DEPARTMENT OF HEALTH AND HUMAN SERVICES

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FOOD AND DRUG ADMINISTRATION

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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VACCINES AND RELATED BIOLOGICAL

PRODUCTS ADVISORY COMMITTEE

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OPEN SESSION

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THURSDAY, NOVEMBER 16, 2006

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OPEN

The conference convened in the Versailles Room of the Holiday Inn Select, 8120 Wisconsin Avenue, Bethesda, Maryland, at 1:07 pm., Dr. Ruth A. Karron, Chairperson, presiding.

COMMITTEE MEMBERS PRESENT:

RUTH A. KARRON, M.D.	Chairperson
SETH HETHERINGTON, M.D.	Member
LISA JACKSON, M.D., M.P.H.	Member
PHILIP S. LaRUSSA, M.D.	Member
JOHN MODLIN, M.D.	Member
CINDY LYN PROVINCE, R.N., M.S.N., M.A.	Member

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COMMITTEE MEMBERS PRESENT (Continued):

WALTER ROYAL, III, M.D.	Member
JACK STAPLETON, M.D.	Member
BONNIE M. WORD, M.D.	Member

FDA STAFF PRESENT:

CHRISTINE WALSH, R.N., Executive Secretary

CONSULTANTS PRESENT:

JAMES COOK, M.D.

SETH HETHERINGTON, M.D.

PAMELA McINNES, D.D.S.

ROBIN ROBINSON, Ph.D.

C O N T E N T S

P R O C E E D I N G S

(1:07 p.m.)

MS. WALSH: I guess we can actually get started maybe and hopefully people will join us, the other few people that we're missing in just a few minutes.

Dr. Karron, do you want to call the meeting to order?

DR. KARRON: Yes, I would. Our meeting is about to begin, and, Christine, I will turn the meeting over to you at this point.

MS. WALSH: Thank you.

Good afternoon. I'm Christine Walsh, the Executive Secretary for today's teleconference meeting of the Vaccines and Related Biological Products Advisory Committee meeting.

I would like to welcome all of you to this meeting of the Advisory Committee. There is a speaker phone for public participation located here in Conference Room C of Building 29B on the NIH campus.

This afternoon's teleconference meeting will consist of sessions dealing with presentations and committee discussions that are both open and closed to the public as described in the *Federal Register* notice of October 31st, 2006.

At this time, I would like to introduce the committee members and ask that they acknowledge by saying "present" if they can hear me.

The committee Chair, Dr. Ruth Karron, Professor, Johns Hopkins School of Hygiene and Public Health.

CHAIRPERSON KARRON: Present.

MS. WALSH: Dr. Philip LaRussa, Professor of Clinical Pediatrics, Columbia University.

DR. LaRUSSA: Present.

MS. WALSH: Ms. Cindy Province, Associate Director, Bioethics Center of St. Louis.

MS. PROVINCE: Present.

MS. WALSH: Dr. Walter Royal, Associate Professor, Department of Neurology, University of Maryland, School of Medicine.

(No verbal response)

MS. WALSH: Dr. Bonnie Word, Assistant Professor of Pediatrics, Baylor College of Medicine.

DR. WORD: Present.

MS. WALSH: Dr. Seth Hetherington, industry representative, Senior Vice President, Clinical and Regulatory Affairs, Icagen, Incorporated.

DR. HETHERINGTON: Present.

MS. WALSH: Dr. John Modlin, Professor of

Pediatrics, Dartmouth-Hitchcock's Medical Center.

(No verbal response.)

MS. WALSH: Dr. Lisa Jackson, senior scientific investigator, Group Health Cooperatives, Seattle, Washington.

(No verbal response.)

MS. WALSH: Dr. Jack Stapleton, Professor and Director, University of Iowa Hospital Clinic.

DR. STAPLETON: Present.

MS. WALSH: Thank you, and welcome Dr. Stapleton to the VRBPAC Committee

DR. STAPLETON: Thank you.

I am Director of the Division of Infectious Diseases, but not the hospital.

MS. WALSH: Okay. Thank you.

Dr. Monica Farley and Dr. Steven Self will be unable to join us today.

I would like to thank all of the committee members for taking time to join us, and at this time I would ask those seated around the table here in Conference Room C if they would please introduce themselves.

DR. BAYLOR: Norman Baylor, Director of Office of Vaccines.

DR. WALKER: Dick Walker, Director,

Division of Bacterial, Parasitic, and Allergenic Products.

DR. VANN: Willie Vann, Chief of the Laboratory of Bacterial Polysaccharides.

DR. FEINSTONE: Steve Feinstone, Chief of the Laboratory of Hepatitis Viruses.

DR. MARKOFF: Lew Markoff, Chief of the Laboratory of Vector Borne Virus Diseases.

DR. BRENNAN: Mike Brennan, Associate Director of Research, Office of Vaccines.

DR. WEIR: Jerry Weir, the Director of the Division of Viral Products.

MS. WALSH: Thank you, everyone.

Two people joined us. Can you just tell me -- this is Christine -- who joined us?

DR. MODLIN: Hi, Christine. This is John Modlin here.

MS. WALSH: Okay. Thank you.

DR. MODLIN: Sorry to be late.

DR. JACKSON: Lisa Jackson. Also sorry to be late.

MS. WALSH: Okay. That's okay. Thank you. Dr. Modlin and Dr. Jackson, welcome to the VRBPAC Committee.

DR. MODLIN: Thank you.

DR. JACKSON: Thank you.

DR. CARBONE: And, Christine, you didn't read my name, but I'm on the phone also. It's Kathy Carbone, the Associate Director of Research for CBER.

MS. WALSH: Okay. Thank you. I was just going to do that.

I ask that all our committee members please identify themselves each time they speak because we have a transcriber present who will need your assistance in order to accurately transcribe all comments to the appropriate committee member.

I also ask that our committee members do not use cellular phones since they may add extra unnecessary background noise to the line.

Should during the teleconference a source of noise occur in your office, we would appreciate it if you would use the mute button on your phone if you have that option. We ask that you do not place us on hold since many clinical centers have background music, and that can be distracting to those remaining on the teleconference line.

I would now like to read into the public record the conflict of interest statement for this meeting.

The Food and Drug Administration, FDA, is

convening today's meeting of the Vaccines and Related Biological Products Advisory Committee under the authority of the Federal Advisory Committee Act, FACA, of 1972. All members of the committee are special government employees, SGEs, or regular federal employees from other agencies and are subject to the federal conflict of interest laws and regulations.

The following information on the status of this Advisory Committee's compliance with federal conflict of interest laws, including but, not limited to, 18 USC 208 and 21 USC 355(n)(4), is being provided to participants in today's meeting and to the public. FDA has determined that members of this Advisory Committee are in compliance with federal ethics and conflict of interest laws, including, but not limited to, 18 USC Section 208 and 21 USC Section 355(n)(4).

Under 18 USC 208, applicable to all government agencies, and 21 USC 355(n)(4), applicable to certain FDA committees, Congress had authorized FDA to grant waivers to special government employees who have financial conflicts, when it is determined that the agency's need for a particular individual's services outweighs his or her potential financial conflict of interest, Section 208, and where participation is necessary to afford essential

expertise, Section 355.

Today's agenda includes a review and discussion of the intramural research programs of the Laboratory of Bacterial Toxins, Division of Bacterial, Parasitic, and Allergenic Products. The committee will also review and discuss the intramural research programs of the Laboratory of Vector Borne Virus Diseases, the Laboratory of Hepatitis Viruses, and the Laboratory of Respiratory Viral Diseases, Division of Viral Products.

Based on the agenda, it has been determined that the committee discussion presents no actual or appearance of a conflict of interest for today's meeting. This conflict of interest statement will be available for review at the registration table.

We would like to remind members that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all other participants to advise the committee of any financial relationships

that you have with firms that could be affected by the committee discussion.

That ends the conflict of interest statement. Dr. Karron, I turn the meeting over to you, but before I do that, can you just -- this is Christine. Can you please tell me who just joined us?

DR. ROYAL: Hi. This is Walter Royal.

DR. JACKSON: Thank you, Dr. Royal.

CHAIRPERSON KARRON: Thank you, Christine.

I want to welcome all of the members to this meeting, including our new members, Drs. Modlin, Jackson, and Stapleton.

As Christine just pointed out, this meeting is convened to review four laboratories in the FDA CBER program. At this point we will go ahead and start with the first speaker who will be Dr. Willie Vann from the Laboratory of Bacterial Toxins.

Dr. Vann.

DR. VANN: Yes. I will give a brief overview of the research program of the Laboratory of Bacterial Toxins, which was reviewed in December 6 of 2005 for the period of 2002 to 2005.

The Laboratory of Bacterial Toxins consisted of three groups headed by three PIs. The Neurotoxins Group, the principal investigator is Dr.

James Keller. Glycobiology Group is myself, and the Corynebacteria Group is Michael Schmitt.

At the time of the site visit, the laboratory consisted of ten members, which you can see on Slide No. 3.

And on Slide No. 4, there's a breakout of the organization of where those members work. The Neurotoxins Group consisted of Dr. Keller, the PI, and his technician, Provina Moto.

Corynebacteria at the time was Dr. Michael Schmitt. He had a post doctoral fellow who is still with him, Lorri Bibb, and a technician, Carrie Kunkle, whom at the time of the site visit had just delivered. So he has only recently replaced that technician a few weeks ago.

In my group there was Justine Vionet and Dwight Peterson, who were technicians; Katarina Anderisheva, who is sitting on the ground, the blond lady who is a post doctoral fellow and a graduate student of Fritzie Jenahara, who has since this time, has left.

Okay. The Laboratory of Bacterial Toxins has responsibility for submissions that were related to toxins and toxoid conjugate vaccines. Submissions that we're responsible for review of IND and BLA

submissions. We participated in IND meetings and numerous technical meetings over the years.

During the review period we had in excess of 200 IND supplements and 15 original IND submissions, two major BLAs, and 96 BLA supplements. We were a chair of at least almost 90 of them.

We also participated in the preparation of policy documents. Particularly Dr. Michael Schmitt was on a committee to develop guidance for production of products using spore forming organisms.

Dr. Keller and I participated in a policy group developing licensing strategies for vaccines to protect against botulinum toxin intoxication. This is part of our counterterrorism program.

We also participated in expert CBER and international workshops and regulatory presentations. Dr. Keller was a very important member of a committee organized by the National Institute of Health, NIAID, on botulinum neurotoxin and counterterrorism, and I participated in a workshop with ECVAM for developing replacement tests for animal tests.

As you can see from the list of collaborators, our work is actually fairly well recognized, and we are part of a larger scientific community.

So if you go to the next slide, and now the research focus. The Neurotoxins Group focuses on the characterization of the mechanism of neurocele intoxication by botulinum toxins. Specifically, Dr. Keller is interested in uptake. I'll talk a little bit more about this later.

The Corynebacteria Group is interested in identification and characterization of putative virulence determinants that are coordinately expressed with iron regulated diphtheria toxin.

And the Glycobiology Group studies the mechanism of capsule olysaccharide biosynthesis and the interaction of neurotoxins with glycoconjugates.

Relevance of the program to the CBER mission. Our research program and the mechanism of neurotoxins has provided a basis for numerous regulatory decisions in the past four years. For example, one is developmental policy for the licensure of potential vaccines against botulinum toxin, review of a recent incident -- well, not so recent -- an incident involving patients receiving misbranded therapeutic botulinum toxin, and there's a case where patients received misbranded botox and were hospitalized on respirators. Dr. Keller's expertise was very helpful for CBER in dealing with that issue.

As a natural consequence of our executing the research program, the Laboratory of Bacterial Toxins has given us extensive experience in molecular biology and biochemistry of toxins in carbohydrates. This has provided us insight in the evaluation of manufacturing changes on the stability and purity of botulinum, diphtheria, and tetanus toxoid vaccines. It is up to us to develop policy on the use of spore forming bacteria in biologics.

And our expertise was instrumental in another case, in the analysis of a misbranded tetanus toxoid vaccine that subsequently resulted in a prosecution in 2003.

The long term objectives of the research program are to uncover new targets for vaccine development and to develop new approaches to vaccine synthesis and new methods for the evaluation of safety and potency.

So what are some of the accomplishments? I've given on the next three slides some select publications from each of the groups, and I will highlight one of them from each just to give you an example of the quality of work that is done by the groups.

Dr. Keller is a very bright and innovative

scientist. In the first publication, one of the things that comes out there is that he's developed a very sensitive, nonlethal assay for botulinum toxin, and he has used this to characterize the response of various animals and recovery from intoxication of the various serotypes of the neurotoxin.

Briefly what he does is he has mice running in a cage, and he measures their voluntary running and the paralysis of these organisms, and then he can look at the rate of recovery and a number of other things.

The sensitivity of this assay is on the order of ten picograms of botulinum neurotoxin Type A, which is about .1 mouse LD-50s, which is more sensitive than the current mouse assay.

And one of the other things that he does that I was going through is that he's really interested in the uptake of various serotypes of botulinum neurotoxins, and he has developed assays to develop models for how the organism takes these and explains some of the differences between the neurotoxins.

Dr. Schmitt is interested in iron uptake and iron utilization by Corynebacteria. Iron is actually very important for virulence, and the thing

about this is he's looking at uptake mechanisms that are coordinately regulated with the expression of the toxin, and the toxin is actually regulated by iron.

He has developed a very novel assay for looking for other regulated systems. One of the things he has identified, which is in the second paper here of Schmitt and Kunkle, is a center of four system that he identified as a result of using this assay system and the recently published genome sequence of Corynebacteria. So he has characterized the biosynthetic system for this center of four and how it is actually regulated and expressed.

And our group is interested in polysaccharide biosynthesis and also toxin interaction with carbohydrates. The last paper here, what we've done is actually used radiation target analysis to define the molecular organization of this polysialyltransferase. Polysialyltransferase is the enzyme that actually polymerizes the sugar to for a polysaccharide.

And that concludes my comment.

CHAIRPERSON KARRON: Thank you very much, Dr. Vann.

I do have one question for you, which is I know that --

DR. VANN: Who is asking?

CHAIRPERSON KARRON: I'm sorry. This is Dr. Karron.

DR. VANN: Okay.

CHAIRPERSON KARRON: I know that when we reviewed the laboratory last year, I know that the plan at that time was actually to dissolve the Laboratory of Bacterial Toxins, and that you would become the new Chief of the Laboratory of Bacterial Polysaccharides, and Dr. Schmitt and Keller would move over to the Laboratory of Respiratory and Special Pathogens.

Has that occurred? Is that what has happened?

DR. VANN: In fact, that has occurred. I'm now Chief of the Laboratory of Bacterial Polysaccharides, and Dr. Schmitt and Dr. Keller are now members of the Laboratory of Respiratory and Special Pathogens. I must say they continue to do fine work in spite of limited resources.

CHAIRPERSON KARRON: Thank you very much.

Are there additional questions for Dr. Vann from the committee members?

Okay. Hearing none, I think we will move on to our next speaker, who is Dr. Lewis Markoff, who

is Chief of the Laboratory of Vector Borne Virus Diseases.

Dr. Markoff.

DR. MARKOFF: Thank you, Dr. Karron.

I'm just going to go over these slides which I made for the occasion thinking that we'd be showing slides.

The first slide shows the organization of the Laboratory of Vector Borne Virus Diseases. I am the only PI or principal investigator in the lab, and I directly supervise Dr. Barry Falgout, who is a staff scientist who has been with me for more than a decade, and Dr. Li Yu, who is a newly minted staff fellow in the laboratory.

And as you can see, Dr. Falgout supervises Dr. Tadahisa Teramoto, who is a post doctoral fellow under ERDA fellowship, and Dr. Eileen Kelly, who is a visiting scientist from Walter Reed Army Institute for research.

And there's sort of an advice and consent relationship between myself and Dr. Falgout on the research that he and these two individuals are doing, and I directly supervise Ms. Stephanie Polo, who is a microbiologist in the lab, along a long term person.

And in this diagram Jin Gao, who was a

microbiologist in the lab, also being supported by an ERDA fellowship. And our lab was reviewed. We had a site visit on January 2006, at which time Ms. Gao was a member of our lab, but she has since been offered a permanent position in CBER in Division of Viral Products, and understandably she took that job. So she's no longer with us. We're currently looking for a replacement for her.

And on the next page, Slide 2, is the personnel history of the lab from the site visit in 2001 to that which occurred in January of 2006, and just quickly you can see that myself, Dr. Falgout and Stephanie Polo and Eileen Kelly are the constants that have been there. We're in the lab for the whole five-year period, and this document is the coming and going.

In essence, in the five-year period we lost two FTE and gained back Dr. Yu, who is a staff fellow, who is an FTE, but is not a permanent, which is not a permanent position yet.

One of the purposes of the site visit in January 2006 was to review the qualifications of Dr. Yu for conversion to a permanent staff. Then he would be a staff scientist if he were converted.

The mission statement for our lab is there

in front of you on the next slide. We view our mission as the conduct of state of the art review of INDs and BLAs through our parallel pursuit of research goals that remain relevant to the regulatory mission of CBER and which enable us to provide insight into scientific issues that relate to the review process.

On the next page, I've summarized the regulatory responsibilities of the laboratory, and first of all, we review all IND submissions related to vaccines to prevent illnesses caused by vector borne viruses, and this includes the Flavi viruses, dengue, West Nile, Japanese encephalitis, and Yellow Fever. VEE, which is an alpha virus, and any other alpha virus related products, for example, recently I got embroiled in a discussion of chikungunya vaccine for emergency use in Reunion where they were having an epidemic through the French government, Hunta viruses, et cetera.

We also represent CBER on issues related to these vaccines in collaborative efforts with WHO and CDC. The WHO has a very active steering committee to promote the development of dengue and novel Japanese encephalitis virus vaccines which meets at least twice a year.

I just came back from a two-day meeting in

Atlanta on this subject.

We also reviewed BLAs and BLA supplements related to these vaccines, and we all have so little outside that category. We have other responsibilities. For example, Barry Falgout in the lab reviews, is a consultant on adenovirus vectored vaccines because Dr. Falgout did his Ph.D. thesis in adenoviruses, and he's one of the few people within DDP that has that expertise

I have been heavily involved in Hepatitis A vaccine reviews, and currently I'm still reviewing anything relating to Twinrix, the bivalent Hepatitis A and Hepatitis B vaccine.

And I and Robert Levis, who has left the lab since 2001, and Jin Gao were involved most recently in development of a rabies, *in vitro* potency assay for rabies virus vaccines, and that's still an ongoing project largely because it's an international effort, and there's been lots of bureaucratic delays in getting that done. But I hope to see that done very shortly.

And here on the next slide I've summarized our work load for the last five years, and I think it's just easier to look at the red print. So we've done a total of 28 pre-IND reviews. Those are reviews

where you get a big folder of what a company wants to do, and usually they are followed by a meeting with a company and some advice goes back and forth. There's a direct interaction.

We've got 49 total INDs that we review of which in the last five years, there were 16 new originally INDs that initiated within the last five years.

There's a lot of activity, I should say, in the development of novel flavivirus vaccines as many of you may already know.

This IND amendments, there were a total of 448 reviewed by the four individuals on the list there, and the BLA that we reviewed or participated in the review of 29 BLA supplements, and eight of those supplements were chaired by myself or Dr. Falgout.

Research activities are indicated on the next slide, and I'll just read what I wrote. A genuine effort is made to maintain a high quality research effort that is consonant with the mission of the laboratory and the nature of its regulatory responsibilities. Thus, the research effort generally makes use of the tools of molecular biology in order to elucidate mechanisms of flavivirus replication as they may relate to strategies for attenuation of

flavivirus virulence and therefore, enhance vaccine safety.

The lessons that we learn in the lab are applied in the review of novel vaccines, and as some of you may know, we are reviewing a large number of live virus vaccines that were attenuated by site directed mutagenesis of the flavivirus genome in one or another of a variety of ways.

So here on the next slide, research projects, 2001 to 2005. I've basically listed some of the major research projects, and in red are the three projects that I'll discuss in a little bit of detail.

So I'll just read these off as a list. We developed and characterized a candidate, live attenuated dengue vaccine candidate, ourselves that's called Mutant F virus. The candidate we did the most work on is Dengue 1 Mutant F. As you may know there are four serotypes of dengue viruses, and everybody agrees that the first dengue vaccine will have to consist of a tetravalent mixture of all four serotypes.

We have shown that when we introduce the mutations, characteristics of Mutant F into dengue 2, 3 and 4 genomes, the viruses have the same tissue culture phenotype. Only dengue 1 Mutant F has been

tested in preclinical studies in monkeys and has proved to be attenuated.

Dr. Yu primarily was involved in developing and characterization of a candidate live attenuated West Nile virus vaccine, which we call Mutant E virus. I'm very hung up on alphabetical designations, Mutant E West Nile. Dr. Falgout and Dr. Teramoto were majorly involved in the study of the mechanism by which flaviviruses can repair three prime terminal deletions of genomic RNA. This is a natural phenomenon that Dr. Falgout discovered serendipitously and that we went to work to find out the mechanism for this repair, and I'll talk more about that.

And then I've been involved in the study of the role of the conserved basic residue and the capsid protein in virion morphogenesis, and I'll just let you read the last two slides so that will save time. The last time projects at the bottom.

I had mentioned the rabies potency assay.

Okay. So I'll talk a little bit in detail on Dr. Yu's project, which ended up in development of a candidate West Nile vaccine.

This was tied to a previous study that I had done in the late 90s on the role of the conserve three prime stent and loop structure in the flavivirus

genome in virus replication.

And so when Dr. Yu came to the lab, he embarked on a study of the three prime SL in the West Nile genome, and the idea of this was to use the two studies to get some information on the role of the three prime SL in flavivirus replication, and when we added the results of Dr. Yu's study to that of the dengue study that was done earlier, we showed that there are bulges in the long stem. There's a, I should say, conserved long stem and loop structure at the three prime terminus of all flavivirus genomes, and the results of Dr. Yu's study indicated that the loci of small bulges in that long stem, or in other words, where there is no base pairing, was critical to virus replication.

That led to a publication in the *Journal of Virology* in 2005. After that Dr. Yu took several of the viable mutant viruses that he had made. These are West Nile viruses now that have mutations in this three prime stem and loop structure, and we tested them all of their level of attenuation, neuroattenuation in mice, and West Nile virus was identified as a candidate vaccine, and that virus is currently in the NIH patent process.

This next slide just shows you briefly

what the mutation is in the three prime SL of the West Nile genome. So what you're looking at is just the three prime SL nucleotide sequence. The whole genomes is over 10,000 nucleotides long. So this is at the very end of the genome, and what Dr. Yu did was simply transfer a set of bulges in the dengue three prime stem and loop structure, which substituted for a bulge in the West Nile three prime stem and loop structure. That virus was viable.

He made many other mutations, but this is the one that turned out to be a candidate vaccine. If you go to the next slide, you can see that that virus -- you see the pink Xes across the top. That's the Mutant E after intracerebral inoculation of two day old mice.

All of these other mutants that Dr. Yu tested and wild type West Nile virus, all kill, had a 50 percent lethal dose around ten or 100 PFU, but at 10,000 PFU, the Mutant D virus didn't kill any mice.

We have since learned that at around 100,000 PFU we get about a 50 percent, is the LD-50 of that virus in mice. We're currently introducing more mutations into that genome to try to attenuate it further.

On the project on the capsid protein, the

flavivirus capsid protein is about 100 amino acids in length. It's very hydrophilic because it has to associate with RNA in the nucleus of the virus and the nucleocapsid of the virus.

It contains a conserved internal hydrophobic domain. That hydrophobic domain has a single positively charged amino acid, and that's conserved in all mosquito borne flavivirus capsid proteins, except that of YF, Yellow Fever, virus.

And this is called Arginine 55 in the dengue 2 capsid. So what Jin Gao did was mutagenize that Arginine 55 and we evaluated the infectiousness of RNAs bearing those mutations in both monkey cells and mosquito cells.

On the next page is just a diagram of a picture, acquire CyroEM, cross-sectional photo of a dengue 2 genome, and from a publication, and you can see the nucleocapsid is all inside, is completely inside. The virus was actually a three nanometer space between the nucleocapsid and the lipid envelope, and so it's not obvious how mutations in the capsid protein, which capsid is entirely in the nucleocapsid, could affect functions of attachment.

But in our analysis of the phenotypes of the mutants, which is summarized on the next slide,

you can see that thee of the mutations that Jin Gao made, substituting that arginine with asmet or leucine, had a host range phenotype whereby the resultant virus particles were very defective for detachment, entry into the endosome or uncoating. We don't know which at this point in monkey cells, but we're actually able to infect mosquito cells.

So this shows that a mutation of the capsid could somehow perturb functions of the envelope glycoprotein, which is actually the major protein involved in virus attachment.

And we're studying this further. I guess there's not really time to go into more detail on that.

And then the next project I wanted to highlight was one which had been the work of Dr. Teramoto and Dr. Falgout, and as I mentioned, the preliminary observation was that Dr. Falgout prepared an infectious DNA for Japanese encephalitis virus, that inadvertently lacked the three prime terminal seven nucleotides of the West Nile genome, or of the JE genome, which both Barry and I expected to be a lethal event.

However, RNA transcripts generated from that DNA were shown to be infectious in cells, and

when we looked at the three prime terminal nucleotide sequence of the RNA in replicating virus, the wild type sequence was restored.

So the question arose what is the activity that is preventing this to happen because there was no known activity of the virus coded RNA dependent, RNA polymerase that could account for this.

So we studied this question further in the context of our dengue 2 infectious DNA because of the safety aspects of working with Japanese encephalitis virus infectious DNA.

And basically Dr. Teramoto introduced a series of deletions with the three prime terminus of the dengue 2 infectious DNA of up to 22 nucleotides. Transcripts were generated and used to transfect LOCM-2 cells which are monkey kidney cells, and Dr. Teramoto looked for virus replication for up to 100 days post transfection. I should say post transfection, not PI, and the genomes of viable viruses were sequenced.

And we also studied this phenomenon in the context of an *in vitro* assay for the RdRp activity, which was all contained on the viral, nonstructural protein called NS5.

The summary of the results were that

essentially that deletions of up to six or seven nucleotides were usually repaired, and on some occasions even up to ten nucleotides could be repaired.

So basically we concluded there does exist a mechanism for three prime end repair and restoration of the nucleotide sequence.

Some of the characteristics, this was heavily studied by Dr. Teramoto and Dr. Falgout in terms of looking at the genomes of viruses that arose, and we concluded that there was a slow evolution of mixed sequences toward the wild type length, suggesting that there was a nucleotide addition mutation process which selected for better replicating molecules.

The short of it is that molecules that were shorted in genome length were obviously able to replicate all along the way, but the selection pressure was toward making longer and longer molecules.

So we are looking at that. We want to map. We think that's a function of NS5, a heretofore undescribed activity of NS5, and we were engaged in a project in collaboration with Dr. Steve Whitehead's group at NIH to try to map the locus of this activity

within NS5.

So I'll stop there.

CHAIRPERSON KARRON: Okay. Thank you very much, Dr. Markoff.

Are there questions for Dr. Markoff?

DR. STAPLETON: This is Jack Stapleton.

Do you have a hypothesis for what directs the sequence's repair?

DR. MARKOFF: In that last project?

DR. STAPLETON: Yeah.

DR. MARKOFF: We have a hypothesis that, as you may remember, those nucleotides, as I said in discussing one of the earlier projects on the West Nile vaccine, there's a conserved three prime stem and loop structure there.

DR. STAPLETON: Right.

DR. MARKOFF: So those six, seven, or ten nucleotides have complementary -- are complemented on the opposite strand of that stem and loop. So our major hypothesis was that the other strand is being copied when the new sequences are generated.

And we have done some experiments to test that hypothesis. They tend to confirm that hypothesis, but it's far from certain. So we're going to look at that further.

CHAIRPERSON KARRON: Are there questions? Okay. Thank you very much, Dr. Markoff. The next speaker will be Dr. Stephen Feinstone, who is Chief of the Laboratory of Hepatitis Viruses.

Dr. Feinstone.

DR. FEINSTONE: Thank you, Dr. Karron.

The Laboratory of Hepatitis Viruses has been in existence since about 1990, and within the last two years, we've added a group that doesn't work specifically on hepatitis viruses, and that's the team led by Dr. Chintomani Atreya.

But the laboratory, as you can see in this slide labeled "Personnel," is divided into three teams. There's my group, with Kathleen Mahalick and Tania Uren, who left the lab in May and has been replaced by Dino Feigelstock.

Marian Major's team has one ORISE Fellow, Dr. Wananabi, and one biologist, Francis Wells, and at the time of our review in January of 2006, C.D. Atreya's team consisted of himself plus a biologist, Ms. Colcarne. Krishna Keith was a visiting association. Dr. Chen, who is an ORISE fellow, and Karen Stark, an ORISE fellow. At this time he has himself plus two members of his laboratory.

Also listed on this page are individuals

who have left the laboratory during the period of this evaluation.

The regulatory activity of the Laboratory of Hepatitis Viruses consists of review of all submissions dealing with all the hepatitis viruses, and we do receive submissions for all of the viruses except I think for delta. We don't have any direct submissions for delta, and there are at this time no proposed delta vaccines.

But we do have pre-INDs, original submission INDs, as well as IND amendments for Hepatitis A, B, C and D. We have BLA and BLA supplements for Hepatitis A and B viruses.

The regulatory activity, you can see on the next slide that deals with the numbers of submissions that we review, and as you can see, we do get quite a few IND amendments primarily, but pre-INDs and original submission INDs are also quite numerous in all of these areas for hepatitis.

Dr. Atreya's group deals with rotavirus vaccines and rubella virus, as well as SARS applications and Norwalk virus application, and they also have quite a bit of activity that's listed on the next page and the following page.

So the BLA activity that we have is

listed on the following page. As you can see, I've reviewed 39, Marian Major 40, and so on. Most of these are BLA supplements, but we do also have some original BLA submissions.

And then finally listed are inspections that our laboratory has been involved in, and as you can see, Dr. Atreya has done one, Dr. Major one, and Ms. Mahalick one.

The research program of the Hepatitis laboratory, again, is divided with the three groups. Dr. Atreya's group reviews, and has programs of rotavirus and rubella virus and a small program on SARS virus.

Dr. Major's team investigates Hepatitis C pathogenesis and vaccine development.

And my team has been involved more with animal models, cell culture studies, as well as vaccines.

To summarize briefly the research activity in the lab in the three teams, which is shown in much more detail in your briefing books, but we've extracted just a few of the highlights, Dr. Atreya's team, as I mentioned before, has focused on rotavirus and rubella virus activity, research, as well as a small project on SARS.

In the past Dr. Atreya's group has shown that rotavirus attenuation phenotype does not involve mutations in the NSP4 C terminal region, and rotavirus NSP5 associates with cellular actin by directly binding to the actin binder aldolase B, and Dr. Atreya believes that this may explain the formation of the viroplasms where replication of these rotaviruses occur, and he believes that this is accomplished by anchoring to the cytoplasmic actin through the actin alloy's B binder.

He has also shown that rotavirus VP4 translocates to cellular peroxisomes by functional peroxisome targeting signal 1 and PTS-1, and using inhibitory drugs, biosynthesis of intracellular lipids was shown to be crucial for the mature virion assembly, release, and perhaps RNA packaging.

In the future, he had plans to study the role of cellular aldolase and actin on the NSP5 viroplasm formation and their contributions to RNA replication and packaging, and further characterization of the nature of the rotavirus particles following drug treatment toward understanding of the role of lipid inhibitory drugs.

So he has actually shown that through the use of statins, he can reduce the replication of

rotavirus in cell culture by nearly two logs, and this is an area of continuing investigation.

His rubella virus project has been carried out in recent times primarily through collaborations. He had previously demonstrated that the N-terminal region of the capsid protein interacts with cellular protein P32, which enhances viral infectivity.

Perturbations in the P32 level is known to be associated with autoimmune disorder, such as arthralgia, and it's known that the rotavirus vaccine is often associated with arthralgias, and the idea was to investigate this interaction with P32 as the possible cause of that adverse effect of rotavirus vaccination.

He has also demonstrated that the -- I'm sorry. Rubella virus -- he has also demonstrated that the rubella virus P90 interacts with the cellular Citron-K kinase, CKK, and affects the host cytokines in cell culture.

At the organismal level this event could explain the teratogenic manifestations of rubella virus that have been observed when patients, when mothers get rubella virus infections during the first trimester of pregnancy.

They've shown through his collaborations

that P90 can have a profound effect on cell replication when it's over expressed *in vitro*.

In the future, he has continued plans to work on rubella virus and using a rat model of *in vivo* interactions with rubella virus P90 and CKK will be to evaluate to test if that is responsible for the associated teratogenic manifestations of rubella virus infections.

And as he notes here, most of this work has been done in collaboration with Dr. LoTurco's laboratory.

It appears that as a side note, it appears that it's very likely that Dr. Atreya's group will be moving again from the Division of Viral Products into the Office of Blood Research and Review where he is likely to take a new position.

Dr. Major's program has been very extensive over the past several years, and she has made extensive studies of pathogenesis, of Hepatitis C virus in the chimpanzee model. She has studied viral evolution in the Chimpanzee model using a monoclonal virus, essentially a monoclonal virus that was derived from the infectious CDNA clone of Hepatitis C virus that we developed in collaboration with Charlie Rice's laboratory at Rockefeller

University.

And so using this virus, we can inoculate chimpanzees with a virus that does not -- with an inoculum that does not contain the full array of quasi-species that one normally finds in the typical clinical sample that would be used to infect chimpanzees. So it's a virus that has only a single sequence is the best that we can determine.

And from that we can follow the true evolution of the virus in the chimpanzee model over time and associate that with various clinical and serologic events occurring in the chimpanzee.

She has also studied the phenotypic effects of mutations arising during infection that she has uncovered by this analysis of viral evolution, and she has also been heavily involved in studying the mechanism of protection from infection as well as applying that information to the problem of vaccine development.

So in the next several slides she has shown just a few of the areas that she's been working on, and I would like the emphasize that this really represents only a small amount of her work, and there is much more detail in the briefing book.

So one of the things that we were very

interested in was to look at the difference between animals that clear infections and animals that become persistently infected, and amongst other things that she's shown is that animals that become chronically infected actually do control virus replication to a very large degree.

Typically from the peak viral levels found during acute infections, these animals plateau off at a viral titer that's about two logs lower than the peak, and so they average about a 98 percent reduction from the peak virus loads, which we believe is due to immunologic control of virus replication.

She has also shown the clinical differences and the virologic differences in the animals that are able to clear the virus compared to the animals that go on to persistence, and as you can see in the slide, in the open circles, the circles and squares represent animals that go on to persistence where the closed circles and squares represent the animals that cleared the infection.

So you can see both the ALT in the squares and the peak RNA levels in the circles occur generally earlier in animals that clear the infections relative to the animals that go on to persistent infections, and we think that this is indicative of an earlier,

but probably a subtly earlier immune response to the infection in animals that clear.

The animals that clear seem to be able to control the infection earlier, which is due either to a more effective immune response or a slightly earlier immune response.

In the next slide you see the rate of mutations occurring in animals that go on to persistence and how those mutations associate with some of the clinical findings. And this is just a small amount of the data that Dr. Major has developed that are represented in this slide.

But what you can see is that the rate of synonymous and non-synonymous mutations that occur over time seems to change, where you see early in infection there are more non-synonymous changes relative to synonymous changes.

I should also say because you don't see this data, that we really don't see mutations arising in these chimpanzees during the first 22 weeks or so of infection. It's only after we begin to see the immune response coming up that the animals begin to accumulate mutations.

So during that first 22 weeks that we've studied these particular animals, they had essentially

no mutations arising. So with the advent of the immune response, you do see the appearance of new mutations, and remember, again, these are all animals that were infected with the monoclonal virus. So this is not selection of preexisting variants that existed in the population at the time of inoculation.

So over time, you begin to see more synonymous mutations arising, and what we think that this represents is sort of a steady state of stability when the virus is no longer under the same immune pressure that is seen relatively earlier in the chronic state of the infection.

In the next slide, you see the results of one T cell vaccination experiment that was done. What we had learned prior to this is that animals that recover from infection can always be reinfected, even if you use exactly the same inoculum, in our case using a virus with exactly the same sequence that the original animal was infected with.

But the animal rapidly controls the infection, and I should also say that animals that clear the infection do not make antibody to either of the surface glycoproteins. We can detect no antibody by either ELISA type assays or neutralization assays in animals that clear the infection.

So they cleared it is believed primarily by the T cell response, and so one of the things that we had done was to try to see what would happen if we induced a T cell response by vaccine and to look at the effect of that on the response to a challenge inoculum, and so this is an experiment that Dr. Major did with a vaccine that she developed along with Monserrat Pruich in the laboratory.

And so this vaccine consisted of a DNA vaccine and a vaccinia virus challenge, and both the DNA and the vaccinia virus had the same antigens coded, which included the viral NS3, NS5-A and NS5-B proteins that we knew were generally potent inducers of T cell responses in the chimpanzees.

And what we saw in an animal that had been vaccinated with this regimen upon challenge is that there was a very rapid control of the viral infection, and as you can see, the level of virus that we could detect in the serum actually went down to zero. It became undetectable.

But then there was a breakthrough and that animal eventually went on to develop a persistent infection, and what Dr. Major did is, amongst other analyses that she performed, she looked at mutations in the viral genome that occurred after that

breakthrough, and she did identify several mutations that appeared to be in T cell epitopes, and as you see in the lower panel there, she analyzed those, and as you can see, T cells from the chimpanzee did recognize the peptides made with the original sequence from that region of where the mutations occurred.

But following the mutation, the mutated peptide was not recognized by the same chimpanzee, and both of those two chimpanzees suggesting immune escape.

She also showed that this was based on CD4 response that you can see when she defeated CD4s of the ability to detect those epitopes was largely eliminated.

Now, for my team, we've been working, amongst other things, on non-primate animal models for Hepatitis C, and we have followed on the work of the Mercer Group in Canada on a chimeric mouse model, which you can see in diagram 4 on the next page.

What this is is a transgenic immunodeficient mouse which is expressing the neurokinase plasminogen activator gene under an albumen promoter so that it expresses in the liver. And what the uPA does, it results in destruction of the liver once it begins to express, and using the

liver destruction as an inducer of regeneration signals, one can then inoculate human or in our case often chimpanzee hepatocytes into the spleen, which can then repopulate the liver and, as I said, under the signals for liver regeneration, you can get a fairly large degree of liver repopulation with human or chimpanzee liver, and the animals then become sensitive to infection with Hepatitis C virus.

There are a major difficulties with the original albumen uPA system, and that is it required that you use homozygous mice to get good engraftment. The homozygotes are essentially impossible to breed, and they are simply not very healthy, and there's a very high mortality rate of homozygote pups, and so you must maintain the colony as heterozygotes, and so it really requires an immense mouse colony to generate just a very few animals that are useful in Hepatitis C experiments.

Antonio Uren in the lab started working on several solutions to these problems. One was a new transgenic system, which I sort of didn't put in here correctly. It's not a uPA system. We had talking about using an inducible promoter for UTA, but instead we used a transgenic mouse that expressed a nitroreductase gene from bacteria, and using a PRO

drug term, CB1954 in the presence of this nitroreductase the PRO drug is converted into a toxic substance that induces apoptosis in the liver, in the cells in which it is being expressed.

The second solution was to use the same uPA transgene, but under a different promoter, in this case the major urinary protein promoter also expressed in the liver. These mice were developed by Dr. Sangrin, the same person who developed the albumen uPA system. And we have also done a very small amount of work on alternative systems to uPA.

So the bottom line of this research has been that these new systems do work to some degree, and in the next slide, you can see repopulation of an NTR transgenic mouse with GFP hepatocytes from a healthy mouse, and so that has been quite successful.

At the time that Dr. Uren left the laboratory, we were will working on when to give the drug, what dose of the drug to give, and issues such as that to optimize this system, but it hadn't yet progressed much beyond that.

The second system that we used was this MUP-uPA system in which the neurokinase plasminogen activator is expressed under the control of the major urinary protein promoter, and the advantage to this is

that it's not expressed until about two to three weeks of life, and so the pups are born relatively healthy, and at the time that the transplantation is done, the animals are much older and much more able to accept the transplant.

And in the next page you do see again the repopulation of mouse livers with health mouse hepatocytes from transgenic mouse that expresses GFP.

And on the next page you see some preliminary data on infecting mice with Hepatitis C, some of these transgenic mice that showed that we can, indeed, infect them with Hepatitis C. However, to date, at the time that Dr. Uren left the laboratory, we still had a lot of problems with consistency in the system and to get a consistent level of hepatocyte repopulation.

A lot of the problems came with our ability to maintain hepatocytes frozen that we really -- it appears that we really need to use very fresh hepatocytes.

In a second project that was done by Deb Taylor before she left the lab, it involved the investigation of the effect of ADAR on HCV replication. Deb had previously shown that the Hepatitis C genome seems to undergo deanimation by

ADAR-1, which is adenosine deanimase associated with RNA, and what you see in Panel A on the next slide.

What she found was that ADAR is induced by interferon treatment, and using the HCV replicon system, she was able to show that by using ADAR inhibitors one was able to rescue the replicon from interferon treatment. So typically as you see in the lighter colored boxes, with interferon the replication goes down very rapidly, but with an ADAR inhibitor, one's able to sort of rescue that.

And to make a long story short, she showed that that was related to ADAR directly by using both siRNA and VA RNA, which are ADAR inhibitors that you see in the next page.

And finally, what I don't show there is that we have been able to use ADAR inhibitors to actually enhance the replication of Hepatitis C virus in cell culture.

And I'll end there.

CHAIRPERSON KARRON: Thank you very much, Dr. Feinstone.

Are there questions for Dr. Feinstone?

DR. LaRUSSA: Yes, one question. This is Phil LaRussa.

Dr. Feinstone, you've had two people

leave, first Dr. Taylor and now Dr. Uren. Who's going to take over or take responsibility for the work on the mouse model?

DR. FEINSTONE: So the mouse model work is somewhat on hold right now. We do have some funding that will support a post doctoral fellow whom we hope will be involved in the mouse project. We've also initiated collaborations, one with Snori Thorgesson and NCI, and he's very actively involved with these mice and doing some very interesting work with them in his systems using mouse embryonic stem cells, as well as with a group at Children's Hospital in Philadelphia, Allen Flake.

And that group is able to do *in utero* inoculations, and what we wanted to do with Dr. Flake's system was to actually develop a non-immunodeficient mouse model so that we could use mice that express these transgenes, but actually inoculate them with the human or chimpanzee liver *in utero* and to which they, of course, would become immune tolerant at that point, but the rest of their system would be intact.

And then once those animals are born, we thought we could further stimulate the replication of the transplant of liver by either treating with the

pro drug CB 1954 or in the MUFF system that in two to three weeks of life they would have this sort of a stimulus for further repopulation of the liver.

And so Dr. Flake has our mice now and is breeding them and is going to try to work on that project, but we hope this year we will hire a post doctoral fellow who will continue the work in our lab.

DR. LaRUSSA: Okay. Thank you.

DR. MODLIN: Dr. Feinstone, it's John Modlin.

I'd be curious as to your own personal and professional view about Hepatitis C viral persistence in humans. I was reading with interest a paper that was in, I believe, the Critical Infectious Diseases within the past week or so from Spain suggesting that in humans it's clear all critical markers of replication, they're still able to find replication in circulating peripheral blood leukocytes and in the liver as well.

DR. FEINSTONE: Well, I haven't seen that paper. there has been a fair amount of speculation that no one actually really clears the virus, that there always is some left somewhere.

However, in the clinical situation, and I'm sure you're aware in Hepatitis B, patients who are

chronic carriers or who have cleared Hepatitis B infection and then perhaps later in life undergo immunosuppression sometimes have a recurrence of Hepatitis B infections.

DR. MODLIN: Right.

DR. FEINSTONE: To my knowledge, and I've talked to several hepatologists about this, that hasn't been observed in Hepatitis C. So at this point we don't really know if the ability to find genomes or fragments of genomes is really clinically significant.

DR. MODLIN: I don't mean to prolong this. In the paper they're actually able to find negative stand RNA.

DR. FEINSTONE: Yeah.

DR. MODLIN: Suggesting that there was at least some very low level of ongoing virus.

DR. FEINSTONE: Right, right. You know, I think it's possible. You know, the assays for negative strand RNA are technically difficult. One has to be really careful when you do them, but I wouldn't dispute it. In fact, we have found some similar things, but you know, it's all very low level, all PCR results which, you k now, it's very hard to be absolutely certain of them.

DR. MODLIN: Thank you.

CHAIRPERSON KARRON: Thank you, Dr. Feinstone.

The next speaker with be Dr. Jerry Weir with the Laboratory of Respiratory Viral Diseases.

Dr. Weir.

DR. WEIR: Thank you.

I'm going to give an overview of the laboratory of pediatric and respiratory viral diseases. You can go ahead and turn to Slide 2 that shows where this laboratory fits into the Division of Viral Products.

As you see, there's seven laboratories. You've already heard from the lab chiefs of two of these. Today I'm going to talk about the laboratory of pediatric and respiratory viral diseases, and there have been quite a few changes in this lab over the last few years. In fact, you've noticed that it's titled in this slide "The Laboratory of Respiratory Viral Diseases." That's because the focus of this lab is now exclusively in respiratory viruses, and we just haven't gotten around to officially changing the name yet.

I'll describe the current organization of this lab in a minute. The lab chief position is vacant at the time and has been for almost a year now

sine Roland Levandowski left the division last November, and I'll also point out now, even though I'll describe the organization in just a minute, that only one team out of this group was actually in the site visit that was conducted in June of '06, but I'll try to sort of put in the perspective for everybody of where the rest of them fit in the scheme of things.

If you go ahead and turn to the next few slides, Slide 3, I'm just briefly going to go through some of the background of the division and this laboratory.

The mission of the Division of Viral Products is to regulate viral vaccines and related biological products to insure their safety and efficacy for human use.

We also facilitate the development, evaluation, and licensure of new viral vaccines that positively impact the public health. You can see the responsibilities on Slide 4, and these apply to all of the laboratories in the division.

We all review an investigational new drug and biologics license applications as well as other pre-marketing activities, such as pre-INDs. We're all involved in BLA supplement review, lot release review and testing. Some of us are involved in lot release

review and testing, as well as other post marketing activities, such as biological product deviations.

We participate in manufacture inspections, both pre and post licensure, and we have a very large role in consultation with other public health agencies, such as WHO, CDC, NIBSC and others.

And finally, last but not least, we conduct research related to the development, manufacture, evaluation and testing of viral vaccines.

Go to Slide 5.

The role of research for us. Our research and laboratory activities are designed to complement the regulatory mission. In general they address issues related to regulated viral vaccines, as well as to anticipate and address issues related to the development and evaluation of new viral vaccine products. This includes general issues applicable to many products or product classes, as well as specific product issues, for example, correlates of protection, the animal models necessary for the implementation of animal rule.

Finally, if you'll look at Slide 6, you'll see the current layout of the laboratory, respiratory viral diseases. There are now four teams in this laboratory. The first one that I've listed is the

influence virus molecular biology team with Dr. Zhiping Ye as the team leader.

The respiratory virus immunology team, Maryna Eichelberger as the team leader.

The influenza vaccine technologies is a vacant team leader. This is was the group that was leaded by Roland Levandowski before he left, and then the subject of today's site visit is the agnogenic structure and function team with Judy Beeler as team leader.

I'm going to go through one slide each for the groups that were not reviewed, again, just to give you a little perspective of what they do in this laboratory.

The influenza virus molecular biology team is headed by Zhiping Ye. The major regulatory responsibilities of this group focus on influenza virus vaccines. The areas of research in this group can generally be described as the molecular mechanisms of influenza virus attenuation and virulence, molecular and genetic approaches to improving influenza vaccine candidates, and the evaluation of new influenza vaccine technologies.

This group was reviewed on a site visit in November 2004.

The second group, Slide 8, respiratory virus immunology. This is one that's headed by Maryna Eichelberger. This group also has major regulatory responsibilities in the area of influenza virus vaccines. Their areas of research focus on the role of neuraminidase and virus delivery, the adjuvant effect of neuraminidase and neuraminidase's role in homo as well as heterotypic immunity.

This is a new research team. All of its members, in fact, came on board just this past September. So they have not been the subject of a site visit at this point. What we plan is, of course, that all of these groups will be site visited the next time as an entire laboratory.

If you go to Slide 9, this is the influence of vaccine technologies group that was originally headed by Roland Levandowski. This group is still here. They function and have major regulatory responsibilities also in virus vaccines, but they have traditionally been heavily involved in influenza vaccine to release as well as the preparation of reagents.

The areas of the research of these folks focuses on improved growth characteristics of influenza viruses, as well as improved assays for

influenza potency and clinical evaluation. This group was also part of a site visit that was conducted in November of 2004.

And now the subject of today's site visit review that's being presented to the VRBPAC is the antigenic structure and function team. Judy Beeler is the team leader of this group, and the rest of the group includes Lynne Crim and Susette Audet.

This group has major regulatory responsibilities basically for the other respiratory viruses, RSV, PIV3, other respiratory virus vaccines, as well as you will see in just a minute, they have responsibilities in the areas of measles virus vaccines and immunoglobulins.

The areas of research, which I'm going to go over just briefly in a few minutes include RSV whole cell interactions and measles immunity, and this was the one that was reviewed in the site visit of June 2006.

Okay. So I'm going to spend the new few minutes basically summarizing some of the results that Dr. Beeler's team presented at the site visit. It can be divided up into two sections, the focus on RSV studies and a focus on measle studies.

Dr. Beeler is here today. So if anyone

has technical questions or questions about her research, I'm sure she'll be happy to answer them. But I'm giving a general summary of some of the results that she presented in your site visit book.

The RSV studies that this group has focused on address the following questions listed in Slide 11, which RSV envelope proteins bind heparin, and the studies over the last few years have focused almost exclusively on RSV-F glycoprotein, which domain is in the RSF-F mediate heparin binding.

And are these interactions specific for cellular glycosaminoglycans or GAGs? And are these virus-GAG interactions important for virus binding and infectivity?

A summary of some of these results are shown on Slide 12. Basically this group identified eight linear heparin binding domains in the RSV-F glycoprotein, RSV F-heparin binding peptides were shown to bind specifically to cellular GAGs. Two of these, FSV-F heparin binding domain peptides had unique reactivity patterns that suggest they may also bind to non-GAG cell surface molecules.

Three of the heparin binding domain peptides blocked virus attachment. Three of the peptides inhibited virus infectivity. The overall

conclusion of some of these studies were that the interactions between RSV-F heparin binding domains and cellular gags facilitate virus attachment and infectivity.

Dr. Beeler outlined, Slide 13, outlined in her site visit book the future directions of this group and these can be summarized in this slide. Basically this group is going to pursue the identity of non-heparin binding RSV-F peptides that bind cells, block virus attachment, and inhibit virus infectivity.

They're also interested in using the RSV-F peptides that bind cells and block infectivity to probe for potential RSV receptors, and finally, to evaluate the peptide specific neutralizing antibody responses.

A second part of this group or the second part of the study described by this group focused on measles. These are outlined in Slide 14 and have been divided up into studies on passive immunity and active immunity. The first one I'll talk about in the next couple of slides, focuses on the passive immunity studies, and this is collaboration between these researchers in OBRR and the Office of Blood at CBER.

The general question being addressed in these studies is will an apparent increase in the

relative number of donors with vaccine derived immunity have an impact on measles antibody potency of immunoglobulin products, and I'll show you a couple of her data slides in a second.

The second part of the measles studies is titled "Active Immunization," and here the group uses a validated measles neutralization assay to essentially provide support for several ongoing studies for researchers in the field who focus on questions about measles immunization. Some of these questions are listed at the bottom of the slide.

What are the optimal ages for measles immunization? How durable is post vaccination measles immunity? And is measles vaccine administered by the aerosol route, immunogenic.

The last one that I mentioned is a WHO priority, particularly in Africa and other areas of the world where needle free technology is important.

Okay. If you turn to Slide 15, the measles study passive immunity, the goals of this study were to estimate the proportion of donors born after 1956, in other words, donors whose most likely exposure to measles was through the measles vaccine, and to measure measle neutralizing antibody in 166 lots of IVIG from seven manufacturers between 1998 and

2003.

Slide 16 shows a little bit of the data from this study. The top bullet points out that there are two types of plasma, one, recovered plasma from volunteer donors, the second source plasma from paid donors. Approximately 60 percent of the donors in the first group were born after 1956, in other words, most likely were vaccinated, 85 percent of the second group.

And if you'll look at the graph that Dr. Beeler provided, in the bottom part of that graph you see various manufacturers and different lots of IVIG that each were tested for measles neutralization. And you see the general trend in each manufacturer is downward as each bar left to right represents a different year, and you see an apparent decreased trend in the measles antibody potency during the years.

If you turn to Slide 17, you see some more results from the passive immunity studies. In this case these were IVIG lots made from source plasma. I mean, these were IVIG lots from both source plasma and from recovered plasma. These were assayed for measles neutralizing titer, and these have shown the different years of the plasma.

And here you see that the source plasma, which are the open bars in the graph, generally have lower titers than the recovered plasma, and in fact, some of these are significant, as shown by the arrow bars, the second and the sixth one in the graph, and basically what this is showing is that the source plasma which has a higher percent of donors born after 1956 generally have lower titers, suggesting that a higher percentage of vaccine immunity correlates with a lower measles antibody titer.

Slide 18 shows some other conclusions from their works. Listed here are measles neutralizing antibody, which was predominantly IGG-1 and IGG-3. IGIV lots manufactured using a unique protease adjustment step at the lowest levels of IGG-1 and IGG-3, and the lowest measles antibody potency compared to other IGIV products.

The conclusion from some of these studies were that changes in donor demographics and unique manufacturing steps may contribute to diminished potency of measles antibody and IGIV products.

The future directions for these studies and some more conclusions include that the quantity of measles neutralizing antibody needed for passive protection is not known. Therefore, it is currently

not possible to reset the lower limit for measles antibody potency for immunoglobulin products.

And in the coming year, 2007, the Office of Blood, Research and Review will have a workshop that this group will participate in to discuss the relationship between antibody potency of immunoglobulin products and successful immunoprophylaxis.

And the last two slides summarize a little bit of their work in measles and the active immunity studies that I mentioned a minute ago. As already pointed out, this group uses their validated neutralization assay to support numerous studies.

Slide 20 shows a group of studies that have already been completed over the last few years and the general outline or the general point of the studies, such as investigation of waning immunity, infant immunization, and so forth.

Future studies for the active immunity focus of the group are shown in Slide 21. Here Dr. Beeler's group would like to develop a measles neutralization assay using a reporter readout, and they also have a set of ongoing collaborations which are listed in the table shown in this slide.

And finally, I'll stop here. As I said,

this was the group that was reviewed on July 29th, 2006 and evaluated for overall progress and future directions.

And, again, Dr. Beeler is here. If anyone has technical questions, I'll be happy to answer anything about the lab and the division if you'd like.

CHAIRPERSON KARRON: Thank you very much, Dr. Weir.

Are there questions for Dr. Weir or for Dr. Beeler? Okay. Thank you. I think at this point we'll move forward. Christine, I believe you have an announcement for us.

MS. WALSH: Thank you, Dr. Karron.

As part of the FDA Advisory Committee meeting procedures, we are required to hold an open public hearing for those members of the public who are not on the agenda and would like to make a statement concerning matters pending before the committee.

I have received one written comment from B. Saku. A copy of this statement has been given to the committee members, has been placed in their viewing notebook at the registration desk, and will be made part of the official meeting record.

Is there anyone in the room who would like to address the committee at this time?

I see no response. Dr. Karron, I turn the meeting back over to you.

CHAIRPERSON KARRON: Thank you, Christine.

I think at this point we will take a five-minute break. This will allow us to clear the teleconference room for our closed session. So we will reconvene in about five minutes.

Thank you, everyone.

(Whereupon, at 2:39 p.m., the open session was concluded.)

CERTIFICATE

This is to certify that the foregoing transcript

in the matter of: Vaccines and Related Biological Products Advisory Committee

Before: Food and Drug Administration

Date: November 16, 2006

Place: Bethesda, Maryland

represents the full and complete proceedings of the aforementioned matter, as reported and reduced to typewriting.

Charles Morrison