who are providing testing for other vaccines have now been able to implement here. They came to the FDA. They presented their validated assay.

Based on this work, we have now changed our policy, and a letter was sent out to all manufacturers of HIV vaccines that they have to replace the old less sensitive RT assays with these highly sensitive assays. So that all new vaccines have to be subjected to this testing before moving forward.

DR. FAGGETT: I hope you send a copy to HCFA so they will pay for it.

ACTING CHAIRMAN DAUM: Other questions for Dr. Golding? That means it was exceptionally clear. Thank you very kindly for taking time.

DR. GOLDING: Thank you.

ACTING CHAIRMAN DAUM: Dr. Huang, can you hear us?

DR. HUANG: Yes. You are much clearer.

ACTING CHAIRMAN DAUM: A tribute to our audio-visual helpers.

We will now move on to the next item, which will be to hear from Dr. Ira Berkower regarding activities in the Laboratory of Immunoregulation.

DR. BERKOWER: Thank you. Could I have

the first slide, please.

ACTING CHAIRMAN DAUM: Do you want to adjust the microphone so you are clear. We need to hear him.

DR. BERKOWER: Let me begin by showing you the personnel of the Laboratory of Immunoregulation. The Laboratory is divided into two PIs -- works under two PIs. One is Dr. Carol Weiss. The other is myself. We are each supported by a Master level microbiologist, and there are two post-docs in the lab, and we are actively recruiting a third.

I'd like to start by showing our regulatory responsibilities that we perform for CBER. We work on the evaluation of a number of products that are under initial -- their initial clinical development under IND. These include, as shown, vaccines and immunotherapies for HIV, including structured drug interruptions, but we are also involved with vaccines for other viral diseases such as Hepatitis A and B, dengue virus which is not shown on there, and even in the past, an allergy immunotherapy.

When products have moved along to the licensing stage, we have been actively involved in serving on licensing committees, particularly for

Hepatitis A and B and for novel forms of Hepatitis B vaccine.

We also serve on CBER policy committees such as the committee writing a guidance document on peptide vaccines, of which I am the co-chair, and on cell substrate issues, which we serve in collaboration with Hana's group and others; and we have taken on such issues as the potential risk that BSE could ever get into any of the vaccines in our vaccine supply.

Our work has been recognized by various awards such as the FDA-wide Regulatory Scientist of the Year Award and the second highest award in the Public Health Service.

We have also competed successfully for grants and awards at the NIH, such as an innovative AIDS vaccine grant award to Dr. Weiss and awards from the Intramural AIDS Targeted Program to Dr. Weiss and myself, and these are part of that external money that was discussed before which has helped support our work.

Now our research work is designed to support CBER's mission in the areas of vaccine safety, potency, and efficacy, the traditional vaccine concerns. Dr. Weiss' work on HIV entry mechanism, which I will be highlighting in a few minutes, her

expertise in molecular virology and also her direct clinical experience, ongoing activity in the care of AIDS patients has contributed to her review of HIV vaccines and therapeutics, and my own work on HIV neutralizing antibodies as well as on the immunogenicity of vaccine antigens -- I teach a course called "Vaccines 2000," for example -- have contributed as well to evaluating novel vaccines for HIV and hepatitis.

In the next -- The whole rest of my talk will be devoted to just giving you two examples of our research projects. One will be from my lab; one will be from Dr. Weiss' lab.

So let me start with a project that we have, our main interest in the lab, on particulate gp120 vaccines. This project is motivated by an observation from other vaccines, such as hepatitis B surface antigen, the vaccine for hepatitis B virus, in which the particulate form of this antigen is 1,000-fold more potent as a vaccine than the same weight of the protein as monomers.

So what we have done to try and apply this to HIV is shown in the bottom of the slide. We have made a hybrid linking gp120, shown in red, with a carrier protein, shown in green, and the carrier

protein was shown because it is capable of spontaneous self-assembly on its own.

What we hoped was that, as the carrier protein assembled, it would incorporate gp120 into a multimeric particle so that we could take advantage of what I call the multimer effect that was previously observed for other vaccine antigens.

If you think about all the viral vaccines, many of them are particulate, multimeric, and a few of them are not.

What this slide shows is two carriers that we have explored rather extensively. The first one, shown in green, was the core antigen of hepatitis B virus linked to gp120, and the second one was the surface antigen in the same virus linked to gp120. Both of these carriers have the ability to self-assemble, but there are very different particles that they form.

The core antigen forms a rigid icosahedron, has a very tight structure, and as opposed to the surface antigen which forms a loose lipoprotein micelle which is pleomorphic -- anyway, we tried both -- and the next two slides will show first our results with core antigen hybrids and the second with surface antigen hybrids.

This slide -- What the previous slide showed were DNA constructs. Those were inserted in the vaccinia and expressed as recombinant proteins, and then assayed for whether they assembled or not, whether they became large or remained small monomers.

The size was determined on a sucrose gradient. The bottom shows just core antigen by itself and, as I said before, core antigen by itself spontaneously assembles into particles, as shown by the fact that they move about 60 percent of the way down a sucrose gradient from the -- this is the top; this is where core antigen particles go.

Our core antigen envelope hybrids just stayed at the top, indicating that they failed to assemble, first of all. And secondly, what this shows is it's easy to tell whether something assembles or not, based on whether it goes to this size or this point in a sucrose gradient.

Under the same conditions of sedimentation, we analyzed our surface antigen gp120 hybrids, as shown in this slide. Now you can see that the surface antigen hybrids move very, very well. They are assembled particles, and it doesn't matter, since it's a hybrid, whether we assay them by their gp120 content or their surface antigen content. They

are dual antigens, and they assemble and move as one particle.

The other thing this kind of result shows is that the amount of material that remains behind at the monomer stage is almost nil, indicating efficient assembly.

This material was purified by cesium chloride gradients subject to electromicroscopy. On the right side of this slide we see authentic surface antigen, just native surface antigen particles, and on the left side we see for the first time surface antigen of gp120 hybrids. This is the first time, I believe, that there's been a regular array of multimers of gp120 on the surface of a particle except in the virus itself.

These materials -- I'm sorry. This is a schematic showing what you just saw in an artist's eye view. If surface antigen is a lipoprotein that is a lipid with surface antigen floating in it, then this has surface antigen floating in it with perhaps, in red, gp120 hanging off.

Our major findings have been that surface antigen gp120 hybrids do assemble. They do it efficiently, and they preserve the native confirmation of gp120 in the hybrid.

The physical properties are consistent with about 200 -- with this being about 200 proteins and about 15 percent lipid, all incorporated into one particle. in contrast, the core antigen hybrid did not assemble, perhaps because it is too rigid to accept the gp120 tail.

Our plan for this material is to express more and immunize mice and monkeys and determine whether the proposed multimer effect really occurs. One of the ways we are going to increase expression is by using optimized codons, and we are working now to substitute for every triplet codon not the one the virus chose but rather the one that is used by the most highly expressed proteins in the cell. You would be surprised how many substitutions that requires. The virus had its own reasons for not being over-expressed.

Now I would like to turn to the work and accomplishments of the Weiss lab and illustrate one of their projects. Dr. Weiss does work on HIV envelope structure and function, and particularly the transmembrane protein of the HIV virus, which has become a very hot area of research both in vaccines and in therapy, as I will show in a moment.

Also, her regulatory duties were listed

earlier, and she provides direct clinical patient care, which I believe helps us to understand what we are seeing when we review proposals for new therapies.

This slide illustrates the prevailing model of HIV induced membrane fusion. This is a process that takes at least three steps, and each one of those steps corresponds to a discrete conformational state of the envelope protein, and I'm just going to walk you through this very quickly.

The first stage is the native gp120 and gp41, this transmembrane part, on virus, on cell-free virus. Once the virus encounters its receptor, its CD4 receptor and/or its second receptor, as Dr. Golding has described before -- Once it encounters its receptor, it undergoes a major conformational change in the surface, in the envelope protein which takes off these head groups, exposes a fusion peptide and, in particular, exposes tow helical regions.

One is shown in green and, because it is the immuno n we call it the N-peptide. The other is shown in this spring structure, which we call the C-peptide region. These are both external to the virus. They are exposed on the surface, and they need to go through one more change to here in which the triple helix becomes a six-helix -- a compact six-helix

structure, which is believed to be the last or the next to the last step before membrane fusion can occur.

Now what Dr. Weiss has come up with is an assay that detects this fusion intermediate and can distinguish the fusion intermediate from the native structure, and serves as a measure of how much activation has occurred, how much receptor mediated activation has occurred.

The assay depends on the observation that this C-peptide, if synthesized just as a synthetic peptide, shown in red, can combine with this structure, as shown here, and can be used in immuno precipitation to detect how many of these were formed. I'm going to show that in just a moment.

The other feature of the C-peptide is that, when C-peptide goes on, this next structure cannot be formed, and viral infection is blocked.

Okay. This is actually a data slide, and I'll direct your attention to the right. The C-peptide, which I just showed you before, is being shown as to whether it can bind gp41 on virus that's in its native state or virus that has encountered its CD4 receptor and is now triggered to the fusion active state.

So whether it can distinguish these two states is shown by these two bands, because when they encounter -- When C-peptide binds gp41, it can be in this assay brought down in immuno precipitation and results in a band in this location. So you can easily distinguish between the triggered form and the resting form.

Now this slides shows an experiment utilizing this assay, and in this experiment what is done is that the wild type which has isoleucine at position 573 in the n-helix has been substituted with single amino acid substitutions, either highly conservative leucine for isoleucine, a little away, alanine for isoleucine, and so on.

As you can see, just a conservative -- the most conservative substitution you can think of drops this down about half. A not very farther off substitution drives it further down.

What this indicates is that the effect of C-peptide is probably due to direct binding to the N-peptide, because mutations in the N-peptide region, even very conservative ones, reduce or diminish almost entirely the C-peptide binding.

The second thing that was measured with these same variants is their infectivity into cells,

with the wild type being considered 100 percent, the very conservative substitution of leucine, one-third, alanine less than five percent.

What's interesting about this then is that the same mutations that disrupt C-peptide binding to N also disrupt N function in going on to the fusion mechanism. We tentatively conclude from this that this result results from the fact that N+C has to form that last compact six-helix bundle as part of the direct process leading to membrane fusion. So the N/C interaction is a direct part of the membrane fusion and is an essential step for HIV infection.

Why is this important for therapeutics? Well, I believe that this very mechanism has potential for therapy and should be potentially much more efficient than something that is devoted to later stages of viral infection such as maturation, the protease inhibitors, relatively early stages such as RT inhibitors.

This occurs even before that. It occurs before any replication has occurred and could potentially lock the door, preventing virus even getting in the cells in the first place.

The major findings of this work is summarized on this slide in which it says that we have

identified -- I should say she has identified envelope glycoprotein confirmations that are direct intermediaries leading toward membrane fusion. And although these are transient, they are, nevertheless, good enough, last long enough that a peptide can block them.

So that potentially they may also be a good target for neutralizing antibodies, an obligatory step in the virus, a conserved sequence -- that N-helix is a conserved sequence, and although it is transient, it may be untransient enough, persistent enough, that it could actually be a good target of neutralization.

In summary, we hope that these projects will advance knowledge in key areas of AIDS research and at the same time provide the scientific base for us to make important decisions concerning new vaccines and biologic agents. Thank you.

ACTING CHAIRMAN DAUM: Thank you. That's a pretty exciting part of what goes on in FDA that we don't get to hear much about.

We have a moment for Committee input, questions, clarifying. Dr. Katz, Dr. Faggett, Dr. Goldberg.

DR. KATZ: When you described Dr. Weiss'

responsibilities, I was surprised that one of the two major areas you listed was review of highly active retroviral therapy protocols. Why does that get thrust on an immunologist?

DR. BERKOWER: Okay, HAART therapy, per se, is dealt with in Drugs, Center for Drugs. We have begun things that are considered therapeutic vaccines. if you think about it, it's not trivial to evaluate a therapeutic vaccine.

For example, everyone who has the infection already has antibodies and probably has a lot of other -- as one measure of being immunized and probably has a lot of other immunologic phenomena going around surrounding the infection.

So what the vaccine is supposed to do above and beyond that is hard to say. Instead of tackling that head-on, there are a variety of strategies for showing that a therapeutic vaccine has actually done anything.

One of these strategies would be that, if somebody is on HAART long term, that by being vaccinated when they are -- when the virus is suppressed, that they might have a better long term outcome.

Another one, which is very controversial,

is to interrupt HAART and measure the return of virus. As you know, there have been some claims that when it's done with the right kinetics that that can be actually a kind of a therapy.

One way to evaluate a vaccine would be to vaccinate someone while they are on HAART, stop the HAART, and measure the time for the virus to come back. So there are all kinds of interactions between the vaccine and HAART, some of which are very, very complex, that could be used to try and demonstrate some form of efficacy of a therapeutic vaccine, and we are involved with those.

ACTING CHAIRMAN DAUM: Thank you. Before we call on Dr. Faggett, let me just inquire. Dr. Huang, are you there?

DR. HUANG: Yes, I am.

ACTING CHAIRMAN DAUM: Can you hear?

DR. HUANG: I hear quite well, thank you.

ACTING CHAIRMAN DAUM: Do you have any questions?

DR. HUANG: No, not at this time.

ACTING CHAIRMAN DAUM: Good. Dr. Greenberg, are you there? I am going to infer that you are not. Okay, Dr. Faggett, you're on.

DR. FAGGETT: My question is the following

to Dr. Katz, really. How many FTEs do you have in your organization?

DR. BERKOWER: Four.

DR. FAGGETT: That's all the positions you do have?

DR. BERKOWER: Yes, and we have obtained outside funding for three more.

DR. FAGGETT: Okay. How many do you need?

DR. BERKOWER: Actually, let me modify that. We have four, plus we have a Fogarty Fellow supplied by the Division, and we have funding on our own for two.

How many do we need? We need about two more.

ACTING CHAIRMAN DAUM: Thank you. Dr. Goldberg.

DR. GOLDBERG: My question has been asked -- My question about this has been asked by Dr. Katz. Can I ask a question of Dr. Golding?

ACTING CHAIRMAN DAUM: Sure.

DR. GOLDBERG: You have this great assay. Do you get royalties for it? I mean, is it patented and sold or, you know, can this bring funding into your group or did I miss something in what I looked at, that it is?

DR. GOLDING: Okay. So when it comes to patent and royalties and so forth, it's rather complicated. We have actually at least one member of our group, Dr. Klinman, who was successful in applying for patent regarding the CpG oligos, and that recently has materialized into some royalties.

Unfortunately, along with the patent and the royalties comes some potential conflict of interest. That's something that we as an agency have to deal with, because if somebody gets a patent and is able to maybe harvest, I would say, what is sold, it also means that he may be conflicted in what he can review or not.

So while we want our scientists to be successful, to develop new assays and to get them patented, and we're really looking forward to as much benefit as possible, we also be extremely cautious of how that may impact the day to day work.

DR. GOLDBERG: No, I was wondering whether the agency had any kind of policy for handling this so that there could be some funding back to the agency for such things. It was developed on Federal money, basically.

ACTING CHAIRMAN DAUM: I would remind everybody of --

DR. GOLDMAN: Yes. I'm Neil Goldman. I'm the Associate Director for Research at CBER.

The way the government now works, when we patent a finding, 50 percent of it goes to the government, and 50 percent can come back to the individuals. So, in fact, that money can come back, and many times it has and has come back to the lab and is used by the investigators to help fund their research or continued research.

As Dr. Golding has mentioned, there are issues of conflict of interest, and those do have to be dealt with, and they will never go away.

ACTING CHAIRMAN DAUM: Thank you very much for that clarification. Thank you very much for a very interesting presentation as well.

We have now reached the end of the open session,a nd we are going to prepare ourselves to go into closed session. Thus, at this point I would like to dismiss all individuals in the room except the voting members of VRBPAC, and we will take a five-minute break for various functions, including, hopefully, getting Dr. Greenberg on line here, and reassemble in closed session.

DR. KOHL: Bob, I misunderstood something.

ACTING CHAIRMAN DAUM: Dr. Kohl wishes to

speak.

DR. KOHL: So does that mean we can -- In the closed session, we cannot address questions to the presenters? Will they not be here for the closed session?

MS. CHERRY: They will not be here.

ACTING CHAIRMAN DAUM: They will not be here.

DR. KOHL: Then I would like to address a question to Dr. Berkower. Is that permissible?

ACTING CHAIRMAN DAUM: We will reopen the session for a moment and ask one last question from Dr. Kohl.

DR. KOHL: I'm sorry.

ACTING CHAIRMAN DAUM: I did look around, Steve, and I --

DR. KOHL: No, no. I didn't understand that the presenters would not be there.

ACTING CHAIRMAN DAUM: Please.

DR. KOHL: Dr. Berkower, the productivity of your laboratory, at least on paper in terms of publications, is a little thin. Can you help us understand how we can help your Division with that?

DR. BERKOWER: Sure. I think there are two aspects to this. First of all, I have not had a

post-doctoral fellow provided by the Division in several years, and I think that's really basically what you're looking at.

The second thing is one of the strengths of the FDA, one of the advantages of working at the FDA in the days when there was intramural support was that it allowed us to take on projects that were risky and that perhaps weren't even the kind of thing that a study section would immediately recognize as something they think is a good idea.

So just to develop that a little bit, back about ten years ago when V3 loop was the hottest item, we were doing neutralizing antibodies and finding another site, and that site was, of course, the CD4 binding site, dependent on the native confirmation, and was not getting adequate response from the vaccines that were then being proposed.

Since that time, that's completely turned around. The V3 loop is a laboratory artifact, I believe, nowadays, and the site that we discovered is the one that everyone is trying to get antibodies to, of those of us who are trying to do antibodies. That's one.

The second thing is particulate vaccines. A number of groups have proposed making particulate

vaccines, but a very few have succeeded until this past year, until a year ago. We hadn't succeeded.

I've made many constructs with the core antigen and finally got to the surface antigen. So I think being at CBER has allowed me to persist and keep doing this until I got a particulate vaccine antigen. That's been a real plus. In the old days, that wouldn't have been -- the cost of that wouldn't have been to lose my staff fellow, but in these days with very tight budgets that was the cost.

So it was kind of a choice between persisting and doing something risky or not. Frankly, one of the advantages of having us do research on the very same thing we regulate is it gives us a lot of respect for those people who succeed in making vaccine constructs that are different from just the thing that everyone else has.

I have improved respect for applications where something truly novel has been accomplished. IN fact, when I get a new application, that's the first thing I look for: What's novel about it? So I think that that's my answer.

ACTING CHAIRMAN DAUM: Thank you very much. At this point, we will take a five minute break.

Dr. Greenberg, are you there? I don't think so.

We will reassemble for closed session, VRBPAC members, voting members, in five minutes.

(Whereupon, the foregoing matter went off the record at 11:25 a.m.)

CERTIFICATE

This is to certify that the foregoing transcript in the matter of: Vaccines and Related Biological Products Advisory Committee

Before: DHHS/FDA/PHS/CBER

Date: March 9, 2001

Place: Bethesda, MD

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