and it should show Group IV over here, and we have no idea what receptor it may be using in humans or in its putative original host.

#### *Coronavirus Serogroups*

I would point out here that the three different serogroups of coronaviruses—these two are human coronaviruses and they cause colds, upper respiratory tract infection—have been very stable over a long period of time, causing 15 to maybe 30 percent of colds in people. There are no animal models. The reason for that is the specificity of this receptor interaction. The spikes that Paul has already showed you are characteristic of the coronavirus, and they are the delivery system for this virus into susceptible cells.

I would point out again that the RNA of the coronavirus that's inside this particle is infectious. So if we take the RNA of any coronavirus and transfect it into any host cell, it will cause infection.

#### *S Protein Characteristics*

In fact, it is the spike protein, and sometimes the hemagglutinin protein, this small yellow one here, which determine what cells can actually be infected with the virus. So these are determining the restric-



tion of host cells.

What I am going to talk about here is mostly the spike protein and how it delivers the viral nucleocapsid in the RNA into cells, and to point out here the hemagglutinin esterase glycoprotein is only present on Group II viruses and is not on SARS. The spike protein has been shown to be a virulence factor in many different coronaviruses. The tip of it determines the specificity of binding to receptors, and we'll talk a bit more about that, and the membrane fusion and the cell-to-cell fusion activity resides in this S2 membrane-bound portion.

Between S1 and S2, for many of the Group II viruses, there is a protease cleavage site, which is essential for infectivity and also cell fusion. Other viruses don't necessarily have that. It's very interesting, and I think Linda Saif will have more to say about this, that sometimes deletions in the S1 region, this receptor binding region, while not changing the receptor specificity of the virus can change the tissue tropism of the virus.

We have recently shown that the conformational change induced by receptors in this spike protein can also be induced a high pH of 8. This is important, because one of the pHs where coronaviruses may find themselves in the small intestine is, in fact, alkaline like that. The virus is neutralized by antibodies to this spike protein, both to S1 and to S2, and so it's a major target for vaccine development.

### *Spike-Host Interaction*

These are the steps in the interaction of the virus, which should be up here. This is the virus envelope shown here, and the nucleocapsid inside the virus. Here's a great big spike protein. Sometimes the first thing that interacts is the spike protein of a virus interacting with some molecule that has a lot of sugars on it and that may dock it onto the host cell membrane. In some cases this can be done by a different protein, such as the hemagglutinin esterase for coronavirus. It's not clear if the hemagglutinin esterase is sufficient to cause entry of a coronavirus into cells. It looks from many studies as if it probably is not, but it's certainly important for this docking to host cell membrane.

Then the virus interacts with a specific receptor, a protein-containing receptor. Binding can occur in the cold, but if you warm this reaction up and allow the bound virus to interact at 37 degrees, you get a major conformational change in the spike protein; in some cases, not a coronaviruses known yet, this may allow interaction with a co-receptor. We have no evidence yet for a co-receptor in coronaviruses. There are changes also in the receptor as a result of this inter-reaction, and that leads to fusion of the viral envelope with the host cell membrane and delivery of the nucleocapsid into the cell.

We're all familiar with this from influenza, and this is drawn to scale, the fusion domain of the influenza HA2, the gp41 of HIV, and so on, and here is the coronavirus fusion domain. I just point out that Paul's already shown you how big the RNA is; the proteins are also enormous. There are probably many interesting variations on a theme of how these work, but I think it's the same thing with two heptad repeats and conformational change.

### *Host Receptors and Specificity*

This slide shows the phylogeny of the nucleocapsid genes of coronaviruses, and nicely groups them into Groups I, II, and III. If SARS were on here it would probably be in a different group. The two human coronaviruses are quite distantly related. All of these viruses in Group I share the same receptor, and that's a different receptor than what we found for mouse hepatitis virus. We don't know the receptors for the avian coronaviruses or SARS.



The receptor for the Group I viruses is aminopeptidase N. You may say, "I thought you said they were species-specific." They are. The only receptor we know for Group II viruses is CEACAM1 of mouse, and also 9-O-acetylated sialic acid, which is the thing that is bound by the hemagglutinin esterase.

This is the CEACAM1A that we recently saw the structure of; it's an immunoglobulin superfamily member that projects from the membrane, and the virus actually binds right here to this projecting region. The viral spike glycoprotein would be coming down from the top of this screen. Why does it bind to this particular molecule? It really binds here because it's available on the surface of the intestine and the respiratory tract, and it has this wonderful projecting available loop to bind to. It has a life of its own. It's a signaling molecule; it's important in homophilic adhesion and tumor cell suppressor activity; it's important for angiogenesis. Interestingly enough, it's also a receptor for Hemophilus influenzae, the human CEACAM1 is, and it is a receptor for the Neisseria, the pathogenic strains. It is an opportunist thing to pick up this as a receptor.

If you were looking down from the top of the receptor, this is what you would see as a virus. We've recently been making mutations in all of these charged residues and this hydrophobic residue, and trying to understand the molecular basis of specificity. Different strains of virus care more if there's a charged thing here than here, and so on. Even though they all use the same receptor, the bottom line is that they use it in subtly different ways, and that can lead to some very interesting differences in the biology of different strains of the same virus in the same host.

Aminopeptidase N, or CD13, is also a huge glycoprotein. This is a 150,000 molecular weight enzyme, and that's the receptor for the Group II coronaviruses. Again, it's in the surface of the enteric tract, which is a target for many coronaviruses, and also in the respiratory tract. It's important in neurology, in immunology, and in tumorigenesis, and the viruses have picked up the ability to use that as an entry mechanism.

This is human coronavirus, it binds to human aminopeptidase N. The porcine coronavirus binds to pig aminopeptidase N, and they can not use each other's receptors. That specificity is amazing because this aminopeptidase N is very highly conserved among different species. Dave Wentworth in our lab recently showed that it is a single glycan that's present in the pig aminopeptidase N that makes it impossible for the human virus to use it as a receptor, a single sugar. So as the hosts are evolving with their virus, the virus is adapting to changes that may occur, putting in a sugar, for example, into the receptor, and the spike has to adapt to that, and that's what causes the specificity.

### *Changes Needed to Jump Species*

That also points out that many of these viruses have probably been with their hosts for a very long time. The questions that we have are, How much change does there need to be for a virus to jump to a new host, using a new receptor system? Down here, this is the feline coronavirus, and it uses feline aminopeptidase N, and Dina Drossin showed that all the Group I coronaviruses can actually use feline aminopeptidase N. That is perhaps the original aminopeptidase N that these viruses all bound to, and then they specialized more and more to efficiently infect their human, porcine, or canine hosts.

How many mutations does it take in a spike protein to allow it to jump to a different host? I don't have the answer for that, but we're beginning to look at this. These are different strains of mouse hepatitis virus—there are more than twenty strains with different tissue tropisms and virulence mechanisms—and we're looking at the receptor-binding domain of the spike protein here. The neurotropic strain is shown



on the top, and you can see that there are three regions identified by Taguchi which are conserved among all coronavirus spike proteins. You might think, Ah, those are the ones that bind to the receptor. But apparently they're not, they're probably ones that are important for the structure of this.

We have recently been looking at other regions here, and I have starred them. What we have done is to put all the mouse coronaviruses up here, and then rat, bovine, and human coronaviruses in Group II, and these starred ones are amino acids that are conserved in all mouse strains and different in the rat, bovine, and human Group II coronaviruses. There are not too many residues like this in the receptor-binding domain, so we have made mutations in these. Using the elegant technique that Paul Masters described, the targeted RNA recombination, in this huge genome it's possible to put a single amino acid substitution into the spike protein and say, What does it do to virus infection? This is the wild type, and what you're looking at are viral antigens in the cytoplasm of infected cells. These are the new recombinant viruses that have the single amino acid change. Here's one with two amino acid changes, and it can get in but it can't spread. So these are critical amino acids, and we are about to put these into other species and see if they in fact have gained host range. We know other examples of mouse viruses that do gain extended host range upon mutation, and some of them confer tremendous ability to fuse.

### *A SARS Coronavirus Natural Host?*

What is the natural host of the SARS coronavirus? You see a lot about this in the popular press, and in fact when the genome came out reporters called me up and they said, "Can you tell what the host is?" No. I have trouble with one virus. But it is a very important question, because the question is whether a virus that was in a host can infect both that host and humans, in which case you have a reservoir and SARS could reemerge again, or whether it was a mutation that lost the ability to grow in its original host and now it can grow in humans. Then, if the epidemic can be contained, it's very unlikely that the same mutation would arise again.

I put a candidate up here, and this is from a recent National Geographic magazine. This is a tiny mouse-like creature that was recently discovered in China, and it is a relative to some of the higher primates because of the structure of its brain and its jaw and so on. The point is, we don't know, but it is very important to know, and for those interested in evolutionary biology it will be fascinating to see how the interactions occurred, and how the virus may have changed during that process.

### *Human Coronavirus 229E Infection*

I just want to talk for one slide about human coronaviruses, and then talk about the targets for antiviral drugs. Human coronavirus 229E has been so innocuous and so stable for such a long time, and it is a problem. It's a major target for the drug industry, not for virus-specific drugs but for things to alleviate symptoms. People have done studies in volunteers, so they just give this virus intranasally and look what happens. Nothing bad happened except many of them got colds.

Here's an example where eleven people were inoculated intranasally with 229E, and then they recorded their symptoms and looked at the nasal epithelium. Many of them didn't have symptoms, a couple might have, and four had definite symptoms. These are their symptoms. All of them, however, had disruptions in the nasal epithelium. Human coronaviruses can infect again and again and again in the respiratory tract, so the same volunteer could get the same virus again if inoculated at once.

This is a human-only virus, so that it's been impossible to have an animal model for infection with this. The importance of being able to use an animal model for SARS cannot be overemphasized, nor can the wonderful luck of having been able to grow this virus in monkey cells. I understand it's still been very



difficult to grow it in human cells. Those are very good, wonderful things that will help to solve this problem.

### *Targets for Drugs and Vaccines*

This is just a summary of what Paul has shown you before, emphasizing what are the targets for development of vaccines and drugs against coronaviruses. Here's the virus particle, and the spike protein is a very good target for vaccines because it will raise neutralizing antibody. Inhibitors of fusion that block the conformational changes in the spike are also good targets. There are some of those that have worked now for HIV, so people are trying very quickly to see if they can develop such inhibitors for SARS. Receptor blockade—we have monoclonal antibodies that block infection with the mouse virus in vitro and in vivo in a mouse system, and also ones against the 229E receptor, aminopetidase N. Those might be developed as the receptor blockade for people who were exposed.

Uncoating is an interesting step, but one of the biggest targets is going to be protease inhibitors that prevent the polyprotein processing that Paul Masters told you about. There's another step once these big polyproteins for polymerase have been processed, and that is that they assemble into a unique polymerase complex, which is a membrane-bound complex that sequesters that huge RNA and has pieces of the polymerase on it and makes these proteins. There may be ways to inhibit this process, which seems to be unique to coronaviruses, or inhibit budding or exocytosis and maybe even virus release by inhibiting secretion. There are a number of targets where this virus may be vulnerable, and if drugs are developed or vaccine strategies, I think drugs, particularly these protease inhibitors, will be applicable not only to SARS but also to a large number of important diseases of animals, which is what Linda Saif is going to talk to you about next.

## **Coronavirus Biology and Pathogenesis: Coronavirus Transmission and Persistence**

Linda Saif, Professor, Food Animal Health Research Program, Ohio State University, Wooster

### *Porcine Coronaviruses*

**Scott Hammer:** Thank you very much, Kathryn. The next speaker is Linda Saif who is a professor at Ohio State University in the Food Animal Health Research Program. As was mentioned, she is going to talk about coronavirus transmission and persistence. Linda.

**Linda Saif:** I'd like to thank the organizers for inviting me. It's going to be very difficult to cover all the veterinary and animal coronaviruses in 10 to 15 minutes, so I am really going to focus on the ones that we've worked the most with, that are associated with enteric and respiratory infections.

The first group of viruses I want to define, because many of you will not be familiar with these terms, so that you know what these stand for. I am going to talk about the porcine coronaviruses. The ones that cause enteric infections are transmissible gastroenteritis virus, which can infect all age groups of swine, with the highest mortality in baby pigs. A more recent virus that's emerged, the porcine epidemic diarrhea virus, wasn't previously known before 1978; it was first identified in Europe and then it spread in the 1980s to Asia. It causes a syndrome in baby pigs very similar to TGEV; it was hard to differentiate until it was then discovered that this is a new virus in the Group I family. Fortunately, at this time we don't have this virus in the United States. After the virus emerged, it became endemic in the swine population, and now it affects mainly older pigs, greater than three months.

Among the respiratory infections, the main virus is the porcine respiratory coronavirus. This was identi-



fied in 1986 in Europe, and then independently in 1989 in the USA. Interestingly, these two emerged independently, because they have different S gene mutations. This virus, from all the analysis, is thought to be an S gene deletion mutant of TGEV, with 621 to 682 base pairs missing near the end terminus. This virus again can infect all age groups, but one- to three-month-olds are the ones that show the most disease.

The other interesting aspect of this is, although TGEV can cause both epidemics as well as endemic infections in the United States, since PRCV occurred in Europe, most of the TGEV infections are not recognized anymore. In other words, it appears that the PRCV has displaced this virus in Europe, but this doesn't seem to be totally the case in the United States.

You've seen many pictures of these coronaviruses. This is a typical picture of the Group I coronaviruses that don't have the hemagglutinin. What you can see here is the spike protein, schematically shown in the yellow. I wanted to mention, we did design specific primers in RT-PCR and nested PCR assays to detect this virus by using the sequence in this area. Because parts of this are deleted in the PRCV strain, we can actually use this test to both detect both of the viruses, as well as differentiate them.

There are two models, I think, for respiratory and enteric coronaviruses in animals. PRCV is the S gene deletion mutant of TGEV, but they're the same serotype. You can not differentiate them by way of neutralizing antibodies. That epitope has been preserved on the spike of both viruses. TGE infects the small intestinal villous enteric sites, and occasionally the upper respiratory tract; it's not exclusively an enteric infection, although it's mainly the upper respiratory tract that is infected. It induces villous atrophy. This is a low magnification of the small intestine. When it infects these cells, the brown cells here (this is immunohistochemistry) are actually infected by the virus. When it infects these it causes these cells to lyse, they're destroyed. It's a cytolytic infection. Then you are missing these long fingerlike villi, so this leads to vomiting and diarrhea and a severe dehydration malabsorption syndrome.

The PRCV infects epithelial cells of the upper and lower respiratory tract. This is a picture of the bronchial of the lung, and you can see here the brown staining is the PRCV-infected cells. These are the respiratory epithelial cells and a few unidentified cells in the small intestine. Interestingly, even though these have the same receptor that Kay talked about, this virus does not replicate in the intestinal epithelial cells, so we think there may be a co-receptor for TGE to be able to infect these cells. With the PRRS virus, the few infected cells that you see are actually in the lamina propria, and not these epithelial cells. It does have a different pathogenesis by losing this portion of the spike gene. It causes anywhere from moderate to subclinical respiratory disease, but, interestingly, even though animals sometimes don't show overt clinical signs, sometimes when you euthanize these animals you still see interstitial pneumonia associated with this virus. This virus replicates to very, very high titers in the lung.

### *S Gene Deletions in PRCV*

This is to give you some perspective about where these deletions occur. These are the TGE virulent viruses, the viruses that cause intestinal disease at the top, and then we see various strains of PRCV that have been isolated from pigs, and the S gene has been sequenced. This shows the antigenic sites on the S gene; I am going to refer to these later, so I am just going to mention these here as well. What's deleted in this region here are the C and D epitopes, shown here. What remains here are the immunodominant epitope, site A, which neutralizes antibody. This site is present, so we still can get neutralizing antibodies with PRCV that will neutralize TGE virus.

You can see with the field strains there are various-size deletions of the S gene, so it's not all uniform.



We don't really understand how this is occurring, but there is a hypothesis that some of these viruses that have the shorter deletion are actually more virulent than the viruses with the longer deletions.

The other region that's changed here is in the nonstructural protein gene, open reading frame 3 and 3-1. For all the PRCV strains that have been characterized, there are changes in that region as well. We're not sure whether or how this region contributes to virulence or pathogenicity.

### *Bovine Coronaviruses and Features*

The bovine coronavirus I want to show, because this is in the second group, and these are the ones that Kay talked about that have the HE protein on the surface, that is actually a hemagglutinin. I think if you look at the electromicrograph of these you can actually see that on some particles you can see the shorter surface projections. These are actually the HE, and the longer ones are actually the spike. This is immuno-M; it's been reacted with antiserum, so you can actually see this fringe around the particle from the reaction with antiserum. The antiserum does bind to these spike proteins and neutralize it to keep them from infecting the cell.

When we set out to find the various bovine coronavirus strains associated with the various disease syndromes I am going to show you, we found that the best region to use in terms of primer design for RT-PCR or nested PCR was the N gene, because there are highly conserved regions here. When we designed these primers, we found we could pick up both respiratory and enteric strains of bovine coronavirus.

Bovine coronavirus, interestingly, is associated with four clinical syndromes in the field. It's associated with enteric infections—a major cause of calf diarrhea, just like I showed with TGE—causing diarrhea, dehydration and intestinal villous atrophy in a similar manner. But this virus also infects not only the small intestine but the large intestine, and the target age groups when this commonly occurs are shown here.

The other syndrome that probably non-veterinarians are not aware of at all, but we're quite aware of this syndrome, is the winter dysentery. I don't know if you can see, this is an adult dairy cattle. It's quite common in the winter months in dairy cattle, and they actually develop a severe watery bloody diarrhea syndrome with this virus. For years the cause of this was not known, but in 1990 we were able to isolate this virus and reproduce at least part of this disease in adult cattle using the bovine coronavirus isolate. In animals that had been post mortem, it is associated with villous atrophy, but we also sometimes see an upper respiratory infection. This usually occurs in the older animals. The respiratory infections were recognized as the cause of calf respiratory disease in calves anywhere from two weeks to six months. The other thing that's very similar in terms of some of the aspects to SARS is the bovine respiratory disease complex, or shipping fever. This occurs in young adult cattle, and can also occur in adult cattle. This occurs in feed lot cattle, and I am going to talk some more about some similarities of this syndrome with SARS. This causes cough, as you can see here in nasolacrimal discharge, and often pneumonia.

It's interesting that all these bovine coronavirus isolates, just like all the TGE and PRCV strains, belong to a single serotype, even though they display these different disease syndromes, with two subtypes. They are, in our experience, pneumoenteric, meaning that the same strain can cause both enteric and respiratory infections. It's not like PRCV that mainly infects the respiratory tract, or TGE that mainly infects the intestines; these can infect both. When we tried to look at whether there were sequence differences between the respiratory and enteric strains of bovine coronavirus, we could only find point mutations in the S gene.



### *Infected Tissues and Shedding*

The issue is what tissues do these coronaviruses infect, and I think what you'll see here is the full spectrum in terms of enteric versus respiratory. The PED infection has been defined as purely intestinal, does not infect the upper respiratory tract or cause viremia. These are all compared here with the recent report about SARS in the macaque. The SARS from this report was isolated from upper respiratory tract, lower respiratory tract, and one out of four monkeys also shed virus in the feces.

There can be two ways that virus is shed in the feces. One is by infecting these intestinal epithelial cells, in which case we'll usually get very high titer shed in feces. The other scenario happens with PRCV that infects only a few cells in the intestinal tract, but occasionally it can be shed in feces, and under certain conditions it's more commonly shed in feces. We think this is due to large amounts of the virus being swallowed. Which scenario are we seeing with SARS? I think the answer is we don't know at this stage, but I think it'll be important to find out in terms of vaccines and pathogenesis.

TGE replicates mainly in the intestine and the upper respiratory tract. There is little evidence of lower respiratory tract infections. The PRCV is mainly in the lower respiratory tract and upper respiratory tract. I want to make a note here that there is viremia, and this virus can replicate in alveolar macrophages as well. There's not evidence that it infects other organs, even though it does cause a viremia.

Interestingly, the vaccine strains that have been developed are intermediate between the wild type TGE, the virulent strain, and the attenuated strain. This virus, then after passage—these are usually passaged over a hundred times in cell culture—has acquired more of an upper respiratory tract tropism than an enteric tropism. This was what Kay was mentioning: there are actually two point mutations that appear in the spike protein gene that appear to be responsible for going from an enteric to a respiratory tropism.

The bovine coronavirus, enteric and respiratory as I mentioned, seems to be more pneumoenteric. We've identified 42 amino acid changes in 38 different sites, with 5 or 6 clustered changes. At this point we don't really know what that means in terms of the phenotype.

### *Animal vs. Human Respiratory Coronaviruses*

How do respiratory coronaviruses in animals compare to those in humans? We compared the various parameters of PRCV versus the respiratory bovine coronavirus, and we can see that these induce similar clinical signs to what's being recorded in SARS patients. These viruses infect similar tissues, except we and others have documented that PRCV can infect alveolar macrophages. We actually haven't looked at these for bovine respiratory coronavirus.

The pathology is similar: interstitial pneumonia, bronchiolitis, alveolitis. The shedding periods are similar, but when you look by nested RT-PCR you see almost a three-week shedding period compared to infectivity assays or lysal tests. As I mentioned, the fecal shedding of the PRCV is variable in pigs.

### *Exacerbating Infection and Shedding*

What factors then exacerbate respiratory coronavirus infections or virus shedding? There are a number of ones that have been studied in animals. Aerosol is one factor. When animals were exposed by aerosol versus intranasal inoculation, several studies have suggested that virus is shed in higher titers in the nasal secretions, that there's a longer shedding period, and there can often be more severe respiratory disease.



The dose is important. Studies that were conducted with a graduate student in my lab, Dr. Van Cott, showed that a higher dose led to high titers being shed in nasal secretions and longer shedding. A Canadian study showed that pigs given a very high dose of PRCV had more severe pneumonia and deaths than pigs exposed presumably to lower doses by contact.

### *Co- or Sequential Infections*

The other factor that is important in terms of SARS is this question of whether there are concurrent or sequential respiratory viral infections, and whether these exacerbate the SARS coronavirus infections. What I want to show here is a precedent for that in animals, a recent study conducted by a master's DVM student in my lab, Dr. Hayes. In this study, he infected pigs with a porcine arterivirus which in the Nidovirales order, which is the same order as the coronaviruses are in. He gave them this PRRS virus first, and then PRCV after five days. What he found in this study was a longer shedding of PRCV after the dual infections, and the fecal shedding of PRCV was more common after the dual infections. We don't understand the reason for that at this time, but we can speculate on several hypotheses. He also found—also the group in Belgium, Van Reuth and Pensaert, found—that there was prolonged fever, respiratory disease, and reduced weight gain after these dual infections.

This shows a normal pig lung tissue; this is what the tissue looks like after PRCV infection, and this is after the dual infection. I want to point out there is a lot of infiltration here of monocytes in the lung. They are some of the major lesions that are seen, as well as some inflammation in the lungs.

The other study I think is quite pertinent with what we're seeing happening the world today is the study from Van Reuth and Pensaert in Belgium that showed if they gave PRCV first and then they infected pigs with swine influenza two days later, they had severely enhanced respiratory disease compared to either virus alone. Often it's hard to reproduce some of these respiratory viral infections in an animal model, so this has relevance because recently avian influenza outbreaks have been diagnosed in the Netherlands, so I think we can imagine what might happen if both of these viruses co-infected people together.

Other factors have been defined from animal studies. These, again, were mixed infections, dual infections with PRCV, or with PRRS, or with PRCV followed by bacterial LPS in five days. These animals were found to develop more severe respiratory disease after LPS exposure and enhanced fever, compared to pigs inoculated with each agent alone. Again, this may have relevance to SARS cases if there are secondary bacterial infections.

### *Corticosteroid Treatment*

A number of SARS patients have been treated with some of these corticosteroids. I'll just show two examples. These are not relevant in terms of the respiratory component, but in terms of the enteric component. There's a paper that suggests the administration of dexamethasone could enhance the severity of TGE infections. In some studies we conducted, trying to reproduce winter dysentery in cattle, we found that if we first established the infection and then treated these animals with the dexamethasone, in one of four animals after treatment there was a reoccurrence of fecal bovine coronavirus shedding.

### *SARS Similarity to Shipping Fever*

I mentioned I'd also talk about some shipping fever similarities. There are factors that seem to exacerbate bovine respiratory coronavirus infections. First we found when we did epidemiologic studies a risk factor was if calves had lower serum antibody titers—these were neutralizing titers of less than 400,



because bovine coronavirus is prevalent in the cattle population, so most animals have at least some antibody to them—the animals with the lower titers were more likely to be infected and develop the disease.

The second thing, these animals are essentially raised as young animals at one farm and then they're shipped to large feed lots for fattening. There is a stress factor when they're shipped long distances from one farm to a large feed lot. We're seeing some of these SARS cases, especially in patients that traveled recently and come back with the stress of travel, so we see something a little bit similar to this. The other thing that happens when these animals arrive at the feed lot is they're commingled together. Once you have animals arriving from different locations and commingling, you have more disease outbreaks. It might be that there are different coronavirus strains circulating, and they sort of interchange some of these strains, so they're actually seeing some new strains that they haven't seen before that can cause disease.

The other thing that's very well established with the bovine respiratory disease complex, or shipping fever, is the impact of other concurrent respiratory infections and bacteria. This complex involves both other viruses and bacteria; there's a synergistic effect to produce the pneumonia.

### *Reinfections with Coronaviruses*

Do coronaviruses cause reinfections? I won't go through all this data, but the bottom line for most all of these is yes, as Kay pointed out. Interestingly for PED, the reinfections were symptomatic, but for the bovine coronavirus and the PRCV they were not symptomatic. I think the take-home message here is that respiratory coronaviruses commonly cause reinfections, but in our experience with animals they're usually mild or subclinical after the primary infection, although there can be exceptions to this.

### *Crossing the Species Barrier*

Kay alluded to this issue, and I am going to show two pieces of experimental data that we did to address this issue: Do coronaviruses cross the species barrier? Is there a wildlife reservoir for coronavirus? We observed bovine-like coronaviruses from captive wild ruminants, and we actually were able to isolate these in cell culture. Then we took these viruses, and we have a [inaudible] calf facility. These animals are derived germ-free, so you can see the effect of a single organism in them. We inoculated these calves with the enteric coronaviruses from the captive wild ruminants. The bottom line after calf inoculation was all of them were transmissible and caused diarrhea, fecal shedding, and seroconversion to bovine coronavirus. Our conclusion was that coronavirus from wild ruminants can experimentally infect young calves, so we have this potential reservoir.

The other thing that's even more dramatic is the transmission from a mammalian host to an avian host. These studies were recently done collaboratively with my husband's research group with Dr. Ismail, a graduate student. We have the bovine enteric coronavirus strain that had been maintained by seropassage in calves. This virus was never isolated from any poultry, and the original isolate was from cattle that had no association with poultry. We're pretty certain that this is actually a bovine coronavirus. We took one-day-old turkey poults (this means baby turkeys) or chicks, and they were inoculated at one day of age with the enteric bovine coronavirus. You can see that the turkey poults developed diarrhea, had fecal shedding, and seroconverted to bovine coronavirus. In the chicks we could not show evidence of infections. Our conclusion here is that bovine coronavirus can experimentally infect baby turkeys, so we have the issue of whether there could be cattle-to-bird transmission.

Vaccines in Animals



The last topic I was asked to address are about vaccines for animal coronavirus. Obviously, this is a huge topic and I won't be able to do this justice, so I am just going to focus mainly on our experience with TGE and PRCV.

Among the vaccines that have been developed, the target population for enteric TGE and the enteric bovine coronavirus is the suckling animal. The major focus here is on passive immunity. The current vaccines are designed to immunize the mother to transfer passive antibodies via the milk to the suckling offspring. This is in monogastrics such as pigs—and humans are also monogastrics—which secrete primarily secretory IgA antibodies in the milk. This type of vaccination can be accomplished by exploiting the common mucosal immune system. Because we know that neutralizing IgA antibodies to TGE in milk are a correlate of protection to TGE, oral attenuated TGE vaccines have been designed to induce IgA antibodies in milk by stimulation of the intestine of the mother and exploit the common mucosal immune system.

For PRCV infections, unlike TGE, it's interesting that we found they only induce partial immunity to TGE, so there is some compartmentalization to the common mucosal immune system. But then in studies conducted by a graduate student, Dr. Sestak in my lab, we found if these sows were monoclonally infected in several different pregnancies with PRCV, this would induce higher IgA antibody titers in the milk and higher protection rates. We think that, in order to exploit the different mucosal components of the common mucosal immune system, we may need to use repeated immunization at multiple sites to stimulate enough memory cells to have an impact on the infections.

What are the problems with the existing vaccines, because they only induce partial protection in pigs? A number of problems have been defined. The attenuated vaccines given to pigs intranasally or orally, we found, as I showed you, replicate poorly in the intestines. A few IgA antibody-secreting cells are induced, leading to low IgA antibody titers in the milk. When this has been tried using killed TGE vaccines and giving them by conventional parental routes, like intramuscularly, they only introduce IgG antibodies in the milk. These IgG antibodies were not protective.

Our experience with working with rotavirus vaccines—now we've started some projects with calicivirus vaccines like Norwalk-like virus—is it is quite difficult to find intestinal immune responses except by using high doses of live vaccines. It is much easier if the animal's been naturally infected or effectively primed to boost mucosal immune responses. Another problem with the commercial vaccines is the dose of virus in the vaccines apparently was too low to stimulate adequate IgA antibody responses.

We've also tried attempts to active immunity, because these infections are also common in animals after weaning. The take-home message that I want to show you is that the TGE virus, the virulent virus, stimulated very high IgA antibody-secreting cell numbers and lymphoproliferative responses as a part of cell-mediated immunity in the intestinal tract. It did not stimulate many antibodies in the respiratory tract, even though we have this common mucosal immune system. When the animals were challenged, they were protected against both diarrhea and highly protected against both nasal and rectal shedding compared to the controls.

When we used the respiratory coronavirus, interestingly, we got very few IgA producing cells in the intestine; mostly IgG and lymphoproliferative responses in the respiratory tract. These animals showed partial protection against diarrhea and complete protection against nasal shedding, and we think this is due to the fact that at four days post-challenge, we saw a huge anamnestic response with a great increase in IgG and a lower increase in IgA antibody producing cells, which we think led to this partial protec-



tion. I think this has a message in terms of vaccine development.

I am going to go through this very quickly, but I think there will be some interest in this, because this is actually attempts to immunize animals to develop active immunity using the TGE spike glycoprotein. Initially this was produced by a recombinant baculovirus system, and we looked at the spike glycoprotein alone, or with the N and M protein, the structural proteins of the virus. What you can see is we made three constructs of this; two of them had all four major antigenic epitopes, with the A being immunodominant, and the third construct only had a fragment of the spike gene without the A or B epitope.

When we immunized and we used a subcu route. We gave two doses before challenge with incomplete foynes oil adjuvant, and then we looked at these for neutralizing antibody titers. The two with the immunodominant epitopes did have neutralizing antibody titers, but not this group [using the third construct]. We challenged these and 100 percent of these animals came down with diarrhea. There was no protection by administering the spike protein by a parental route, no protection against the enteric disease.

We did this, then, by combining the three major structural proteins of the virus and administering this interperitoneal, because there is a commercial vaccine that's licensed for this route. This time we put in a more potent mucosal adjuvant. This is a mutant LT heatlabile toxin from *E. coli* that's essentially been engineered so that it's not toxogenic. This was obtained from Dr. John Clemens at Tulane. This raised neutralizing antibodies, it raised IgA responses in the mesenteric lymph node, and there was again partial protection against fecal shedding. The virulent virus, you can see, raised more IgA and IgG antibodies in the intestine and complete protection.

I think the conclusion here is that systematic S vaccine given subcu with incomplete foynes did not induce protective immunity to TGE, but when we combined these with other proteins of the virus, giving it IP with a potent mucosal adjuvant, it did induce partial protection against TGE shedding. If we look at the literature, there is precedent that in vitro studies suggest that both recombinant N proteins with the T-cell epitopes and S proteins may be required for maximal antibody responses.

### *Conclusion and Summary*

Finally, my concluding remarks are that enteric coronavirus alone can cause fatal infections in young animals. However, the respiratory coronavirus infections that we've seen are more often fatal in adults when combined with other factors. An example here is shipping fever in cattle. But remember, these infections are occurring in animals that have some preexisting antibodies, so it's not a naïve population like we're experiencing with SARS.

There are no commercial veterinary vaccines at this time to prevent respiratory coronavirus infections, except for infectious bronchitis virus infections in chickens, so vaccination for infectious bronchitis virus is done using either killed or live vaccines. But this is complicated in terms of the efficacy of this vaccine by the existence of multiple serotypes or subtypes of IBV, and there are many. The other thing that doesn't make this analogous to some of the other animal models is that only short-term protection is needed in the chickens because of their short life span. The live attenuated TGE vaccines, over a hundred passages in cell culture in our experience have been stable over time, so I think that's important to note in terms of these potential for live attenuated vaccines.

I hope I have convinced you that knowledge of SARS pathogenesis—how this virus is infecting, what



are the tissue sites for infection—with an appropriate animal model is extremely important to design effective vaccines. We really need to know what type of infection we're working with. Is it systemic, is it localized respiratory, is it respiratory and enteric? I think I have shown you that priming for mucosal immunity in naïve subjects is difficult, especially using non-replicated or killed vaccines such as the spike protein, but boosting may be more effective. I think others may speak about this, but the influenza literature suggests that IgG antibodies in the lung, either serum-derived or locally produced, may prevent pneumonia, but on the other hand IgA antibodies may be essential to prevent the upper respiratory infections and shedding. Thank you.

## **Coronavirus Biology and Pathogenesis: Technology in SARS Discovery**

Thomas Ksiazek, Centers for Disease Control and Prevention, Atlanta

### *Early CDC Involvement with SARS*

**Scott Hammer:** That was simply terrific. I think we are impressed by that, and we want to congratulate Linda for being named a fellow of the National Academy of Sciences just two weeks ago. I think you can see why. The last talk in this sequence is from Thomas Ksiazek, who's the acting chief of the Special Pathogens Branch at the CDC, who's going to talk about SARS coronavirus discovery and what we've learned from this. Tom.

**Thomas Ksiazek:** I want to thank Ian and the rest of the Academy for inviting me. What I am going to try and do is tell the story of discovery of this, and try and perhaps leave some time for a little discussion in the panel about how technology can be applied. This is certainly a story of the mix of old and new technologies, and there's a lot of serendipity and hard work involved in these things. So let's see.

The story at least for us at CDC, as these things usually do, begins on a weekend. People know the story of this, probably perhaps some better than I, because there had been something going on in China for quite some time. On a weekend there was some suggestion that what was thought perhaps to be linked to what had been going on in China had popped up in Hanoi, Vietnam. In this particular instance there was some direct evidence of transmission from hospital cases to hospital workers, and perhaps among the staff. That story played itself out as very important, and perhaps the conclusion of that has been successful in that Vietnam now apparently has no further transmission.

Because of the H5 M1 story, and a lack of knowledge of what was going on in China, the Flu Branch had the primary action on this. We became involved because of the evidence of person-to-person transmission in preparing a member of the Flu Branch, Tim Miyake, to take off for Hanoi. Keiki Fakuda was already in China, and soon went to Hong Kong because of the way the story was playing out. Our branch normally becomes involved in hemorrhagic fevers and other things that have person-to-person transmission as an important component, or at least a potential component. I think that's probably why we initially became involved.

### *Determining SARS Etiology*

The etiology of this, of course, was at the time unknown. I think there was again some suspicion that it was H5 M1, and one specimen from the index case in Hanoi did make it to the Influenza Branch for some antibody testing to rule in or rule out H5 infection. It very soon became apparent that there was a paucity of real diagnostic specimens upon which to try and determine what the etiology was. One of our chief goals was trying to attain some useful and pertinent materials to do some of this. I should say, perhaps by way of introduction, that we've done this a few times before with other new diseases, such as



the hantaviruses in 1993 here in the United States, and Nipah virus in 1998 and '99 in Malaysia, so there's sort of a litany to this that makes some sense.

Very often there's a very critical role that pathology plays in terms of setting the direction that one proceeds, and our group, Special Pathogens, and the remainder of DVRD works very closely with infectious disease pathology activity that can help frame the direction of one of these investigations by performing not only specific diagnostic tests, but also by looking at the pathologic process and using that as a guide to where one wants to go in terms of the possible agents, and which tissues to focus on, etcetera.

This is a photograph showing Dr. Zaki and his associates Dr. Shieh and Chris Paddock. Pat is one of the cytotechs that's very important because she does most of the immunohistochemical staining in the branch.

The etiology itself, once we obtained some materials, the general approach is that of applying a broad-based mix of both classic and molecular techniques, and trying to focus on a list of pathogens that the initial clinical history, clinical laboratory, and sometimes most importantly the pathology findings tend to focus on.

### *Molecular and Classical Techniques*

In this instance there was at least some evidence that perhaps a viral etiology was involved. This is a lung from one of the early cases, showing the presence of some giant cells in the lung. The other thing that should be noted is that there's diffuse alveolar damage. This is a lung, not a liver; it doesn't look much like a lung. The presence of these giant cells, I think, convinced many of us that it was worth looking, or continuing to look, for the presence of a viral pathogen in this. This is just a close-up of that same view.

In this instance, the breakthrough really didn't involve any very elegant molecular technique. The first thing that happened at CDC was that we saw this in Vero E6 cells. There were a variety of different cell lines that were used, all of them targeted at different groups from respiratory viral diseases, because there was at least some suggestion that this bore some clinical pathology similarities to hemorrhagic fevers. We persisted in inoculating Vero E6 cells, a cell line that's often used for the isolation of hemorrhagic fevers, and this happened very early in the course with one of the patients that had been exposed in the Hanoi hospital. Here you can see normal, more or less, Vero E6 cells with a lesion in the rounding and death of cells in the middle.

This then led to the application of some more classical techniques. This is an electron microscope, and this is Cynthia Goldsmith, who works for Dr. Zaki's group, looking down the tube of an older electron microscope. This is what was actually the first key identification in our laboratory that this was a coronavirus, the presence in thin-sections of morphology and morphogenesis resembling that of coronaviruses. Negative stain was prepared around the same time, which has the characteristic look, although this one's somewhat damaged, of pictures you've seen in the previous presenters' photographs. This all happened over a matter of a couple of days, and the fact that this looked like a coronavirus then led to the application of some more elegant and modern techniques which could be applied.

This is actually something that was done a little bit later, that shows the presence of these same virions in clinical materials from one of the initial patients: a bronchial aspirate from a patient from Hanoi, showing the presence of the same virus we saw in the cultures I just showed you.



With the new information that this was a coronavirus, Dr. Dean Erdman and his associates designed a set of primers that would then be used initially on the virus material, and eventually as a diagnostic technique on clinical materials. This then allowed a small piece of the polymerase gene to be sequenced up, and I'll show you that. We also had collaboration with another laboratory, and RNA derived from both clinical materials as well as from the virus itself was sent to Dr. DeRisi's lab in San Francisco. Their findings confirmed essentially (this is Dr. Erdman) these findings. Essentially this virus, representing the virus isolate is, as others have pointed out, equidistant from the previously known coronaviruses and probably now should be considered Group IV on the coronaviruses. I think all these things in a sequence of two or three days are what suggested that this was a coronavirus, and indeed a unique coronavirus.

### *Possibility of Cofactors*

There were certainly other candidates, and I think the possibility still remains that cofactors may be involved in causing the disease that we call SARS. This was on or about the same time in Hong Kong, where paramyxoviruses, in this instance human metapneumovirus, was a leading candidate for the cause of the syndrome. I think that there certainly have been found human metapneumovirus in some of these patients, and indeed in patients here in the United States, but I think that the predominance of evidence in other laboratory-generated data now suggests that the coronavirus is indeed the etiologic agent of what we're calling SARS. I just hasten to add that certain human metapneumoviruses are pretty common infections. I think that we found them, but I think we have not indeed found any joint infections here in the United States, whereas in some of the other sites where they had access to many more patients that was in fact the case.

### *Looking at Cell Cultures and Tissues*

Some of the indirect evidence and some of the staining that also related these two coronaviruses was done by Dr. Zaki's group. In culture, although it doesn't seem to predominate, we do in fact see the formation of giant cells. Through the collaboration of some other groups who had worked on coronaviruses, we were able to obtain some hyperimmune and reference sera from other Group I coronaviruses that could be used to stain cell cultures. There was a pretty gallant effort to try and show that some of these giant cells, for instance in patients, still bore the antigens of the coronavirus. Unfortunately, I would suggest that the course of this disease is rather prolonged and the post mortem materials available are taken probably, in the instances that we've had access, after the twentieth day. Even in other respiratory infections, the antigens don't persist for that long a time. There has been, other than a few spare monocytes found in these tissues, a lack of ability to demonstrate specific staining, at least with the cross-reactive antibodies that we've so far tried.

### *Diagnostic Techniques*

Now the isolation of the virus led to the development of some specific diagnostic techniques. Other laboratories have done the same thing. This shows infected Vero E6 cells lit up, stained in an indirect fluorescent antibody, with a convalescent patient serum, showing the presence of the viral antigens in these cells. We developed, sort of in parallel to that, at the same time an ELISA test by extracting the antigens from infected cells, and that's currently what we're using, working hard on moving to express antigens. This virus grows quite well in E6 cells, and these are some of the first attempts to purify and demonstrate the protein profiles of the virus, which some of the coronavirologists in the audience will probably recognize as being somewhat similar in the M protein, the glycoproteins. I couldn't point out, from my own experience, the M protein. This is an EM of the gradient-purified prep, and the growth at a very encouraging rate and yield of this virus in these cells has made it somewhat simple to get some of the early diagnostic tests out.



This is an early Western blot demonstrating the response of some of the convalescent patients. It looks like, at least in the early response, the N protein seems to predominate, and that's being targeted in some of our early efforts to make recombinant proteins. Some of the later samples indeed do have what appears to be spike protein reactivity.

This is a rather complicated slide, but it just goes to show using the ELISA and the indirect fluorescent antibody, and using convalescent and acute sera from patients from a variety of sites in which there were outbreaks, that one could convincingly demonstrate that patients, if one had a sample taken late enough in the course, could demonstrate conversion from negative to positive. This is one of the means of demonstrating that they had in fact been infected, and that the diagnostic technique, either indirect fluorescent antibody or the ELISA test, has some value in diagnosis. We continue to use this in surveillance and are now in the throes of sending this out to state diagnostic labs in the United States, as well as some international partners.

This shows some of the early sites where we had either had panels sent to our laboratory, and were part of that table I just showed you, or where other serologic tests that we had provided to labs had been utilized to demonstrate the presence of patients.

We also were fortunate to have some collaborators who had done experimental or had derived known infections of both C43 and 229E infections in humans and were able to demonstrate that, even when titers were quite high to these human upper respiratory coronaviruses, they were unreactive with the new SARS coronavirus. In terms of doing at least some early demonstrations of the specificity, we found in fairly large populations of so-called normal sera that we could demonstrate essentially no previous infection in people who had not had any previous exposure to the SARS virus, or in the population of the United States. Both suggested that the test was pretty specific and also suggested that there had been no previous circulation as far as the population of the United States, and somewhat similarly I think the same thing has been demonstrated in Hong Kong.

### *Genome Sequences are Highly Similar*

Everyone knows that the virus has been sequenced in its entirety from a number of sites, and essentially the virus has been, in these various outbreaks, shown to be quite similar in sequencing, in the entire genome and earlier attempts to look at smaller pieces of the polymerase gene. It's been found to be essentially identical from location to location, although I note that in the popular press there's a lot of speculation about the variations of the virus, and I'd leave it to the Toronto virologist to talk a little bit more about that, perhaps in the discussion.

### *SARS in Cynomolgous Monkeys*

The disease, in an article that just appeared in Nature this last week, has been reproduced in cynomolgous monkeys, thus fulfilling Koch's postulates, a respiratory infection leading to interstitial infiltrates and disease in the monkeys having fulfilled a modern version of the postulates.

### *Coronavirus Agent and Detecting Antibodies*

This novel coronavirus in a variety of sites has been isolated, and I think is now generally accepted to be the etiologic agent for the disease we're calling SARS. The presence of the virus is noted in respiratory secretions and respiratory tissues primarily by a variety of techniques. The sequencing suggests this is sort of a common outbreak originating, I think now common knowledge, from China, probably late last year into the first part of the year, and then moving on through Hong Kong to a variety of sites.



Patients respond with measurable antibody to the new coronavirus by a number of techniques. I should mention that we have done some neutralization tests, and patients, including those who unfortunately succumb, also do have neutralizing antibodies, suggesting that it does induce, as one would expect, neutralizing antibody. There are a number of questions.

### *Origin of SARS CoV?*

Many of the previous speakers have already speculated on this, and I am sure we'll do some more of that: where did it come from? I think geographically everyone believes it's from southern China. More specifically, and we've seen tables of this, there's a variety of previously known coronaviruses from a variety of animal species. But this virus bears little in terms of its being closely related to those, so there's not a lot of hints given by that, so there are certainly other animal species. Most of these, as you'll note, are economically important or somehow husbanded by man, and therefore observed closely. There's a lot of wildlife species out there that probably people really haven't looked for coronaviruses in, and if I had the opportunity to throw my two cents' worth in, it'd be my bet, I guess.

Once again, I emphasize that the virus is not very closely related to the viruses that have been previously identified in this family of viruses. What we're still up to here in the United States is we have lots of specimens coming in from around the United States. We're attempting to get the diagnostics out there to be kinder to ourselves, I think, and also to make the diagnosis more rapid. I think that certainly the sequencing of the genome so rapidly is a good example of the use of modern technology, and that will certainly help in making diagnosis, treatment, vaccines, and everything else possible in the near future. With that I'll close, and thank you for the opportunity to speak with you.

## **Coronavirus Biology and Pathogenesis: Panel 1 Discussion**

### *Jumping Species*

**Scott Hammer:** We're running a little behind, but actually the session got started late. We don't want to miss this opportunity for a panel discussion, so we'll sort of back things up unless Ellis gives me the high sign to do otherwise. Can I ask the speakers from the first session to come up, and we'll start this discussion session? I have a couple of questions to get things started, and then I am going to open this up to the audience. There are microphones—where are those microphones?—for people to raise their hands, please stand up and identify yourselves. Let me get the ball rolling as Kathryn sits down.

Knowing that we're in the realm of speculation, and it's come up, is there a speculative consensus, that this was likely a trans-species jump, or do we really not know and should keep that question completely open? Kathryn?

**Kathryn Holmes:** It does not have enough similarity to any of the known viruses in its sequence to believe that it could have come from them. I think it's much more likely that it evolved quite separately.

### *Sequence Variation and Mutation*

**Scott Hammer:** How broadly has the molecular epidemiology of coronaviruses in animal species been done, particularly in the area of the world that we're interested in, as far as sampling of animals and full genomic sequencing? This is an open issue.

A question that Tom raised about sequence variation, and what, if anything, can be drawn from the couple of reports that have appeared about sequence variation in humans, and are there analogies with vet-



erinary populations? If you passage a virus in a particular host repeatedly, how much variation do you see? I guess the issue has come up as to whether we're going to see sequence variation in humans, are we already seeing it, and what does that mean for pathogenicity and ultimate escape from controls? This is the realm of discussion of speculation. No one is asking you for facts at the moment. If you've got facts, great, but really part of this is stimulation.

**Kathryn Holmes:** I feel comfortable speaking about the issue of mutation. I think one of the things that troubles me is that the press has focused so much on mutations, when this is, after all, an RNA virus, and they do have a fairly high frequency of mutation. The important thing is really the selection of the mutations that occurs, and I think what we've seen when people look at these viruses coming out from all over the world is they're really very, very similar. There are very few mutations. Importantly, none of the mutations has in any way been linked to any function. I think that genomics is one thing, but if you're going to talk about pathogenesis you really have to have functional evidence. I think that those mutations have been quite properly used for epidemiological markers, and that's helpful if they're trying to trace who gave which virus to whom, but I don't think yet we could say anything at all about their significance biologically.

**Scott Hammer:** Linda or Paul, do you have any additional comments on this aspect?

**Linda Saif:** Actually I would agree with Kay's analysis here. I think, like I showed with the differences between the respiratory and enteric coronavirus, these are scattered point mutations, and at this stage we don't know what that means phenotypically, in terms of disease expression or even tissue tropism. I think just looking broadly and saying that there are mutations in these different strains, that's to be expected, because we isolate a lot of animal strains and we see a lot of point mutations throughout the genome. But trying to translate that into a functional aspect, or a phenotype in terms of a commonality, I don't think we have the information to be able to do that. I think eventually what people are going to start doing is looking at what the range of mutations is in the antigenic epitopes, once those are characterized, and maybe the receptor areas and things like that, to see if there's any commonality. But right now I don't think we have enough information to put any picture together for that.

**Scott Hammer:** Paul, any additional comments?

**Paul Masters:** I don't think I have anything to add to that, except that obviously we need a good animal model, and that might provide a correlation between various mutations and pathogenicity.

### *Persistence in the Environment*

**Scott Hammer:** One other question I had before opening it up is the issue of persistence in the environment, for which we've seen some data presented, or at least spoken about, and a lot of speculation. What do we know from the current human coronaviruses or animal coronaviruses about the role of persistence in the environment, as opposed to organism-to-organism transmission? Basically, what does persistence mean for acquisition of disease, infection of disease, or is this an open area? Has this been studied in animal populations, environmental contamination versus close contact of one animal with another?

**Linda Saif:** Well certainly in TGE with fecal transmission, the virus is quite stable in the winter months in frozen fecal material. When you apply disinfectants, if it's sequestered in some of these specimens, it is much more difficult to disinfect. The virus is also inactivated by UV radiation, so in the summer the infection seems to spread less dramatically than it does in the winter months. So there's definitely a seasonality for the enteric TGE infections. The other thing with stability is reservoirs. The other possibility



that has been examined with TGE is that when there are open swine facilities, and there can be birds or flies and things like that, they can perhaps mechanically, but maybe by also ingesting and just shedding the virus again in feces, possibly transmit the virus to other farms. We have to think about possible fomites or mechanical vectors. I think a big issue is whether this is shed in feces so that you have the potential for sewage contamination and things like that, or is it really mainly just droplets? Then you'd have drying, and you'd have spread by droplets on surfaces, because there is some stability to the virus once it's dried onto the surfaces.

**Scott Hammer:** Any other comments on this issue? Tom, is CDC looking at persistence in the environment?

**Thomas Ksiazek:** Well, I think people have, in some early studies with persistence of the virus and trying to get a handle on whether it goes away in hours or days. It doesn't go away in minutes certainly, it lasts for probably a few days. I think probably the lesson learned is that disinfection practices and infection control in hospitals—and maybe Larry's going to address this more in talking about the epidemiology—remain important in trying to control this in the environment where we've seen it.

## Open Discussion

### *Antibodies and Protection to 229E or SARS*

**Scott Hammer:** Let's open this up to the audience. Please, microphones. Please stand up and identify yourself and then ask a targeted, single question so we can get egalitarianism here.

**Bing Lu:** My name is Bing Lu, I am an internist, I was previously trained as a molecular virologist. I have two scientific questions. One is regarding the bovine 229E strain that Dr. Holmes mentioned, about those human volunteer experiments. Repeated infection did occur, and what was the experience regarding mucosal immunity, IgA protection in those situations? That's one question.

**Kathryn Holmes:** It varied considerably from person to person how soon they could be reinfected. Those were very limited studies, many of them done a long time ago, and I think much more work is needed to answer that question.

**Bing Lu:** In relating to Dr. Saif's comment, I thought this would be a very interesting experiment to carry out. The other question is for Dr. Ksiazek. In those convalescent patients, SARS patients, you demonstrated some anti-S antibodies being present. Is any clinical correlation, in terms of the development of those antibodies, say between people who succumb versus people who recover; does that antibody seem to be responsible or not?

**Thomas Ksiazek:** I just hasten to say that the numbers of samples that we've tested are so low I don't think we probably can draw any real conclusions. The later the sample the better the antibody, and people who've succumbed have succumbed around the twentieth day. Some of the better samples there were taken a little bit later than that, so I don't think I could draw any conclusions from that.

### *Virus Titers in Human Stool*

**Scott Hammer:** Next question. Please identify yourself.

**C.J. Peters:** C.J. Peters, Texas. Specifically, actual titers of virus in human stool and, secondly, presence of viremia in any coronavirus model?



**Thomas Ksiazek:** Let me just say that, in participating or hearing about people who participated in WHO calls, that virus has been found in stools, and I think there may be some papers now just coming out in which there are some not infectious virus, but rather RNA copy data that's becoming available. But we've not any experience. Larry, maybe you.

**Larry Anderson:** I don't think that's been done yet, or at least we haven't seen the data.

**Scott Hammer:** Other questions?

### *Noninfectious Persistence in Hosts*

**David Pearl:** David Pearl in Public Health Research Institute in Newark. Is there any evidence for carriage of virus, especially when you do specificity experiments; if you don't see infection, is there any evidence that the virus will persist in the host, noninfectious?

**Kathryn Holmes:** What we see with bovine coronavirus when we survey animals in the field is repeated infections in the same animal periodically, and especially with the respiratory infections. At this stage what we don't know is are these new strains being introduced, or are these strains changing and evolving so that the animal is susceptible, or is there a reservoir or some tissue that's infected where there is recrudescence of shedding?

When we treated the one animal that had been infected with the winter dysentery strain of bovine coronavirus with dexamethasone, we did get recrudescence of fecal shedding, suggesting that it was there at low level, but that was within about two weeks. The bottom line answer is we're not sure, but there's also evidence from infectious bronchitis virus that this virus can persist in the kidney of chickens, and how it persists and the sporadic shedding is not known. There really is no research that I am aware of on coronavirus persistence.

**Linda Saif:** May I add to that? There's been some very recent studies that are very nice on the feline coronavirus by Herrewegh and Rottier and their colleagues, and they isolated cats and could show that cat kept in isolation from all other cats should shed feline enteric coronavirus for months. I think it's an interesting point that, during that persistent infection in the animal, mutations in a nonstructural gene could arise which then allowed the enterotropic virus to become systemic and cause the fatal feline infectious peritonitis syndrome, which does have virus in the bloodstream. This is an example where probably the animal itself is the source of reinfection.

### *Neuropeptides or Neurotransmitters*

**Scott Hammer:** David, and then over there. I am sorry, go ahead.

**David Laurence:** David Laurence, Wadsworth Center. This may relate a little bit more to the epidemiology, in regard to the dual infections that Dr. Saif was talking about, as well as the transportation stresses, because of the sensitive innervations of the respiratory tract and the gastrointestinal tract, have you done any experimental viral infections in looking at potential neuropeptides or neurotransmitters in modulating changing co-receptors for infection?

**Linda Saif:** The short answer is no, but there is a lot of background with some of that with TGE infections where that has been studied, and it's known that it can amplify the diarrhea through these neurotransmitters.



*Host Range in Cell Culture*

**Scott Hammer:** Next question.

**Tom Monath:** Tom Monath, Acambis. It's for Tom Ksiazek. Tom, you mentioned you looked at many different cells in vitro in the initial search for a susceptible cell. Can you tell us something about host range of cells in culture, or laboratory animals, or what about hemagglutination with different cell types in different species as a way to get a clue about the origin perhaps of the agent?

**Thomas Ksiazek:** Well we haven't gone through the entire periodic table of cells, but we've tried quite a few, both initially in trying to isolate the virus, and I can tell you that the only cell lines that we've so far been able to grow the virus in have been Vero and Vero E6, LLCMK2s, and primary monkey kidney. These happen to be rhesus. The question arose whether people who are doing this primary respiratory isolation were at hazard if they were using that cell line. I think we've got one additional line, PK15s, that's replicated the virus. We've probably tried a couple dozen largely human cell lines in addition to that, and not been successful.

**Tom Monath:** No other species?

**Thomas Ksiazek:** No other species. We haven't, again, done this extensively, maybe somebody else—the virus has been disseminated to other laboratories, there may be other people who can shed some light on that.

**David Ho:** Answers from Hong Kong

Scott Hammer: Okay, David.

**David Ho:** David Ho, New York. Having spent some time with the scientists in Hong Kong perhaps I could pass on their answers to some of the questions.

**Scott Hammer:** Fantastic.

**David Ho:** Concerning antibody seroconversion correlation with the disease, I think they're seeing, as long as you follow the cases out, seroconversion by IFA. There's no correlation in terms of mortality and that particular parameter. The problem is, even in the cases that died, there is control of virus [inaudible] dying of alveolar damage. In terms of the issue of virus in various places, certainly by PCR one finds lots of RNA copies in stool and sometimes much higher per swab than respiratory secretions. Of course, for the particular outbreak in Hong Kong, diarrhea was very a prominent feature in two-thirds of the cases. In terms of viremia, one could amplify in some cases virus from the plasma, and it's not consistent and it's probably low-level. Is there another question?

**Scott Hammer:** Since you're standing up and are just there, what about pathologic evidence from autopsy data, now that we've got the virus in situ hybridization studies, localizing virus beyond the respiratory tract, for example the gastrointestinal tract?

**David Ho:** Unfortunately the autopsies in Hong Kong are rather limited. They're limited to lung and upper airways primarily. They haven't shown really any gastrointestinal.

Thomas Ksiazek: There has been some in situ hybridization at CDC, and it was also unsuccessful in



localizing respiratory tissues. No GI tissues were available.

### *Vaccine-Introduced Antibodies Enhancing Infection in Cats*

**David Ho:** I do have a question for the panel, and that is there are some reports suggesting that in certain, I believe, feline models with coronavirus infection, vaccine-introduced antibodies actually enhanced disease, is that correct? Do we understand what's going on there?

**Linda Saif:** I am not an expert on the feline infectious peritonitis virus, but there are some studies that showed that the feline infectious peritonitis virus can infect the macrophage, and there are some experimental data that shows that certain types of antibodies could enhance the infection, the uptake presumably of the virus into the macrophage. I don't think the mechanism is fully understood, and I am not sure if the mechanism is understood in vivo in the host animal, how big a role this plays. Maybe Kay can give you more information about that.

**Kathryn Holmes:** When people worked at epitopes on the spike protein of feline infectious peritonitis, they found most of the monoclonals did have the antibody-dependent enhancement phenomenon, but there was one epitope they found that did not, so people are trying at this time to develop vaccines that will be appropriate for cats, because this is a very serious, very serious problem in cats, and they would like to have a vaccine. But the risk of antibody-dependent enhancement is real. There were three, I think, different types of vaccines for FIP that showed that.

### *Concept of Super-Spreaders*

**Scott Hammer:** Other questions, Ellis?

**Ellis Rubinstein:** Since it's apocryphal that there are different forms of the virus so far, is there any speculation about these so-called super-spreaders, if they exist? Was there an implication perhaps, Dr. Saif, that there might be a combined virus at play with certain people, where they might've had another kind of normal flu or something and then it becomes worse, or do we know nothing about it at all?

**Linda Saif:** I would think maybe CDC wants to first address whether there are super-spreaders, and then I would just comment after that about the animal studies.

**Thomas Ksiazek:** Well I think the data from Singapore and other sites clearly demonstrates that there are a lot of people infected from certain patients, and few people infected from other cases of SARS. I think it doesn't look like that's a virus-specific phenomenon, since we see few changes in the genome, and the fact that virus transmitted from a super-spreader to others doesn't necessarily convey that property to the person that gets infected. It usually doesn't occur that way. The possibility of co-infection, other factors, host factors, immune suppression, are clearly there and important questions to address, and we just don't have answers to those questions yet.

**Linda Saif:** I think what I tried to address with our animal models was factors that could be involved in exacerbating the respiratory infections. Those were the ones that I listed in my talk, that we have experimental evidence can play a role. Whether these are the same things that are going on in SARS patients, as Larry said, there's probably not enough evidence to know.

### *Peak Viremia Correlates with Symptoms*

**Scott Hammer:** Can I ask a question about some of the veterinary models, whether peak viremia correlates with symptoms, or not particularly in the respiratory syndromes, or do we know that?



**Linda Saif:** I think in our experience the peak viremia usually does correlate with the peak of respiratory shedding as far as the clinical signs. But our experience is that when we look at those correlates, the correlates are better if we're using a test to detect infectious virus—a test like an ELISA for viral antigen, rather than maybe RT-PCR or nested RT-PCR—because many times the nested RT-PCR is so sensitive it'll pick up positives before the animal actually shows symptoms, or for several weeks after the animal is no longer showing symptoms.

**Scott Hammer:** One more question, I think just one more. Fred? And for the record.

**Fred Hayden:** Fred Hayden, University of Virginia. I was wondering, in regard to pathogenesis disease and what's been learned in the animal models, if you could comment on the possibility of immune enhancement, either based on humoral responses or if there's information regarding the pro-inflammatory cytokine responses in the respiratory infections?

**Linda Saif:** Immune enhancement. The main model that has been looked at is the FIP model, the feline model. As far as the viruses that we've looked at, especially TGE and PRCV, I don't think we have evidence for that, but in some cases maybe we haven't looked, especially with PRCV, where we have infection of the alveolar macrophages. People are just starting to look at the cytokine profiles. Actually, the group that I mentioned in Belgium, Van Reuth and Pensaert, have done quite a few examination of the cytokine profiles, and I think at this stage some of that is preliminary and some of that is published, and it varies probably with the respiratory virus. With influenza in pigs, they did find inflammatory cytokine responses, and at this stage with the PRCV I know they found high elevated levels of alpha-interferon, and I am not sure about the other cytokines in comparison with an influenza infection.

**Scott Hammer:** Corollary to Fred's question, we're talking about the serological, humoral responses—what's the role of cytotoxic T cells or ADCC in animal coronavirus infection, do we know?

**Kathryn Holmes:** With TGE, NK cells have been shown to play a role. There is evidence for antibody-dependent enhancement in terms of the immune response. It's been very difficult to do the cytotoxicity studies because, just like humans, we work with outbred animal populations, so these studies are much more difficult to do. There haven't been very many of these studies done.

**Scott Hammer:** I think with that we should take a break. Fifteen minutes, please, come back and we'll start, and let's thank the panel.

## Session II: On the Front Lines

Moderated by Scott Hammer

### On the Front Lines: Clinical Spectrum of SARS Infection

Larry Anderson, Centers for Disease Control and Prevention, Atlanta

#### *Introduction*

**Scott Hammer:** While people are sitting down, I'm just going to mention, the second session is going to be shorter than the first, so we're going to try to complete it before lunch. Obviously lunch will be a little delayed, but I think we'll catch up as the afternoon goes. Also, sessions II and III have a fair amount



of overlap, since we're getting into the clinical issues, and so whatever questions we don't really address in the panel discussion of session II we will take care of in session III.

Without further ado, the first speaker in session II, which has the catchy title of "On the Front Lines," is Larry Anderson from the CDC, who's chief of the Respiratory and Enteric Virus Diseases Branch at the Centers for Disease Control. We've asked Larry to talk on the clinical spectrum of SARS and related issues, such as diagnostics, if he wishes to. Larry.

**Larry Anderson:** I'm going to take a little liberty in what I actually talk about, but first of all I'd like to thank the organizers. I think the first session has been really outstanding, and I think it's a great opportunity to exchange ideas and for us at CDC to learn more about coronavirus, since we actually haven't paid a lot of attention to it, since other viruses seem to be more important. But it certainly has come to the fore now.

### *Early Epidemiology of SARS*

What I'm really going to do is to first talk just a little bit about the epidemiology of SARS, and I think that kind of sets the stage. I think it also highlights some of the issues related to control and, to some extent, the disease picture as well.

The recognition of SARS as a significant disease began to be recognized in China, but the event that really brought it to global attention was when a visitor from China stayed at the Hotel M in Hong Kong over one night. Related to that, the time that he was in the hotel, ten people were exposed, acquired SARS, and then transmitted to multiple countries.

The initial event was a physician from Guangdong province in China visiting Hong Kong, staying in Hotel M. That led to his hospitalization and later death, and multiple health-care workers were infected, and they then infected subsequent individuals.

The event in Vietnam, a WHO physician there, Carlos Urbani, treated patient B. He brought the recognition or the concern about this to the attention of WHO, and the subsequent very rapid response there. The outbreak in Singapore, some cases in the United States, a case in Ireland, and then the outbreak in Canada—all were related to this initial event in Hotel M in Hong Kong.

### *Transmission Modes and Settings*

One of the distinctive and consistent features of the epidemiology of SARS is the frequent and predominant instance of transmission in the hospital setting. This illustrates data from two settings, Canada and Singapore, and the number of patients that were infected related to hospital exposure. Often health-care workers, but also other patients and visitors to the infected patient, or to other patients in the hospital, have also become infected in the hospital setting, with most of the other instances having a clear link to a SARS case, which is also very important in the epidemiology of this virus.

Some of the observations: primarily health-care workers and household members, infrequent instances of endemic community transmission. This has really been critical in the ability to control SARS in some settings, and the likelihood that with good infection control practices we actually can control the spread of this virus; at least at this point in time, it appears to be. The modes of transmission that follow from the epidemiology, close contact being probably the most important mode of transmission, can include droplets, fomites, direct contact and autoinoculation. It appears in some instances that airborne transmission may have occurred. The instance in Hotel M, in which people staying on one floor where there



wasn't reported close contact, is consistent with airborne type of transmission in that setting. With the fact that virus has been isolated from fecal tissue, and the cluster or large number of cases associated with one apartment complex in Hong Kong, one of the possibilities is that origin of virus, possibly fecal-oral transmission, was important in the transmission in that setting. Other factors probably are important in transmission, particularly the super-spreader concept, which we've commented on earlier. It'll take a little bit of time to sort out which of those factors are actually going to be most important in transmission.

### *Super-Spreaders in Singapore*

The super-spreader concept is illustrated anecdotally in a number of settings: Canada, Hong Kong, Vietnam, etcetera. But the data from Singapore, I think, illustrates it most clearly. What this shows is the number of secondary cases linked to each one of the cases in Singapore, 201 cases. The vast majority of cases had no secondary cases. This is not the natural situation, because Singapore instituted early on in the course of the outbreak stringent infection control practices in the household setting, and over the course of the outbreak enforced quarantine in the household setting in addition to isolation procedures. With those infection control practices, it's very clear that you can limit transmission, and I think that is the reason that transmission appears to have been stopped in a number of settings. You can see one case, secondary case two, three, and then there's a few super-spreaders, five in which they had more than ten secondary cases, and up to forty secondary cases associated with an individual case of SARS.

### *Rate of Spread and Controlled Regions*

The outbreak has continued in a very steady fashion globally, and this just illustrates the number of cases in yellow, and, on the left-hand side, deaths. Note that we're looking at tenfold lower number in deaths versus cases, so it's not the same scale. It continues to increase in a very steady fashion globally.

Just a note in terms of transmission, and that's the red stars. These are settings in which transmission appears have been controlled or stopped: Canada, Singapore, Thailand, US. We really have never had much in the way of secondary transmission, but have prevented that, although we haven't probably had any of the misfortune of having any of the super-spreader type of cases in the United States and Vietnam. I think for a respiratory transmitted virus to be able to be controlled with infection control, isolation and quarantine-type of measures, is not what you would expect for rhinovirus, respiratory syncytial virus, certainly influenza, and the other viruses. I think it tells us something about the transmission, and I think it probably means that transmission occurs when the individual is sick. They may be infected, they may have virus earlier on, but they're not transmitting virus. It also suggests that you're not getting transmission, which is different than infection necessarily, with asymptomatic or mild cases of infection. We don't know how often that does occur, but the epidemiology suggests that it's not important in transmission of the virus, or maintaining transmission in a community.

### *Clinical Features*

Switching to the clinical features of SARS, and since we're going to have some of the folks that actually have been on site looking at this, I'm going to go through this pretty quickly. I think this slide illustrates a very important feature that early-on suggested that this was different than the usual respiratory viruses. It's really kind of the progression of illness. In most instances it starts with fever, then you get non-productive cough (often but not exclusively), then shortness of breath, and then you'll have the interstitial pneumonia noted on chest X-ray. As you look further down, upper respiratory symptoms are infrequent. I think Ian's description of his illness was reassuring in the fact he started out with fever and rhinorrhea, and when you get the fever and rhinorrhea it sounds more like classic respiratory virus and does not actually sound like SARS. So that's reassuring. Lymphopenia seems to be a common feature, but I think



I'll leave that for the later discussions, which on sight can give more detail.

Another feature of it has been the age-related severity of illness. In general, it's been older individuals that have been more likely to have severe illness and die; not exclusively, but it's been more common. This just illustrates some data describing ten children, and what you see is less severe illness in the younger children, more severe illness in the older children, and I think this is consistent with some of the other data that you see later on.

The other point is that there's a high rate of severe illness with SARS cases. This just illustrates the fairly consistent finding of progression to respiratory failure and a case fatality rate that varies from site to site. Some of the risk factors that seem to be associated with severity of disease, which may be discussed later, include co-morbid conditions and age, as I mentioned earlier.

### *Case Fatality Rate*

I think one of the things that's worth noting is the case fatality rate. Initially, we saw case fatality rate more in the range of 3 to 4 percent, and I was thinking that the case fatality rate would actually go down as we learned more about asymptomatic and mild illness. In fact it may. But one of the things that has occurred in overall SARS cases, most of which have been hospitalized, is that the mortality rate has actually gone up. The reason for that is that a progression of disease, that death does not occur early on. It's a matter of the early-on symptoms and then progression of respiratory failure, so early on in the surveillance we didn't have evidence on what the course of the infection was. As we're beginning to do that, the case fatality rate is continuing to go on. In a recent study in *Lancet* they suggested that the case fatality rate in Hong Kong was likely to move towards the 2 percent range, and this may ultimately be the case fatality rate that we see. Of course, this is excluding the mild and other illness that we may find out about with time.

### *Diagnostics*

Switching to diagnostics. As always, diagnostics—the sensitivity, specificity, and ability to detect infection—is first based on the type and timing of specimen collection. I think it's important to recognize that we do not yet know what the best specimen is at certain times of the illness that's most likely to detect evidence of infection. There's a lot to learn yet. I think over the next month or two, particularly from the sites that have had a fair number of cases—Canada, Hong Kong, Singapore, etcetera—we'll begin to get data on that, but right now we're not quite sure.

Also, the types of assay and, another point, interpretation of results. As we get evidence of infection in mild, asymptomatic, or persistent of virus in different specimens, that of course becomes a real issue. What does this mean in terms of the individual patient, and also ability to transmit to others?

So far, electronmicroscopy, isolation of the virus, detection of antigens in tissue have been applied. These are not helpful yet diagnostically, but certainly will be important in thinking about the pathogenesis of disease in animal models, and, maybe as we get better probes, also in human tissue. Detection of viral RNA by polymerase chain reaction has been really a key part, in conjunction with antibody assays, in what we know about the infection in site of replication, or at least presence of virion RNA to date. Virus has been detected in respiratory secretions, stool specimens, and sometimes up to thirty days after onset of illness, both by isolation and PCR. It's also been detected in urine specimens and by PCR in lung and kidney tissue, in addition to the bronchial lavage specimens. I think the types of assays, the sensitivity, are improving as we refine the primers and probes that we use in the PCR assays, and hopefully develop some of the subclass or class-specific antibody assays as we develop additional reagents.



Other tests have also been used, and I think this just illustrates some of the data early on in terms of detection of virus by PCR, serology, one or the other. I think this also underlines—this is data from Hong Kong—the fact that antibody has not been detected in non-SARS patients. Dr. Ksiazek has found similar results in the United States, and I think these data, although not complete, certainly suggest that this virus or related virus has not been circulated in human populations, or at least circulated extensively.

### *Conclusion and Summary*

I think SARS has unique clinical and epidemiologic features. The good news is that the SARS outbreak has been controlled in some settings, but not in other settings. We have some things that could cause us problems in the future: the possibility that SARS in immune-suppressed patients may be different clinically, more difficult to detect, identify, or co-factor in super-spreaders, lead to persistence of virus and transmission later on, and the ability to detect SARS. We still have a great deal to learn. Thank you.

### **On the Front Lines: Clinical Experience in Toronto**

Donald Low, Mt. Sinai Hospital, Toronto

#### *Toronto Index Case*

**Scott Hammer:** Thanks, Larry. The next speaker really was on the front lines, and was in fact quarantined for a while, if I recall correctly. We heard from him through the New York Times down here. In this hemisphere, the most affected country, of course, has been Canada. Donald Low, who is the microbiologist-in-chief at the Mt. Sinai Hospital in Toronto, is going to talk to us about the clinical experience with SARS in Canada. Thank you for coming.

**Donald Low:** Thanks very much. It's a pleasure to get out of Toronto and to be part of this symposium.

What I'll share with you today is a bit of a personal experience, but also some things that we've learned. You can't even start to recognize all the individuals that are involved with this; it's been literally hundreds of people, as you can imagine. I'll tell you a little bit about the outbreak.

As Larry said, it was the Metropole Hotel, not the M Hotel, and that was February 21. I think one of the interesting things is, at the Marco Polo, I was there on February 21 with my son, and it's sort of ironic that I was reading in the news about avian influenza that morning, thinking, Oh, we might have a problem here.

Our index case was at the Metropole, and she came back on February 23, thank God on Continental Airlines (I fly Air Canada), and she arrived in Newark, New Jersey. A good screening process in Canada would not have picked her up anyhow, if it had been recognized. She came back that Sunday and developed pneumonia and lived at home with several family members, one of which was her son. She died at home on March 5 with pneumonia, and was not admitted to hospital. Her son, who cared for her right around the time of the funeral started to become symptomatic, and he saw his family doctor and got started on some antibiotics. Eventually he was so sick that two days after his mother's death he went into Scarborough Grace Hospital on March the 7, where it was recognized that he had pneumonia, and was put in an observation unit for about fourteen hours.

On March 12 a warning came out on Promed about a global alert, and on March 13 he died. That morning, Allison McGeer, who's part of our group at Mt. Sinai Hospital, got a phone call from Sandy Finkelstein concerned that now they had two patients in a family that had died of a respiratory illness,



being concerned could this be avian influenza as a possibility. That day, on March 13, Allison brought the whole family into the hospital and put them on isolation. As they came in and were evaluated, it was evident that four other living family members had pneumonia as well. We admitted all of those to hospital.

The first week it was interesting. I remember that Friday. Thursday we learned about it, on the 13th, and phoned the WHO and phoned CDC, and on Friday we're getting the body, because the first patient they didn't want to do an autopsy on, which is this patient here. This is the now-famous Tora 2, and we had to get the body and found a pathologist that would do an autopsy, which wasn't easy. I remember that Saturday getting the stuff together, so we sent some down to the CDC and we sent some to our national microbiology lab, as well as getting other specimens. That was a busy, very interesting weekend, not knowing what we were in for.

### *Case B, Spread Beyond Families*

Probably there are several lessons in this outbreak that you can take away. Case B, Mr. P, happened to be in the bed beside Case A at the Scarborough Grace emergency department for about ten hours. He came in minding his own business, had a bit of atrial fibrillation, got sent home on March 8, but at home he developed a respiratory illness. He went to see his family doctor, took a chest X-ray, it shows a nice little infiltrate in his right upper lobe, started on some antibiotics, but he didn't get better.

At this time we recognized we had a problem, contact tracing was being carried out. We looked for this person who was then admitted back to Scarborough Grace Hospital before he was recognized as a contact. That is, when Public Health went to his house he was already on his way to hospital, so he was admitted without protection. That weekend he came into hospital and had pneumonia, I saw him on several occasions. He died on the 21st. That was sort of when it hit the fan, when all of a sudden we realized that we just didn't have a problem within a family, we were having hospital workers reporting, phoning in with fevers, EMS, emergency, the paramedics, ambulance drivers with fever, visitors who had been in the hospital that were sick, family members. In fact, the place became so overwhelmed that they closed the emergency department at Scarborough Grace Hospital, and I opened up a temporary unit at an old TB hospital called West Park. We did that on the night of the 23rd of March.

We created a floor where I admitted 14 health-care workers in a period of about 48 hours. Since then, one of the nurses that was helping me, her husband is dead, and she's on a ventilator and she'll probably die in the next while. She's been on a ventilator for several weeks. At the time we were pretty cavalier in dealing with this infection; God knows how I ever got away with it, and I might have seroconverted. You had to get volunteer nurses to come work, you didn't have the proper isolation, you didn't have anterooms, you didn't have anything, but you were just trying to do the best you could.

### *Early Lessons*

In any event, a lesson from Mr. B was to remember the family members, because his wife was actually sick. She came into the emergency department with him, sat in the emergency department in the waiting room, and probably was responsible, as you can see here, for infecting 24 other people. Mr. Case B himself was also responsible for infecting another nine individuals.

Another lesson here is that you might misdiagnose this infection early on. Case C was a patient who was in the same observation unit on March 7, but he had congestive heart failure, got sent home on the 10th, and came back on the 13th. They thought he had congestive heart failure, and he was admitted to the coronary care unit with a diagnosis of congestive heart failure. While I was out at West Park admitting



patients that night, I couldn't understand why I was admitting cardiac-care nurses. They weren't in the ICU, they weren't in the emergency department, why were they sick? Were we being oversensitive in our case definition? In any event, we found out that this person was not only responsible for infecting 21 persons at Scarborough Grace Hospital, but also infecting the person in the bed next to him, who got transferred to our hospital with pneumonia. He couldn't have had SARS because he wasn't in ICU, and then he infected seven health-care workers at our hospital, where he was for about eight hours, not on protective isolation. He also, before he was diagnosed, got sent over to York Central Hospital, sat in the ICU for about a week not being recognized, and infected 15 persons in that hospital, including a visitor, a friend of his who died.

Another problem was back in that emergency department the night that Mr. B came in. A man, an older gentleman, fell and hurt his knee. His two sons brought him in, and they sat next to Mr. B's wife. Unfortunately, they happened to belong to a charismatic religious group that became known as the BLD Community. They sat in that emergency department, and then went on to develop disease, and this was our BLD outbreak. This was our only community outbreak, within this charismatic religious group. The patient, after being in the emergency room on the 16th, became symptomatic on the 19th. They had a lot of religious events and celebrations around the Easter weekend, and his father also developed it died on April 1. They had a funeral, which all five hundred of the BLD community came to, and you can imagine a lot of close interactions and exchanges, kissing and hugging. That resulted in 31 BLD members coming down with SARS, and I think it was stopped because our Public Health put all five hundred on isolation. Unfortunately several of them went to see their family doctors, not being recognized what the disease was.

### *Prevalence in Health Care Workers*

What is it clinically? This is a paper from our group at Mt. Sinai, recently published in JAMA. You can see here, this is just the first 144 patients, a sense that the distribution may be more female because of health-care workers, and 45 years of age, reflecting a lot of patients that became infected. You can see here that a lot of health-care workers, 51 percent, making up mostly nurses, but physicians, even us who don't put our hands on patients that can get infected. Of that 144, it really is a hospital-environment type of disease, and very few cases actually related to travel in our experience there.

### *Clinical Symptoms and Initial Screening*

What do you present with? This is probably important, especially with in the midst of an outbreak and trying to identify people who may have the disease. Typically you'll develop a prodrome first of muscle aches and pains, just not feeling well, quickly followed by fever. Patients are developing symptoms within three to ten days of known exposure to an infected person. Diarrhea also occurs later, as does cough, as Larry mentioned.

Where are they seen? That's important, because if you do have an outbreak, who do you have to warn, who do you have to make concerned about seeing these patients? Prior to hospital admission they were evaluated and sent home; the stories come time and time again, people being sent home with disease, not being recognized. This tells a bit about this transmission in the community business. Why it doesn't really occur that frequently is in the early stages of the outbreak, we were pretty cavalier about saying, well, if you don't have pneumonia, even if you have disease, go home and spend your time on isolation. A lot of things fell through the cracks, and people were going out to malls, they were going to saunas trying to sweat out this fever, there was a lot of interaction with the community. Despite that, we didn't see disease in the community other than the BLD community. What I think it says is that this is not a good virus for transmitting outside of certain settings.



Emergency departments are important for being able to recognize disease, whether this is travel-related or not, as you can see here in doctors' offices and occasionally with hospitals. The median time from visit until admission is three days, so lots of people coming in, being sent home, still being sick, and coming back before admitted to hospital.

### *X-Ray Features, Lymphopenia*

On chest X-ray, what do you expect to see? It is normal in about 25 percent early on, and that would then be a suspect. Pneumothorax occurred in four of our patients in this group of 144 that I'm talking about right now. A unilateral presentation: this was a young emergency room nurse that presented feeling quite well, other than this fever and this hacking cough, that I admitted with an infiltrate, right middle lobe. About 30 percent of patients on admission will have infiltrates on both lung fields. This was a respiratory therapist that I admitted March 23-24.

Other observations. The lymphopenia is interesting. We don't really know how often, though, it occurs in regular viral pneumonia, or whether it's really predictive. The LDH being high in about almost 90 percent of patients may be reflecting lung disease. Creatinine kinase was an interesting observation, being abnormal in about 40 percent of patients.

### *Treatments Used*

Treatment, we obviously don't know what we're doing with treatment. There's a paper that I had reviewed previously, and Fred said has just come out in *Lancet*, looking at ribavirin and steroids, suggesting that this is the way to go. I'm not really convinced of that. We really need a clinical trial to evaluate it. We used ribavirin almost in all patients early on in the outbreak, and for myself, clinically it wasn't making any difference. We had lots of side effects, and we were using the big dose—hemorrhagic fever kind of doses of ribavirin—so pretty well everybody had to get transfused. We started using steroids later on in our experience because of what was coming out of Hong Kong, and of course they're on antibiotics, so you didn't know if they did have an underlying treatable cause of pneumonia. And it was all IV ribavirin, a little bit PO.

### *RT-PCR on Lungs from Autopsy*

We were able to beg or borrow about eleven bodies from this outbreak to date. It is very difficult getting autopsy, and in fact our coroner had said there's no need to do autopsies on these patients, we know that they died of SARS and it's not going to help us. There was a lot of work getting these bodies, I'm telling you. I managed to get 11 so far, and we hopefully will get another couple before it's over. There will be a lot of very important information that should come out of that. In this little study that we did, Kevin Kain and Tony Mazzulli at our place had access to the new Artus system, this is something that's available commercially now. We looked at lungs from 11 patients, 14 controls, 4 of which during the outbreak didn't have SARS and 10 that had been collected prior to 1998. Of multiple samples from multiple sites in these lungs, all 11 are positive using the Artus RT-PCR.

### *Duration of Illness and Viral Load*

The main duration of illness before these patients died was 20 days, and you can see that if you look at those that died shorter than 20 days, they were more likely to have high copy numbers, high viral load, as opposed those that lived beyond 20 days. It didn't appear in this small sample that the use of ribavirin or steroids really had much effect on the viral load. Here are patients, despite being ill for 20 days, on a ventilator for usually about 5, 7 days; when you look at their lungs at autopsy, you're still able to isolate a high viral load, suggesting that probably it's not just an immunological effect. We really need something



to treat these patients with.

Of that small group of 144, 8 died, 6 of these had diabetes. The mortality rate appears to be going up, but I think what we're defining as the denominator is making part of the difference, because some studies are just using probable cases to determine mortality.

### *Infection Control Procedures*

Infection control. I think that we have been convinced that contact and droplet spread is the mode, although we can't, in some circumstances, as Larry mentioned, rule out airborne contact with a contaminated environment. Reaerosolization, when you remove the mask after coming out of a room, how do you prevent transmission? I think the successes we've had in Toronto is in rapidly identifying patients and managing them with precautions, but more importantly getting their contacts and ensuring they don't transmit, whether you put them on isolation precautions, quarantine, or whatever. This is really critical, minimizing the opportunities of exposure of staff and other patients to SARS patients. It's unfortunate, but you can't have traffic into these rooms. These are obviously dirty rooms, and the more traffic, the more likelihood there's going to be an occult break in technique, and people are going to get disease. Minimize the number of droplets. When you're coming into a patient's room you don't want to be coming in when they're coughing or if they're vomiting.

What we've been using underneath this mask is an N95 mask, a face shield. The hairnet is because these things get all tied up in nurses' hair. When they're trying to take them off, the things are flying sideways and you're putting your hand on a dirty thing, and you might get an itchy eye, and of course gowning and gloving. We have no cases left, but I think we're still seeing in Hong Kong transmission to health-care workers that are using precautions. This is probably the most disturbing thing about the outbreak, this occurring. High-risk activities, intubation, anesthesiologists—that's a high risk job, it's worse than being a fireman. We've got about five anesthesiologists that have developed this disease.

For low risk activities, why is it occurring? Probably occult breaks. I think this is something we just haven't emphasized at our place, and probably everybody is not. We tell people how to go in a room to protect yourself, we don't tell people how to leave the room. I can't remember all that—you're supposed to take your gloves off first, then you take your gown, then you wash your hands, then you open the door, then you leave, then you wash your hands again, then you take off the face shield and throw it out, and then the mask, and change it, and wash you hands. So it's not surprising that you could have breaks in this technique, and that might explain why we have 15 health-care workers that have come down with disease while they have been using precautions.

This was just reported on Friday from MMWR, from Sunnybrook, a 50-year-old physician that looked after one of those BLD patients who became ill, was admitted to the ICU, intubated on April 13, positive in sputum and stool. That was Easter weekend, another weekend from hell, that 9 health-care workers became ill during that weekend, and 6 of them had been involved in the intubation.

### *Lessons Learned in Toronto*

So it's over. As our Mayor said, "Who's on first?" It is safe to come to Toronto, the outbreak is over, we have not seen a case now in the community for over 35 days and not a case in the hospitals for over 20 or 25 days. I wonder, these things that we call travel cases, if they really are cases, or somebody who's got pneumonia with no diagnosis that happens to come from Asia.

What have we learned? I think that it's primarily contact and droplet spread. It's a disease of tribes, and



whether that tribe is a hospital tribe or a family tribe or a close community like BLD, that's where transmission occurs. I think, fortunately, we haven't seen transmission outside of those tribes in the community, and we've been watching, believe me. I think other indirect evidence suggests it, except for the few cases we've seen in airplanes.

It's a different life when you're dealing with an outbreak; your life changes in hospitals and it is surreal to screen staff, to waiting in line to get in the hospital, to talking to your health-care workers. You could imagine looking into an audience of two hundred people with masks on, it's just such a bizarre time. Thanks for your attention.

## **On the Front Lines: SARS: An Update from China**

Chen Zhu, Shanghai Second Medical University, People's Republic of China. Delivered by Dr. Scott Hammer

### *Introduction*

**Scott Hammer:** Thank you, Don, for that very vivid description of what Toronto and you went through. It gets back to what Ellis was saying also about health-care workers truly being on the front lines of this disease, and paying for it with a big price.

I'm twice removed from the speaker, again I'm subbing for Ian who had gotten slides from Professor Zhu Chen in China. He couldn't be here for obvious reasons, tied up with work in China, but wanted to participate in this Academy event, and to demonstrate a little bit of what is going on in China. I'll try to do this justice, but forgive me since I am twice removed from the presentation.

What this presentation is about from Zhu Chen is really to illustrate, I think, the awakening and the approach, the really multidimensional approach, that China is now taking toward trying to control SARS. I think everyone has read the New York Times, as well as those of you who've been in direct contact over there, you know that this has been not just a medical and public-health event, but a major economic and political event for China.

For those of you who don't know, Professor Zhu Chen is vice president of the Chinese Academy of Sciences, and was named the vice director of the Task Force Group for Science and Technology Research on SARS in China.

### *Impact in China*

We've talked about the numbers earlier this morning, about well over 7,000 [cases] in the world, but I illustrate again that China is the hardest hit. At the time these slides were sent, 5,000 cases with 2,300 in mainland China, 267 deaths, with Beijing being the most affected area, with nearly 2,400 cases and 139 deaths.

One positive note, and this is reillustrated by the Promed data from this morning, the numbers of new cases being reported from China on a daily basis is beginning to decrease, and I believe it's either five or six days in a row where there are less than fifty new cases. This is encouraging, but still extremely preliminary. I think we need a much longer trajectory of time before thinking things are under control in a country that large and that populated.

### *Task Force Commitment to SARS*



The wake-up call, I think everyone knows about, and now it's become the number-one priority, as David, who is just back from China, can probably reemphasize to us as well. There's a central command under the State Council and ten task groups, including the task group on science and technology. There's a huge dedication to this, and everybody has the responsibility and it's being labeled directly by the Chinese as a national disaster.

The science-and-technology task force is really a multidimensional approach to look at identification, helping to identify the pathogen—obviously many of the samples have come from Hong Kong and China—the epidemiology, diagnostics, treatment, understanding the disease mechanism, biosafety- and infection-control procedures, and education.

His slides are really illustrative of the breadth of activity that's going on. Looking more closely at sequencing of the viral genome in multiple isolates is going on in the human population, and I believe animal coronavirology is also taking off to look for the source.

### *Modeling*

There's a lot of interest in modeling and predicting the trajectory of the epidemic, looking again at this issue we talked about in the last session of whether there is variation in the viral genome and what the relevance of that is. The data are really still preliminary, and the methods of spread. As we've talked about, and Elisabeth Rosenthal's article was the most intriguing about that in the New York Times a few weeks ago, showing the proximity of multiple species to one another, and multiple species to humans.

### *Diagnostics and Treatment*

China is also working very hard on diagnostics. This is part of Ian Lipkin's brief, but very intense role over in China; it was PCR-based, if you will. Everything we've talked about before, looking at chip technologies, RT-PCR, serum antibodies, and antigen detection, are all being done very furiously. There's a lot of interest in treatment, of course, and some of the data that have come out of Chinese experience, and particularly the Hong Kong experience, with steroids and ribavirin, as Don mentioned, which was also used in Canada. There's been talk, and I don't know of any data, maybe David does, about looking at convalescent serum to be infused into acute cases. That obviously is a difficult technical issue, and raises some infection control issues of its own.

### *Drug and Vaccine Development*

There's a lot of interest in drug and vaccine development, obviously, and some issues have already gone on in the prophylactic mode of intranasal interferon, but I haven't seen any data on this. Again, with preparation of immunoglobulin, preparations from convalescent serum, there are issues there related to human products. A lot of drug screening is going on there, and we're going to hear about what's happening at the NIH and USAMRIID from Dr. Laughlin later. Biotechnology, looking into traditional Chinese medicines, although I don't know specifically which these are, and obviously interest in vaccine development, partnering with a number of scientists.

### *Passive Immunotherapy*

The passive immunotherapy concept is important, and humanized monoclonal antibodies are being investigated. I don't know their state of production, but there certainly are technologies that are known for developing humanized monoclonal antibodies for other pathogens, and this is going full force, and certainly is probably a more interesting way, if you will, in the long run, if passive antibody is important and can be helpful acutely, than convalescent serum.



### *The Latest Science*

They are also interested in the latest in science, and so antisense oligonucleotides or RNA interference are being thought about, but I think similar to these have been thought about, at least antisense, for other viral diseases have been thought about for a long time, and only come to fruition for a limited use for CMV. This is a ways off for the SARS coronavirus, and RNA interference is being looked at for every pathogen that I think we're aware of, and I think is part of the future but still an important issue.

### *Infection Control*

There's a lot of interest in the bioprotective gear, and infection-control issues. I won't go through the details of this, I think they're obvious and Don touched on a number of these things. I think also Don's photographs and his description show us it's one thing to talk about infection-control issues, the sheer inconvenience of taking care of people with this kind of protective gear on for single individual patients, but a hospital full of these individuals is a torturous issue for medical care. I think can't be underestimated and you can only realize it if you've done it.

There's a lot of interest in the immunologic response, and I think one of the things we didn't hear too much about yet, because we don't know it, is really what the host susceptibility issues are. We've got some analyses of this, age-related responses, etcetera, but really the host determinants of acquisition of infection and disease progression besides age or underlying illness is still to be thought about, and is going to take us a while to elucidate.

### *Capacity-Building and Collaboration*

On the capacity building front, this is part of the openness. I think every epidemic has its good sides, the AIDS epidemic has many good sides as far as the international cooperation to battle a pandemic as we've seen. This in a short amount of time has illustrated how the world really can come together in collaborative scientific relationships, and what the power of the Internet is. Within China, a number of comprehensive research centers are being built up or established that are listed here, new biosafety regulations, and increasing numbers of P3 facilities to actually look at the virus. I know that China's reached out to a number of scientists and organizations around the world for sharing of resources, training and technology transfer, networking their research teams, putting in strategic plans, and partnering with industry.

### *Thanks*

In particular, Professor Zhu has wanted to thank U.S. scientists who've actually been directly on the ground. Ian and David Ho are listed here, and I hope Ian is still on the phone so he can see that he's listed high on the professor's slides, and David Ho's group, and then there are, of course, European and other collaborations that are trying to work on the diagnostics and the therapeutics.

Being as polite as the professor is, wanted to specifically send his written thanks for the New York Academy of Science and wish us a successful meeting, which, so far so good. I think it's been a terrific morning so far. His last slide is here, so in absentia I think we need to thank Professor Zhu and realize that China's facing one of the biggest challenges that any country could face. With that, thank you, as a second removed.

## **On the Front Lines: Panel 2 Discussion**

### *Spectrum of Disease*



**Scott Hammer:** What I'd like to do is ask Larry and Don to come have a brief panel. We are actually not doing too badly. We will probably have a 15-minute or so panel here, and then break for lunch. We will only be 15 minutes behind schedule.

I would like to start, as you are sitting down, and please speak into the microphone for the audio recorders. We have seen the clinical spectrum of disease, that helps us not just with case definitions but with diagnosing probable or possible cases. The question probably is unanswerable, but have we seen the full spectrum of disease, and what is the asymptomatic reservoir out there? Does the CDC have data and does Toronto have data on serologic profiles of potentially-exposed but well individuals? The spectrum of disease and the asymptomatic reservoir of previous infection.

**Donald Low:** There has to be a [inaudible] for this disease which we probably don't recognize yet, but we had admitted patients who had contact who clinically you would think that they had disease, but never really developed pneumonia, and just had a flu-like illness with myalgia, muscle aches, pains, a convincing story but only a serological test will really tell us. The study that we're doing right now, one of the little studies we're doing in our hospital, is trying to do a seroprevalance of a hundred people that had any kind of contact and a hundred that didn't, to see if we can identify people that had mild disease that just didn't present clinically, or come to our attention, or didn't meet the case definition.

**Scott Hammer:** Are you using IFA or an ELISA? Which is your serologic study?

Donald Low: We're going to wait until we feel that this is getting it; now we're trying to gather this data and the clinical histories that go with it, because memories are short. The decision on eventually what test we'll use will be what's best available when the time comes.

**Scott Hammer:** Larry, do you have any additional comments?

**Larry Anderson:** I think these are very important questions, and I suspect that in Hong Kong and Singapore, that are doing some natural history studies, and in Canada as they get through the testing and so forth, we're going to get information. We are doing some of those in the United States, looking at the course of infection in individuals and contacts. We don't have that many cases, so it's going to take a while for us, or I think other places are better to answer these questions.

### *Correctly Assessing Fatality Rate*

**Scott Hammer:** Next question is, you brought up the mortality issue, and I think that's been one of the issues in the popular press. The mortality first is 3 or 4 percent, then it jumps to 7, then it jumps to 20. When you look at the case fatality rate from the cumulative world numbers now, it's about 7.8 or 7.9 percent. Where do you think this is going to settle out? You addressed that a little bit, but I think people focus on the case fatality rates day to day, and that's problematic. When do you think we'll really have a time course that we can actually assess what the case fatality rate is? It's really quite remarkable to have a 20 percent case fatality rate in Hong Kong and 3 or 4 percent in other places.

**Donald Low:** Again, it might be the denominator. In trial we've had 250 suspect and probable cases, and 24 deaths. Probably at the end of the day it'll be about 28 deaths. You can see, if you take away those suspect cases—part of the definition of suspect sounds like they're not really a case until they get pneumonia, but for me they've got it. Knowing the epidemiological history, the clinical presentation, the fact that they have the lymphopenia and the LDH, and all these things clinically look like these people really have disease, but they're suspect. Do you exclude those, and if you exclude them then the mortali-



ty rate jumps from 7 percent to 15 percent. And then there is this other issue about taking time for patients to die. I think only when we get a good serological test are we going to be able to say really what the mortality rate is, and I would bet that it's someplace around probably 15 percent or less, maybe 10 percent.

**Scott Hammer:** Larry, do you have any other comments?

**Larry Anderson:** I agree. I think there are two factors. One is taking a cohort of patients and actually understanding what is the natural course of disease. In many of the patients, we haven't gone through the time to understand what the outcome is. The other is we don't yet know what asymptomatic non-classic SARS coronavirus infection looks like and how frequently it occurs. I think that one factor means the mortality rate will increase; the other will give us a lower overall mortality rate. Right now we're finding out the first, the natural course in classic cases of SARS. We don't yet know what the other contributing that will lower that rate. I suspect it's going to be 10 to 15 percent when we include all those factors.

**Scott Hammer:** Let's open this up to the field.

### *Infection Control Confounds Super-Spreading*

**Bing Lu:** This is Dr. Lu again, Association Chinese American Physicians. In light of the concept of super-spreaders, if we can visualize the slide Dr. Low showed from Case A to Case D, every one of them seems to be a super-spreader. But from there, apparently you did very successful work and the new cases have stopped. Is that because dying off of super-spreaders, or is that because of the preventive measures that you used? What's your speculation, for both professors?

**Donald Low:** I think it's a great observation, that once we're able to put these people in isolation they didn't become super-spreaders because of using precautions. Early on, the way we got into such trouble was we had all those initial four patients you mentioned died. They all had bad disease, and there was extensive spread within their families. That was right around or before the time that the warning came out. I think subsequently people that may have become super-spreaders never did. If you look at the Singapore story, the three individuals that got sick at the Metropole Hotel went home to three different hospitals, and only at one hospital was there transmission. The other two patients with SARS were admitted to two other hospitals where there are no secondary cases that I'm aware of, or if there were there are very few.

**Larry Anderson:** I think the concept of super-spreader is confounded by infection control. If you think about what happened at the Metropole Hotel in the early spread from there, it was very efficient transmission. Once we started instituting infection-control practices, we've been able to control. Infection control is key in what's happened in the course of this outbreak and the ability to control spread. It really needs to be done very carefully. Some of the super-spreaders are maybe actually shedding more virus and more efficient transmission. The other piece that comes into place is the diagnosis was missed, and they did not institute infection control practices early on. I think both are contributors.

**Scott Hammer:** Peter, can you take a microphone? Right in front of you.

### *Diabetes Association*

**Peter Palese:** Peter Palese, Mt. Sinai in this city. Just a question in terms of the connection with diabetes. First, what is your thought about this, and second, has it been also observed in other countries?



**Donald Low:** I think it has been. In the other studies the association has held up. I'm not sure why this association, and Larry would know better than I. With influenza, diabetes is not a risk factor, think. Is it? Okay, it is. From what I remember from other countries, the observational thing has held up as well. I don't know why.

**Scott Hammer:** Singapore had a fair number of diabetic patients, but they have a fairly high rate of diabetes in their population. I think we need more data to really understand what the risk factors are for severe disease.

**Donald Low:** Do you know why, Fred, the risk factor for diabetes?

Fred Hayden: I'm not certain about it, but there has been clear association with diabetes in hospitalizations for flu complications, and lack of control with ketoacidosis. They're included in one of the groups with chronic metabolic disease as indications for immunization, and that does reduce their likelihood of hospitalization. I'm not sure about the mechanism involved, though. Similarly, I'm not sure why chronic hepatitis B virus infection in Hong Kong has been recognized as association for progression of SARS.

### *Sequelae*

**Scott Hammer:** Go ahead, Kathryn.

**Kathryn Holmes:** I wanted to ask you about the sequelae in the patients who survive and in all organ systems that are affected.

**Donald Low:** Psychologically is probably the biggest one, the difficulty they've had in fear of going home and infecting their family, their kids, fear about going back to work, fears about relapsing. That's going to be a whole aspect of this thing which is going to require some study. Again, we're just starting to see people in follow-up and getting back—and this is probably true after most serious viral infections—getting back to your exertional energy that you had previously. We're anecdotally hearing about people with hyperactive airways and cough, probably cough being the most significant. Again, this is just anecdotal, we just haven't gathered that information prospectively yet.

### *Social Harm of SARS Fear*

**Scott Hammer:** Don, what about social harm, the fear of the SARS patient and whether it's discriminatory activity or other shunning, which we've sort of read about in other world circumstances, but you're right on the ground with a number of cases?

**Donald Low:** In the Chinese community, it just closed it down. We have five, six major Chinatowns in Toronto. Our Asian population is over 500,000. It was just a devastating effect there because of the stigma associated with the early cases. But in fact the number of patients who actually [were] Asian were very few afterwards. A more interesting phenomenon is that people in Toronto have come to live with this disease and accept it much more. You talk to people outside of the city, people who otherwise would surprise you. Physicians and ID physicians in other parts of the world, would not come to Toronto. Medical conferences got canceled, and there is fear still of coming to Toronto. I remember interviewing somebody from Le Monde who came to visit, and his coworkers said good-bye to him like they'd never see him again. This was it. He left the airport and everybody was in masks and wearing gloves and he got to Toronto and he wondered where the hell he was, because nobody was wearing masks and everybody was going on with their normal lives, except within the hospitals. It's a fascinating observation.



## *Likely Site of Initial Infection*

**Scott Hammer:** Why don't we start with Fred, then go down the line. Fred, you've got the microphone, so why don't we go?

**Fred Hayden:** A quick comment and a question. The comment is that even in uncomplicated influenza you can see abnormalities of gas exchange or hyperactivity in tracheo-bronchial clearance that will go on for weeks or months, and that's without pneumonic disease. I imagine these individuals will have much more protracted abnormalities.

**Donald Low:** Expect that to happen.

**Fred Hayden:** And a delayed recovery because of that. The question really is to ask you to speculate, if you would, about what you think is going on in terms of viral pathogenesis, the initial site of acquisition, based on what you know about modes of transmission. Is it likely upper-respiratory in the absence of upper-respiratory symptoms, or maybe is it swallowed virus, gastrointestinal and then viremic spread to the lung? What do you think is going on in terms of how the lower respiratory tract is being infected? Or is it top-down, like most of the conventional respiratory viruses?

**Larry Anderson:** That obviously is a good and very important question, and we don't know the answer to it. I can speculate. I think what's happening is the initial infection is the upper respiratory tract, and whether it's progression to the lungs via what we think for RS and some other viruses, just transmission of particles down, versus viremic spread, the clinical course of illness is certainly consistent with viremic type of spread, but I don't know that that's the case. We don't know what cells are infected, if in fact it's the reticular endothelial system versus the mucosal system in the upper respiratory tract. Who knows, I don't know.

**Donald Low:** Clinically when you admitted these patients, it could've just been cytokine response, but it was like they're viremic. They had this intense temperature, this fever that just wouldn't go away, then the cough would come later, and then the infiltrates would come later. But maybe that's all cytokines.

**Larry Anderson:** I think it is curious, or interesting, or leaves lots of room for speculation, the fact that we were unable to identify virus in pulmonary cells, although we can detect it by PCR. Maybe the possibility is that it's actually not in the alveolae and the respiratory epithelial cells, but other cells with the virus. But it's all speculation, we just don't know.

## *Accounting for Recovery Rates in United States*

**Scott Hammer:** Question.

**Martha Enserinin:** Martha Enserinin, Science magazine. Whatever the true mortality rate turns out to be, it seems really remarkable that there have been no deaths at all in the United States. I wonder, is there anything that might explain that, for instance, are there any differences in treatment in the United States compared to Canada?

**Larry Anderson:** I think the real difference is we haven't had the serious illness that others have had. We've had very few patients that have been intensive. I think one of the six patients was in intensive care, I think that's correct. We haven't probably seen that many true SARS coronavirus infected patients.

**Donald Low:** You might not have seen any.



**Larry Anderson:** We do have some antibody and virus positive patients.

**Donald Low:** I'm teasing you.

**Larry Anderson:** But not the serious type of illness that you've seen.

**Donald Low:** It's a problem is that right now you couldn't get SARS in Toronto. If I give you a million bucks you couldn't SARS. You can't get it. And yet people who arrive from Toronto in New York and develop a lower respiratory tract infection with a fever, if you don't have an etiology, you're called probable SARS. We've had 15 travel cases in Toronto. I don't think we've had 15 travel SARS cases, but if they come back from Asia with a respiratory tract infection, now they're called probable SARS. We're stuck until we get a diagnosis.

**Larry Anderson:** Right. It's important to recognize that gastrointestinal and respiratory illness are the most common illnesses associated with people coming back from travel. It's probably because they're the more common illnesses that humans get. Most of these are not SARS, they're other illnesses, but as Don was suggesting, they will be classified as SARS if they have fever and respiratory complaints. A lot of the cases in the United States really aren't SARS, they're other illnesses.

### *Pathogenesis Findings in Hong Kong*

**David Ho:** This is David Ho again. Let me pass on some information that may address some of the questions. First, Fred's question about pathogenesis. The scientists at the University of Hong Kong have been looking at the nasal secretions and looking at the pathology. You definitely see virus particles in the epithelial sheds that are shed. However, there seems to be very little clinical symptomatology nor any pathologic findings in that part of the airway. Obviously, it's quite different when you go below. So there's virus there but it's not seeming to cause any CPE within the cells.

In terms of the issue of super-spreaders, I think obviously infection control plays a role, but if you take that away, we really have to look at the data that have been generated by the folks there looking at viral load in oral washes. A more extensive study is ongoing now, but if you look at what's available, preliminary results, if you look at patients within 2 days of developing symptoms, and look at the oral washes you get a range of viral load, perhaps differing by 2 to 3 logs, and then if you look at patients between days 7 and 10, it's 2 to 3 logs higher. Again, there's a range of 2 to 3 logs. Then if you look at days 14 to 21, it's gone back down substantially, even for those cases that have progressed on clinical basis. So you have viral load going up in the first week following onset of symptoms, and then coming down thereafter.

If you think transmission is related to load of virus, actually, as you look across individuals and across different times, you will have a six-log range in amount of virus. That certainly could go a long way to explain why some cases are super-spreaders. The other issue is whether there's a unique virus for super-spreaders. If you look at the sequence, there are now sixty-some full-length envelope sequences available in Hong Kong, and there's not a "unique" sequence for super-spreaders.

**Scott Hammer:** Thank you. One last question before lunch? If not I wanted to thank the panelists.

## Session III: Approaches to Vaccines and Drug Development



Moderated by Scott Hammer

## Approaches to Vaccines and Drug Development: Blocking SARS Virus Fusion

David Ho, Aaron Diamond AIDS Research Center, New York

### *Perspectives from Visiting China*

**Scott Hammer:** Session III is entitled "Approaches to Vaccines and Drug Development," and we recognize that this is early to talk about successes in this field, obviously what the directions are, what the targets are, what the realistic time lines might be if everything went perfectly, and we've got a great panel to start this discussion off. The first speaker is David Ho, everyone knows him. He's professor at Rockefeller and head of the Aaron Diamond AIDS Research Center; a good friend and colleague for many years, he's just came back from Hong Kong, and I think he's made three trips to Asia in the past month or so. He's going to talk about possibly blocking SARS fusion and share his other experiences. David.

**David Ho:** Thank you, Scott, and I thank you and Ian for putting this session together and for inviting me to participate. You're right, I actually have been to Asia three times in the last six weeks, with great deal of distress on the part of my wife, but perhaps even more on the part of my mother. She's telling me, "everybody's running away from those places, and you seem to be headed in that direction too many times."

I should just make a few comments before I go into this very brief presentation. I've been there several times, I've seen the impact on the population. We already see it as a crisis from such a great distance here in the United States, but having been on the ground in Hong Kong all three times—in Guangdong once, in Beijing twice, and in Taiwan once—people are just consumed by this epidemic. As you know, the socioeconomic impact is huge as well. I think I was one of five guests in a hotel that would accommodate close to a thousand people, and then the last time it was one of ten guests that would accommodate five hundred. It's nice to dine in the restaurants alone, you get very good service.

### *Action and Mobilization in China*

The other thing I've noticed is that obviously China realizes that a mistake was made early on in this epidemic. In particular the lack of urgency and lack of transparency by the CDC there resulted in this explosive epidemic, a great deal of international embarrassment, and in fact lost opportunities, and this is fully realized by the leadership there now. I'll just give you one example of lost opportunity. The coronavirus was actually isolated in a lab back in February, long before others had detected this new virus, and it was in a Chinese military medical academy where people just by chance put some of the samples from Guangdong on Vero cells and turned up this virus. The EM pictures are available. We've seen it on this most recent visit. The great academicians, senior ones, declared that this was chlamydia, not a viral infection, and therefore that was brushed aside for some time, and only brought back as others demonstrated that this was probably the causative agent.

I'm also impressed by how, in the last trip in Beijing, people have really galvanized. As you said, this has been a real wake-up call for the country, and I certainly sense that everyone is very determined to turn this challenge into an opportunity. The greater transparency we're now seeing I think will, I hope, ripple through and effect other areas.

We've seen examples of how people have been mobilized. Scott, your indirect presentation suggests that



there are a lot of people who have been brought in—the Chinese Academy of Science, the Chinese Academy of Medical Sciences, and so forth. It's been orchestrated not in the Ministry of Health but in the Ministry of Science and Technology, and the effort is really quite well organized, and it's massive. When China decides to do something it does it on a pretty grand scale. We've seen efforts to build P3 and P4 laboratories, and some of the design for the P4s are impressive. Just to give you an idea, there's an effort to sequence a hundred full-length genomes, all in the next few weeks, so that molecular epi and other things could be done. There are already labs making all sorts of vaccines, including, of course, killed vaccines, sub-unit, and various light viral vectors. In fact, some of the killed vaccines have already gone into animals. There is a mobilized effort to get human serum, so that convalescent serum could be used as perhaps initial prophylaxis or therapy. When I was there last week, a delegation was sent to the south to look for the animal source that might be responsible for this epidemic. The effort is pretty impressive, but of course we wish it had happened a bit earlier.

### *S Gene Characteristics*

I'm a little embarrassed to be up here talking about this—I've worked on HIV for 22 years, and I've worked on this for maybe four weeks, so I'm a little embarrassed to be up here talking about it. But I learned a great deal from other people in a rather short period of time, and it was on the second visit that I was asked to participate and make some contribution, so we've been trying to carry out one or two projects since that time. The one I will talk about specifically is a joint effort between our group in New York, largely led by Linqi Zhang, and the group at University of Hong Kong. As you know Malik Peiris and K.Y. Yuen were probably the first to see the coronavirus in clinical samples, and their associates [inaudible], and another postdoc in our group, Fengwen Zhang, who's in the audience here.

We just decided to focus our energies on the spike protein. As has been discussed, it is encoded by the S gene, previously called the E2 gene, and this introduction has been given this morning already, so I will not elaborate. When the genome was posted on April 14, Linqi Zhang in our group immediately tried to track it down and analyzed it very quickly. Within a few hours we realized that it is a typical type I membrane protein with a huge ectodomain, so you could see this is over twelve hundred amino acids, and most of it is on the outside; the transmembrane region here; and as with other coronaviruses there's a short intracytoplasmic tail. We focused on the S protein initially because we wanted to get involved in making some sort of S gene-based vaccine, or S-subunit vaccine.

If you look at the predicted N-linked glycosylation sites, you could see that in the ectodomain there are 22. For this Toronto sequence there are 22 N-linked glycosylation sites. We know from the work of many virologists, some in the audience, that the S gene could be subdivided into S1 and S2, with S1 being principally the receptor-binding domain and S2 perhaps the fusogenic domain. For many of the other coronaviruses there is a cleavage site that's obvious, so S1 and S2 would be cleaved. But for this one, at least, we could not find an obvious cleavage site. To us there is not an obvious fusogenic peptide sequence either, but I saw it on Kathryn's slide, so it must be there.

I'm really not going to talk about the vaccine work, other than the fact that we wish to play around with the S gene and do some work related to that area. But upon further analysis, Linqi Zhang in our group saw that the SARS spike protein also has two regions that are predicted to have alpha-helical structures with heptad repeats. Obviously this would be the transmembrane region right here, and obviously this part would be S1 and so this part would be S2. We, of course, do not know where that split occurs for the SARS virus right now.

### *Heptad Pattern in S Resembles gp41*



You could see this pattern of heptad repeat is very reminiscent of what we have known for some time in the HIV field, with the gp41 fusion. So gp160 for HIV is divided into the ectodomain gp120 and the fusogenic transmembrane protein gp41, and of course we know the story for this. I should also say that this heptad repeat, 1- and 2-motif, is certainly found in many, many envelope viruses, including orthomyxoviruses and paramyxo-, Ebola and other things. Certainly for HIV and for the paramyxovirus- es it's well known that peptides derived from these repeat regions could specifically inhibit virus entry and subsequently viral replication.

I failed to mention that we also carried out this particular analysis on a number of other coronaviruses and, as has been reported, many have two heptad repeat regions, and some have three.

For the HIV people in the audience, this is well-known to you, but for the non-HIV people let me just point out what's going on here. Here is the viral membrane, the target cell membrane, gp120 is known to bind CD4 and subsequently the co-receptor. This results in the uncoiling of the transmembrane protein to reveal an N-heptad repeat sequence and a C-heptad repeat sequence, with the N-terminal fusion peptide inserted into the opposite membrane. As has been shown in the HIV field, this structure is trimeric, and then the N and C or heptad repeat 1 and heptad repeat 2 will collapse in an anti-parallel fashion, forming a six-helical bundle. As this hairpin formation occurs, the two membranes are approximated and fusion occurs.

This structure has been well-defined for some years now by two laboratories, Peter Kim, formerly of MIT, currently of Merck, and the late Don Wiley. You could see that this is the gp41 sequence—transmembrane, cytoplasmic tail, and the fusogenic peptide. But there's HR1 and HR2, also known as N36 and C34. These form this six-helical bundle as they collapse into the anti-parallel structure. This picture is simply a sort of a top view of the bundle.

### *Peptide Fusion Blockers for HIV*

Actually, in the early '90s Dr. Jiang and others at the New York Blood Center fortuitously observed that peptides from this region actually could block viral infection, HIV infection specifically. As you know, subsequently scientists at Duke and then Trimeris and later Roche have shown that this peptide DP178, later termed T-20 and currently termed Fuzeon, is a specific anti-HIV inhibitor, and this drug was licensed a few months ago.

Of course we believe, and there's actually evidence to suggest, that these C peptides, like T-20, actually interfere with this process by locking the virus in the intermediate conformation, so that fusion can not occur. Knowing all this, and knowing there are these heptad repeat sequences for the SARS coronavirus, it is a no-brainer to then try out the idea with the SARS virus.

Basically it's just a matter of doing it quickly and doing it right. Linqi Zhang very quickly designed peptides that would overlap the HR1 region, as well as peptides that would overlap the HR2 region. You could see the peptides are depicted by these lines; they range from about 27 amino acids to 46. These were all synthesized in a matter of about ten days, and purified to greater than 95 percent purity.

### *Peptide Blocking Assays for SARS*

Not having the virus, we needed to do the assay somewhere else, and for some time now we have had ongoing discussion and collaboration with the group at University of Hong Kong. We in fact just a week ago went to Hong Kong with these peptides and put them into testing in two different assays on Vero cells. It was only a matter of 72 hours before the results were apparent. This is just one picture from



what we call a simple qualitative CPE assay, where Vero cells were challenged with the SARS virus, and you could see the obvious CPE. In some of the cultures that included peptides at varying concentrations, it's clear that the peptides can protect the Vero cells from infection—not all the peptides, but five of the 12 peptides that I showed on the previous slide had anti-viral activity, with varying degrees of potency.

Similarly, a second, more quantitative assay, was performed, and that's based on plaque reduction. So Vero cells were challenged with a hundred plaque-forming units of virus, the experiment was done in quadruplicates, and then peptides were added, ranging from 0.5 nanomolar concentration all the way to 25 micromolar concentration. Essentially the two experiments agree in terms of the outcome. I don't want to show the real curves, because these results are preliminary and we have repeat assays coming out actually tomorrow. But it is safe to say that these peptides have the following properties: there's no toxicity whatsoever on the Vero cells in a number T-cell lines that we tested. We have also put all of them in the HIV assay and the activity certainly is specific, it doesn't extend to HIV. On the other hand, we have taken T-20, T-1249, and other HIV C-peptides and actually shown that they do not block the SARS coronavirus infection.

As I mentioned, five of the peptides blocked at varying activity, but two of them specifically block in the nanomolar range. We're titrating this down more carefully so we could actually determine an accurate IC50 and IC90. It is pretty clear just from the initial set of experiments that this concept is valid. As has been noted for HIV, for parainfluenza type 3, for respiratory syncytial virus, Ebola and others.

I personally do not like peptide therapeutics that much because they're difficult to make and they're generally going to be injectibles rather than oral pills. But it's certainly something that could be developed quickly, and one thing good about peptides is generally they're not very toxic.

What we're now doing is six additional peptides are being made and will be finished next week, so that we could see if activity could be improved upon just by overlapping different regions of the HR1 and HR2. Also we're in the process of thinking about how to best optimize the peptide sequence; we do know that from work in the HIV field you could actually change amino acids to lock the peptides into particular conformation, therefore leading to greater potency. The T-1249 peptide that Roche has is one example of an T-20 improvement.

The collaborators in China are very anxious to see if such an approach could be taken right away in a short period of time to do monkey experiments. They have inoculated rhesus macaques with SARS virus recently, and they're going to work out the model conditions ASAP. It might take a few weeks to scale up and purify, say, ten grams of such a peptide so one could do a proper in vivo experiment. There are many other obstacles in the way, but this is just an example what one could do in a fairly short period of time. Thank you.

## **Approaches to Vaccines and Drug Development: Lessons in Interventions for SARS**

Frederick Hayden, University of Virginia School of Medicine, Charlottesville

### *Existing Human Vaccines and Antivirals*

**Scott Hammer:** David, thanks very much for sharing the exciting information. Questions again will be held until the panel discussion. The next speaker is Fred Hayden, who is extremely well-known for his work in the control of respiratory virus infections, drug development, drug testing in clinical trials, and vaccine development. He's a Professor at the University of Virginia in Charlottesville, and is going to



share his expertise with us and perspectives on some of the lessons learned from what he's been doing as how we might address the SARS issues. Fred.

**Fred Hayden:** Thank you, Scott, and thanks to the other organizers for the opportunity of participating. It's very humbling to think that I'll be talking about developments that occurred over periods of years to decades, and David has just shared with us a dramatic story measured in two weeks in terms of coming up with a potential candidate for intervention.

I wanted to also add that what I'm going to be talking about really are personal perspectives; they're pretty highly selected and the intention is to try and stimulate discussion. Because this is a heterogeneous audience, I thought I'd start with some general comments, and then really focus down on a couple of points that are more relevant to SARS specifically.

With regard to where we stand now, of course the greatest progress has been made with regard to influenza viruses. We've had inactivated vaccines for over fifty years, and intranasal live-attenuated vaccine is close to approval. Part of the success relates to the fact that influenza virus induces strain-specific durable immunity, and that we have a fairly good understanding at least of antibody to hemmagglutinin correlating with protection against illness. The problem here, of course, is the virus keeps changing.

In regard to respiratory syncytial virus and human coronavirus, we know that natural infections induce incomplete protection, so that there are these repeated infections throughout life. The immune correlates are not nearly as well understood, to my knowledge, although it's noteworthy that we do have antibody in the form of monoclonals to the fusion protein, which are effective for prevention of lower respiratory tract disease in high-risk infants, so that that strategy of passive immunoprophylaxis has been demonstrated to work.

There are several vaccine approaches which are in clinical trials, but this area was severely affected, I think, by the experience in the 1960s where a formalin-inactivated whole virus vaccine, when given to young infants who were previously uninfected, appeared to induce aberrant immune responses such that, when the infants then acquired natural infection, subsequently they had worse outcome with enhanced disease expression and about a 16-fold increase risk of hospitalization. That has given real pause to trying to understand better the immunology of RSV infection in terms of searching for effective vaccines.

With regard to antiviral development, of course we have two classes of antivirals for influenza, and an agent in terms of aerosol ribavirin, which has probably generated more controversy with regard to its potential therapeutic value. So far, to my knowledge, we really don't have anything that's proven to be useful as a vaccine or antiviral for human respiratory coronaviruses.

One important point is that, even though one has effective interventions in the form of the vaccine and antivirals, it's of course incumbent on us to use them appropriately. I just want to remind you that, according to CDC statistics—Larry Anderson was involved with this study—we still are experiencing on an average annual basis roughly 36,000 influenza deaths in the United States alone, and a substantial number of RSV deaths, so that effective application of existing modalities is really the important message here.

### *Flu Antiviral Results*

In terms of the number of studies that have been done for influenza antivirals, I've just tried to summarize again some personal thoughts about what we've learned over the past decade in the development of



the neuroaminidase inhibitors. First, rapid development is possible, when linked to effective virologic and clinical surveillance, access to populations, and accurate diagnosis. I think all of these things are coming to be in place with regard to SARS. Early treatment is essential for optimal outcome, and I'll return to that in just a few moments. Antivirals can provide a viable adjunct to vaccine, or in the instance where a vaccine is not available for prophylaxis, as a substitute, so that there are a number of potential strategies where an antiviral drug could be used. Again, many of these would be applicable to SARS, and also would be applicable in the event of our next influenza pandemic. I think one of the points I would like to editorialize about is that it would be important for us in terms of our public health planning to incorporate stockpiling of existing antiviral drugs in our influenza pandemic plan. To my knowledge that is currently not in place.

The pharmacologic and tolerance profiles of drugs are key, obviously. I think the fact that inhaled zanamivir has not received much clinical use is an example of this particular point. Finally, resistance emergence can be clinically and epidemiologically important, depending on the particular drug and virus combination one is talking about.

Let me illustrate a couple of these points for you. First, early antiviral treatment of influenza not only reduces symptoms, but can impact on lower respiratory tract complications, as well as the likelihood of hospitalization, so that in studies that have been done with oseltamivir, the risk of hospitalization is reduced by roughly half, whether one is a healthy adult or a high-risk or elderly individual, in the one month after enrollment in the placebo-control trials that have been done to date.

#### *Treating Households to Control Flu*

As you've already heard, households are probably an important site of transmission of SARS, and indeed other respiratory viruses. A logical strategy in such close-contact circumstances is treatment of the ill index case, combined with post-exposure prophylaxis to the healthy household contacts who maybe have been exposed to that particular virus. This combination approach has been tested with both classes of influenza inhibitors to try to look at the potential for reducing secondary influenza illness in the context. When studies have been done with the older M2 inhibitors, you can see that there's very little evidence of protection against secondary influenza transmission, and this appears to relate, at least in part, to rapid emergence of drug-resistant virus and spread to contacts, resulting in failures of drug prophylaxis.

In contrast with the newer neuraminidase inhibitors, whether one is talking about inhaled zanamivir or oseltamivir, there are high levels of protection observed in healthy household contacts when that combination approach is used, and no resistance emergence or transmission has been recognized to date. So the particular drug does really make a difference in terms of the likelihood of problems with emergence of resistant variants.

#### *Ribavirin and Steroids for SARS*

As you've already heard, the standard of care for SARS patients in many of the medical centers in Hong Kong, at least, has been a combination approach with the systemic ribavirin, as well as relatively high dose glucocorticoids. There are various different kinds of drug doses and specific agents that have been used in terms of the glucocorticoids, but in general this is the strategy that's been used. For patients who have progressed in terms of their pulmonary status, it's not uncommon that they've been given pulse glucocorticoids, again in quite high doses. It's important to bear that in mind in examining the natural history data, in terms of both clinical and virologic course of infection that's being published currently.



Indeed this is some of the data from Malik Peiris' group recently published in Lancet, where they looked at nasopharyngeal viral RNA levels at these different time points after onset of symptoms in a cohort of 14 hospitalized patients. As you've already heard, the titers were relatively lower in week after infection, peaked on day ten in this sample, and then declined thereafter. Bear in mind that in uncomplicated influenza in adults, basically for nasopharyngeal recovery virus, most individuals would be negative for virus by day five. This is a very rapid loss of virus relative to this protracted period of replication that's seen in SARS patients. Indeed, 90 percent of individuals would be positive in the upper respiratory tract or feces out here on day 15. As you've already heard from Don Low, it's not uncommon that in individuals who have died of SARS they'd still be virus-RNA positive in autopsy lung samples.

There are several points here. First, there's a protracted period of virus replication. This clearly means that there's an ample window of opportunity for intervention with an antiviral drug when we have it. Second, this peak in viral replication occurred in the face of 14 days of systemic ribavirin therapy in this particular study. It clearly was not exerting a substantial antiviral effect.

### *Steroids Enhancing Viral Load*

Finally, there is the concern that's already been raised about the potential that the corticosteroids, which were given a three-week tapering course in these patients, may have actually contributed to this protracted and increased viral replication at this time point. There are human examples of this, and this is one study done in a placebo-control blind fashion in RSV bronchiolitis and pneumonia in infants who had severe disease and had to be mechanically ventilated. You'll see that, as indicated by the dash line, the dexamethasone recipients, and again this is a fairly high dose given for four days, had a delay in the clearance of respiratory syncytial virus from the tracheal aspirates compared to placebo administration.

There are also animal model data from the pneumonia virus of mice, which is a paramyxovirus, where steroids will enhance replication and mortality under these experimental circumstances. Even intranasal steroids, we now know, will delay viral clearance in rhinovirus (colds in adults), and will increase the risk of acute otitis media in children given that intervention. I think one has to be very cautious about corticosteroid effects on viral replication, particularly in the absence of an effective antiviral cover.

### *In Vitro Ribavirin Testing Against SARS*

One of the things that has fostered the development and testing of drugs for influenza and RSV has been the availability of cell culture system, multiple animal models, and the ability to conduct studies in experimentally infected humans, due to proof-of-principle studies and some dose arranging. Already it's quite remarkable that we have with SARS coronavirus availability of cell culture assays, as well as at least one animal model in which putative antivirals could be tested. However, it's unlikely that we'll see of course experimental human infections by this, or probably even the recognized human respiratory coronaviruses because of some safety considerations.

Let me share a couple of examples with you. These are data from John Huggins at USAMRIID, looking at increasing concentrations of ribavirin on plaque numbers due to SARS coronavirus. You can see that, even at very high concentrations of ribavirin, there was no inhibition of replication by this virus. This has now been found in several other laboratories, so that under in vitro circumstances, at least under these test conditions done in Vero cells, there's no evidence of a selective antiviral effect. Of note, the same cell system with ribavirin will show activity against other RNA viruses, often in concentrations down in the 20 to 40 microgram per mL range. So it's not just a matter of the cell system alone.

### *Interferon Protection Against CoVs*



Using this particular assay system, John has also screened a number of other agents, and you're going to hear more about this from Cathy Laughlin, but one of those that turned up to be positive, and this was I think predictable, these were type 1 interferons, both interferon alpha and beta have activity. This then reminds us to look back at what's been learned about activity of intranasal interferons for prophylaxis against experimental coronavirus infection. I was able to find two particular studies that address this question under experimental conditions, where at the common cold unit, a relatively high dose of intranasal interferon, 12 milliunits a day for four days, was significantly protective against both infection, with a 55 percent reduction compared to placebo, as well as the development of coronavirus colds, with an 85 percent reduction. Not surprisingly, symptom scores were also markedly reduced.

A lower dose of intranasal interferon, two milliunits once a day, did not protect against infection, but moderated the frequency of colds. It appears that intranasal interferons, at least for human respiratory coronaviruses, could potentially provide protection against illness. This is certainly something that, as we've heard, warrants testing and appears to be undergoing testing right now in China. On the other hand, not knowing where virus replication is initiated in this syndrome, it's hard to know what the outcome might be, but it's certainly a testable question. It's not clear to me that protection in the nose will be sufficient to protect against SARS coronavirus infection.

Another issue, of course, that the interferon activity raises is what are its potential uses as therapeutic intervention? This, I think, needs to be predicated on a better understanding of disease pathogenesis with regard to the endogenous interferon responses. I just wanted to share with you this one older publication, dating now over forty years, from Sam Baron and Alec Isaacs, the discoverer of interferon, where they studied fatal cases of influenza and found an absence of interferon activity in the lungs of these individuals. We know that there's a brisk interferon response in the nasal passages and blood of uncomplicated influenza sufferers, but there may be a deficit in endogenous interferon responses, so that this again is an important part of the story in terms of trying to understand better what are the host innate immune responses to SARS coronavirus, and are there over-exuberant responses, or deficient responses that one might be able to supplement?

### *What We Need to Know*

To summarize for you, we need a better understanding of the natural history of infection. The fact that there is protracted viral replication in illness, I think, tells us that an antiviral could do considerable good in this particular disease. The approach of trying to impact on host immune responses, and the question of immunopathology, will depend again on a better understanding of the mechanisms of injury, and these host responses. We do have test systems that are available for both screening for potential antiviral drugs, as well as pre-clinical assessment in the form of at least one animal model. As you've heard, several potential antiviral targets exist, and indeed selective antivirals are already in the pipeline.

Ultimately, I think a combination of antivirals and host-response modifiers will be required for optimal therapy, and I would just like to again restate that controlled clinical trials are going to be essential for better understanding of what really works in this particular illness. Thank you for your attention.

### **Approaches to Vaccines and Drug Development: Status of Drug Screening vs. SARS**

Catherine Laughlin, National Institute of Allergy and Infectious Diseases, Bethesda

#### *A Collaborative Screening Program*

**Scott Hammer:** Thank you, Fred, that's exactly what we wanted to hear, the perspective from you. That



leads perfectly into the next speaker, Catherine Laughlin Chief of the Virology Branch at NIAID, in charge of the drug-screening program that the NIH and USAMRIID are undergoing for the SARS coronavirus. She's going to update up on the status of that drug screening. Catherine.

**Catherine Laughlin:** I would like to thank Drs. Hammer and Lipkin to inviting me to make this presentation to you, the other organizers as well, and also Fred Hayden for really introducing the overview of the potential use of antivirals. I am going to talk about specific types of pre-clinical testing but I am going to reinforce, I hope, a number of the themes that Fred raised.

First of all, I have to correct Dr. Hammer. I'm not in charge of this program; I'm here as a representative of a truly collaborative effort between the CDC, USAMRIID, and NIAID. This collaboration fell together really almost instantly, very quickly, and I think that was because we have a long experience, starting in the late 1990s, of working with smallpox, working with West Nile, and other pathogens as they've become an issue where protection and defense from the pathogen has been a concern. We have definitely worked out how to work with our colleagues, and I think things are pretty efficient right now.

I'd like to mention that the FDA, while not a formal part of this collaboration, has made it very clear that they would appreciate being made aware of anything that looks as though it should go into the clinic as quickly as possible, so that they can proactively work with any pharmaceutical sponsors and with the government to make things happen quickly.

### *Protecting Intellectual Property*

The first obstacle really to getting a large screening program underway was the development of a way to protect the intellectual property of the sponsors who provide the compounds. We actually are feeling rather pleased that, as a result of the really energetic efforts of our tech-transfer people and their colleagues at the USAMRIID and the first few pharmaceutical firms that we've worked with, we now have one simple agreement that gets signed by the pharmaceutical sponsor, by USAMRIID, by NIAID. It's a combined material transfer agreement and CRADA, which is very novel just by itself, and our hope is that most pharmaceutical firms will find this default template suitable for their needs, because if we can get this document signed quickly there's no delay in getting drugs into pre-clinical screening.

A number of companies who all have lawyers—and I apologize in advance to any lawyers who may be here, but it's the lawyers' responsibilities to protect their companies' rights—we have found that a number of companies actually do want to negotiate minor modifications to the agreement. When I left on Friday, our tech-transfer people had thirty files of agreements in process of negotiation, most of them ready to be signed. I'd like to encourage anybody here who has compounds that they would like to be screened to get in touch with us as soon as possible. I'll have contact information at the end of the talk, and let's get this legal process rolling as quickly as possible so we can get on to the important business of looking and seeing if drugs have activity.

### *CPE-Based Screening Assay*

The assay itself is performed up at USAMRIID. It uses virus provided by the CDC. As I think Fred mentioned, it's an in vitro CPE-based assay; it's based on Neutral Red uptake which measures living cells. Right now the capacity is about 400 compounds per week. With active compounds, or when we have questions, we do follow-up assays that are a little more labor-intensive for plaque reduction or yield reduction. We're exploring right now other possible mechanisms of getting some high-throughput screens established, but those are not reality yet.



## *Roles of Government Agencies*

The various roles of the government agencies. What we do, listed under NIAID, is we've been negotiating all of these MTA/CRADA agreements with the help of our contractor, which is Jack Secrist, whom some of you may know at Southern Research Institute, who is well known certainly in the antiviral community. He has an activity that's been ongoing for tuberculosis and for bio-defense agents that we've now adapted to SARS, where he will contact any potential sponsors, handle the logistics of acquiring the compounds, he puts them under code, so they all go to USAMRIID under code, although we tell the investigators at USAMRIID what they are as soon as they have given us the results of the data. Then he also returns the data to the sponsor so that we can really make sure that the people who are providing the drugs get the data that they need for their future planning as quickly as possible. Last, but certainly not least, this is clearly the most important part of the whole process, the actual in vitro testing right now is done at USAMRIID.

## *Possible Viral Therapeutic Targets*

We've already had some discussion of possible viral therapeutic targets; this is clearly just a partial list, and I'm going to modify it a bit from what I've learned this morning. Clearly cysteine protease inhibitors are of extreme interest. There's the RNA-dependent RNA polymerase, which is not a mammalian enzyme, so it would be a target that would be expected not to result in toxicity to the cell. As part of the RNA replication, there's a helicase activity; there are other types of targets involved with genome replication and transcription. Some of these involve compounds that really have cellular effects, such as ribavirin and alter nucleoside pools in the cell. Kay Holmes mentioned the assemblyosome this morning, and that certainly would be a target. Someone mentioned the N protein, and that that has potential activity in regulation translation of viral RNAs. That would clearly be a novel target.

Then the very exciting large category that we've just heard about is that of fusion inhibitors. As I think was clear from Kay Holmes' presentation, there are many steps of the viral life cycle where fusion inhibitors might play a role, both at blocking the actual fusion between the virus and the cell. By blocking the spike, you could block the cellular receptor and thus prevent cellular fusion, you can block cell-virus fusion, and you can also block cell-to-cell fusion in the formation of these giant cells. Since those are showing up clinically, I think is a potential important target.

These are the classical antiviral targets. But I think as Fred mentioned, it's my belief also that probably optimal ultimate therapy will involve a combination of an antiviral with a compound that acts on some step of the pathogenesis of the virus. Here we're really limited, because we don't understand the pathogenesis. Again, I want to echo Fred that these initial natural history studies that are being done right now are crucially important, and it's important to do them in both immune-competent and immunosuppressed populations, so that we can understand the contribution of the innate and potentially adaptive immune response to the virus.

I think that we heard from Linda Saif this morning also that, although steroids have been used as something that would seem to be a logical thing to test, there are animal models in which they actually exacerbate the disease, so that in the absence of natural history data I think we have to move very carefully about proceeding clinically with compounds in these last categories. The last point I think that I want to emphasize as a result of this is, because it's difficult to experiment on people, and we really don't like to be in that situation, the development of animal models where compounds which are not strictly antiviral, ones that impact the immune system of the host are exceedingly important to develop. We're very encouraged by the preliminary results with the macaque systems, and hope that those will continue, and that other hopefully smaller models will be developed as well, although possibly not as small as the



mouse that Kay showed us this morning.

### *Testing Priorities*

What we came up with as our testing priorities. The obvious first class of drugs to go after are those that have been approved by the FDA for other viral indications. There wasn't really any rational reason to think that these would work, but if we were really lucky and got a home run, these are drugs that clearly we understand their safety profiles, they could go into people immediately, and it would've been lovely. It has not transpired. The next priority has been to go after drugs that are in clinical development, generally for other indications, that address the targets that I had on the previous slide. Those are proceeding apace.

Another priority—and again, this is not a rational way to approach drug development, but it's with the potential advantage of getting it into people quickly—if we can find any other drug that is already FDA-approved for anything and happens to work against SARS as well, that clearly would be wonderful. We're starting by looking at a standard library that has a selection of different drugs that are FDA-approved with different types of indications and chemical structures. It is called the Preswick library, but we'll go from there when we've done that.

Also, and I think very importantly, we're looking at anything. There are a tremendous number of pharmaceutical firms and individuals with an incredible amount of good will that want to do something to help. They are all calling and offering what they have and explaining the reasons that they think things might work, and we're accepting all of those into the screening program. Some of them are natural products, including Chinese herbal medicines, which I think we're a little excited about, but very interesting. Some are not quite so exciting. Dried broccoli I think was one, and I apologize if the person that offered broccoli is here also.

### *The Method of Testing*

The way the testing is done is in 96 well plates, and the initial testing is done with four drugs on a plate. For that we can do five dilutions per plate, and cover a range of about 625 full dilution-range concentrations, generally from one microgram per milliliter to one hundred. All the testing is done in Vero E6 cells. For the initial confirmatory testing, we essentially do the same tests, but with one drug per plate, and this allows us to get a few more drug concentrations in.

I'd like to really commend John and his group, because they have developed an assay and are working on assay validation all at the same time, while running replicates of the initial compounds. This is just unheard of speed for this type of activity.

What the data typically look like, what we measure is cell death, and live cells are up there, and the increasing drug concentration is down there. With this drug, you can see that low concentrations did nothing, but it starts protecting the cells there. The way we measure it is by a 50 percent inhibitory concentration, which is right there. At the same time, on the same plate, you run uninfected cells in parallel, but they're also exposed to the drug, and you can see that the cells do just fine until the concentrations get this high. We figure a 50 percent killing number for this drug this for cells; you can see there's a window of concentrations between which you can kill the virus without killing the cells, and that's called the selective index. Generally, with antiviral drug development, you'd like a selective index of ten or better. We're considering encouraging really anything over two. As we get more positives we may get more selective.



Fred showed this also, but I think we both really wanted to make the point that we and lots of groups—again, these are John Huggins' data—have looked at ribavirin very hard. It does not work in vitro.

### *Screening Results to Date*

Where we are right now with the testing program is about 120 compounds have gone through. The only ones that have really shown any degree of anti-SARS activity have been beta-interferon, rimantadine, and some cysteine protease inhibitors. Unfortunately, I need to emphasize the point that all of these showed activity that is not clinically useful right now, so that we consider these hot leads and are actively pursuing these categories, but we don't have anything to help people right now.

As I said earlier, I wanted to give you the contact information, and if we get a participant list we'll e-mail it to all of you to make sure that we can disseminate this as widely as possible. The primary contact is Jack Secrist, the Southern Research Institute. Terry Greenfield in my group can also help with that. Any questions regarding the actual mechanics of the assay should be directed to John Huggins, and he is very happy to give the details of the assay to other people who would like to set it up.

Finally, I just want to thank the collaborators, and you can see there's a large group of people at USAMRIID all working very hard for John right now, another group of people at Southern Research Institute with Jack Secrist. In my branch, the people who are actively engaged in the antiviral program are listed. I also want to mention two other program people, one in my branch, Ng Jung Park, and another in the division, Linda Lambert, who are knocking themselves out arranging research support for other aspects of SARS virus control and containment, and we all work for John La Montagne, who you will hear from later. Thank you.

### **Approaches to Vaccines and Drug Development: Adenovirus Vector Technologies for Vaccines**

C. Richter King, GenVec Inc., Gaithersburg, VA

#### *Starting to Build Vaccine*

**Scott Hammer:** Thank you. It was very informative, and it also illustrates one of the reasons to have this meeting, and that is to create contacts and interpersonal knowledge and program knowledge to foster collaborations from this meeting. In that vein, the next two speakers illustrate the importance of public-private partnerships in moving ahead against the outbreaks such as this and a new pathogen. Rick King, who's Vice-President for Research at GenVec, has agreed to discuss with us adenoviral technologies, vector technologies for vaccine development. Rick.

**C. Richter King:** Thank you for inviting me. This is really an exciting meeting. It's great to see how much progress has been made in just a few weeks, and to see how motivated people are. I think that's just a terrific example of how fast you can move.

On our side, we've really just started the process of building a vaccine, and I'm going to tell you about the technology platform that we're using. The progress that we have made, though, is not insignificant, and that is that we do have all the agreements signed, and the sort of paperwork out of the way to get going. I credit Gary Nabel and Phil Gomez at the VRC for a lot of persistence on this, and we have a lot of great collaborators at the contracting group, the SAIC, who also put through a lot of paperwork to get this out of the way and get us onto the scientific side of things.

#### *Adenovirus Vectors as a Vaccine Platform*



I'm going to tell you, because it's way too early to talk about data relating to SARS, the information about the technology that's behind the approach that we're taking. Not all the methods that we're using that I'm going to talk about today are going to necessarily be applied to SARS, and in fact I'm happy for ideas coming from the audience in the coffee break time and so forth as that seems reasonable.

We think, and we've come to this field only really in the last couple of years, that adenovirus vectors can be used as a vaccine platform for several different types of disease, and this meeting is obviously about viral diseases. There's a number of reasons why they have advantages. There really are potent immunogens, and they trigger a strong and potentially medically significant immune response through a variety of different cellular pathways. There's been a lot of publications on this, and I'll just show one very quick graph on that. I won't dwell on this because it's really the work of other people.

Something that's important to recognize is that adenovirus vectors are really very well tested in the clinic now. There are thousands of patients that have received adenovirus vectors for a variety of different types of diseases. They've been injected into the heart, they've been injected into the eye, the muscle—they're really quite well-tolerated as a vector system themselves, and even in the vaccine setting they're well tolerated.

I'm going to spend a little bit of time on versatility. You can do a lot with these types of vectors, and I think you can do a lot to generate a better vaccine. Finally, and very importantly I think, the methods to take adenovirus vector vaccines into the clinic are well-established, manufacturing and quality control procedures are in place. Not only that, and I think just as important, should things look promising, the ability to take these further to a real commercial phase process are also becoming more well-established. Of course, there's not an approved product yet based on this, but there are products that are far enough along in the pathway, into phase two testing. Thinking about the approval process, many other companies including GenVec are investing in the methods to really generate the commercial phase manufacturing for this. This is a non-trivial hurdle for anything that would need to move forward for really wide-spread application as a vaccine.

I should mention that the commitment to the platform of adenovirus vaccines is really not unique to GenVec. We have Dr. Emimi here in the audience, who will be on the panel, who has been really leading a terrific effort at Merck as well, and a tremendous amount of great data has come out of that group as well.

### *Previous Results with AdVectors*

This is really just to remind people that a variety of different publications, this one happens to be on an Ebola virus antigen, suggest that if you immunize animals with plasmids you can get a little bit of a boost with a secondary plasmid immunization. If you follow that with a boost using an adenovirus vector, you get really gigantic increases in immune response. This is just measuring antibody titer, but the same thing can be measured at cell-mediated responses as well.

I think it's basically these kinds of reports that have stimulated the interest in a variety of different disease targets. As I mentioned, we signed an agreement in April. I think two weeks after the cancellation of the AACR meeting in Toronto we had signed an agreement. That's how fast the paperwork went. I think part of that is that we had had a long-term and highly productive interaction with the vaccine research center to build a multiclade HIV vaccine with them, that will focus on a variety of different envelope clades and multiple other HIV antigens. We also have an existing and very active and productive collaboration with the NMRC and their agile vaccine program, looking at generating a vaccine for



malaria. We'll be using multiple antigens in different parasitic stages, and we also have the same kind of approach with the NMRC looking at dengue fever virus. This is something that has become a developing interest and agenda. We are not really initiated as a vaccine company; we've had applications of delivering other types of genes for so-called gene therapy or gene transfer applications.

### *Manipulating AdVectors*

What are these technologies that I'm talking about? They really all derive from the ability to very quickly and flexibly move things around inside the adenovirus genome, and do it under controlled conditions. We can end up with optimized vectors, which change the performance of the vector itself, and I'll show you a little bit of information on that. The other thing that we can do is really try out a lot of different conformations of the antigen itself, or try multiple antigens themselves, and then test them quickly. Supporting that is a couple of technologies, which I think shouldn't be overlooked, and that is that you need cell lines to grow these adenovirus vectors. These are now well-characterized. The FDA has looked at them for clinical studies, and they're available now for production. An area which I'll just touch on very briefly is that I think you can begin to think about using libraries of adenovirus vectors potentially to select for the vaccine of interest.

I'll just go through a couple of examples of this, just to sort of stimulate people to think about different ways of using this technology. I mentioned that you can flexibly move pieces of DNA around in an adenovirus vector, and that's done by homologous recombination in *E. coli*. These are methods that were worked out in our situation by Doug Broff at GenVec. Basically, you can take pieces of DNA that have short regions of homology on each end to the adenovirus genome, recombine them into a full-size vector genome, and then simply transfect that into the recipient cells that complement for the deficiency in the adenovirus genome. All of what I'm going to tell you about are adenoviruses in which essential genes have been removed, so they no longer can replicate. What comes out of those transfections is a large number of highly homologous vector, which can then be purified and taken rapidly into the immunological screening.

This method had undergone the scrutiny of a clear documentation, so we have a paper trail all through this process. As you are developing a candidate for human use, we can move it quickly into the clinic.

### *Using AdVector Libraries*

I wanted to bring up the idea of using libraries of adenovirus vectors. The same base technology, can be applied, but instead of starting with a single gene here and inserting it into the backbone of the adenovirus, you can put a collection of genes, a library of genes, or a variety of different modifications of a single gene. When you do that, you start first with a library of genomes, and then when those are transfected into your conversion cell, you get a library of adenovirus particles, each containing a different transgene. From that, they can be purified and tested for a variety of different characteristics. At GenVec we didn't start this as an immunological screening methodology, we started it as a method to screen for genes with interesting biological characteristics. I think it could be applied to immunological methods as well, or perhaps to optimize an antigen for the performance that one wanted.

### *The Base AdVector*

The base vector that we have been using is a so-called second generation adenovirus vector, and by that I mean it has multiple deletions in essential gene product. What GenVec has done is take out all of the early region 1 genes, a portion of the early region 3 genes, and much of the E4 region—all the functional regions of E4. The advantage of doing this is really two-fold. One is that, when it's put into a complementing cell, 293 cells containing the E4 open reading frame 6, this genome really has no chance of



recombining back into a replication component vector system. In our history of using this vector, and we've done many, many, many viral conversions, we've never seen any generation of replication-component adenovirus. That's an advantage from the safety perspective or the purity perspective, I suppose, but it's also an advantage from the space and flexibility perspective, because what those kinds of modifications allow us to do is think about putting expression cassettes into several different regions within the adenovirus genome. We've done this, again not necessarily for vaccine applications, but it's certainly applicable to vaccine applications. You could think of putting multiple antigens into different portions of the adenovirus genome, you could put immune-stimulatory molecules or APC maturing factors.

### *Broadened Ad Tropism Improves Response*

I want to mention one modification that we've made to the adenovirus genome which is to actually begin to think about retargeting the vector to antigen-presenting cells. For this work we started with a simple hypothesis, and that is, can we increase infection or transduction of antigen-presenting cells using tropism-modified adenovirus vectors? What I mean by tropism adenovirus vectors, this is not a coronavirus in this case, this is an adenovirus particle, but it has an extension called fiber. On the end of fiber is the receptor-binding components for adenovirus, and we can add to those. In this case, we've added a RDG sequence which will bind to integrins. This vector, which now has a broadened tropism, can now get into a larger number of different types of cells. We wanted to test the hypothesis whether this RDG-containing vector could actually present antigens more effectively following transduction of antigen-presenting cells. This is work that was actually done just around the corner here at Cornell, in the laboratory of Ron Crystal with Stephan Worgall.

In these two graphs, you can see that the transduction of some murine dendritic cells by a beta-galactosidase gene containing vector is better, almost an order of magnitude better, when you use the RDG-containing adenovirus. The same thing is true with another vector that carries a different marker gene. We were confident based on this that we thought we were getting better transduction of antigen-presenting cells, and they showed, in fact in this experiment, that if you immunized with that RDG-containing virus and compared it to a wild type adenovirus vector, and then looked using an ELISPOT assay for the strength of the cellular response to that antigen, they could see a difference in a fairly standard ELISPOT assay where the target was CT26. We think that this is, at least possibly, a way of augmenting the immune response even better than adenovirus is doing right now.

### *Speed and Scalability*

I want to just quickly go through another advantage that I think adenovirus technology provides, and that is that we can move quite quickly. There really are good, well established ideas about using, manufacturing, and testing methods for Phase III in commercial process. They really have been designed to do that, they're not just a quick modification. A lot of money was spent on generating these kinds of methods. They've been discussed with the FDA; we had these conversations around our gene therapy applications, not around vaccine applications, but we think that much of the same kind of information will apply here. The documentation is well-advanced, quality systems are coming along well. We're confident that that can move ahead.

In addition to that, with a vaccine application you really need a scalable process. Something that's manufacturable on a laboratory scale is not really sufficient; we're going to have to make a lot of this. Especially for the worldwide AIDS vaccine, there's going to be a lot of that that's going to need to be made, and we've taken the time to adapt our manufacturing methods to bioreactors. We're currently at a relatively small scale, but the advantage of using bioreactors is that they can inherently grow, you can add a step and make the things much, much bigger. So far, in all our efforts to scale the process up it's



been very well-behaved.

In addition to that, the methodologies are in place to rapidly purify the adenovirus vectors using methods which really can just get bigger with the size of the method. These are concentration steps, and then finally column purification method, which avoids some of the cumbersome old fashioned gradient-separation methodologies. These methods are well-adapted, well-established for making commercial-grade protein products, and we think that they'll be easy to adapt to the virus systems as well.

### *Analytical Tests in Place*

Finally, there's a whole collection of analytical testing which is now in place that will assure that, should we be fortunate and find adenovirus vectors which will be useful against SARS, they will have the appropriate quality systems in place such that they can be released for use in normal humans, in their initial clinical tests, and have a variety of safety testing: identity, purity, potency, and other tests as well. These are being developed, and one nice byproduct of this is that the methods that one generates for both manufacture and for testing will be similar for all adenovirus products. A vaccine that's generated against SARS will use many, many of the same tests as one that's generated against HIV, so there's some nice synergy there.

That's really where we are, we're really just at the very beginning phase of the process. But we think there's some technological advantages that we have at the beginning. It's very potent, it's a safe system, we have a lot of different things we could do to increase its activity, and we think it's really a commercial-type product. That's where I'm going to stop.

## **Approaches to Vaccines and Drug Development: Some Approaches to Vaccine Development**

Thomas Monath, Acambis, Cambridge, MA

### *Considerations for SARS Vaccines*

**Scott Hammer:** Thank you, Rick. The last formal speaker in this session before we head for the panel is Tom Monath, who has had a wonderful career in virology and is Chief Scientific Officer at Acambis, who's going to talk to us also about vaccine strategies, potential approaches. Tom.

**Thomas Monath:** Thanks, Scott. I'm very pleased to be here. I'd like to commend the Academy for again being proactive in organizing a meeting on an important emerging disease.

I'm unfortunately, like others, unable to show you any hard data on a new vaccine, so I'm going to have to share with you some of the thinking we've done on this topic, and perhaps it'll have some general relevance. We started about a month ago trying to decide whether, first, and then how we would approach development of a new SARS vaccine. Some of the considerations were: it was clear that we would have to explore multiple approaches, there was no single technology that kind of stood out, it would be a significant effort, we couldn't do it halfway, and we'd have to develop a team that incorporated molecular biology, virology, immunology, animal experimentation, and clearly we would need collaborators as well.

Currently the only available model, of course, are monkeys, and that means one has limited array of facilities and collaborators. There are many other areas, for example, in selection of an appropriate adjuvant for a vaccine where we would need collaborations. Probably most important, we felt that a strong collaboration with our veterinary colleagues was going to be important because, as we're all aware, coro-



naviruses have not been particularly important human pathogens, but there's been a wealth of experience with respect to a variety of animal diseases.

Where this disease is going to be in the future is obviously a big question for any decision on whether to embark in a program to develop a vaccine. I don't think we can answer that question now; perhaps the default is healthcare workers—they seem to have clearly been impacted by this disease—travelers, military populations affected by outbreaks. Beyond that I'm not sure, and I think only time will tell. Our strategy is basically to get started and then to reassess the epidemiology and how this epidemic plays out.

### *Ideal Vaccine Qualities and Features*

What should a vaccine look like? Clearly, I think we're looking for a vaccine that would be administered pre-exposure and would induce protection against clinical disease, perhaps against infection and transmission, or the ability to transmit to others. Preferably the smaller number of doses the better. This could be the same product, or some kind of a prime boost strategy with different products. A big question is about mucosal immunity which we'll come back to. I think clearly the vaccine needs to elicit neutralizing antibody response, and probably also a CD8 T-cell response, with the appropriate T-helper orientation. The immunopathology issue will have to be addressed; the vaccine should not obviously enhance infection on exposure to wild-type virus in the future. It will have to protect against the major circulating strains, be safe and immunogenic, particularly in the elderly, and will have to be manufactured efficiently in large scale, alluded to by my predecessor here, preferably without BL3 containment, which is a big issue for manufacturing. Of course, it should be affordable.

Some of the anticipated complexities have already been mentioned at the meeting. The neutralizing protective antigenic determinants are likely to be conformational, at least in part, heavily depending on glycosylation in the case of the spike protein. There's a possible requirement for robust mucosal immune response, which has generally been difficult to achieve with many vaccines. The whole issue of whether or not a vaccine would enhance subsequent infection is an issue. What happens with regard to the antigenic variation of this virus in the future is a concern for vaccine development. As has also been mentioned before, immunity induced by vaccine may be problematic because it is may not be durable, or protection may be incomplete.

### *Existing Animal CoV Vaccines*

Let's turn for a moment to what we know about veterinary coronavirus vaccines, and Dr. Saif has given us an elegant discussion of this earlier. Of course, these viruses affect a variety of animals and are respiratory or intestinal infections. Protection afforded by vaccines and natural infection seems to be mediated, at least in part, by mucosal immune responses, and particularly passive maternal antibody, which is also mucosal, or lactogenic in the case of mammals. Passive administration of neutralizing antibody to the spike protein has been shown to confer protection in some species, which is important, of course, because that is a relatively simple immune correlate of protection, and would be nice if it holds true for SARS.

However, there are few examples of effective veterinary vaccines, and I wanted to just kind of run through some of what's been learned. This is a very high-level kind of review, but it's instructive because it gives us perhaps the way forward. In the case of infectious bronchitis virus disease of chickens, a variety of different strategies have been tried, including fowlpox-vectored spike protein, which induced antibody and protection in some experiments. Recombinant S1: not very effective. Inactivated and live-attenuated commercial vaccines: partial protection, relatively short duration of immunity. Priming and



boosting looks quite promising in some experiments, with fairly high-grade protection in some studies where the priming has been done with a live vaccine and boosting with recombinant spike protein.

The canine coronavirus: inactivated vaccines have shown some promise, in some experiments quite complete protection. This is probably the best example of that, and hopefully would be true for SARS, but who knows at this point? The bovine viruses: hemagglutinin has shown partial protection and induction-neutralizing antibody, same with live vaccines. The pig respiratory coronavirus in an activated vaccine: again, just partial protection.

Dr. Saif spent a lot of time on probably the most complex of these, and best-studied, which is TGV in the pig. A wide variety of approaches, including oral immunization with plant recombinant vaccine, DNA adenoviral vectored S1, recombinant S1, recombinant S, M and N with the mutant LT that she mentioned, inactivated whole virus, and various priming and boosting strategies. In all of these cases, I think one can say that protection has been less than optimum.

The cat and feline infectious peritonitis virus vaccine development has been plagued by this whole issue of immunopathology and enhanced disease, particularly when S1 spike protein or inactivated whole-virus has been given. Even with vaccines that have eliminated the S protein, live-attenuated virus or pox vectored-virus has shown somewhat better protection, at least in some studies. This immunopathology thing is not an all-or-none in this model for vaccines.

What do we take away from all this? There's a huge amount of data, and I haven't really given it justice, but I think we can say that mucosal immunization strategies, particularly in mammals lactogenic immunity, is required to protect newborn animals. Partial protection has been demonstrated with live or inactivated vaccines against some of these diseases. Protection is often not durable. Subunit vaccines have not been particularly effective, except perhaps in the context of a priming and boosting strategy. Antigenic variation is certainly a problem with IBV. Live vectors have shown some promise in some models, and of course we have this immune enhancement with feline infectious peritonitis.

### *Possible Targets*

If one is to develop a vaccine, say a live vector vaccine or recombinant vaccine, I think we would all agree that the S protein, which would induce neutralizing antibodies, is the primary candidate. Conformation may be critical, although there are linear epitopes that have been described in murine hepatitis and IBV, giving some promise perhaps that your expression system may not require fully native conformation of the protein. Other proteins, particularly M, might be also considered in a vaccine strategy.

With regard to the immune correlates, I'm intrigued with a newspaper report, and I don't think there's anything other than that, of successful treatment with convalescent serum obtained from patients, again indicating perhaps that induction of neutralizing antibody would be an effective strategy with respect to a vaccine.

We clearly are going to have to know about the pathogenesis of this disease better. I would like to think that systemic immunity, IgG-based immunity, would be sufficient to protect the lung tissue, but I think this whole issue of mucosal immunization, whether it's necessary to establish a barrier at the site of entry of the virus in the upper airways, is going to be useful or necessary to prevent an infected individual from transmitting. That's certainly something that's going to have to be looked at.



### *Inactivated Whole-Virus Vaccine*

Let me run through various approaches. None of this is very novel, but it's helpful to think through what are the pros and the cons of the traditional approaches to vaccines. Clearly, one starting place might be a simple good old-fashioned inactivated whole-virus vaccine. One of the interesting issues with SARS, of course, is that it grows in an acceptable cell substrate. There are a lot of vaccines, live and killed, that are being made in Vero cells today, and Aventis Pasteur has contributed their LS10 Vero cells—these are free from any bovine spongiform encephalopathy issues—as a substrate for isolation of vaccine strains. The titers that Dr. Ksiazek and others are reporting, pushing  $10^{9-10}$  TCID<sub>50</sub> per milliliter, are right at the limit where one would need to make a killed vaccine, so that's promising.

Obviously such a vaccine would contain all the structural antigens. The method of inactivation may be important to preserve the native structure. Formalin is not good at that, and there are many examples of why one might want to try other approaches to inactivate the virus that are selective for nucleic acid and not for the antigenic proteins like the ethyleneimines. I think concentration and purification of a whole-virus vaccine is a relatively trivial matter to remove host-cell DNA. Such vaccines, however, may not do a good job at induction of mucosal immunity or T-cell immunity. They may not provide long-lasting immunity, and would require multiple boosting. Of course, they may sensitize to immunopathologic responses, as Fred Hayden mentioned. This was a problem with measles and RSV vaccines. Probably the biggest issue is that they would require large-scale production under BL3 conditions. With viruses as dangerous as SARS, there are very few places in the world that could do large-scale production under BL3 conditions.

### *Recombinant Subunit Vaccine*

A more attractive or simpler approach may be recombinant subunit vaccine, principally the S protein. There are multiple available expression systems. I would principally favor eukaryotic expression systems that could be used, but glycosylation is likely to be important and could be a problem. Would one want to express proteins as soluble spike protein, or incorporate them in some kind of a particulate structure, a virus-like particle structure, for which there are many approaches that could be taken?

Certainly, the purification of this large glycosylated protein is going to be a challenging matter. Undoubtedly an adjuvant would be required, probably one better than LM. Mucosal delivery may be required. Such a protein may be useful also in a priming and boosting strategy. Some of the issues we've talked about with an inactivated vaccine are certainly going to be the same for recombinant protein, although the manufacturing is much less of an issue.

### *Live Vector or Replicons*

Live vectors or replicons are an interesting approach. Adenovirus has been mentioned. The MVA, modified vaccinia Ankara, is an interesting vector. Perhaps a heterologous coronavirus or an alpha-virus replicon. All of these are interesting approaches, but one of the underlying issues is going to be anti-vector immunity, and that may be the biggest single problem with a live-vector approach.

### *Live-Attenuated Virus*

What about a live-attenuated virus? If one could make it, it probably would be the best approach. One could introduce mutations or deletions into the SARS genome, but we really don't understand enough about the molecular determinants and virulence at this point to do that, I don't think. Empirical approaches, passage in cells or random mutagenesis could be used, but without a suitable animal model to select the attenuated virus it would not be possible to make a live vaccine. Also, there's no real established model at this point for safety testing a live-attenuated vaccine, although macaques might turn out



to be appropriate. This could be the best way to induce mucosal as well as systemic immunity, or could be part of a priming and boosting regime, and would not be necessarily a manufacturing issue.

### *DNA as Vaccine*

Finally, DNA. DNA alone has not been very successful as a vaccine, in humans at least. Multiple-dose is required, induction of mucosal immunity problematic. It is probably best thought of as part of a prime-boost strategy which has already been mentioned. There still are regulatory issues with DNA vaccines.

This list could be much longer to develop a suitable vaccine, but I just really wanted to focus on the animal model issue. It's been mentioned many times, both for immunization and protection, and in toxicology studies. It is a daunting task to think that, until we have something easier to work with than monkeys, we're going to make much headway. We can do some work on immunization strategies, certainly, but protection, if it depends on monkeys, is going to be difficult. Of course, any lab working on a SARS vaccine is going to have to have containment.

### *Timelines and Costs*

Finally, and this is the last slide, to share with you how one thinks about this from an industrial perspective, and what the timelines and costs are likely to be like. This is a very aggressive view of what it might look like. Clearly there's a research phase, maybe as short as a year, in which one would develop the suitable candidate or candidates. That's relatively inexpensive, perhaps three to five million dollars. Then one enters the development phase: you know what your candidate is, you go through the business of process development, figure out how you would make it. Once you have made something that's close to the final product, the FDA is now very interested in seeing sort of classical toxicology approaches applied to biologic products, and clearly that will have to be done.

We've sort of drawn our go-no / go decision line, you can see it there, about 18 months from now, when we think we'll be able to understand more where the disease is headed in terms of its epidemiology, and we won't want to spend a huge amount of money. At that point we would be in a better position to commit. The manufacturing and then scale-up to industrial scale, often requiring a separate building, is an expensive issue. Of course, the clinical trials take a long time and are also expensive. At a minimum, we're looking at 60 to a 100 million dollar investment, and perhaps five or six years at minimum. I'll stop there, and thank you for your attention.

## **Approaches to Vaccines and Drug Development: Panel 3 Discussion, including Richard Colonna (Bristol-Myers Squibb), Michael Dunne (Pfizer) and Emilio Emini (Merck)**

### *Timeline and Steps to Drugs or Vaccines*

**Scott Hammer:** Can I ask all the speakers to please come up for the panel discussion? In addition to the speakers I'd like to ask Emilio Emini of Merck, Rich Colonna of Bristol-Myers Squibb, and Mike Dunne of Pfizer to come up, three of the senior scientists at those companies, who've agreed to be on the panel. While everyone's sitting down, I might ask Mike, Rich and Emilio, since it's your first opportunity to speak, whether you've got general comments about either drug or vaccine development for SARS.

I think the lead-in from Tom was perfect, about what the reality test is for drug development and vaccine development with any pathogen, but what's the best case scenario for drug or vaccine, and do you agree with Tom's timeline, which is aggressive but still daunting? I think we should give you an opportunity to make any general comments before we open it up for questions. Emilio, Rich, Mike. Please bring the



microphones close to you, because the audio people are having a little trouble hearing us. Thank you.

**Richard Colonno:** I'll start with antivirals versus vaccine. Similar types of timelines, but actually could be shorter. I think it really comes down to a bit of luck and what priority of resources are put on it, but what you're really looking at if you're starting today to come up with a new and effective selective antiviral, you're probably talking a minimum of three years in the pre-clinical, and I'm giving very aggressive timelines. The right resources and the effort put in and a little bit of luck, three years to probably get to the first demand, just the discovery, early development, safety, etcetera. Clinical studies, I think you could gain some time; I'm sure the FDA would be very aggressive in terms of approving a drug.

First-demand studies, again, single ascending doses, multiple ascending doses, can be done in less than a year. But then where you go from there is really open-ended. I don't know how you would actually test things, what would be required to get approval. It would depend on at that point, and we're talking four years from now, what the number of cases are, is there enough going on literally to test the drug at that point, etcetera. Assuming that this becomes more of a chronic event, seasonal, that it occurs every year, it may not be very large outbreaks but clearly sufficient outbreaks where there are enough patients, I'm sure you could probably test it fairly quickly. What we could use as our example is the influenza drugs, the raminidase drugs, where the actual clinical testing can be done probably within two years or less, depending on how long you would have to treat, etcetera. Unlike, again, the raminidase drugs, if you're not going after a preventive kind of indication, you're just really treating and not trying to prevent, then again the treatment period could be very short. It could be two weeks, it could be three weeks, one doesn't know. Those studies I think could go fairly quickly, and Fred could probably comment much more on those. Again, you add that all together, and not mentioning cost—I won't get into the cost of doing that, but far from the cost—you're still talking that probably four- to five-year period.

**Scott Hammer:** Mike or Emilio?

**Michael Dunne:** Thanks. I agree with everything Richard was saying, that's pretty much the outline. You could get lucky and cut a few steps out along the way. There might be specific circumstances for the highest-risk patients to see drug a little sooner than, say, less sick patients where the risk-benefit ratios might be a little better and some of the earlier toxicology steps might be kind of put on the back burner for a while. But in general, I think the comments that everybody's been making are right in line. It does take some time to get it all to come together. Meetings like this and the New England Journal publication this month, all of these pieces of data all coming together in a focused way, really help us make our plans. I think when the information just kind of spills out in dribs and drabs it's hard to really get a focused group to get onto the topic, but now that there's been focus to this, we do have a good group of people that can pull all the pieces together, and we need to come up with something.

**Scott Hammer:** Thank you.

**Emilio Emini:** I agree totally with everything my colleagues have said. Let me just specifically address one of the vaccine questions. Obviously, and again this reflects what the earlier speaker said as well, we know very little, practically nothing about the immunobiology of the virus at this point. The approach that I think we're all essentially taking is an empirical approach. You can pretty much look at the virus, figure out what the potential targets might be, and immediately begin, whether it be by looking at recombinantly expressed S-glycoprotein in any one of a number of viral vector delivery systems.



I think what we'll see over the next six to twelve months is that immunogens, using most if not all of these technologies, will have been developed, and we'll at last know whether or not in animal model systems it was neutralizing antibodies. Then the hard work starts. The hard work is, does that make any difference? We'll have to see how the primate model that's been just described for infection is going to turn out. It's not going to be an easy model to work with, simply because of the nature of the virus that you're dealing with. If systemic neutralizing antibody turns out to be ineffective or insufficient, as you very well know, Scott, then it gets very difficult, obviously.

It's almost impossible, obviously at this stage of a research effort that literally only began a few weeks ago, for most of us to make any prediction in terms of how long it's going to take to do anything. But as Dr. Monath said, you titrate this along carefully, you see what you can do technically, how you can begin empirically, and then you rely on luck and a combination of events and knowledge as the field progresses over the next year to two years. Not the least of these is also getting a better understanding of the actual epidemiology of the infection. So we'll see.

**Richard Colonno:** As a virus, okay, it's got a very good high feasibility index in my view, simply because you have a virus, unlike some of the other ones we work on, it does grow well in cell culture, you have a plaque assay, you can titer it, you can grow it to high titers, it's got good targets. I think the assay is relatively quick, a couple of days versus some of the other assays that go for a long time. You don't need replicons, you have an infectious RNA. I think all those things will accelerate the process, and especially having these two proteases to work on, I think the odds are high, if you really wanted to do, this that it probably could be done.

#### *Screening Chinese Herbal Medicines*

**Scott Hammer:** We have outstanding scientists already in the coronavirus field, human and animal, that we saw this morning, that gives us a basis to put some ideas together. Before I open it up for questions from the audience, Ian, are you there?

**Ian Lipkin:** Yes.

**Scott Hammer:** Do you have quick comments or questions? We're all interested to hear your voice again at the end of the day. You were on mute earlier, so we couldn't communicate with you, but if you had any quick comments or questions for this panel or burning questions from earlier in the day, now's your time.

**Ian Lipkin:** I think it's an extraordinary presentation, and I was delighted particularly to hear Tom talk in a very generic way about what needs to be done as we think about potential vaccine strategies. The other thing I was wondering was whether or not Cathy had any interactions with people at the Beijing Genome Institute, who are beginning to explore many traditional Chinese medicines. They don't yet have a high throughput screening system, but they have great interest in exploiting the one at USAMRIID.

**Scott Hammer:** Dr. Laughlin, did you hear that?

**Catherine Laughlin:** Yes. We've actually started relationships with a number of investigators and personnel in China, starting I think just a week ago last Friday. I'm embarrassed I don't know if any of them are at the Chinese Medical Institute or not, but we have received some herbal extracts, I think at the beginning of this collaboration. That began within the last two weeks. We will develop quickly more



extensive ties with the investigators in China, and definitely are very interested in trying in the in vitro system compounds that they are eager to test clinically, and other compounds as well.

### *Protective Responses with MHV System*

**Scott Hammer:** Ian, you're granted a second question, given your special status. Do you have one?

**Ian Lipkin:** No, that's okay, I'll wait until the next session.

**Scott Hammer:** But please stop coughing. It's like a little background music. Periodically we hear this mild cough, it sort of reminds us what we're doing here clinically.

**Kathryn Holmes:** I'd just like to make one comment, and that is that there is a good deal of information about things that affect the immune response and protective responses in animals from the MHV system, so Steve Stolman, Mike Bookmeyer, Stan Perlman, and others have got quite a lot of data on T-cell effects and so on, and I think that could be directly translated, since this virus seems to be, at least to me as an MHV person, somewhat MHV-like in some characteristics. But I think the power of the inbred mice really has been helpful.

**Scott Hammer:** Thank you. Questions? Can we get a microphone back? Please stand up and identify yourself, please.

### *Information Needed for Treatment*

**Marilyn Larkin:** I'm Marilyn Larkin, from Lancet Infectious Diseases. Probably the wrong panel to ask this to, but in light of everything you have said . . .

**Scott Hammer:** We're all interactive and integrated here, so it's okay.

**Marilyn Larkin:** What do we tell people now? What do we tell our readers that you are recommending in terms of treatment now? I know infection control for prevention, but what strategies are you suggesting be used for the next five years?

**Scott Hammer:** Does anybody want to tackle that? I think actually we probably should shorten the timeline. Can I rephrase the question? On the available data, I think we've sort of heard that in bits and pieces today, is there any specific treatment that could be recommended, or in fact is it supportive while we wait for advances? I think my question shows my bias, but Fred, you're probably the best person to answer this.

**Fred Hayden:** Reiterating what I said earlier, I think that the effort right now should be directed at trying to develop a much better understanding of the natural history of this infection, both in terms of virology and host immune responses, and engaging in controlled clinical trials. I would personally be unwilling to make a recommendation based on what I know for empiric therapy. I think that there's been an abundance of that already, and we haven't unfortunately learned as much from that experience as we really need to. It's time now to engage in controlled trials. I hope that there will be such forthcoming, and to try to answer the question, are steroids doing good or harm, or having an indifferent effect? Is there possibly a role for other interventions like interferon, either for prevention or for treatment of established disease? These are testable questions right now.

**Scott Hammer:** Right. I think it's obvious but worth stating for the record that one can't fault any of the



empiric therapies that have been used when faced with a frightening outbreak, not knowing where it was going, with people dying. I think picking the antivirals off the shelf and thinking about steroids in this disease were logical clinical decisions. The issue now is really trying to bring rationality into development from this point on, so that anecdotal experience is minimized. Questions? Ellis, you've got the microphone.

### *Pharmaceuticals Collaborating*

**Ellis Rubinstein:** I read recently about the notion that the pharmaceutical companies might try to create a kind of a collaborative early research thing, sort of like the Summit Tech. Is this the perfect opportunity to try to do it for a problem that exists where, instead of all of you quietly doing your own thing, for the basic, early research you could somehow gang together and do something a little faster?

**Emilio Emini:** I mean the answer to something like that obviously, Ellis, is that there are times when that kind of collaborative effort—go back to the early or the middle years, as it turned out, of HIV research. There were collaborative efforts like that put together because those were very difficult, intractable problems. I think at this stage, given the early point where we are right now, the fastest way in is for each of us to bring to bear the technology and concepts and the expertise that we have already, internally, and we'll see where that brings us. Obviously, as time progresses, if the basis for collaborative work develops, all right, because collaborative work among the pharmaceutical industry happens all the time. A lot of people don't see it, but it literally happens. In fact, I know very few fields in which you don't see that kind of collaborative work. It's just the early days right now, and so I wouldn't be surprised if it develops here as well.

**C. Richter King:** I'd just like to say that I agree with that, and say that already I think that's going on between academic scientists and government scientists and biotech and pharma scientists. I think that those things are well-advanced for this disease and other diseases, and I think it works very, very well, because it does require kind of creative work that we saw earlier in the morning to get to the point where you can even begin to think about generating a screen or a vaccine or anything like that. It's really built off the science that's occurred. So I think that the cooperative approach is a very, very good one.

**Michael Dunne:** Just to segue off of that, I think a collaborative approach also makes a lot of sense when we get to clinical testing. As Dr. Hayden had mentioned, it may be necessary to use combinations of therapies at some point, there may be a restricted patient pool, so it's worth talking to each other about how to study medicines if we can get to that point.

**Scott Hammer:** There was a question back here.

### *Inducing Mucosal Immunity*

**Bing Lu:** Bing Lu. From Dr. Monath's talk and also this morning, Dr. Saif's talk, it seems that because immunity is going to be very important for vaccine development. So my question is in terms of aerosolized vaccine delivery through respiratory tract or metered dose, inhalers, is there any preexisting information for peptides or virus to be delivered?

**Thomas Monath:** I'll just say that I think the field of mucosal immunity was extremely exciting about a decade ago, where everybody kind of rediscovered IgA, and many of the promises have not matured. We all recognize that for some infections this is very important, but the methods we have for officially inducing mucosal immunity are quite limited, the best success being administration of a modified live agent to the root at which it normally infects the host. That being said, there certainly are approaches



that have shown some promise, and when you get away from a live agent you typically come back to, depending on a mucosal adjuvant, a different class of agents than we normally think of as adjuvants for typical [inaudible] immunization. That has been a very problematic area, with mutant labile toxin of *E. coli*, for example, being one approach. My personal hope is that we're not going to need it. I think, at least for amelioration of disease, induction of humoral antibody, neutralizing antibody, ought to be the first objective, and see if that works before we worry about whether we have to induce mucosal immunity. Dr. Saif is smiling, she may want to comment further.

**Scott Hammer:** Other questions, of did you have a comment, Dr. Saif?

**Linda Saif:** I just make the comment that I agree with that assessment, and that goes back in my mind to the pathogenesis. Does this viremia contribute to the virus getting to the lungs? If so, maybe systemic immunity will make a lot of sense. Or is the primary site of replication the upper respiratory tract, and that's what contributes to it getting to the lungs, in which case maybe mucosal immunity will make more sense? Again, it goes back to understanding a little more about the pathogenesis.

### *Likely Successful Drug Targets*

**Scott Hammer:** Other questions from the floor? I'd like to just close with two questions. One, is there consensus or not that on the drug development side the two most promising targets, or the ones that intrigue people the most, would be fusion or entry and protease inhibition? Is there any disagreement on the panel with that, from what we know?

**Emilio Emini:** Let's not forget the RNA polymerase.

**Scott Hammer:** And polymerase.

**Scott Hammer:** So we have three targets, thank you. Okay, entry in the broad sense. Entry in the broad sense, protease, and RNA polymerase. Can we draw any consensus, besides wanting to induce neutralizing antibodies, if that's the protective correlate of immunity? It sounds like there's a fairly broad perspective on where to go with vaccine development. Do we want to just go down the panel and ask where people want to think the likely success might be? We're trying to be controversial here, or stimulatory, so you won't be requoted necessarily.

**Emilio Emini:** It's okay, I get quoted out of context all the time. I would agree with what Tom just said. I think that I actually personally give it a fairly reasonable likelihood—I won't put a number on it—that circulating neutralizing antibody is going to have an effect here. If that's the case, if it turns out that way, then the path to getting the vaccine is going to be not easy, but at least more straightforward than it would be otherwise. As I was saying earlier, there are a number of different approaches that one could obviously use to try to get there, and those will be the first approaches that will hopefully be tested, and will be tested in fact, as we make our way through the next twelve months. So we'll see.

**David Ho:** I think it's safe to say in terms of humoral responses, it's likely to be easier than HIV. It's not saying much.

**Scott Hammer:** Mike, Rich, any disagreement with that, Tom, Rick?

**C. Richter King:** Going back to the comment earlier, I think we're in the empirical phase. The quality of the animal models and what we're going to be able to learn from that is key. I think you can pick



good targets, a lot of them came out at the meeting, and we'll have to see how they go.

**Scott Hammer:** I think on that note we should—I'm sorry.

**Fred Hayden:** I'm just commenting. I think you may get an early read on the importance of neutralizing antibody if you can do further studies with convalescent sera or monoclonals when they're available, both in the animal models and potentially as therapeutics in early stage disease. Looking at viral load reduction in the respiratory tract, the blood is a marker for that.

**Scott Hammer:** Catherine, I skipped you. Do you have any additional comments on that?

**Catherine Laughlin:** No, other than just to repeat what many people have said all along, that it's really early days. I think we need to understand the natural history of the disease, and develop appropriate animal models, and that will allow us to make better rational decisions about approaches.

**Scott Hammer:** Great. I think we should take a break. Thanks to the panel.

## Session IV: Future Perspectives on Emerging Infections

Moderated by Allan Rosenfield

### Future Perspectives on Emerging Infections: Special Pathogens in Three Cultures

C.J. Peters, University of Texas Medical Branch, Galveston

#### *Removing Geographic Barriers to Disease*

**Allan Rosenfield:** Ellis, maybe somebody could try to get Ian, because I know he wanted to be part of this. Good afternoon. It's been a fascinating day. Glad to see so many people have stayed for the end.

I know Ian wants to join in moderating this session, and hopefully we'll have him back on in just a moment. The last session is to focus on the water, Galveston, look at what the world looks like from Texas. We're going to actually talk about future perspectives on emerging infections. First speaker is C.J. Peters from University of Texas Medical Branch at Galveston. He's going to talk about special pathogens in three cultures, academia, CDC, and USAMRIID.

**C.J. Peters:** Thanks. Ian suggested that I talk a little bit about emerging viruses and what it's like to see them from the different spots where I've been hanging around at different times. I just put this up to show that we have an island, too, that Manhattan is not the only island. We're all competitive, and David Ho had the newest data, so I thought I'd try for the oldest data. The point I wanted to make, though, is that for 50 million years or more we've been separated into different continents, and these continents have been separated by geographical barriers. In areas of these continents different viruses have been evolving and often transmitted by animals to their own species or between species by arthropods. These viruses are ecologically constrained, they can't just pick up and go, but there are a lot of them.

This is some work that has been accumulated over the years by study of arthropod-borne and rodent-borne viruses. There's a huge pool of genetic material out there, not all that well-characterized, and this doesn't even touch the viruses like the coronas and the other viruses that each species may have as it's own complement. I bet you that every mammal has a cytomegalovirus, but we've only characterized a



handful of them; similarly for herpes viruses and so on.

There's a lot of genetic material out there; it was separated but now it's coming together. After whoever started moving around the world—the Chinese, or the Europeans—the so-called age of exploration started mixing these viruses. Yellow Fever was probably the most disastrous mixture, because we carried *Aedes aegypti* wherever we went. That was a very efficient transmitter, and Yellow Fever killed lots and lots of people, as an arthropod-borne virus moved out of Africa. West Nile is only the most recent.

### *Case of West Nile Emergence*

I mention West Nile because I think it shows us two things about these emergences. First of all, we'll never know why and how West Nile came, but there are usually things going on that make us think we understand, and we talk about them. For example, in the case of West Nile there are several factors that must have been important. First of all is a phylogenetically-related virus that uses the same mosquito, *Culex pipiens*, as well as other *Culex* mosquitoes, as a vector, and of course they are both using birds as their vertebrate amplifier. There was an epidemic of West Nile Virus that occurred in the Middle East; it happened to be a strain that caused exceptionally high viremias, so it was a prime candidate for introduction. *Culex pipiens* becomes chronically infected with West Nile Virus, loves to hide in the toilets of airplanes, and air travel is increasing greatly. I'm sure that somehow this all came together to bring us West Nile Virus. It doesn't just happen.

### *Looking for a SARS Reservoir*

I think when we start looking for the reservoir to the coronavirus, some of the lessons are that (a) we may need to study coronaviruses in a more general way in the animal world around us, and (b) when we go to that market with a beautiful photograph of all the things there, there have been a lot of different critters in that market for a very long time. I don't think Chinese culinary habits have changed enormously. We ought to be looking for some perturbation in the animal population prior to that, or perhaps this may be a recombinant virus between a couple of viruses that we don't know the mother and father, but it's just a bad offspring.

The other thing I'd like to contrast to this are the viruses that have probably evolved during and after human urbanization. This data is pretty sketchy, and it mostly comes from some hypotheses and some nice studies on an isolated human population where these diseases die out if they're not continually reintroduced. This process goes on today. HIV is the most recent example, SARS may be even more recent than that. These viruses 3,000 years ago stayed put. But then in the 15th and 16th Centuries, sailing boats brought these viruses, for example measles and smallpox, from their foci in the Old World to the New World. Today you don't have to wait for Columbus, there's an airplane. These viruses can have a tremendous impact.

### *Factors Leading to Emergence*

I don't have to show this, but I did, because it shows potentially we can be looking at something that would affect the longevity of a relatively developed country in a big way. The factors that go into the movement and emergence of these viruses involve a whole list of things. About ten years ago, the Institute of Medicine put together a fantastic report on emerging infections, and we got together as a sort of anniversary and looked at what happened in the last ten years. The conclusions were—the whole committee, everybody was behind this—things have gotten worse in the factors that were previously identified in emergence: travel, transportation of vectors and reservoirs, ecological disruption, crowding, changes in human populations, the nature of the food supply, and so on. This has come out and is not yet fully published, but this is an ILN report that goes through that, and I would recommend it to you, par-



ticularly if you're looking for funding, you're going to talk to your Congressman. It's a dandy tool, because it really lays out the factors. I used to have a graph for each one of them, but it all looks the same, so I just made one graph and I use it for all of them.

### *New Diseases Appear Constantly*

When I was at Special Pathogens Branch, for about ten years, something happened every year. There was a new virus, new to science, there was an old virus that wasn't supposed to be there, or there was an old virus that was doing something it wasn't supposed to be able to do. Hendra and Nipah viruses represent viruses that belong to a completely new genus within Paramyxovirinae. In other words, they not only took us by surprise individually, but they took us by surprise as a taxon. They're particularly scary viruses. They're not human-to-human viruses as far as we can tell, but Nipah virus, for example, came somehow from bats to pigs. The changes in pig agriculture probably fomented its dissemination among pigs, and it can spill over into humans, and also kills cats and the occasional dog. This is a virus that's pretty flexible in its species requirements for disease, not just for infection.

There's plenty of things out there that are problems, directly and potentially, and even some of the vector-borne disease like rift valley fever virus can be exported out of sub-Saharan Africa into the Arabian peninsula and cause epidemic disease. We have every reason to believe, based on laboratory studies, that the vectors in North America would support its transmission as well.

You add all this up and there's real evolutionary opportunities for the viruses. Generally these are not, in my experience anyway, particularly driven by genetic change. The genetics seems to help the adaptation of the viruses to new circumstances. Influenza, of course, is the obvious exception to that, canine parvovirus probably, and who knows, maybe SARS.

How do we typically deal with these problems? The individual practitioner makes the diagnosis of disease, CDC then collates this through the local and state health departments, identifies the threats, NIH builds a research and knowledge base through academia, industry picks up on the needs and makes the product. I made this slide for BT, but the same thing goes for emerging infections. What's the emerging infection threat? We know there is one, but no one will predict what the next one is, except pandemic influenza which I would agree with, of course. Many times the organisms have not been funded as thoroughly in the research to lay the groundwork for our understanding, and there is no market.

We've got to find a way, I think, to get ahead of this. SARS is a good example. We can't wait around until the bodies pile up and eventually we get there. I think that there's been a tremendous outpouring of effort on the part of CDC and NIAID and others to make this happen.

### *Likely Trajectory for SARS*

What's going to happen with SARS? Nobody wants to say this, but since I'm in one of those sessions that you put the more decrepit guys in, I can say something about this. This is what I call public health's dirty little secret. This is longevity in years from birth, and this per capita GNP. The countries that have dealt well with SARS so far are mainly up here. There are some exceptions down here where you see public health at a given GNP can make a difference, but when you get down here it doesn't matter what kind of public health you have. I think that in rural China and if this, God forbid, gets into certain other areas of the world, we will not eradicate it. It will probably maintain itself, and we will begin to see foci of countries that are endemic, perhaps with seasonality. Countries that are capable of fighting this, but like Vietnam it takes an all-out full-court press to be able to deal with it one time, and they won't be able to keep it up. Then we'll see the developed countries, which will change to a degree that you'll think that



going to an airport scanner is easy. I think it'll change to a degree where, for example, in hospitals you'll come in the emergency room, and if you cough you go down another corridor and you go into a negative pressure cubicle until your SARS test comes back. I'm somewhat kidding, I'm trying to provoke you, but I'm also somewhat serious.

Dealing with these issues in the Third World is not going to be easy. We've heard a lot about the political organization, but the infrastructure is a huge problem. If the price of ten N95s is your annual healthcare budget per capita, that's does not portend well. Local culture is going to be extremely hard to change.

### *Risk-Benefit Equation for Remedies*

We have a certain risk-benefit equation for remedies, and we have always insisted that our standards must apply to products for the Third World. I think that that's going to change, and I think that David Ho had an excellent model. We made a vaccine against Argentine hemorrhagic fever when I was with the Army in USAMRIID in '85. It works. Randomized double-blind control trial. We sold it to the Argentines, we put it into 250,000 people who were at risk. They've had good protection, they've had no serious adverse events, but we can't use it in this country because no one will pay for the freezers to keep the vaccine lots that were made here, and also because now the standards for vaccines have changed here. The 250,000 people in a relatively-developed country with a good public health system like Argentina don't count, we have to start over again with a lot of the safety testing. I think that it may be that things would be developed to a point here and then manufactured and produced overseas if we can't find a better industrial model.

### *Activities at Various Institutions*

To try to tie some of this back to institutions that I've been associated with, I'm actually in some ways pretty typical, except it looks like I probably couldn't hold a job very well. Like many of you in this room, I started out with NIAID. I was a Fellow at the laboratory they maintained in the Canal Zone for a while, and I got my training and I got started. I've been in a couple of academic institutions. I don't think I have to say anything about the tremendous amount of technological and scientific support that we've seen through NIAID, and the funded research in academia by NIAID in the foundations.

I would like to say something about USAMRIID, because it represents an institution that brings some things to the table that other people don't have. One of these became apparent with the Reston episode in 1989. USAMRIID had a cadre of veterinarians who were accustomed to working at biosafety level four with nonhuman primates. It had a plethora of containment space, and Tom Monath and others were building an antiviral program there at the time. To be able to have this put together, containment space three and four antiviral drug program, nonhuman primates at three and four, is an extremely valuable situation. In addition, they had scientists there who were actively involved in vaccine research. A lot of you know Connie Schmaljohn.

The CDC is an institution which I don't have to talk very much about, except to say that it is the national focus for looking for new agents. We found new agents while I was there. The good things come to you, there's a pretty good system built up for looking for the new agents, and it's moving more and more smoothly. There are some things you didn't see. There are, for example, quarantine issues. Those were handled fairly smoothly, and the thinking and the legal groundwork was laid by the CDC programs for dealing with bioterrorism and smallpox. I think a lot of this bioterrorism effort will resurface in dealing with SARS and other emerging infections, as a very strong plus.

I had a couple of experiences there which I think are sort of pertinent to SARS. We started using rib-



avirin in HPS on a compassionate use basis. That was probably not a good idea, but there weren't enough cases and we were naïve enough to start it. It became apparent very soon that the disease went very fast, and there wasn't much virus around even in early stages of HPS, yet it was already started. It's very hard to stop, but you've got to go across and get into controlled trials immediately.

The last thing I want to say is that there are two things that are not on this list. One is, where's the National Vaccine Authority? I don't want to work for them, but we need somebody who's nationally responsible for antiviral drugs and vaccines, or anti-infectives and vaccines, and that once again was one of the conclusions of this panel. The other thing is I have never worked for WHO, but I think there are some things that we can do to enhance their effectiveness. Thank you.

## **Future Perspectives on Emerging Infections: SARS and Public Health Systems**

Marcelle Layton, NY City Department of Health

### *A Public Health Perspective*

**Allan Rosenfield:** Thank you. Ian, are you there?

**Ian Lipkin:** Yes.

**Allan Rosenfield:** Would you like to introduce Marci?

**Ian Lipkin:** How do you introduce Marci?

**Allan Rosenfield:** You want to say anything?

**Ian Lipkin:** What we tried to do in putting together this panel was to give a perspective on local, national and international views of what it means to address such an outbreak. You've heard from C.J. Peters, who represents obviously three separate cultures: academia, the CDC, and USAMRIID. Marci was the obvious choice when we wanted to think about a large city, because she's confronted so many different outbreaks, ranging from West Nile where we first met, to anthrax, and of course now SARS. She's one of our own.

**Allan Rosenfield:** Let me just add, Marci and the City Health Department in New York have done an extraordinary job ever since 9/11 on a whole range of things, particularly during SARS. I'm delighted Marci's here, and also that she does have an appointment at our school. Marci.

**Marcelle Layton:** Hi, thanks. It's an honor to be here, though I'm not sure I characterize myself as old and decrepit (thanks C.J.), though it's always an honor to speak after you.

What I'm going to talk about is to really focus the first half of my presentation on the public health infrastructure issues that need to be in place ahead of time to deal with the threat of a disease like SARS (the same infrastructure needs that we need for bioterrorism), and then I'll end by talking about what we've been doing at the City to prepare, and continuing to prepare, for the possibility that this might come here.

### *Recent Prominent Diseases in NYC*

I actually started officially working for the Health Department in 1994, in August, and about a month



later got a call from our Division of Quarantine out at JFK Airport about the concern with the brewing plague outbreak overseas. It was sort of my first recognition of the things that needed to be in place to respond to the threat of imported infectious diseases into a place like New York City, where every day we have about 30,000 international travelers arriving at one of the airports in the tri-state area. Even at JFK alone there's a hundred flights coming in a day.

If you think about the most prominent imported disease that we've dealt with, at least during the past ten years that I've been here, it's obviously been the West Nile outbreak. It made me realize that diseases that I really didn't spend much time studying during medical school or NIAID Fellowship residency are things that we do need to be aware of in a place like New York City. The possibility is that they could be imported here, depending on the disease, and all the factors are in place for them to become established, which was the case with West Nile.

The other thing that West Nile did for us is that, though we were starting to prepare for the possibility of bioterrorism—that was already part of my job—West Nile really in many ways accelerated our emergency preparedness down at the City Health Department. We really were primarily a nine-to-five City agency up until then, and we've mostly since then developed a system that tries to deal with public health emergencies with an all-hazards approach, realizing that the components that need to be in place are pretty much the same, regardless of what the threat is. We've adapted an emergency response command system; it was extremely helpful within minutes of the World Trade Center attack to have a system in place where people knew what their role was going to be and where they should report. That hadn't been in place several years before. We worked very closely with our sister agencies in emergency management, law enforcement, as well as with the hospitals through the Greater New York Hospital Association. Recognizing that any issue in New York City is probably going to impact our neighbors as well, the need to work with all the regional public health agencies.

### *NYC Public Health Surveillance Systems*

I'll talk a little bit about our surveillance systems, the need to have thought through ahead of time what needs to be in place to mobilize a large scale surveillance and epi response. I'm taking great efforts to improve our lab capacity on both volume and the range of diagnostics that they offer. I'm thinking through ahead of time and having plans in place for mass prevention, emergency communications, and practicing a lot through tabletop exercises and drills. All of that really helped us be better prepared, having done that ahead of time. I can't tell you how helpful that was for all of us, both for the World Trade Center, and the anthrax outbreak of 2001.

I'm going to keep showing this slide, but sort of walk you through. There are more steps to this, but I think these are really the key issues that we always have to continue to focus on in dealing with any biologic emergency, whether it's intentional or natural. Detection I think is our primary responsibility in public health, in partnership with the medical and laboratory community. We sort of focus on several different ways of keeping alert to new or recurring disease issues.

The first and most important is what is termed traditional surveillance—depending on medical and laboratorians to recognize something unusual and to call us, knowing when to call us, knowing how to call us, and making sure that we're responsive when they call. Though there's a number of diseases on the New York City Health Code that are legally reportable, there's also a phrase about the importance of reporting any unusual disease manifestation or cluster, with the recognition that we can't know ahead of time everything that's going to occur, and that if a physician sees one or two unusual cases they make not recognize they're part of a citywide pattern unless they report to us.



We also partner very closely with the Medical Examiner's Office to alert us to any unexplained potentially infectious death, and every day we get a pile of death certificates from our Vital Records of people who've died of what potentially could be an unexplained infectious disease, to look for any unusual pattern. I'll talk about syndromic surveillance in a second.

I always show this slide: West Nile keeps coming up. To just emphasize how powerful a single physician's phone call can be for recognizing a new disease or an emerging disease, the doctor that called about the four cases in blue, originally two and then four cases of an unusual encephalitis with muscle weakness, really deserves the most credit for recognizing West Nile in New York City in 1999. Of more concern, though, were all those orange boxes, which really weren't recognized until we started looking for them. Though encephalitis was reportable no one had called them in. We recognize that traditional surveillance is something we need to continue to promote in our healthcare provider community.

Syndromic surveillance is getting more attention over the past year; it's sort of the sexy new surveillance system. For those of you who aren't aware of what it is, it's the concept that if you can detect an increase in a prodromal-type constellation of symptoms, you might detect outbreaks earlier, compared to waiting until patients are sick enough to present to the hospital, get diagnosed, and then hopefully have the physicians call, so that patterns could be recognized. It just recognizes that most people don't necessarily go to the physician or doctor right away. Initially they may just feel sick enough to go to a pharmacy or call a hotline to get advice, their managed care system if they're sicker, may call in sick to work, or if they're really sick, call an ambulance and then arrive at the emergency room. All of that is a little bit earlier than traditional surveillance.

In New York, we've invested a lot of staff resources into developing several systems, and there are real benefits to having more than one system. If one goes off, it could be an artifact, but when they all go off we get much more concerned. Though we haven't yet detected a bioterrorist event using these systems, we haven't had a big, large event to detect. It wasn't really meant to detect seven cases of skin anthrax, but it is our earliest indicator of flu each year, and this past Fall was the earliest indication of the Norwalk virus outbreaks that soon began to be reported in cruise ships. Actually just a week or so ago it was our first indicator that the pollen counts were up before we actually heard it in the news, with a dramatic increase both in pharmacy sales and in emergency room visits.

### *Methods of Detection and Response*

We detect an outbreak. There's a lot of people who want to know right away. From my viewpoint, my most important audience to get information out to is the medical community, and we've developed a broadcast alert system that allows us to communicate, especially to hospitals. We now realize the need to expand it beyond that; when there's an acute event, I don't want my providers in New York City to read about in the papers. I want them to get much more detail and information from us at the Department, including when to call us, how to collect specimens, and to provide guidelines. This is just one of the many alerts we sent out in 2001 for anthrax.

With bioterrorism, we have a lot of new friends that we work with. The cartoon just says, "Baking flour spilled, who did you just call?" and there are all of these other agencies showing up besides Public Health. We found that, even for natural disease outbreaks, there really needs to be a partnership. We need to know each other ahead of time; things go so much smoother when we do, including both law enforcement and emergency response.



As far as the epi and surveillance response, detecting the outbreak is almost in many ways the easy part. You sort of see something unusual, the first thing you need to do is figure out if it's something you need to be concerned about, and that requires having trained medical epidemiologists. In my program, being ID-trained or ID-aware is extremely important to be able to triage these calls—having them available twenty-four hours a day, seven days a week, with the ability to go in, facilitate getting specimens to our lab and down to CDC, as well as doing the initial epi investigation, with the recognition that sometimes it's not even an outbreak. We investigate a lot of false alarms, thankfully, through the syndromic system, and even if you confirm it's an outbreak, you need to figure out what's causing it, and nowadays you need to think about whether it's intentional or natural.

Once you decide there's an outbreak, especially once it's a prominent outbreak like anthrax or West Nile, you need search capacity to sort of mobilize that response. You need hotlines to triage the calls that will start pouring in once things are public, teams to send out to interview patients and review charts, database systems to track the data, systems in place to get and test laboratory specimens and link that data to the patient, and the ability having the staff on board to analyze the data, to inform decisions.

Watching what's occurring overseas, sometimes people just think these numbers magically appear. They don't. You need accurate data, you need complete data, and it's much harder to do than I think many people realize. I really feel for the folks in Asia and China that were dealing with hundreds, if not more, cases being reported a day.

### *Preplanning Public Health Interventions*

Obviously there's the need to preplan for both medical and public health interventions, the need to sort of think through ahead of time search capacity issues. This is from the 1918 flu pandemic, and we've been trying to work with our hospital systems to improve individual hospital preparedness. Again, the importance of an incident-management system, maintaining awareness among clinical staff of what to report, when to report, how to report, good triage protocols in place at the points of entry so that potentially contagious patients are recognized, having thought through ahead of time within each hospital how to deal with a surge need for contagious patients, communication, training staff. The last bullet is probably the most challenging: having individual hospital systems, since we don't have a single system in the United States, but private, public, academic institutions working with public health to come up with a regional mass casualty plan. Easier said than done.

Mass prophylaxis I'm not going to dwell on. This is just from the last time we needed to mass vaccinate for smallpox in 1947. I have a feeling it won't be as easily done today as it was then.

Lastly, before I transition to SARS, the importance of communication. This is obviously from 2001, but I think it really illustrated the fact that effective communication really underlies every aspect of a successful response. If you don't communicate well, and especially if you lose trust with misinformation, it's extremely hard to regain, and government especially needs that credibility in the time of a crisis. The best way to maintain that is to have a clear and effective spokesperson who's available to the press and communicates clearly. Something I give Mayor Giuliani a lot of credit for is he never spoke without having a physician, a public health physician, by his side. That was my Commissioner at the time, Dr. Cohen.

I think the most difficult issue as a new outbreak is emerging is the ability to communicate uncertainty. If you don't know, you need to say that, and then explain what you're doing to get the information that everyone is demanding.



The other thing is sort of behind the scenes; everyone sees how we communicate with the press, what they don't see is that those press stories generate a lot of public concern, and that you need the ability to overnight set up hotlines to handle the extreme demand in information that those media stories tend to generate.

In the United States we got a tremendous infusion of funding to improve our public health infrastructure after the events of 2001, and we've used that to improve surveillance, laboratory capacity, emergency communication systems, and I always emphasize the fact that we in New York City try to use them for things that will help us both for bioterrorism and natural disease outbreaks. I think most people in the audience are old enough to remember double green stamps; sometimes when I talk to students they have no idea what I'm talking about. I think the ability of that funding, and what we've been able to do over the past year, has made it much easier for us to sort of mobilize this response to SARS and prepare for it, even though, to be honest, it really hasn't come here, we've mainly been watching the outbreak unfold from afar.

### *Introduction of SARS to New York*

As far as our introduction to SARS, I, like probably many people in the room, can remember reading the Promed announcement in February about an unexplained pneumonia in China, the concern that it might be chlamydia, and I now pay much more attention to Promed overseas than I did before. My very first sort of recognition of what was coming is I got called on March 12 by CDC about a New York City businessman who had died in Hong Kong of an unexplained illness after causing outbreaks in both Vietnam. With his return medical transport back to Hong Kong, he had died in Hong Kong, was obviously the index patient in the Vietnamese outbreak, and the request was to get assistance in arranging an autopsy as they were trying to get his body brought back here for examination. That was my introduction to SARS, the day the first WHO alert went out.

The day the second alert went out I again got woken up—the other one was in the evening—by CDC about the physician from Singapore who was detained in Germany after leaving New York City because of the concern that he had SARS. He was an ID physician who had actually cared for two of the three index patients in Singapore. The information we initially got is that he was here at a pharmaceutical conference, that ended up not being the case. They weren't sure where he had been, where he had stayed, but they thought he had gotten some medical care here, and that's the information I had to go then track down. Quite challenging on a Saturday. We were able to quickly verify that he had minimal exposure to other people, had not been seen in a hospital, we were able to identify the few people who had seen him here, and that day we sent our very first SARS alert out that acknowledged his case, because it was already in the news, We mainly tried to mobilize our local community to be alert to this new disease, SARS, what to look for and when to call us.

### *Newest Public Health Efforts in NYC*

This just sort of summarizes what we've been doing over the past two months, obviously emphasizing surveillance, the need to rapidly identify and isolate any potential cases, identifying their contacts and monitoring them. We actually, ironically enough, had just proposed some changes to the New York City Health Code in March to strengthen our ability to do detention, isolation and quarantine, and give us more flexibility, and where we can do that. We drafted some detention and isolation orders as well that took into account the process needs. We've been doing a lot of outreach to the medical community through alerts, through our Web site, and through talks. Public outreach is especially targeting both the media and community groups in our Asian community, given the impact that it's had locally, similar to



what it's had in Toronto, and then spending a lot of time planning for a more worst case scenarios.

This is a quote from one of Dr. Gerberding's press conferences not too long ago, something that I think the epidemiology, especially from Asia, continues to emphasize, and something we continue to try and keep in the back of mind is as things have become quiet. "It just takes the combination of one highly-infectious person and an unprotected exposure to start a chain of transmission." I think this is one of the most beautiful representations of epidemiologic data I've ever seen, that just so visually tells you if you miss a hot case, the potential that it has both within the hospital where the patient is admitted, as well as the potential for community transmission.

To talk in a little bit more detail about our surveillance efforts, we do what we call sort of reminder surveillance, and hands-off passive surveillance Citywide, where we send out almost weekly alerts and updates to sort of keep this in the back of people's minds. We did some targeted surveillance in hospitals and among providers serving our Asian communities, knowing that there's a lot of travel back and forth, tried to get protocols out to hospitals for targeting those who are first to see the patient, patients with fever or cough, and to have them recognize the importance of taking a travel history, and distributed posters to hospitals. We also have SARS teams, we have four of them I think, with about four or five people on each team, two of whom are here with me today, that have spent a lot of time doing active case and contact management. All of our staff needed to get fit-tested in the event that we needed to do a home visit. We've also kept a more close eye on our death certificate data, looking for unexplained respiratory illness. Syndromic surveillance—people think about it of detecting outbreaks early, it also gives us some peace of mind because there is concern about unrecognized transmission and the fact that it's remained quiet has been reassuring to me that we're not missing anything.

Dr. Don Weiss is in the audience, who's the artist behind this particular poster that we are getting out to hospitals now. We had one that wasn't as snazzy looking before, because we are concerned that we need to remain vigilant, as long as the outbreaks are continuing in Asia. We aren't just asking to hear about patients who meet the probable or suspect criteria that CDC has promulgated, but recognize that there could be cases that aren't recognized as SARS and called secondary cases, and to be alert to any health-care worker who has unexplained severe pneumonia or respiratory distress, or any cluster of illness. We want to hear about that immediately.

We have met with our hospitals and have gotten guidelines out, based on the CDC guidelines, trying to emphasize the need for taking all precautions early, to err on the side of caution. With all that effort, you ask people to call you, we do get calls. We've triaged over 180 calls since mid-March about potential cases. Most of them, obviously, did not meet the case definition. We've had 21 cases in New York City as of this morning, 18 suspect, three probable. All of them are travelers to areas that were affected, of different ethnicities, mostly mild illness, no secondary spread, all fully recovered, no lab positives to date. There was one patient who actually was a tourist, not a New York City resident, who was transiting through—a young gentleman, who did not like the idea that his vacation was going to be cramped by us, and required a legal detention with guards outside his room in order to make sure he stayed inside in isolation for the ten-day period. To date we haven't had any community transmission, which I think is more just good luck than anything else that we actually haven't had a case.

### *We Cannot Be Complacent*

I just want to end by acknowledging that we can't afford to be complacent, to think, well, we're out of the woods. We are continuing to spend a lot of time contingency planning. We met with the hospitals on Monday to start to share with them some draft materials we have, for them to think about all the surge



capacity issues and protocols that need to be in place in case we start to see nasocomial transmission, learning from what happened overseas and in Canada. We have started to develop a policy as far as isolation and quarantine, as well as when we would implement certain community-wide measures that uses graduated criteria from where we are today, with no evidence on any secondary spread, to if we started to see widespread transmission. There's another group working on developing criteria for the types of physical requirements we would need for isolation and/or quarantine facilities, and have actually started visiting and surveying potential sites.

We've been working with other agencies to deal with the legal, security, and logistical issues that we would face in the event that we would need to implement widespread isolation and quarantine. I actually feel that—I raised this yesterday—once we have a more solid plan, that was still a work in progress, that we need to advertise this, we need to get people to understand this may be coming and to get their buy-in ahead of time.

Obviously, with what we do, there's a lot of things that may change that—the magnitude, pace, epidemiology of the outbreak, our resources at the time, the health of our workforce including our healthcare workforce, communication and politics and social issues.

I just wanted to acknowledge, I think something we've been really impacted by at the Health Department is the effect this has had on our Asian communities, and the stigmatization and stress that this has caused. We've been trying to counteract that with education and communication, but it's been challenging, and we really need to think through some of these issues in the setting of a large scale outbreak. I think we're also heartened by Vietnam, that a rapid response can give you rapid control, that Vietnam was able to basically stop the outbreak with just sixty cases, using all of the mechanisms that I just talked about: contact monitoring, case isolation, etcetera, as well as doing a good job with just tracking the outbreak.

### *Difficult Remaining Questions*

I just wanted to end acknowledging sort of the questions that we struggle with. How long do we continue the surveillance efforts, especially from travelers from countries who are no longer on the WHO list? As least right now, CDC continues to include them in our surveillance criteria, as we have, not knowing where this virus is going, the concern we're going to have triaging flu-like illness next Winter. We recognize we haven't had any community transmission or secondary spread, but maybe we don't have SARS and are our current isolation and quarantine measures sufficient?

We try and assess the home situation before we let someone go home, we follow the case and the contacts for ten days after they're illness. We're not quarantining contacts yet, but we might if we had a more concerning case. Then, the issue I raised before, how best to prepare the public for a potential wide-scale quarantine measure, recognizing that we have not implemented quarantine and/or isolation in this country in a large scale for a long time, except for isolated cases of TB. This is a cartoon from outside Bailey Seton Hospital, our original quarantine facility on Staten Island, one of the boats going out, recognizing that in this day and age people are less accepting of government trying to interfere, impact on their personal freedom, compared to when we used to stick signs like this outside their door. Then the whole concern of will people take this concept seriously? This is my last slide.

On that note, I appreciate the opportunity to talk at this symposium today, and I guess I'll be back in a few minutes [for the panel discussion].



## **Future Perspectives on Emerging Infections: A View from the Field and the Bridge**

John La Montagne, National Institute of Allergy and Infectious Diseases, Bethesda

### *Importance of the SARS Problem*

**Allan Rosenfield:** Thanks, Marci. The most troublesome point on that slide is next Winter when flu season does arrive, and trying to differentiate flu from anthrax, from SARS, and from almost every other bioterrorist agent, all of which start with the same symptoms, is a challenge. Our last speaker has three minutes to speak—but it's government, it's okay. We're delighted John La Montagne is here from NIAID, "A View from the Field and the Bridge." What is the bridge?

**John La Montagne:** I honestly don't know, Allan. I was given that title. First of all, I'm very mindful of the fact that I'm between you and a reception, so I will be brief.

I don't have any slides, in part because I really didn't have time to think about putting together a nice PowerPoint presentation. But I did want to make a couple of points. The first is really to thank the New York Academy of Sciences for putting this meeting on. I think it's very, very important to start the dialogue about the scientific activities that we need to undertake in the future to get control of this. The second is to extend Tony Fauci's greetings to all of you. He is following this issue very intensely, as you know, and I think would've been here had his schedule permitted. It is at this point in time I think it's just difficult to predict when he might be called to other more pressing issues, but he did want me to extend to you his best wishes.

I think that there are only basically three or four points that I want to make, and one is I think an echo of what Marci just talked about, and that is that we really are facing an important problem. It's an important problem because it's unpredictable, and it's a serious cause of disease already that we see. The disruption that has occurred in China with this infection, and in Canada, are very, very dramatic impacts. They could've happened here actually; if you think about it for just a minute, we're very lucky. This could've happened in New York City or Los Angeles or Houston or even Galveston. I think that really means that we have to very, very seriously take this problem. The worst case scenarios are of course difficult to contemplate. I guess I've associated myself with that remark that I think was made by Yogi Berra that it's difficult to make forecasts, especially about the future. I don't want to stand up here with this lofty title for my talk about the view from the bridge. I could be the Captain of the Exxon Valdez, and not see that reef before my ship hits and releases all the oil.

### *Needing Collaboration and Cooperation*

I think there are some things that we need to be thinking about, and one of them is the uncertainty that we're facing. Because we're facing that uncertainty, I think we need to prepare ourselves as comprehensively and as completely as we possibly can. Not simply from the perspective of our public health colleagues, who are facing this problem on a day-to-day basis, but also from the perspective of the research community. I think we have an obligation to do whatever we can to find solutions for this disease that result in effective vaccines, therapies, diagnostic tests, that can assist the control measures most productively. That is exactly what the NIAID and our partners in the public health service, the CDC and the FDA, are really trying to do.

This is another point that I want to make: this cannot happen without the kinds of collaboration and cooperation amongst agencies that you've seen. I think it is an unbelievable testimony to the effectiveness of our public health institutions, not just nationally but globally, that so much work and so much



progress has been achieved in such a short period of time with this particular disease problem. I think a lot of that has to do with the fact that there's been an expansion of interest in infectious diseases in general. I remember in 1990, when we had a meeting in May down in Washington. C.J. I think was at the meeting, and we talked about emerging viral infections. It was a meeting that Steve Morse and I organized. At that time, the topic was viewed somewhat suspiciously. I remember that in the audience, Howard Teman, who was one of the participants, had very heated debates with Josh Lederberg and Bernie Fields about their view, which they held pretty steadfastly, that we needed to prepare for other emerging viral infections, and I think the prescience of Bernie and Josh on this issue is really very, very apt.

I think one of the lessons that has grown out of this last ten years or so has been the important role that collaboration plays; not just simply collaboration between academic and government investigators, but collaboration and cooperation, I think especially so, between the basic and public health research communities and the private sector. By that I'm particularly referring to the pharmaceutical industry. We need to try to develop mechanisms in which that can be accomplished and be more successful.

International collaboration is key, I think, in this arena. We need to be able to work effectively with our colleagues overseas. I think you've seen some evidence of that already. I think that this example has enhanced the role of collaborative research and cooperative activities, and the importance of international relationships, for these kinds of research projects and for these kinds of public health projects, and that's very, very important.

I think communication, which Marci talked about very effectively and others have spoken about more eloquently than I can speak about, needs no further elaboration from me. But I think that that's a very, very key part of the equation.

#### *NIAID SARS-Related Activities*

The last thing I wanted to tell you was a little bit about what we're doing specifically, and where we're headed. One of the things that we're going to be doing is we're convening a meeting on May 30 to talk about research activities related to SARS. If you want additional information on this, we just put a Web site up yesterday. We're organizing this meeting rather quickly. The secretary of the Department is quite interested and has told us he will be there, schedule permitting, so we're looking for, I think, an important discussion which will help guide our efforts in this area. If you just want to just take down the web page, it's <http://SARS.IQsolutions.com>. IQsolutions is our contractor that's helping organize this meeting.

We have also initiated activities within both of our extramural program activities; that is, our grants and contracts programs that we administer and through which we make awards to the academic and private sector. For example, the partnership grants for diagnostic, vaccine and therapeutic development that we've initiated for biodefense, we've also added SARS to that list. That I think just happened recently if my information is correct.

As you've heard from Cathy Laughlin, we have expanded our antiviral screening activities quite rapidly, and I think we will look to expand in a variety of different areas in the very, very near future, as resources and time permit.

At the intramural side of the campus, we have begun with a number of activities. The Vaccine Research Center, which is part of the NIAID, has begun activities on vaccine development, as has the Laboratory



of Infectious Diseases under Brian Murphy. We have also begun discussions in collaboration with the CDC about the admission of patients potentially to the clinical center, and we have, I think, come pretty close to finalizing the clinical protocol and other details associated with that.

In summary, I think that we are prepared to do what we need to do. This is a very, very serious problem in our opinion. It's one that has to be taken seriously, it's one that we're taking seriously, and it's one that will require, I think, the commitment of all of us to its solution. It's not going to be an easy path, but I think it's one that we can and must pursue. I think it's also an important challenge to us, because it is in a way a test of our abilities to deal with other natural or other kinds of emerging infectious disease problems. Influenza is the one that most commonly people talk about, but there are many others that one could conjure up and imagine. I think it is really the scientific community, if you will, that is going to be judged by how it responds to this challenge. So far, I think the response has been admirable, and I'm very optimistic and hopeful that we're going to have the kinds of solutions we need very soon. Let me quit with that.

## Future Perspectives on Emerging Infections: Panel 4 Discussion

*What We Might Have Done Better*

**Allan Rosenfield:** Thank you, John. We are actually almost back on schedule. We have fifteen to twenty minutes before the reception for discussion. Let me start and see whether, Ian, are you still there?

**Ian Lipkin:** Yes.

**Allan Rosenfield:** Would you like to start off?

**Ian Lipkin:** I have a general question. I think the

**Allan Rosenfield:** Excuse me. Panelists, come up to the front. Sorry, Ian. Go ahead.

**Ian Lipkin:** It is truly remarkable how well people have collaborated internationally in the course of this outbreak and we congratulate ourselves. I'm wondering if people on the panel might want to comment on things that we might have done better or differently.

**Allan Rosenfield:** C.J., Marci, someone want to start, John?

**John La Montagne:** Ian, that's a question that's going to be asked, I think. I honestly don't know that we could've done anything differently. You know, 20/20 vision, as they say, is always 20/20—is that Yogi also? Obviously we would've liked to know perhaps sooner about the extent of the disease in certain parts of the world, but that's the nature of public health now. I'm not sure that much time would've been saved.

**Allan Rosenfield:** C.J., Marci?

**C.J. Peters:** I think one of the things that always gets overlooked in these outbreaks, time and time again, is getting a good sample set. Once the balloon went up, we needed good sample sets that were collected. We needed that just as bad as we did case-counting, so that different laboratories would have acute and convalescent samples preferably from the same patients. I think that would have given us a



solider movement in that sort of middle ground.

### *Understanding Animal Diseases Better*

**Allan Rosenfield:** Ian, you have any comment on that?

**Ian Lipkin:** The only other point I'd like to raise was one that came up repeatedly on some of the early conference calls that we would have at CDC on Wednesdays. Kay Holmes probably remembers this comment that came up over and over again, and that was that we know very little about diseases of animals, and if we were to invest more time in studying diseases of wildlife and domestic animals before they hit the human population we might be in a better position to address them once they do.

**Allan Rosenfield:** Comments?

**C.J. Peters:** This may not be a disease of animals. I mean I agree with Ian, but I just hasten to point out that it may not be a disease of animals, like arenaviruses are not diseases of rodents. It's only when they get into humans that they cause hemorrhagic fever.

**Allan Rosenfield:** There was an interesting theory by an epidemiologist in Hong Kong who felt there was good evidence that rats could easily have been the mechanism of transport, particularly in the original apartment houses. We don't know that WHO followed up on that during their visits in Hong Kong, because there's nothing in their report that just came out.

**Ian Lipkin:** C.J.'s point is well taken. I think what we're saying there is that probably the best reservoir vector is an animal which doesn't show signs of clinical disease. It still raises the same issue as to whether or not one shouldn't be doing surveillance and determining what's actually out there in bush meat and in wildlife and so forth, so that we could at least recognize these agents and so that we could pursue diagnostics and early recognition.

**C.J. Peters:** I tend to think of it as a biodiversity issue. We need to understand the biodiversity of those little critters called viruses, and some of the technology that you've developed, Ian, would be very helpful to apply, and not just to human samples, but to try to understand what's out there in what we sometimes refer to as the critters.

### *Concern about Convalescents Shedding*

**Allan Rosenfield:** From the floor, any questions? I guess we have one back there and then you're next.

**Maggie Fox:** I'm Maggie Fox with Reuters. I want to ask if any of you all are concerned. It seems that the patients, especially the convalescent patients in Hong Kong, have been sent home. You talk about the difficulty, Dr. Lipkin, of obtaining samples, serosamples before and after. Is anyone concerned that with the veterinary evidence that animals who have been infected may continue to carry virus and shed virus, that it's possible that SARS could reemerge through convalescent patients?

**Allan Rosenfield:** Anybody want to take that?

**Marcelle Layton:** I'll just make a comment to start, that here in the United States at CDC they're doing a number of studies, one of which is a natural history study of cases that meet certain criteria. They're trying to get convalescent specimens on a regular basis. My concern is, though, that because so few of our "cases" are likely cases, that similar or exact studies are really needed in areas that are affected to



help answer that question. I do think there's a lot more epidemiologic investigations that need to be done in areas that have been more severely impacted than the United States.

**Allan Rosenfield:** David?

**David Ho:** That question, I think, is being addressed. The full answer is not out yet, but certainly after 21 days their shedding of virus, at least by PCR in the stool, we for the moment have to assume that's potentially infectious. I think a more detailed analysis will be forthcoming in a short period of time, exactly to answer the issue of isolation for the discharged patient.

### *Concluding Remarks*

**Allan Rosenfield:** Other questions. Scott, do you have any?

**Ellis Rubinstein:** If you're going to end, I have to say one thing. I really want to thank, once again, Ian and Scott for working extremely hard to put this thing together in three weeks. I know better than anybody how many hours they've spent on this, because we could only give them so much support. I think I've heard enough from everybody here that they've enjoyed the opportunity to mingle with each other, and I think that they deserve all the credit for that. So thank you very much, Ian and Scott. Thank you all on behalf of the Academy and the staff. Our staff worked hard and Rashid Shaikh is our Program Director, and he gets some credit, too. If you're interested in our future events, he's right there. Please enjoy some drinks if you have a little bit of time, and if not, we'll see you at the next one. Thank you.

**Allan Rosenfield:** Thanks Ellis.