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IMMUNE CORRELATES OF PROTECTION AGAINST INFLUENZA A VIRUSES IN SUPPORT OF PANDEMIC VACCINE DEVELOPMENT

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P R O C E E D I N G S 8:30 AM

Agenda item: Introduction

DR. WEIR: Good morning. I am Jerry Weir of the Division of Viral Products at the Center for Biologics, and I am going to quickly introduce our morning session, our speakers and make a couple of announcements.

First of all, thank you all for coming and participating in this, and what I am going to do is mention the Organizing Committee so you will know who these people are, and if you need anything you can seek them out, and we will try to help you with anything.

As I said, I have a couple of announcements to make and then we are going to have introductory remarks this morning from all three of the participating agencies. Dr. Jesse Goodman is the Director of the Center for Biologics Evaluation and Research at the FDA. He will come up after me and then make a few remarks, and then following that Anthony Fauci, the Director of the National Institute of Allergy and Infectious Diseases will speak for a few minutes and then finally David Wood, the Coordinator of Quality, Safety and Standards for WHO will end the introductory session.

The Organizing Committee, as I said, this was a joint enterprise of three agencies, CBER, NIAID and WHO, and our Organizing Committee consists of Maryna

Eichelberger over here, Hana Golding, over here, Maureen Hess who may be outside. Oh, no, she is at the back there, myself, Catherine Luke from NIH over here, Kanta Subbarao in the front down here and for WHO Martin Freed in the center and David Wood up front here.

All of these folks will be happy to help you with anything you need. So, just let us know and we will try to make the meeting as enjoyable as possible for you.

Okay, I only have three brief announcements this morning. First of all, as I think most of you know are aware we had such an overwhelming interest for this meeting that we ended up webcasting it. So, it is extremely important that everyone use the microphones at all times. That is the only way any of that will be able to be transmitted over the web.

So, they have a couple of microphones in the center. Take a couple of extra minutes to go to the microphone and use it for questions or comments. It would help if you would identify yourself as well. I don't think we have the cameras set up so they will zero in on you but I think it is more important that they hear your questions.

An announcement for the speakers, we have succeeded in loading most of the slides for the morning session but please come see one of the organizers or the audiovisual fellow well before your talk so that we can get

them on the computer and get them loaded before the session, and finally I am going to mention this in a second, we want all of you to really interact and make this a workshop and so what we are looking for is all of you, any of you to submit ideas for the panel discussion that is at the end of day two.

So, if you have ideas, you have comments seek out one of the organizers and let them know what you would like to hear in this panel discussion.

I will remind you that the goals of the workshop are pretty simple. The idea is to identify gaps in our knowledge and abilities to address the challenges for development and evaluation of pandemic influenza vaccines and to facilitate implementation of a global research agenda to improve the efficacy assessment and basically to address these gaps.

We have four sessions, as you know or you can see on your agenda. We are hoping to review and discuss the current knowledge regarding correlates of protection against seasonal influenza, talk about the immune responses to avian influenza infections and vaccines for novel influenza viruses in humans, discuss assays to evaluate vaccine immunogenicity and evaluation of avian influenza vaccine efficacy, and again, I emphasize there will be this panel discussion at the end of day 2.

Please think about this, submit your ideas. We will accumulate them. We will actually write them down and make slides of them and we would like this to be a real workshop.

With that, welcome one more time, and here is Dr. Jesse Goodman.

Agenda Item: Welcome and Opening Address

DR. GOODMAN: Good morning. I really think this is a tremendous workshop, and I just want to welcome everybody and thank you all for participating.

I am just going to make a few comments about why I think this is so important and what we see from our perspective looking at a number of vaccines, both existing ones and vaccines in development, what are some of the important questions and observations and I will quickly go through that.

It has sort of been this area. I have been fascinated in the last few years. It is not at all sexy. You know what are the assays? What are the correlates, but every single thing we try to do in the real world about influenza vaccines we come up against the limitations of existing assays and their performance or the lack of understanding of correlates of protection.

So, what are some of the things we need? We truly need better assays, and we also need correlates of

protection meaning assays that can be easily performed in a variety of settings and some of the people in this room have done the studies that both point the way toward that but also show some of the difficulties and with results that are reproducible and comparable across studies and sites and then very importantly the results of which are predictive and can ideally serve as surrogates for clinical outcomes and one thing I have been very struck by in thinking about influenza is that while for regulatory purposes and it is an easier thing in clinical trial design we focus on protection against infection and infection is well defined, in reality particularly for pandemic it is not just infection but protection against illness, hospitalization and death that are important and perhaps more achievable.

So, I think we have to look at ways of understanding data both for HA type correlates and for others that focus not just on infection but on these clinically significant endpoints.

In terms of performance obviously some of the issues are reproducibility, the speed of assays and cost. This is an area where many people have kind of labored by hand in the vineyard over the years but it really hasn't been a focus of modern biomedical science to improve these assays and people have sort of done this on the side.

Not only do we need the clinical prediction as I said about infection and about hospitalization and about death but particularly pandemic vaccines and also the effort we hope to improve annual vaccines raised the issue of how do we even begin to measure heterologous protection, and I am very struck every year when we go through this and hear from our colleagues at CDC and WHO and we look at the cross protection and by neutralizing assays in serum how little we understand about what those data mean and how they translate clinically.

So, I think we want to try to get that information so not just defining a simple surrogate but begin to extend the idea of the surrogate to include heterologous strains and then of course the issue of priming of the kind of memory response and protective response against hospitalization and death may be very, very different from the response against a homologous infection.

Often left out here but a cause of problems and again we have seen this in the regulatory arena and all those in this room who do these assays know that we don't understand that much about the antigen but not only the assay but the antigen itself can affect the assays and I think there may be issues about how the protein is conformed.

We think about that in terms of vaccines but we also probably need to think about that in terms of assays and then there are a number of talks on the agenda as you will see about what are non-hemagglutinin determinants and what are their relevance and I won't go through this.

The other thing although the focus here now is on the pandemic vaccines obviously our understanding of correlates and of protection is deficient not just for H5 which has been a particular challenge but will the knowledge we get for H5 be applicable and how applicable to other sort of neo-antigens and what about seasonal vaccines; do we really think that the requirements both biologically and then from a product point of view should be the same?

There is clearly a deficiency in the basic science in the models and I think we need to tie our work on surrogates to these models and ideally you would have animal models which were reasonably predictive of what you felt the clinical surrogate measures would then be, and we know that none of our animal models currently, well, let us put it this way data from the animal models suggest certainly that that is not going to be a simple correlation.

So, you know, observations such as protection and the apparent absence of antibody, etc., what does that

mean? What are we measuring? What are we seeing, and how do we instead of just shake our heads about it or conclude that the animal model is fine and it tells us everything is fine, how do we understand this in some kind of way we can hang our hat on?

Then another thing we deal with frequently in the regulatory agencies and certainly our colleagues in Europe have dealt with the same thing is that we also need better potency assays and obviously the understanding of surrogates is intimately tied to the potency assays.

So, what do we need to do moving forward and what is the purpose here? I think what we are all trying to do is move this area of assay development and immune correlates to the front burner and that is what I see as an important catalytic event at this meeting.

I have heard for several years our colleagues at WHO and CDC, NIDFC, etc., have said that this is important but how can we now accelerate the pace of moving this forward, and what are some practical suggestions? Certainly we ought to find a better way to support lab quality and standard efforts so that we can be better prepared with the current types of technologies or improve those.

Lack of samples has been a real issue and can we think of ways as a global community to bank samples and share samples? I think that one of the great things about

investment in pandemic preparedness and renewed interest in production of annual vaccine, things we have really been trying to achieve is that there are a lot of studies being done. How can we fully take advantage of those studies to evaluate assay issues and generate samples, etc.?

So, I would just ask that since we have an incredible group here of influenza experts, people working in influenza vaccinology, people with a lot of experience that we try to identify practical opportunities and next steps because I know that those of us at WHO and Tony at NIAID and myself we really want to try to have our institutions support practical ways of moving forward in this area.

So, that said, thank you all for the interest and participation and to the people listed for supporting this and many for organizing the meeting, Jerry, Maureen and others and just to say that we will work with you moving forward and I in the spirit of international cooperation we have the Swiss Matterhorn up there for our European colleagues. I hope there is more snow on it now. David perhaps can tell us.

Anyhow I am sorry we don't have anything locally to compete with this, but again thanks for being here and we really, really, I truly think this is incredibly important and it reminds me you know we are all engaged in

consciousness raising here but this is a perfect; it is sort of you know the FDA critical path initiative, if you want to tie it to that.

What NIH is trying to do would translate in basic science into clinically meaningful stuff and what we are all trying to do for WHO at improving global health, I mean this is the place where the scientific needs and the practical needs are really coming together in a clear-cut way and there is a tremendous opportunity.

So, thanks a lot and welcome.

I am happy to ask Tony to come up and also share a few perspectives.

DR. FAUCI: Thank you, Jerry and Jesse. It is a pleasure to be here with you this morning to just take a minute or two to make some welcome, thank you and opening remarks for this very important conference.

I really don't want to take too much time at all just a couple of minutes but I want to leave you with one message from the standpoint of the way I have been looking at this issue over the last several years. The title of what we are doing today is immune correlates of protection against influenza A virus in support of pandemic vaccine development and it is interesting that I know we know it in the back of our minds but the fact is that it is now the tenth anniversary of the first known human H5N1 that was

isolated in Hong Kong in 1997, the original 18 cases, six of which were fatal.

So, it has been 10 years now we have been trying to get our arms around how we are going to handle the potential for a pandemic flu. The reason that there is still such intense interest is obviously the threat still looms. This is the latest numbers that you are all familiar with of a continually percolating number of infections that tell us every day that we look at the numbers that although there has not been the explosion that everyone is very concerned about and might not ever be, the fact is the problem is still here and what looms in everyone's mind that has catalyzed us all over the years is the threat of this which we all know this famous slide which depicts the New York Times magazine cover reminding us of the events of 1918 where more than 50 million people died.

You know this led several years ago to some very aggressive approaches on the part of the United States Government not just the Department of Health and Human Services but in fact the entire government including the Homeland Security Council and Department of Homeland Security.

There are a number of national strategies. HHS's Pandemic Influenza Plan had a strategy and implementation that really set the road map for the kinds of things that

were done within the department and that impacted so many of us in the room including and particularly the issue of a vaccine which is the broad general topic of what we are talking about today.

You all know that within the Department of Health and Human Services there are complementary roles that are played by the various agencies and I don't want to take any time going over the details in each and every one of them, but they are listed on this slide as you see with the major role played by the CDC in their surveillance and their detection capabilities, their training of local response teams and their important responsibility of maintaining vaccine and antimicrobial stockpiles.

The NIH, particularly NIAID's major role is to conduct the basic and clinical research leading to countermeasure development.

The FDA you heard from Jesse just a moment ago with their important role in regulatory approval and guidance through the regulatory project and the overall coordination of this by the Assistant Secretary for Prevention and Response which takes care of HH wide coordination emergency preparedness.

NIAID is a little bit unique compared to other institutes in that we have the same mandate to maintain a robust, basic and applied research portfolio and as I say

when I talk about our relationship to other institutes you could plug in depending upon the institute microbiology and infectious disease if you are us, cancer if it is cancer, heart, lung, blood, etc., but the thing that we have to do that is a little bit different than others is that we have to rapidly respond to new emerging and re-emerging threats which is somewhat unique in that there is always an emergency aspect, very much the way the CDC every day has to think about what they are going to read about in the paper what is going on the following day, we have to look at how we are going to plan for the research endeavor for those emerging threats and influenza really is the prototype of both an emerging and re-emerging threat, and I think that is the reason why it is so important from the research standpoint to do what we are doing because we are talking about two things at the same time.

You notice the title is Immune Correlates in Preparation for the Development of a Vaccine for Pandemic Flu. So, the one message that I want to leave you with is that in fact it is not just for pandemic flu which as you know the burden of which is substantial. These are numbers that everybody in this room is very familiar with. So, I don't need to dwell upon it but what we have done over the last several years is remarkably accelerated our influenza research funding and if those of you who have seen me talk

about the NIH budget in general you might recall that from the year 2000 and two and one-half to 2003 until 2007, it is a total flat line of the NIH budget.

So, of any of the endeavors that have accelerated I mean it isn't billions and billions of dollars but it has gone from a pittance just in the beginning of the turn of the century to now a considerable robust portfolio and the kinds of things we do as you know are fundamentally based in the basic research but the ultimate goal is to be a part of a team to develop the vaccines, therapeutics and diagnostics.

If you look at the major vaccine development challenges in 2007 Jesse mentioned several of these. One very important one is the subject of this meeting, a better understanding of immune correlates, assays to measure immune responses, new vaccine approaches, dose-sparing strategies, the adjuvant work that is going on, the refinement of new approaches to vaccine development, namely cell culture versus egg based, cross protection strategies and I might say again all of us in the room are aware that although we don't have any definitive answer to this now over the last 3 or 4 years that data both on adjuvant dose sparing as well as cross protection strategies has actually looked rather favorable.

I always say that with a little wince when I look

at Jesse because every time I say that he always gives me the "Show me the definitive data," but the fact is it does look good at least from several of the countries. So, we are continuing to put efforts into that as well as the surrogate measures of efficacy.

I want to close in this very brief introduction by re-emphasizing the point that I alluded to in the beginning about the fact that we are simultaneously doing two things.

A true story, last night, pure circumstance; it was absolute happenstance I had the opportunity at a holiday small dinner to be sitting next to a guy that I have know before he went into this position, Mike Chertov who you know is the Secretary of the Department of Homeland Security and he was saying how frustrating it is to have to put so much resources into trying to prepare for something that might not ever happen and how you are always up for the criticism that you are putting resources and it might not happen; why aren't you putting resources into something that you know is going to happen?

It is a little bit different for us. So, I gave him a little bit of indigestion. I said, "Mike, maybe you have that problem but with influenza we really don't have that problem because everything that we are learning about including correlates of immunity, including advancing the

platforms with new platforms, new ways to make vaccines, understanding the relationship between the immune response and protection against influenza vis-a-vis vaccine, everything we do for pandemic influenza preparedness is going to have a major impact on what we are going to be doing for something that we absolutely know is going to happen every single year is seasonal influenza.

So, again I would like to join Jesse and David in thanking you all for being here and welcoming you and wishing you a lot of exciting times over the next day or so so that we can push this field forward.

Thank you, and, David, I am supposed to introduce you.

DR. WOOD: Good morning, everybody. It is a pleasure on behalf of the World Health Organization to add a few words of welcome to both the participants here in the room and also those who are joining us on the web and also to thank Jesse and Tony for their introductory words, and I can assure Jesse that yes there is more snow around in Switzerland at the moment. It looks like it is going to be a good ski season this year. So, that looks good.

As Tony was saying pandemic influenza will be a major global public health emergency should it occur. History tells us that it will occur at some point. We just really don't know when and the 193 member states of the

World Health Organization have urged the organization to help them get prepared to respond appropriately to this threat.

WHO is therefore engaged in a range of activities geared towards helping countries develop pandemic preparedness plans and as Tony was saying these pandemic preparedness plans include a range of measures, antivirals, non-pharmaceutical interventions, social distancing, etc., and until recently I think it is fair to say that vaccines did not figure very highly in these plans simply because credible vaccine candidates were not available and I think over the last 12 months or so this landscape has changed dramatically .

This has been due I think in large part to a concerted effort from the industry not only in the developed world but also in developing countries and that it has been catalyzed by the global public health community.

I think it is true to say that we now have several credible vaccine candidates which have emerged over the last 12 months and this really gives us now a new set of problems that we are facing. From a global perspective WHO is striving to ensure the equitable distribution of the benefits that will arise from having these vaccines available and particularly in the context of what we are

going to be discussing over the next 2 days it is critically important to help the public health planners to understand the proper role that vaccines will play in the response to the pandemic. We need to be able to answer questions such as just how good are these vaccine candidates and can we do better. In order to help us answer these questions we need bench marks to help us assess the likely efficacy and effectiveness of the vaccine candidates and obviously a clear understanding of the correlates of immunity will help not only the vaccine developers, regulatory officials but also the public health officials all to make better evidence informed decisions about the vaccine candidates that are coming forward and the role that vaccines will play in the overall pandemic response. Hence I think the importance of this meeting and it is very good to see I think an alignment of priorities coming together for the convening agencies because I think that what we will discuss over the next couple of days will I think be a very major benefit not only for all of you as participants in the scientific community but ultimately the general public who should benefit from having better vaccines available.

So, in terms of WHO expectations for this workshop we firstly expect to be able to further develop our guidance to countries concerning specifications for the

regulatory evaluation of pandemic influenza vaccine. We very recently established through our expert committee on biological standardization what we call regulatory preparedness for pandemic influenza vaccines which include a whole range of issues but a section on the markers that are used to evaluate influenza vaccines and clearly we need to be able to, we recognized at the time we developed these guidances that this would be an evolving field and this meeting is an important next step along the way to help us further evolve and develop the guidance that we will be offering to countries.

We expect also to develop plans of action to address the gaps that we know exist in our current knowledge concerning correlates of immunity for influenza vaccine. So, we hope to go away from this meeting with a clear plan of action as to how we are going to address some of those gaps.

We expect as both Jesse and Tony have alluded to that this meeting will also have important implications for the evaluation of seasonal influenza vaccine. Finally, I think we expect this meeting to further develop the strong sense of working together, the strong sense of collaboration that is developed amongst the scientists, amongst the regulatory officials, amongst the public health officials who are all trying to combat the issue of

pandemic influenza.

Clearly there is a need for more sharing of information and again this is I think one of the benefits that we hope to get from this meeting.

To this end WHO has been pleased to be able to support a number of scientists from a wide range of countries to attend this workshop and I would like to acknowledge the generosity of a grant from the Gates Foundation that has enabled us to do this and so we have participants from as I say a wide range of countries who will be able to take back the benefits of this discussion and feed that into their own local circumstances.

Just a housekeeping note if I may, those participants who are receiving support from WHO, if they could see Claudia Alfonzo during the coffee break she will assist them with the matter of per diem. Claudia is at the back there.

So, I think that is enough of the words of introduction. I think we should hand over now to the moderator to get us going on the scientific session, but I would just like to thank before I do the local organizers who I know have done a tremendous job actually in getting us all here today and tomorrow. I know that they have had a tremendous job to field the huge number of telephone calls, e-mail queries that came in once the workshop was announced

and so I think I would very much like to thank the local organizers for the work that they have done on our behalf and so I think now we get on with the business and hand over to the moderators.

Thank you.

Agenda Item: Session 1. Correlates of protection against seasonal influenza - Moderator: Robert Couch, MD

Agenda Item: Plenary Talk: General overview of immunity to influenza A viruses in humans and surrogate markers of protection

Dr. COUCH: I am Robert Couch. I am the moderator of the first session and also the first speaker, and as I told this young gentlemen, I said, "I am not computer literate enough to do this on my own." So, he has given me some help here, but while he is doing that I think a few comments might be worth giving up front. I know that Jerry Weir had told you to store up your questions and comments for the panel. I would say that he sent an e-mail to each of us and I am sure each moderator got the same one I did. He wants this to be a workshop not a series of lectures and if it is going to be a workshop it has got to be a workshop in the morning, the afternoon, tomorrow morning, tomorrow afternoon and at the very end and the only way you make something into a workshop is for the people out there to be participating in this.

So, I have viewed the speakers as introducing these topics and not the persons who are just presenting information for you to look and write down, and there are all sorts of experience in that audience that need to be brought to bear on this discussion. If I say anything needs to be improved maybe it would be that it is easier access to microphones. We have got two in the middle and Jerry has told you have to have microphones.

So, I think we ought to turn around the front end, too, to make it a little easier as well and I know some of you but not all of you and if you don't stand up I am going to call on you. So you may be sitting there thinking about the comments that you might make on some of these sessions. So, that is the concern you will have to give to my moderating session I guess. So, if you don't do it I will call on you.

Now, this is I think the third time I have done this topic, and I know Kanta Subbarao is the one who gave me this assignment because she has heard me probably I think both of the other times, but each time I do it I tweak it a little bit and I agree that seasonal, I thought seasonal influenza was very appropriate to start a meeting with, that that is the problem currently and pandemic doesn't present anything in that way that is unique. It just presents another extreme of what we have to deal with

on an annual basis with seasonal influenza and that is where most of the data is on the subjects you have on the table.

That was very clearly enunciated by Jesse Goodman and Tony Fauci in their introductory remarks.

Now, another comment to make is that Brian Murphy and I have exchanged slides here and I will tell you that we are going to do two versions of the same thing.

So, what I am planning to do here is that I will present and then without stopping for discussions and questions Brian will present his and hopefully we have been short enough so that we will have enough time and then the whole subject will be opened up for this audience to comment and ask questions for the two of us. So that is the plan at the present time, and I have got a timer here. I am not going to time myself. I will just warn the other speakers I will time everybody else.

(Laughter.)

DR. COUCH: All right, seasonal influenza and first of all an overview on immunity. Homotypic immunity after infection with influenza virus is potent and lasts for years. The immunity is associated with the persistence of serum anti-hemagglutinin and antibody.

Actually I feel a little bit like I am getting ready to lecture to the choir but at any rate this will at

least remind everybody of a lot of these principles that we do know, and this is the first one I want to focus on. This is data from a set of volunteer studies that we did a number of years ago. You can see a number of years ago with the dates up there but this was following the 1968 introduction of H3N2, and we followed individuals for 4 years after a documented infection three, two, one and then brought in a new control and challenged all of them which challenged dose by the way 1000 TCID 50s amounts to three to ten HID 50s if you want to think about it that way and these individuals were selected to be free of any detectable antibody and you can see 17 of them, 14 virus isolation, 13 antibody responses, 13 ill and seven of them febrile.

This was when we were doing volunteer studies in the Texas Department of Corrections. So, we knew exactly everything that had happened to those individuals. Those individuals 4 years ago there had been no reinfections in that interval. So, we knew them very precisely and then when they were challenged again you see with that same virus or related virus with which they were infected the most obvious thing is all these zeroes up here. There were two antibody rises here but virus isolation zero, antibody rises two here and no illnesses, no fevers. So, that is a very potent protection from homotypic that lasts for

greater than 4 years. That is what we get from that data, and that was associated with persistence of antibody.

Here are the same individuals. Here is the post-infection GMT's neutralizing antibody. We exchange both neutralizing and HAI and basically except for being somewhat more sensitive are measuring the same thing.

Six and one-half post-infection. That would be a month after the documented infection and then pre-challenge you can see down a little bit but if you go across here it is really say less than twofold or maybe threefold over a 4-year period there of persisting antibody and that persisting antibody was clearly associated with protection.

Now, we will say that repeatedly at this meeting but you talk about correlates there is no correlate that is solidly established as serum antibody to the hemagglutinin in relating to the infection response on challenge.

Now, persistence of antibody. This is persistence of antibody to HAI in this case equal to or greater than 10 and 76 when swine appeared. You see most people know if you were looking at this age group it had been 20 years since they had seen H1NI and yet 92.2 percent of them had measurable antibodies.

If you look at the younger age group, 3.8 percent. At that same time Victoria had been in or the H3N2 viruses had been in the community for 9 years, and this is

the kind of thing you expect, a lot of antibody in both age groups, a little bit more in the younger than the older individuals.

So, it is fitting right where it is and yet this antibody has persisted. Now, A/USSR came in in 1977, and this is a cross-reacting mostly presumably antibody against that one. Those viruses there are similar to one that was present at 53 and this was the year after it first entered the community so that part of this is accounted for by infections with H1N1 previously but you can see this very high number of percentage of individuals who had antibody against that virus.

One of the problems with a hearing aid is that all of a sudden batteries die. I had one that just died. I will replace it in a little bit. At any rate here we have this persisting antibody and now when we looked at our community in that year of that outbreak you see here is the previous year, 1977-78, a community outbreak with an H3N2 virus primarily A/Texas and there were a little over almost 700 isolates in the community, 77 percent of them in younger individuals just as you would expect, 23 percent in older individuals.

On the other hand if you look at the Brazil the subsequent year almost I would say 240 isolates, only 1 percent in those older individuals. So this persisting

antibody that we measure in the serum as anti-HA was clearly associated with persisting immunity for at least 20 years.

So, you can't get more potent than that in terms of a homotypic community. Now, unfortunately influenza doesn't give us the luxury of being able to deal just with that and that creates the problem. Heterotypic immunity after infection with an influenza virus is reduced in potency with increasing time since infection and is primarily attributable to antigenic variation. The reducing immunity correlates with reducing serum anti-hemagglutinin antibody to the infecting virus.

Now, we have similar data to what I showed you a minute ago with challenge of volunteers for those heterotypic viruses but I like the data from a pair of family practitioners in Australia illustrating this data very clearly. They followed their practice very closely from 1968, and thereafter, and they had a group of individuals when the Victoria epidemic occurred 9 years after the introduction of H3N2, they had a group who had never seen it. They had seen it and no evidence of prior infection or illness, 94 of them. So, we call that one zero. Fifteen percent of them of them had some measurable antibody which is not surprising. The infection and illness attack rate was 27 percent and then there was a group that

they had documented infection with 6 to 7 years earlier with the first introduced H3N2 Hong Kong. Thirty-one percent now, twice as many had antibodies, 17.9 percent. Those who were infected with A/England, 72, the first variant, again 4 years now, and now we are up to 52 percent antibody reduced to 8.3 percent, Port Chalmers, the next one 2 years 86 percent had antibody, 4.3 percent, very clearly showing that with this antigenic variation as it varies you lose antibody coverage and you increase infection and illness and that is associated with time since the initial, a very clear statement of influenza and this is a summary of some of the clinical variables that we know relate to seasonal incidence that we associate with those immunological findings, age, children, increased infection, infection rates. That we say if primarily the immunological basis.

Very young children in addition to this infection risk have an increased illness severity risk, increased hospitalization and that is a little dip up in the mortality curve as well.

In the elderly increased complications risk and increased death is associated with the complication. You see we don't need a virus laboratory for knowing that sort of thing. Health status, the healthy are low complication risk, the unhealthy high complication risk. We feed that

into our recommendations for vaccines.

Prior antigenic exposure. Recently we just went through a high level of immunity. The more remote the lower the level of immunity attributable to antigenic variation the greater the attack rate of infection and illness.

Now, with that overview of immunity of influenza I am going to move on now to consideration of the second assignment here and that was surrogates or correlates of immunity to influenza and starting with a repeat.

This slide I have labeled some truisms of human influenza. Immunity to reinfection develop following infection; immunity to reinfection with antigenically similar virus is very potent and lasts for decades.

Immunity reinfection with an antigenically different virus is reduced with time and degree of antigenic variation. Thus, the natural history of influenza defines the dominant basis for immunity to influenza in humans as the immune mechanism directed towards the HA and the NA glycoproteins, those proteins that exhibit antigenic variation and that is fundamental to influenza.

Now, there is an adverse correlation as we have indicated between pre-exposure serum anti HA and occurrence of influenza virus infection on exposure and here are two examples. This is an outbreak in the military with H2N2 in

1960. It is a very nicely described one, pre-epidemic titers less than 8 to 64, number of individuals, percent ill 43 percent with increasing antibody reducing number of infections and illness and this is data with five different antigenic variants in our challenge studies. In relation to increasing neutralizing antibody there is reducing infection and illness for each one of these.

By the way in order to get a zero at all there had to be at least six individuals in that category. Port Chalmers, Scotland never became an epidemic virus was an intermediate variate and Victoria. So, it is a very clear principle that has been stated repeatedly but is well-documented.

I like this from the writings of Thomas Francis. Those of you who are not as old as I am you know at least this name you should recognize. If you haven't go back to the literature. In this country he was Mister Influenza for a few decades.

This comes from the Harvey Lecturers 1941 and 1942. Serum antibody titer of individuals who take sick fall within the lower ranges. Higher titers in unaffected subjects were indicative of resistance.

It is not possible to predict on the basis of antibody titer whether a given subject will develop a disease. So, there are two points I want to make on that.

One is that it reinforces what we have been saying. It was present in 1941. It is present in 2007.

One of the things that we sometimes say in our group, what we do is we keep discovering what Tommy Francis already told us. That is not an entirely inappropriate statement and the other is that you can't predict it. There is no single level. We have looked at it. It is a gradient and that gradient and that principle is what is important as opposed to a single level and the fact that you can't predict the single level tells you that it is not only serum anti-hemagglutinin antibody, that there is redundancy in the system, that there are other factors. That is where we go on with the question of surrogates.

Now, this slide summarizes mediators shown to convey or correlate with immunity to influenza infection and disease and the major mediators, serum antibody to the hemagglutinin and the M2, secretion antibody to the hemagglutinin and neuraminidase cell-mediated immunity, cytotoxic lymphocytes, the effector mechanism NPM1. There are others that have been described, roles not clarified and cytokines.

There are cytokines that release specifically as a result of antigen stimulation that have antiviral effects. Now, the specificities, we have already emphasized that the hemagglutinin and neuraminidase are variant. The

M2 is type specific and so are the cell-mediated cytotoxic responses. So, that is type A as opposed to a variance within type A.

All of these have been very clearly described as having significant ability to convey immunity in animal models, rodent models primarily mice. So, there is no question about that in that system and that has provided a lot of our guidance.

Humans for a long time it was hemagglutinin neuraminidase and these two sites and recently Janet McElhaney has made it possible to put a check at this point.

So, that is something that we also will have on the table in this discussion. A brief review of rodent data that brought us to this. So, this is where a lot of our principles come from. I am not a mouse doctor. I am a human doctor but those mice have taught us an awful lot about influenza. Sometimes they have misled us. Let us not make that 100 percent but on the other hand they have taught us an awful lot.

Passive administration of IgG anti-HA antibody can prevent infection very clearly. Passive administration of anti-neuraminidase and anti-M2 antibody does not prevent infection but reduces the intensity of that infection, reduces the severity of disease, promotes recovery and

reduces complications.

Antibody to internal proteins does not mediate immunity. Anti-HA antibody can cure an established infection once it is under way. Antibody to the neuraminidase and M2 cannot. IgA knockout mouse infections are the same as those in normal mice. That doesn't say that IgA can't convey a role. It just says that it is not essential, and it begins to enunciate the fact that there is a lot of redundancy in this system with alternative correlates for us to consider.

Now, when contiguous preferential antigen uptake and antibody responses on restimulation is for the hemagglutinin as opposed to the neuraminidase.

That is the Ed Kilborn and Burt Johanson and I left this one in primarily because I wondered if that might not be true to M2 because of its proximity and I am going to show you some data in a minute that says that M2 responses do have a little bit of a problem.

Now, for those cellular functions CD8s are the major T cell in the lung and lower airways during influenza. CD8 CTLs can mediate recovery from pneumonia. That is the basic mouse model of mortality. CD8s, CTLs alone can reduce the level of infection in the nasal mucosa. Some of you know I had a little concern about the mathematical data we will show a little bit later because

of the circumstances and wondered if those cells can work at the mucosal level across that epithelial barrier because that is where they have got to work if they are going to be of any value in humans. Human pneumonia is a rare finding. It is a respiratory tract infection. Our cellular immunologist did an adoptive immunization study with those active cells and showed that they cab reduce the virus titer in the nasal secretions and in the nasal turbinates

So, I am reassured that CTLs can actually work at the respiratory track where they gave got to work if they are going to be of any value in influenza. Now, another question is how much value are they but at any rate and for action they have got to have direct contact with that cell you see. So, they had to be able to work at that mucosal surface.

Normal recovery can occur in the absence of CD4 or CD8 but not when both are present. That just is one of the general findings. There is a lot of fine tuning of CD8 and CD4 function but at any rate again it emphasizes the fact that there is redundancy in the mouse system. There is redundancy in the human system and it is a lot of those other correlates that we don't give consideration to.

Now, back to my summary slide and I want to take M2 you see has not mark here for humans; CTLs just recently got marked for humans and consider those type specific

correlates first.

The potential role for M2 antibody and CTLs is not for prevention of influenza but in hastening recovery and preventing complications. Preventing complications, Dr. Wood emphasized the fact that that may be as important to focus on in the severity of these disease as preventing infection but if you prevent infection you prevent infection-related illness, too. So, that accounts for a lot of us having that focus on what goes on with the infection.

All right, antibody to the M2 protein is present in low amounts in adults, requires repeated infection that is antigenic stimulus for induction in mice at significant levels, I will show you that data in just a minute, capable of reducing the intensity of infection and hastening clearance of virus in mice. That has very clearly been demonstrated in the mouse system. It could contribute to hastening recovery and preventing complications of influenza in humans but has not been proven given any proven value and that is its major deficiency for our major consideration. It should be considered but there is no data indicating that the M2 antibody has effect in humans and that is a major deficiency at the present time

Now, this is data from Walter Gerhardt's laboratory. Serum antibody responses, what he did was to take the M2. Walter should be out there somewhere, took the

M2 and expressed it on the surface of the cell and looked for the antibody responses in that circumstance. First infection in mice you can see good MP antibody responses, micrograms per milliliter, very low M2e antibody responses.

After a second infection it got up to 30, 51 here, 63 and then after a third infection it got up to what Walter says is a good level for being able to have a significant effect in the mouse system.

This is human data. We sent Walter acute and convalescent sera from documented acute febrile influenza in healthy adults and this is what he got. You see almost no antibody in the acute phase whereas the MP antibody was present in sizeable quantities. MP antibody is still present in both of these and 21 percent of those got a rise to infection. Half, about half of these got a rise to M2e and a four-fold increase but only up to .56. The majority of the rest of them got a twofold rise. So, it was tweaked in these individuals.

Now, these would be individuals say 20 plus or minus 2 to 3 years. So, they have already had at least two, three, maybe four infections with influenza A and this is what we are looking at with the baseline antibody. If you are going to do anything with antibody in humans it is very clear you have got to stimulate it to the levels in which it would be effective. I couldn't find the number but

Walter is here. He is correct me and my recollection was that he liked it in the mouse. He liked 20 micrograms as his standard for good protection. If that is a standard for humans we are a long way from it you see with what occurs as a result of a natural infection.

So, the task of what M2e has to do is very clear. Now, cytotoxic lymphocytes in humans. Inducible CTLs are present in all healthy persons but are reduced in the elderly.

You see any one of you as long as you are healthy and you are an adult it is there. it is 100 percent. It is not even 99 percent. It is 100 percent. You have got it. So, we were talking about this last week. Gossi Rimerhaus is out there. That is your starting point for thinking about CTLs. Maybe young infants you can talk about starting it up you see but otherwise that is your starting point for this modality.

Reported to hasten the clearance of virus in nasal secretions of infected volunteers, I alluded to that data a little bit ago. The manner in which that was done was the virus shading went down in volunteers who were challenged, no effect on illness if they had CTLs before the challenge. So, the correlate was there. The problem that bothered me was that he eliminated antibody by using HI in serum and that is a pretty crude test for eliminating

antibody but I was reassured by that mouse data I was giving you a little bit ago that these CTLs can work at the nasal mucosa which is where they have to work if they are going to be of any value to humans. That level of value is a totally different question, but correlate with protection to clinical influenza in elderly and that is data that we just recently got from Janet McElhaney and maybe there is more data now. The problem with it is it is elderly and small numbers. Maybe it is bigger than that now.

So, it is a start at any rate saying they can work. I should have said up here that I looked at that and said that reduced in the elderly is not quite right because you test the elderly. A better statement is heterogeneous because we will have responses in elderly individuals that are no different from healthy young adults but they are much varied. Then we will have the reduced responses. So, it is very heterogeneous is a better way to describe it rather than reduced.

Now, the CMI can contribute to immunity in humans and I think that is certain now but the relative significance of that contribution in relation to other mediators of immunity and the different age groups and infection and vaccination circumstances have yet to be defined and again the deficiency of that correlate and that possible mediator.

Now, if we go back to our general slide again I want to take up HA and NA and these very briefly because there is where the action is right now in humans. This is an outline of the occurrence of these antibodies in the nasopharynx, the mid and the lower respiratory tract. What everyone knows, IgA is the dominant one up here. Usually we quote that as 90 percent IgA, 10 percent IgG. That is a pretty well median for it, for immunoglobulins. This is the actual distribution of antibody to influenza A in a group of volunteers that we did at this site and this site and that is an actual gradient that relates to specific data. It was about three-fourths of the IgA up here and about one-fourth IgG.

As you go down in the lower respiratory tract IgG becomes dominant. That is considered to be the major reason for serum IgG standard vaccines protecting against pneumonia in severe disease. It is a perfectly reasonable postulate. On the other hand if you want to protect the nasal pharynx you have got to focus on IgA but one of the other things to point out is that both antibodies are present in both locations. Now, when you look at how they relate to correlates this is a sizeable set of volunteers but these are not independent variables. They correlate with each other, but if you look at them individually the antibody type and location percent infected according to

antibody titer is low, intermediate or high, with serum neutralizing antibody the same thing we have been seeing. As it increases the occurrence of infection goes down, nasal secretion neutralizing antibody the same pattern. Serum anti-neuraminidase antibody the same pattern. Nasal secretion anti-neuraminidase antibody, that really totally is absent or present. If it is present at all we had no detectable infections in that group. So, all of these are correlates, these antigenic variants that we have been talking about and all can contribute to immunity of influenza and the redundancy in the system with the question of M2 and CTL is a little bit out there in humans. It is absolutely amazing.

You see this virus can, I once said to Sir Charles Stewart Harris, "I think this virus knows how to think," but that virus can vary and is tricky and clever but the human is also pretty tricky and clever and has a lot of options to fight and our task is to figure out how to use them optimally.

This is the conclusion on HA and NA. For maximal optimal protection against influenza serum HA neutralizing antibody is essential. Actually I didn't present you all the data to support that strong statement but trust me, it is there. Anti-neuraminidase antibody in serum and anti-HA and anti-NA and nasopharynx secretions are highly

desirable. That is the redundancy and the help we want wherever we can get it.

Now, this is another one of my tweaks for this version. We have recently started back working on secretions having done this decades ago fairly extensively, and we started back and one of the things to look at is heterotypic and heterosubtypic immunity. Suzanne Epstein is going to deal with heterosubtypic lately, but heterotypic is a seasonal consideration. Both of these immune responses were reported decades ago in humans who were infected or had been given various vaccines. So, it is not a new finding and the focus here that I want to awake in mice the relation is to that immunoglobulin. In mice it correlates with the cross-reactivity measurable in vitro. That gives us our rationale for looking just at the antibodies. Secretory IgA is clearly greater than IgG for demonstrating this and that is one of the foci of all of the mouse studies increased by adjuvants not surprising, CT in the mouse and heterosubtypic has now been reported in mouse systems at least for H1, H2, H3, H5, maybe others that I don't know about, and you can't ignore it. This is in parentheses. It can also be mediated by cell-mediated immunity, but our focus here is the immunoglobulins and those antibodies and this is some data we have recently developed and that is cross reactivity in nasal secretions

for 2H1N1 viruses. This is New Caledonia '99, Taiwan '86. Taiwan '86, that virus has not been around since 1991 and then here is 1999. These are secretions from a random group from an individual in 2002. If you look at the ratio of New Caledonia to Taiwan for IgA you see the IgG. They should be the same, same specimen and they are in the two tests. Nanograms of antibody .8 percent antibody 1. You see the way to think about that if you don't deal with nanograms and get used to this kind of thinking is that the titer of New Caledonia and the titer of Taiwan were the same by the IgA in that secretion, so completion cross reactivity between those two.

On the other hand if you look at the IgG antibody in this case the two tests had a slightly higher G here, nanograms .3 and .2 much less cross reactivity for that IgG antibody in secretions than for that IgA antibody in secretions. That is not new. That was demonstrated a long time ago very clearly worked up in mice but that IgA antibody has a potential for contributing to heterotypic immunity in seasonal influenza which is one of our concerns. So you can sort of get a reason for partly why we have gone back to focusing on that particular immune response.

So, in summary homotypic and heterotypic immunity to influenza that follows infection can be potent and last

for years. The degree of immunity correlates with the magnitude of the serum anti-hemagglutinin antibody to the infecting virus. Antibodies to the HA and NA in serum and secretions are proven as powerful mediators of immunity in humans. CTLs appear capable of contributing to immunity but a role for M2 immune response in human influenza has not been described.

I didn't time myself but at any rate I am going to turn the podium now over to Dr. Murphy and we are going to do the same subject.

Come on up Brian, and then when he gets through it will be opened up and so you be thinking about your comments and questions to contribute to this subject of correlates of immunity with a focus on seasonal influenza.

Agenda Item: Humoral immune responses: Viral targets of antibody-mediated immunity

DR. MURPHY: Bob is correct in that we have a lot of overlap but I think some of the points will be important to say again. Now, this is a result from an experimental infection of humans with influenza A virus and you can see the important point of this is that the virus replicates extremely rapidly in humans. You get peak titers within 2 days. The consequences of that, you know the reason for that is the single cycle growth curve is very, very short and you can attain high titers within 24 hours after

experimental inoculation.

The immune mediators present at the time of exposure are the major players in resistance to this virus. Immune factors, either cellular or humoral generated from memory that require infection to be initiated, immune cells replicated and activated make less significant contributions. The other point that I want to make is that the illness that is experienced by the host infected with influenza virus is a function of the amount of virus that is replicating in that individual.

Individuals who have low levels of virus might have minor illnesses. Those with high levels of virus can have the more significant severe forms of influenza. The consequences of the findings that we observed in our challenge study is that the illness correlates with peak virus titer, low asymptomatic, higher titers high fevers and the peak titer is achieved. So, the job of the immune system really is to keep your titers less than 10 to the 3 or in that order of magnitude. Just for those interested in live virus vaccines it replicates to the titers that are associated predominantly with the asymptomatic spectrum of this illness.

Now, for secondary infection the homotypic immunity you can get reinfected with this virus as Bob indicated and basically the antibody previous experience

decreases the level of replication of subsequent infections.

Now, I will just, Bob went over a lot of these points but in 1977, we learned that there was a tremendously long duration of immunity to influenza A virus's homotypic immunity. The virus needs both antigenic shift and drift and it escapes immunity predominantly by changing its hemagglutinin. It has to change the hemagglutinin in order to be either a drift strain or a shift strain.

In 1968, the epidemic was slightly milder than the 1957 epidemic. This suggested an N2 immunity likely played a role in resistance and we had very severe epidemics in 1957 and 1968 despite the fact that everybody during that period of time had been infected with other influenza viruses of different subtypes. Therefore based on this experience we think heterotypic immunity is weak. So, what are these mediators of immunity to influenza virus? I will discuss these different factors. The protective antigens from animal studies, this was a study in which viruses were made in vaccinia recombinant viruses that had the individual influenza A proteins expressed. We had a control vaccinia virus. We immunized animals and then we challenged them with a wild-type virus and I think you can see very simply here that the HA and the NA expressing

vaccinia recombinants provided a high level of protection. These others were very weak if at all, really marginal levels of protection. This really indicated that our focus should be on HA and NA. So, a summary of some of the observations we have to date were that antibodies to HA which can prevent infection as well as prevent penetration are major players.

Antibodies to NA we thought based on the 1957 and 1968 comparison are moderate players and the animal studies were moderate players and this prevents release of virus in infected cells and also likely prevents the penetration of the virus through the mucosal barrier.

We think the other two N2 antibodies and cell-mediate immunities play a very small role in resistance, not to recovery from infection but resistance to reinfection.

Now, I will provide some data on the role of these antibodies and what is the evidence that the HA is actually having an effect, that the NA is having an effect, but this is just to make the point of we all know that this is data from respiratory syncytial virus because I am presenting this here because of its completeness. This is a study in which passive antibody post-infection serum was given to animals and they achieved different levels of neutralizing antibody in the animals and then the magnitude

of replication was measured in the lungs and the nasal turbinates. The major point I wanted to make and this just supports Bob's previous information that serum antibody at titers of around one to two hundred, one to four hundred here completely prevented replication of this virus. Similar data exists for influenza A, not as complete as this.

In contrast the same levels of the antibody only reduced the titers in the upper respiratory tract about 10-to-100-fold.

So, there is also evidence for serum antibodies and immunity to influenza in humans and the nicest experiments of nature are the maternal antibodies and if you have a high titer maternal antibody the infant will develop influenza later. That suggests that influenza antibodies to the HA are correlating with infection. I am going to focusing mostly on these challenge experiments that were done, and I am going to be first talking about anti-NA antibodies.

We did a study back in around 1970 in which volunteers were challenged with a wild-type H3N2 virus but because these were adults, they all had been alive to the H2N2 they had varying levels of serum antibody to the neuraminidase and it was very clear that individuals who had high titers of or titers of greater than 1 to 4; this

is measured by an NI assay; a lot of these illnesses, 60 percent of these individuals did not develop illness. In contrast the ones with low anti-neuraminidase antibody developed illness. Importantly none of these individuals had antibody to the H3. They were selected to be seronegative, never had been infected with the Hong Kong flu.

When you look at this again break it down a little further into individuals who were not ill, had afebrile illness or febrile illness I think you can see that those with the febrile illness, with the low titers of anti-neuraminidase antibody had high titers of virus replication. In contrast the not ill group had high titers of anti-neuraminidase antibody and very low titers of virus.

However, they all were infected. In a second study that we did this was done around 1980, this is a huge study where we actually gave wild-type virus to 163 volunteers and then we looked at, this is a study Mary Lou Clements and I did and it summarized around 3 or 4 years worth of work but what we did was we did a quantitative measurement of the amount of virus that each of the individual, each of the 163 individuals had in their upper respiratory tract and we looked at what immunological factors correlated with this virus index score, and we

measured antibody to the HA with an ELISA, measured IgG antibody. We looked at nasal wash antibody. We are just talking now about NA. We did not have a nasal wash test for neuraminidase. So, we only looked at serum and we were able to because of the large numbers and the large data set, we were able to say that this particular factor in this location here independently contributed to a reduction of replication of the challenge virus. When we did that it was very clear once again you could see a role of NA antibody in the serum and I have no doubt if we had the test we would have been able to demonstrate the same thing for the nasal wash. We will revisit this slide and talk about the HA shortly.

So, the conclusion on the role of the NA antibodies is that they are clearly associated with resistance. They prevent disease but not infection and you will see this differs from HA which can prevent infections. They have a moderate strength and they prevent disease by restricting replication. They affect both the magnitude and the duration.

I didn't show you the duration but it is also affected and again it is the antibody in the serum that is clearly identified with resistance but presumably mucosal antibodies will mediate that as well.

Now, we will go to the HA antibodies and we will

look at the contribution of serum and mucosal HA antibodies to resistance. First we will look at some evidence that suggests that nasal wash IgA antibodies are mediators of resistance, in this case resistance, they prevent infection and so we had a group of individuals. In this case these individuals were challenged with an Alaska cold-adapted virus. This was back probably around 1979, and we selected volunteers to have no antibody or very low antibody to the HA although you have it here. This is ELISA antibody and these titers are the HAI titers would be less than one to eight in these groups.

We had a group of individuals who were not infected, okay, with this vaccine. We had a group that were infected. Those who were not infected had higher titers of nasal wash IgA antibody, again associating this specific factor and they were comparable in terms of their serum antibodies to the HA.

Here is another. If you do enough trials you can get data that will support any point that you want to make. So, this is very easy. I just had to try to remember one or two trials that would make an individual point. We gave vaccinees an H3N2 10 percent to the virus and we challenged them with a wild-type virus and our control individuals all became, they all shed virus. Six out of seven became ill with febrile illness, good challenge virus.

We had one group of volunteers who just had neutralizing antibody which measures antibody to the hemagglutinin who had anti-neutralizing antibody nasal wash but they had no anti-antibody to the neuraminidase and almost no antibody in the serums in the HA. So, we could look at antibody in the nasal wash to the hemagglutinin as an independent contributor and I think you can see that these individuals were completely protected against replication of the virus and illness and three of the individuals were completely protected from infection, and in the same study where we did the large number of individuals we were able to demonstrate presence of protection mediated by the HA serum antibody correlated independently with protection, restriction of replication and then antibody to the HA in the nasal wash we had evidence suggesting that IgA as well as IgG could independently mediate resistance.

So, the conclusions on the role of HA antibodies are that they are clearly associated with resistance. They prevent both disease and infection. They are the strongest antibody because they are able to do both. They prevent disease by restricting replication of virus and they affect the magnitude and duration of virus replication and you have serum and mucosal antibodies independently contribute to resistance.

So, I have developed a little index. It is called the relative strength of the immune-mediators index and as we talked mostly about these antibodies I have developed a little scoring system. This has not been validated for use by the FDA. I want everybody to know. This is the immune strength scoring index. It is in dumbbell units, okay, and the point here is that serum antibodies which are predominantly IgG have a very high strength. This is a five dumbbell score for this particular immune-mediator. In the mucosal site the IgA antibodies are major mediators. Both of these antibodies can prevent disease in the majority of the individuals associated with it.

Anti-NA antibodies, they get a moderate score. All other anti-M2 CD8 T cells which have to react which are as Bob said the M2 antibodies are present in very low quantities. CD8 and CD4 T cells have to be generated from memory and have a very small contribution to the peak titer of virus that is achieved. So, we think that they make a minor contribution.

Now, immunity then really is the sum of one, two, three and four. There is no single correlate or surrogate of immunity. I mean if a vaccinee has very high titers of serum IgG antibody to the HA they will most likely be immune, the same thing with nasal wash antibody but really it is the sum and it is very difficult to develop a single

test that can determine what the sum is.

I just want to show you a clinical trial that we did that addressed the question of heterosubtypic immunity. As I said, if you do enough of these you can have data that supports any point that you want to make. We tested, we did studies in children with live attenuated virus vaccines. These are two different types of vaccines but that is not important. They both were attenuated virus vaccines and we gave them to children who had different qualities of immunity.

We had individuals who have HAI antibody to H1N1. These are kids who got H3N2. So, these were children who had been previously infected within the past season with an H1N1 wild-type virus and then we had some individuals who failed to have antibody to this and we had relatively large numbers of these subjects and there is almost no difference whatsoever in the parameters of infection in these individuals and I think this indicates very nicely that heterosubtypic immunity therefore that immunity that is induced by one subtype against another subtype is weak. If this was a homotypic situation they would be completely protected.

We have the same type of information within an H1N1 vaccine but in any case the immunity, the heterosubtypic immunity, that should be heterosubtypic

immunity was mediated by a live virus vaccine, an H3N2 vaccine. Actually some of these vaccinees and I think you can see again that the pattern of infection was identical in this case.

So, this is immunity induced by wild-type or heterosubtypic immunity just by wild type or by live virus vaccine. Against a weakened virus you can't see a significant difference. So, this is the reason when I say that I think heterosubtypic immunity is weak it is based on observations like this.

Now, that is all I have to say. I just wanted to indicate that NIAID is having a clinical tenure track program. If anybody has scientists who are interested in that tenure track program contact Carol Baron. Sorry to take a couple of minutes but I am required to do this --.

(Laughter.)

DR. MURPHY: -- under the threat of being shot. NIAID is beginning to play hard ball nowadays.

DR. COUCH: Thank you, Brian. According to my schedule we have got at least 20 minutes and it may not be appropriate with the camera but it sure would be nice otherwise if we had the lights on so that we can have the whole audience out there and I am going to threaten you again that this is now to be a comment, not just to stand up and ask us questions, but this is an open subject now

on correlates and immunity but the focus is seasonal influenza. We did not focus on vaccines but they are not excluded for comments on this, and I know that there are a lot of people out there who have information and Harry Greenberg has got his hand up. Please just stand up and walk to one of the microphones if you will. They want to be sure it gets recorded, Harry, and the others of you go ahead and think about your comment and your addition here and stand up and go to one of those microphones. Otherwise I will have to call on you.

PARTICIPANT: This is a question more than a comment, but I was intrigued by your data on heterotypic immunity being greater with IgA than IgG and I am just wondering about the basis for that, and does anybody have any data on the question I am asking which is basically in a nasal wash are there more molecules that are directed at different variants or are there more single molecules that react with different variants; in other words if the basis of heterotypic immunity that you could neutralize many different strains of an H3 because you have very homotypic antibodies but you have a lot of different ones that react with different isolates or do you have antibodies that actually are more floppy and can neutralize the whole bunch? Does anybody have any data on that question?

DR. COUCH: If you know the precise answer to

Harry's question please stand up. In reading the articles on this and the proposed explanations the authors who have written on the subject biologically at any rate don't answer the question that you just raised.

On the other hand if you take mouse data from a single infection and then you look at that antibody, which IgA against a number of variants then that should have been one source originally and yet it is cross reactive. So, it must be the same antibody that is capable of cross reacting. I have assumed that with regard to the IgA but exactly what the mechanism is and why it does it better than IgG you are speculating unless we have an answer, hopefully.

PARTICIPANT: No, I don't have an answer. On one of the tables that Brian showed he showed IgA slash IgM, and I was wondering if there is any specific information about IgM since you might expect a lower avidity antibody response. Is that perhaps what cross reactive in a way, is there any specific evidence about IgM responses that might contribute to that effect?

DR. COUCH: But the IgM does cross react as well. Is that right?

PARTICIPANT: I am just alluding to I am thinking of Brian's table where he showed that IgA slash IgM responses correlate with protection.

DR. MURPHY: I was just talking about the sum of the antibodies and we did not look at it as isolated both IgA and IgM but we used antibodies that would detect both IgA and IgM anti-FAB reagents but IgM as you know like IgA is secreted by the secretory immune system and goes up a concentration gradient and achieves higher titers in the nasal wash than it does in the serum.

DR. COUCH: It may have some benefit in those first infections, too. Kanta?

PARTICIPANT: I do have a couple of questions. Do you know how long nasal secretion antibodies last?

DR. COUCH: That is the weakness of nasal secretion antibody is duration. No, it doesn't last as long as IgG. I had another slide I didn't use here that shows that you see that that ratio of A to G goes down proportionately with time afterwards. We learned that back in the rhinovirus studies and it is not surprising that you can also demonstrate it with IgA whereas the IgG is much more durable but when you think about it the majority of that IgG or Brian may think all of it; our data doesn't say all of it is derived from serum which has much greater durability.

Now, when you talk about IgA though don't knock it too quickly you see because that antibody in serum you know has got a 28-day half life. That antibody up there in

secretions in a matter of minutes you know, 15 or 20 minutes so there is a lot of IgA antibody being generated and the mouse studies say that there is a lot of IgA cells capable of producing that are lining that mucosa in a hurry so that actually I think the way to think about it is the way I did with rhinoviruses years ago. Our problem is how to get it up there and keep it up there for that duration and we don't have a solution to that yet but duration is not as good, but good.

DR. MURPHY: I think there is some data. Phil Johnson did a study where he challenged live virus vaccinees a year later with a second dose of vaccine and was able to demonstrate, Peter did that, was able to demonstrate IgA mediated protection after a year. In adults Mary Lou and I did a lot of studies where we timed challenges 1 month or 6 months and we definitely saw reductions in IgA you know of the protection over a 5-month period. It wanes and it goes away but it can persist for a long time but not as frequently as, it does not stay up like the serum antibodies do.

PARTICIPANT: I was, also, intrigued by the data that you had of people that had nasal secretion antibody but not serum antibody. How often do you see that disconnect and why do you think it happens?

DR. MURPHY: Most of the time you see both. I

don't know why it happens okay? I think what you have is an infection that is largely restricted to the upper respiratory tract. It is a weaker infection. The level of virus is 10 to the 3 rather than 10 to the 6, 10 to the 7. So, I think you really are stimulating predominantly the local immune system in subsets of individuals but generally we see in pediatric individuals both serum and nasal wash antibodies rising in concert. As I say if you do enough of these things you see situations where you get dissociation.

DR. COUCH; If you hyperimmunize that nose you are going to find a lot of IgA that stands there a longer time and those volunteer challenges you know are right there into the nose. Fazacos told us that in mice several decades ago with his hyperimmunization.

Walter, find a microphone.

Walter, please comment? Walter Gerhard.

DR. GERHARD: You pointed out that the indication such as toxic T cells if they have to have an effect on the resolution of the virus infection they have to be at the site of the infection. It is probably true in general although they could also act if they are a little bit away from that through cytokine secretion. However, this is obviously, absolutely has to be the case if you look at serum antibodies. Serum antibodies only can be effective as much as they translate into the respiratory tract

secretions. So, only the local antibodies that derive from serum are effective and in that context I would like to point out a number of studies have been done by transfer of antibodies and what these studies showed and I see primate type experiments also, what they showed is that passive antibodies can be highly effective in terms of protecting the lung, the lower respiratory tract. They become less protective if you look at the trachea and they become least protective if you look at that nasal epithelium and that has been related actually to the rate of transfer in relation to these sites. So, I think that should be kept in mind if you correlate serum antibodies with protection.

DR. COUCH: Again, Walter has enunciated the slides in the upper and the lower of those immunoglobulins where that IgG is at maximal concentrations and that has to be kept in mind for sure.

Please tell us who you are before you speak. You see I am naming some of the others.

DR. MILLER: Mark Miller from NIH. Could you make a comment about the assays used in these studies whether or not they were hemagglutinin inhibition assays versus ELISA neutralization and the variability between them was my first question, and the second one is natural protection is related to age groups which is also related to prior exposure from the first exposed viral type or the age

cohort and there was some mention about the heterogenous response for CTLs but you didn't really comment very much about the antibody responses and I am not sure if any of the studies that you were commenting on were ever powered to actually look at the various different ages and to take into account the decreased immune response.

DR. COUCH: Actually if I remember all of them assays if you will permit me I would have to say quickly I know there is an assay section. So, I am not sure that it is appropriate for us to be dealing a lot with assays but HI assays people may do their own variety. That is fine. Otherwise they are all exactly the same, done with the CDC criteria with exactly the same methodology.

Now, your red cells may be a little different source and your antigens may be one grown in house but otherwise they are exactly the same.

Numbers don't necessarily compare. John Wood is going to tell us about that a little bit later. Neutralizing antibody assays, there are a lot of different neutralizing antibody assays. In fact, we have changed over a period of time in doing ours in three different ways. You can't talk about the advantages or disadvantages of each of those very quickly here.

Most everybody does an enzyme immunoassay much the same although Brian does a kinetic assay. So, what I am

really saying you know there are a lot of differences in assays and if you want to try to standardize and compare assays that is a subject in itself. That is almost for later. So, I don't think that this would be the time to deal with that. If you want to ask me about the ones I talked about I will be glad to talk to you about it later.

Brian, do you want to comment on that?

DR. MURPHY: I would just make one comment. When you are looking at an enzyme, an ELISA assay to the HA the titers that you get in that assay correlate perfectly with either neutralization antibody or by HAI. It is almost a straight line function so that you know you are measuring the same group of antibodies. So, I think that those tests in that way have been validated to show that they are measuring the same thing.

DR. COUCH: We didn't deal with different age groups on responses as I made the comment of CTLs but you can make the same comment with regard to antibody. It is heterogeneous in the elderly age group. Some respond well. Others do not as opposed to almost uniform responses in the younger healthy individuals.

I have forgotten your last question. So, if you will permit me, let us move on to somebody else anyway.

DR. BELSHE: Hi, Bob Belshe from St. Louis. I wanted to relate the experience that the vaccine centers

had during the pivotal field trial that we conducted with the live vaccine because one of the things, one of the opportunities we had during that field trial was to look at H1N1 immunity because H1N1 had not occurred in several years including the field trial years, and so, when we conducted that trial at the end of it we didn't have any protection data on H1N1. So, we asked the children to give us a nasal wash and a blood and then challenged them with monovalent H1N1 vaccine strains and were able to develop some correlates of immune protection in these young children who had not previous experience with virus at all or had only H1N1 vaccine virus, and so when we looked at the correlation between serum antibodies and secretory IgAs we were surprised that these were independent, that there were some children who had secretory IgA and if they had any secretory IgA they were absolutely protected against challenge. The same was true for serum IgG antibodies. If they had any serum IgG to H1 they had absolutely no shedding of vaccine virus on challenge and these were completely independent.

We were, also, surprised that there was a small subset of children who had received vaccine but had neither IgA nor IgG detected. So, we went back and looked at that subset of serum using microneuts and there were only six such children and four of those six children in fact had

microneut antibody to HA which sort of reflects what you said. It is a slightly more sensitive assay, and so I think in this population we gained a lot of information about what is important and I think it is reflected in Brian's dumbbells. You have to add up all these things and if you have any one of them you are substantially protected if it is antibody directed against that exact hemagglutinin.

DR. COUCH: Thank you, Bob.

Peter, is this somewhat along the same lines?

PARTICIPANT: No, I thought I would change the subject because I think we have not exhausted but perhaps plumbed the available evidence on this.

I just would be very interested in at some point during the meeting having some discussion of the role of innate immunity, cytokines, antigen induction. These are things that do occur early on and may influence both the symptomatology and the recovery from disease and I think somehow in this field we are not focusing as much on those as we might and they maybe deserve a dumbbell or maybe even two.

(Laughter.)

DR. COUCH: No disagreement, Peter, but you will have to concede that is a new subject and maybe a little tough for our discussion here.

Dr. Wood?

DR. WOOD: I was intrigued by a comment that you made, Dr. Couch that there was no titer that you thought that correlated with protection and I wondered if you meant that in the context of the individual, if you tried to predict protection for an individual or if you are looking at it more on a population basis because as we try to give guidance when we are looking at evaluation of vaccine candidates then to have a sort of a seroprotective level that we achieve in a population is potentially helpful and I just wonder is that something that we can try to achieve or it is just not doable.

DR. COUCH: Criteria is always desired by regulatory authorities for sure I just reiterate I think Francis was right. You know you can't pick a titer that would guarantee immunity. There is no question about that. I think everybody in this audience will agree with that.

So, when you pick a titer like one to forty that has got an established background of understanding. It doesn't guarantee immunity and some people call it seroprotective. I dislike the term but at any rate it is the gradient and the profile that is important and the higher that profile goes toward the other end the better off your population is going to be.

You can pick any number. It could be 20. It could be 80. These tests differ. John is going to tell us, but

they still give you guidelines. The more people who are above that the better off you are going to be. I have trouble with sometimes using that as an absolute guideline but you know I am a biologist not a regulatory authority.

Arnold Monto?

DR. MONTO: Just a further comment about the same subject and first of all I just wanted to thank you, Bob for bringing up Tommy Francis' comment which I was actually not aware of back from 1941, about the difficulty in individual protection versus population protection. I think that is a key issue, and we are just now analyzing the data from our study of inactivated live attenuated vaccine and placebo and we are finding that nearly everybody to the circulating virus of let say H3N2 has antibody in the inactivated vaccine group to both the circulating antibody and to the vaccine that was in the virus, and it was a drifted year. However, we do find failures in spite of having seroprotection and more than seroprotection by the EMEA criteria where you know above one to forth, this is even above one to sixty-four titers, and therefore there are other components involved in failure of the vaccine in these individuals even though it is a protective vaccine that 70-odd percent of the population is protected and you have to go back to the studies on these levels and remember that these are 50 percent endpoints. These are not absolute

endpoints. That is one factor, but you also have to realize that there are other components involved in protection.

Now, if you look at the live attenuated group only about 20-odd percent of individuals who received the vaccine got HI titers. In spite of that there is protection but in those that failed none of them had antibody to the circulating virus.

So, it is an intriguing difference between the different vaccines and just emphasizes the point that there are many other aspects and we are working with a number of people in the room on trying to look at what is going on this situation.

DR. COUCH: Thank you, Arnold? Kanta?

PARTICIPANT: Do you think we need to look at the quality of the antibody differently in the nasal secretions as well as the serum?

DR. COUCH: All of us would say, "You don't me. You have the answer to that yourself." The more data you can get about your antibody the better off you are, and we don't ordinarily do avidity or even on the single antibody binding affinity. There is on question that I think that would help our understanding, not routinely done though. I don't know data but it ought to be better. The higher the avidity the better the antibody.

Janet, tell us about the CTLs and the status.

DR. MC ELHANEY: I think one of the important points that you brought up, Bob is this whole thing about heterogeneity of the response and now we have got multiple different things that can contribute to what we are measuring in terms of CTLs, but I think that the other important part of this is why we haven't found antibody titers to be particularly helpful and this population actually gets to Kanta's point because I think that this whole thing around avidity whether you are talking about nasal mucosa or serum antibodies is going to be really important in terms of an age-related change an when you have to go into populations and look at what predicts protection I think the point that I have to make is we have to be very careful about defining which subset of older we are actually looking at. We can't combine data from healthy people in the community to those in the nursing home and put it all into one bag and say that this is our correlate of protection. These are going to have to be individually studied. So, I think that is the point that I would like to make.

DR. COUCH: That is very well said. We have said that for decades that children are not the same as adults but certainly adults are not the same as the elderly. That needs to be kept in mind.

DR. BRACIALE: Tom Braciale. I wonder if both of

you would comment on the concept of sterilizing immunity after natural infection or vaccination in the homotypic circumstances or in heterotypic circumstances particularly as a function after time of previous exposure to influenza whether through vaccination or through infection?

DR. MURPHY: Bob demonstrated data showing the fact that you do get sterilizing immunity following natural infection or following challenge of individuals. There is a time dependence on this in terms of the antibodies in serum and the peak of this would be around 24, I mean would be around 28 to 1 month to 2 months. That is when you see most of your sterilizing immunity. After that it goes down after 6 months to a lower level. You have declines in both serum antibody that occur from 1 month to 6 months and you have declines in nasal wash immunity over that same period of time.

Now, effector cells, so those will come into play and when Bob talks about spectrum you always have a spectrum of responses. You just shift the spectrum down all the time but I don't think it is anything other than that. So, you have sterilizing immunity. It is less percentage of your population will have sterilizing immunity as time goes by.

PARTICIPANT: How do you define sterilizing immunity?

DR. MURPHY: No evidence of infection.

DR. COUCH: That is a term that came out of the HIV program.

DR. MURPHY: No evidence of infection as defined by shedding of virus or the development of immunological response. It is not a simple thing. It is very difficult to rule out the presence of an infection in an individual.

DR .COUCH: We are getting into the coffee hour, and the things that we have been talking about and the questions and assays and what have you, they are all going to come up again in these sessions.

Actually don't go away. I was going to recognize the last person before we go but to tell you that he is the last one, and then we are going to take a break and you can store your questions and things for later and for the panel. Go ahead, please?

PARTICIPANT: For the coffee break to jump start the CTL discussion.

DR. COUCH: Make is a short question.

PARTICIPANT: Actually it is more of a comment. So, we saw that evidence for heterosubtypic immunity in humans is weak but you brought up the point that the T cells need to be at the site where the infection is occurring in order to function properly. We, also know from our mouse data and have been led or misled by them that

after a primary infection the number of specific T cells drop over time and that after about 6 months they lose that heterosubtypic immunity.

So, the question is in humans is the same thing happening; are we not looking in the right place when we look in the blood and you see frequencies of CTL but they are not in the right place and you really need to get them in the lung and the question is why aren't they staying in the lung; why aren't they being maintained and is that the key to this heterosubtypic immune question?

DR. COUCH: I don't answer your question. I mean I understand the same as you. How do you get them there and keep them here? It is a little bit the same thinking I had that I put into IgA antibody. How do you get it there and keep it there in the right place to work when the exposure occurs, and it is only out of mouse models that we are going to be able to I would assume get specific guidelines as to how to best do that.

Do you want to comment here? All right, at any rate good audience. Thank you and continue your discussions and bring them back here for after the break.

(Brief recess.)

DR. COUCH: Welcome back. We are continuing the discussion now with Tom Jefferson from the Cochrane Collaboration who will be speaking on an evidence-based

review of the criteria for regulatory assessment of seasonal influenza vaccines.

Agenda Item: An evidence-based review of the criteria for regulatory assessment of seasonal influenza vaccines

DR. JEFFERSON: Good evening. I say, "Good evening" because it is evening for me. I would like to thank the organizers for inviting me. I would like to thank David Wood for telling me what I have to say and I would like to thank specifically Vallie Rodriguez for her patience in organizing all my travel and patience with strange things like Dunn's numbers. She told me I needed a Dunn's number. I asked my statistician, "What is a Dunn's number?" and he said, "Never heard of it. It has got something to do with Shafer's test probably. Anyway I know about a Dunn's number.

What I was asked to present this afternoon, this morning, this night, tonight, tomorrow night, whatever it is is the evidence which we have of the validity of the NRA criteria for the assessment of influenza vaccines.

I was given the brief on seasonal influence of vaccines. However, I have a confession to make. The last slide is on pandemic vaccines.

This is a protocol violation which has been agreed with the organizers. So, I think we are let off.

So, what we thought is first of all we thought let us look at what evidence there is that the NRA criteria make sense. The second question we asked is what does the evidence show and third because this is a workshop what can we do to improve the situation and the situation always needs improving in research. If you read the Cochrane Library 95 percent of Cochrane reviews end up with "We need more studies."

So, everybody is used to that. The media fall asleep when you say that you need more studies but I think in this case as you heard already from the previous speakers we do need more studies and we will go into that in a minute.

Now, I am a person doctor. I am not a mouse doctor either. I am not a ferret doctor and I am not a laboratory doctor. I am a practical doctor.

What I would like to know is if I shoot people full of influenza vaccines, in this case seasonal influenza vaccines and I assign half of the population or half of the sample which is comparable to placebo or do nothing or a control intervention do I actually witness a change or is there a difference in impact of a vaccine and what is this difference? Are the harms worse than the benefits or are the benefits better than the harms and what are the benefits I see? These are the questions we ask.

I think these are practical questions for people who are thinking about embarking in research or are already embarked in research in pandemic vaccines and indeed in seasonal influenza vaccines. We want to know that vaccines, these influenza vaccines actually prevent a certain number of conditions. So, we looked at our database and we looked at our study register. We looked evidently principally we looked at randomized controlled trials because we are looking at this with the perspective of registration of potential pandemic vaccines.

When we looked at those trials in which serological outcomes and clinical outcomes were evaluated prospectively on the same population of course you looked at that, you say. Well, all trials look at the same population. That is not so, unfortunately.

We started off with all comparative studies of vaccines against, comparative studies against naturally acquired influenza. Of influenza vaccines the number in our database is 338.

Now, I think the earliest trial dates from the forties and the most recent, sorry not trial, study, dates from the beginning of 2007, because we haven't updated this searches. We do that once a year.

Then we moved on. Of these 338 we took 281 which compared the effects of seasonal influenza vaccines with

placebo or do nothing. There are 281 of those still with registration in mind. Then we looked at studies of which of these 136 were studies which looked at the serology and the effectiveness in the same population, 136 of these but only 59 in 50 publications were randomized controlled trials. Only four of these were randomized controlled trials at low risk of bias. What does that mean? It means that these were trials that can actually tell me something. I could read them. I could understand and they were likely not to have any interpretation problems.

So, we end up with four. "My God," you say, "Out of 330-something-or-other only four." Yes, and the most amazing thing about these four trials is that they tell us something that we have already heard. They tell us something that Tommy Francis mouthed in 1941-42.

Let us take it one by one. We start off. We have the first one from Holland 1967-68, 374 school-age children. However, the one problem with this trial was a follow-up problem. In our classification which has been published and rehearsed and has been validated high risk of bias or low risk of bias means a number of items which is unsatisfactory. In this case the one unsatisfactory item although it was a high-quality trial was the follow-up. So, we end up with three.

So, we are now back to three. Well, the one trial

on 697 asthmatic children aged 6 to 18 over two seasons carried out in this country and then we have 793 children age 6 to 24 months in 1999 or 2000, sorry. The asthmatic trial was carried out in the Netherlands whereas the healthy children trial was carried out in this country and then 55 people with chronic bronchitis from 1960-61. So, a little bit of history and archeology there; however, the vaccine that they considered in this trial was a bivalent whole variant which I understand is no longer made although whole variant I understand from David Wood is made at least by one manufacturer in Eastern Europe in the eastern part of the European Union.

So, we look at these two trials that we are left with contemporary vaccines and we have one trial on asthmatic children which runs over two seasons in Holland and which shows the usual curves of antibodies that you would expect. The serology you would expect reaches protective levels but the trial reports that the vaccine was ineffective.

Seven hundred and ninety-three children though in this country, they also had, the intervention arm also had some serological responses which you would consider protective and in one season they were protective. In the other season there was no viral circulation. In both seasons they were not protective against otitis media.

So, what do I make of all this? Throw away the baby with the bath water? Wait. We must make this judgment but this judgment is made on two randomized controlled trials on children. One is the trial on asthmatics. The other one is the trial on children around the Pittsburgh area. There is substantial uncertainty about this but we have already heard this. However, what this shows is that there is an absolute requirement for good quality randomized controlled trials comparing the effects of vaccines present, past, future with placebo or do nothing but preferably with placebo which have a serology and effectiveness outcome and we can design some of these prospectively.

I understand from the WHO web site that there are over 300 trials, prospective trials registered on this web site. Let us hope that these are good from the point of view of design and most of all the point of view of reporting.

So, we have got some work to do but then I understand this is what the point of the workshop is. We have some work to do. Let us get our brains together and let us design some of these studies, and most of all let us just take a little bit of time to think about reporting.

You know what I do for the Cochrane Collaboration is a hobby. I work in the evening. So, to me it is very

hard when I work at nighttime. It is very hard to understand trials, which have got bits or studies which have got bits missing, which have got half the population missing at the end of the study. It is very, very hard for me to understand what goes on and my colleagues as well and they are not mother tongue. So, let us just invest a little bit of more time and brain power designing some of these studies that can give us the answers.

Like Tommy Francis was saying maybe one plus two plus three makes five. So, let us be careful. Yes, there are some determinants. There are pretty strong pointers as to the effectiveness of these vaccines from literature but there is something else. There are some other conditions. So, let us design some studies. We have still got time. Let us design some studies which can give answers, more attention to reporting. On my knees this is a plea because I am a reader, okay? In real life I do something completely differently. I read what you lot publish, and it is a plea, please don't send me to bed with a headache because some of these do, not all of them, some of them.

There must be more accountability. We cannot have a lack of transparency in publicly funded trials, Tommy Francis again. If you take the public's shilling, if you take the public' money you are responsible and accountable to the public.

So, you see there have got half Italian and half Brit, and quote Tommy Francis the whole time. It says something about giants.

Also, we need some methodological research into evidence-based criteria of study quality and reporting. What I am giving you today is a cartoon version of what there is.

As I was saying to Bob before we have got no money but if we were in the United States we would probably have six or seven PhD students doing methodological research on our vaccine register and all our data extraction sheets. There are determinants of quality and determinants of understanding of comprehension and data interpretation of some of the trials and some of the studies that we have which are crucial and could illuminate future studies, future trials, future comparative studies.

It is so important that we invest in methodological research but we know almost nothing about this.

Okay, this is the last slide you will be glad to hear and this is the protocol violation. What is this? These are two meta analytical screens, okay? They are known as Forrest(?) plots. Now, do you want me to describe them or are you all familiar with them? Do you know what all this garbage means, all these squiggles and all these

strange things mean? Do you want me to describe them?

Okay, fair enough. Here are the outcomes, influenza-like illness and influenza. These are pandemic trials. What? Yes, pandemic trials from 1968-69, the last pandemic. By the way I am survivor of the last pandemic not that I was aware of it but I am a survivor.

Okay, on the left, so you can ask me anything you want really. I know all about it.

(Laughter.)

DR. JEFFERSON: On the left are the subanalyses. You can see we have got vaccines matching and vaccines not matching and we have got four data sets from three, I beg your pardon from four studies on here and they are comparing what was in effect a pandemic vaccine versus placebo do nothing in some very interesting circumstances. The Morgapgap(?) studies have been carried out in boot camps in military training camps, so just the kind of situation that you would look at in a pandemic, and what we have here are the estimates of effect with vaccines matching and vaccines not matching the pandemic virus and look at what we have got.

We have got against influenza-like illness we have a relative risk and a random effects model of 0.34 which equals 66 percent effectiveness.

Now, that is very high against influenza-like

illness. Can anybody tell me why that would be high like this? A very high percentage of these influenza-like illnesses cases were due to the pandemic virus. That is what that shows and this is very important.

So, I can tell you what worked the last time because look at this. This is the same on influenza and when the Morgapgap studies, when the vaccine was matching the circulating strain efficacy was 93 percent. It was very high. Unfortunately we don't have any data on complications. That is absolutely right. We need data on complications. So we need to think about that very carefully, but what these slides show is what worked in the last pandemic, and it was a monovalent variant vaccine of the old kind that has been shunned because I understand was causing one or two febrile reactions or certainly some reactions.

So, I can tell you now and I can finish with this with what worked in the last pandemic. Unfortunately last night we had a power cut. Electricity was turned off in the hotel. So, my crystal globe that I brought from Italy was actually out of order. I can't make it start up again. So, I can't tell you what is going to happen in the next pandemic and what is going to work in the next pandemic.

However, here we have got a clear indication of what could be. I think I will stop it there.

DR. COUCH: You left us plenty of time for discussion and not only did Tom say that we don't know how to do studies but some of the people sitting out in that audience I know did some of those studies that didn't show up on the screen, but at any rate he has charged us for improving the quality of the studies and he has also raised the subject of homotypic and heterotypic immunity and shown it in what he considers good studies to be an example of homotypics more powerful than heterotypic but heterotypic is measurable as significant.

So, I would say we are open for methodologies, studies, epidemiology, suggestions. One of the things Jerry said was that we want to promote collaborations and new efforts. So, this may be a place to talk about that. I can tell you that I have not ventured much in doing this kind of studies but when I have they are tough and they take a lot of time and effort, too.

We have a comment back in the back, and we have for those of you for this session the front mike you can walk up to. The back mike is mobile. So if you don't want to get up just hold up your hand and we will get the mike to you.

PARTICIPANT: I just have a question on the efficacy of the last study you mentioned. How was that measured.

DR. JEFFERSON: Which particular study?

PARTICIPANT: The one at the bottom. Did you mention it? I can't read from here, but I think you mentioned it was 93 or something in the high nineties percent efficacy, how the efficacy of the vaccine was measured.

DR. JEFFERSON: These were military camps. So, the follow-up was pretty good. They had to report sick. This is a British army expression. You probably have a different expression in the American US forces. They had to report sick. They didn't have a choice because these were military. I think they were marine recruits. So, they would have had corporals and sergeants after their skins if they didn't report sick. In any case they needed a chit from sick parade, from the MO to say that they were sick.

So, the follow-up on these studies is actually very good.

DR. COUCH: Do you know if they had specific illness criteria and whether or not they had a marker for infection, virus or serologic responses?

DR. JEFFERSON: I don't. I would have to go upstairs to my database to have a look at that, but I can answer that later.

DR. COUCH: The tighter that illness definition the greater the protection. Most of you know that because

it is an awful lot of mild illness that is not caused by influenza viruses in the middle of an epidemic. If you make it a classic case of influenza, I don't like, I have got another term I don't like and that is influenza-like illness but at any rate if you are tough on what you call influenza in the middle of an epidemic then you can find highly significant protections.

DR. JEFFERSON: Sorry, I misunderstood your question. The influenza outcome that has serology and/or culture confirmation.

DR. COUCH: Both.

DR. JEFFERSON: Both or no, and/or I think it was, but I can answer that later on.

DR. COUCH: And most people know that if you have that criteria to go with it you increase the specificity of your finding and the greater the protection from your vaccine.

PARTICIPANT: I think you may have answered one of my questions and that is you were saying that you required serologic outcomes. There was a debate whether serologic outcomes is the most appropriate way to evaluate vaccines which produce high levels of anti-hemagglutination inhibition antibodies.

So, were the studies, for example, that did not use a culture or culture PCR outcome instead of a serologic

outcome, were those excluded and what about all the studies that were done in the US military over all these years which I understand were randomized controlled trials with a serologic outcome? They seem not to have been included.

DR. COUCH: What was the starting point for your time period of looking?

DR. JEFFERSON: We went as far back as we could go. I am not claiming this is exhaustive but that is what we did. Now, we didn't exclude anything. We did not exclude any randomized controlled trial against placebo do nothing. What I was showing is not excluded studies but the studies that are the ones where the reporting or the conduction of the study was clear. That is all.

DR. COUCH: Harry, you had your hand up. Microphone, please? Just hold your hand up back there. We have got a mobile microphone.

HARRY: So, we all agree that randomized placebo-controlled trials are the sort of goal. Given the current environment where the highest risk and the place where we need correlates of immunity are in our most vulnerable populations, the elderly and the very young children in those populations at least in the United States how do you do placebo-controlled trials in this day and age? I don't think that is possible.

So, are we ever going to get the data that you

are looking for at least in the United States?

DR. JEFFERSON: That is not a question for me to answer. That is a question for the people in the States to answer. I mean randomized controlled trials, placebo controlled randomized controlled trials don't have to be carried out only in the States. They can be carried out anywhere in the world but whether you are going to get an answer or not or whether you can conduct that is not for me. I am not a US citizen.

DR. COUCH: I was going to say, Harry, if you will permit me I will I will take the prerogative of the moderator. Let us don't take that one on. That is an ethical question more than a scientific. So if we stick to the scientific I think we will be a little safer and everybody agrees that the randomized controlled trials are ideal.

I would like to point out though that while he took apart all those, actually maybe I am a little tougher than you are, Tom. You are a purist. I believe there is such a thing as consensus and momentum you see. They can't all be wrong even though they don't all agree and when we are looking at efficacy but of those that he accepts homotypic immunity is significant. Heterotypic immunity is present but not as significant. So, I think he has verified in that clinical trial from vaccines the kind of things we

have been talking about.

Now, can we get the microphone? Oh, you have got it already.

PARTICIPANT: Tom, I very much appreciate your presentation. One thing you didn't comment on as well as the randomized controlled trial, the only one that I am familiar with in the elderly is the vaccine which is used primarily for the elderly and yet there is only one study that was performed that is the Dutch study and it is fairly inconclusive especially for those of the elderly, amongst the elderly.

In the States we have a problem in that a number of cohort studies show amazing efficacy, a 50 percent reduction of all cause mortality of the entire population which is quite outstanding relative to what we are finding on a population basis. We just don't see that. So, there is a discordance there. There is a lack of randomized controlled trials at least in the US. We talked about the ethics of potentially doing that. How about in the UK?

DR. JEFFERSON: Nowhere, the normal ethics stance would say that nowhere has a policy of immunization of the elderly can a, you are specifically referring to the elderly here; so, I will take the elderly, can a randomized controlled trial against placebo be carried out? So, that would rule out the 91 countries I think there are, maybe

more, maybe someone will know this that are covered by that policy. What I think of the policy is irrelevant. It is the conditions in the countries that would have to be, the people in the countries that would have to be convinced but we have got WHO here. So, I would ask David if it is at all possible to carry out placebo-controlled trials in certain age groups.

DR. COUCH: And in a country that does not have, use or recommend vaccine.

PARTICIPANT: Could I ask a clarifying question? I wanted to make sure I understood that you weren't confusing an endpoint with a serologic correlate following vaccination. So, you were looking at studies that had a vaccine given and then a post-vaccine antibody was determined?

DR. JEFFERSON: The paired sera.

PARTICIPANT: As opposed to endpoint and that is when did a patient or when did a participant develop influenza which one would preferentially use a virus positive case either culture or PCR to actually determine the virus as opposed to looking at serology there; am I correct in that assumption?

DR. JEFFERSON: Whatever the investigators had actually reported that they were doing. The classic design is for the follow-up to take place, a baseline titer to be

taken and then a titer to be taken either once or twice after that 4 to 6 weeks and in some cases for antibodies to be taken or blood to be drawn when this person reports sick.

PARTICIPANT: Having done a lot of these trials our FDA likes to see virus positive cases. We are really not interested in looking at cases that are defined serologically right now. We want to know what specific illness was associated with a virus-positive case.

We are of course interested in serologic correlates of protection and perhaps secretory IgA correlates of protection. I think this is a very important message for the sponsors in this room because typically our clinical trial design as you pointed out would not fit your criteria. We don't take paired sera. We don't do post-vaccine nasal washes in our subjects. We simply vaccinate a bunch of people and have a bunch of controls and then look for virus-positive cases later on.

You are suggesting we need to go back and get those samples so that we can develop the correlates. I think that is a really important message.

DR. JEFFERSON: Thanks. I think that is very constructive. Yes, that is what I am suggesting and also I am pleading for people to carry out follow ups.

Now, you may collapse laughing on the floor

saying that of course we follow up people. Well, some of these studies follow up different populations from the ones they vaccinated. So, it is a little bit difficult to understand what goes on in some of these and it is not a pejorative comment. It is a comment that I make trying to look forward. The criteria that we used here are the criteria which we think were logical within the study question that was given to us.

DR. COUCH: Last comment and I said, "Let us don't' do ethics," and then we gave you an ethical question.

Dr. Wood?

DR. WOOD: Just before I come to that I would like to pick up on the last point by Dr. Belshe. I think that is a critically important message to come out of this session that sponsors in particular could make arrangements to ensure that there are adequate samples taken to enable these types of follow-up studies to be done. I think that is a critically important message to come out.

Going back to the ethical question I think it would be very difficult indeed for WHO to recommend doing randomized controlled trials in countries that don't have policies in place if it is not ethical to do so in other parts of the world. I don't think that is going to be feasible for us to do. However, what I think we can

possibly do and I think it is a good challenge to us all is we can look at the opportunities that we have as influenza vaccines do get introduced into countries because there is not 100 percent coverage straight away and I think those are the types of situation where you may be able to generate data but I think for us to recommend doing randomized controlled trials in countries it is going to be difficult but I think of how do we use the opportunities that may present as vaccine programs get rolled out maybe to generate some of these type of data.

DR. COUCH: Outcomes need to be further discussed. I said no more talks and Dr. Goodman gets the last moment.

DR. GOODMAN: Just a really short comment. I think we shouldn't underestimate what we can learn even from studies that aren't placebo controlled but that are well conducted randomized trials, for example, in terms of correlates of protection because the population is not one person. It is a continuous population where we will see a different series of values and levels. Also, presently placebo-controlled studies are occasionally conducted in populations for whom the vaccines are not currently in the recommended age groups and again we may get extrapolatable information from those.

So, the criticality of having studies that are

done done well and having samples remain available for analysis can't be emphasized enough.

DR. COUCH: This has to come up again in this meeting I am sure. So, we will have further discussion.

Our next speaker is Dr. Bennink who is going to address some fairly specific data on cell mediated immunity, T cell responses in mice.

Agenda Item: Cell mediated immunity: description of T cell responses that correlate with protection and epitope specificity of T cell responses in mice

DR. BENNINK: I sort of feel like the odd man out here I think, you know speaking about mice first of all or mice responses and second after Brian's talk stating that cell-mediated immunity has nothing to do with it. At least we had some correlates here in the last in terms of heterosubtypic immunity but I was given this topic in terms of talking about correlates of protection and the specificity of the T cell responses and so I want to speak specifically on this sort of aspect.

I decided for better or worse and I think after talking with Brian a little bit probably I should have put some data in because some of it is better than probably you have seen in the other aspects and a little bit stronger than what you have seen, but I decided not to, to sort of try to do this topic more generally and broadly and try to

describe it in that sort of way.

Before I want to do that I want to give you some of my opinions as a T cell virologist in a sense but I also see even though I have worked my whole career on T cells and stuff like this, in terms of influenza I have no questions about what Brian has been saying or what has been said this morning.

The ideal is to immunize for specific neutralizing antibodies to the virus. I don't think there is any question to this at all, if you can do that.

At the same time I do believe however, that cell-mediated immunity can provide protection and I am going to describe what I mean by that protection against morbidity and mortality, and I think this can be clearly seen if we remove antibody responses to HA. You see it much more clearly. If you remove CD4 responses you can see that the CD8s do something and if you remove CD8s you can still see the CD4s will help clear virus with the neutralizing antibody.

Also, I think which has also been said this morning as well that for optimal immunity really I think there is a what I would call almost a whole matrix of protection that is set up and that immunization if you can generates a memory in a sense, from all effector arms of the immune system.

So, why focus on the T cell immunity in influenza? Obviously it is because the responses are heterosubtypic and this was shown I think from this T cell responses that we did about 30 years ago in Peter Dori's lab, with Rita Efrosh and Walter Gerhard as well as real soon after that Tom Braciale showed the same thing that these responses can be heterosubtypic.

Now, to really talk about specificity I want to briefly just mention this. I think everybody, most of the topics I am talking about here I think most everyone in this room probably already knows but I just wanted to deal with it so that you see where I am coming from in some respects, but T cells recognize the antigen. So, what they see is this peptide which is 8 to 10 amino acids long in the groove of the MHC molecule.

So, although most immunologists call these things epitopes, okay, it is really a misnomer and we have fought this but we sort of have given in on it, but in most respects, okay? But whereas antibodies actually recognize epitopes on proteins, the globulars and they can neutralize the HA and everything really the T cells recognize if you want to call them that, they could be endotopes or what we like to call them is determinants because they can be anywhere in the molecules and this is part of the reason also in terms of the conservation in terms of

heterosubtypic immunity.

The characteristic particularly in mice and these things is that characteristically there are immunodominance hierarchies that characterize the T cell responses to these, okay, and that is that the T cells respond only to a tiny fraction of the potential peptides that are encoded by the virus genome itself.

So, if you are trying to calculate through these 8 to 10 that could be done throughout the whole genome of the influenza obviously it is a high number. If we were just taking one of those numbers it can be, because the genome is close to 5000 amino acids that you could have many peptides in some respects of different things. Even some of the virus responses can recognize peptides that are as long as 15 mers(?) in some cases such as in EBV. That is much rarer in those cases but the responses tend to be limited to a tiny fraction of the total peptides that can be encoded by the virus and they are ordered into highly reproducible hierarchies that are based on the magnitude. So, by the quantitation of the T cell responses that you get you get these hierarchies and this breaks down into what we call immunodominant determinants that are being recognized and the immunodominant determinants of what is recognized are dependent upon in a sense first of all the antigen presentation, okay? These are although there are a

lot of other factors that also get involved in it, these are primarily dependent on whether these peptides can bind to the MHC molecule and second of all whether they can be processed and how well they can be processed, whether they are degraded and the other major impact I think that plays on this is the repertoire of T cells that can recognize these peptides so that T cell precursor frequency isn't there. Do you have a good naive or memory precursor frequency that is there and that will help dictate or determine some of the, what the hierarchy as well and for heterosubtypic immunity there has been much more emphasis placed on the internal proteins, okay, in part due to their greater conservation. In other words the internal proteins have much more of it conserved between the different subtypes of virus and so besides that in terms of statistical numbers obviously you are only dealing with two glycoproteins on the surface. You have more proteins and more of the amino acids are coded for in the internal as well. So, you are going to expect from that just on a statistical basis, okay for any given MHC molecule more responses based on the internal protein but the importance of it is in terms of heterosubtypic immunity.

So, what do we mean by T cell protection if we are going to call it this, and I think I feel I have to define that a little bit because if you don't it gets a

little bit difficult. First of all because T cells cannot prevent infection, I have been describing to you a little bit what T cells recognize, and I did that to some extent so that I could set up this in a way. They have got to recognize peptides that have been processed from full-length proteins in some ways and most of these are if you want to think of virus-infected cells or it may be processed from virus that comes exogenously in some way which I don't want to really get into too much but it crossed thiamine or in some other way that way but in other words in a sense you have to have an infection before you can actually get these T cells to really function. So, the best expectation in this sense in terms of protection that we could talk about would be to limit or attenuate the morbidity or mortality and from that standpoint in terms of what we talk about in terms of protection when we talk about cell-mediated immunity where really most of the assays that are being looked at it is in terms of weight loss, in terms of reduced mortality, in terms in some cases of reduced days to death or in terms of the reduction in the virus titers and most of these are in the lungs or in the upper respiratory tract.

Okay, so what I am going to do and try not to insult many people in the room as well as outside that have things, and I told you I wasn't going to present data but I

would like to describe some of the many studies that have been done to try to describe what has really has been looked at in terms of these, and I think the area where the most studies have been done is in terms of different virus injections into mice, okay and looking at homo or heterotypic subtypic immunization, okay, where they immunize with one virus and come back and challenge with another virus.

Most of these studies that have been done in mice have been done in either Balb C mice or in B6 mice or other strains of B6 mice, knockouts or transgenics or some other deficient mice in that way.

There are a few of the studies that have been done also in CVAs or C3Hs but there are many fewer studies in terms of that.

So, most of them are done in the H2D haplotype or in an H2B haplotype and as I said both of these have their own characteristic immunodominance hierarchies that are set up in these cases.

A second part of this is that many of the studies, not all, many of these studies have been done with PR8 virus and also with a reassortment of the PR8 virus called X31. So, a lot of the challenges, the X31 has the hemagglutinin and neuraminidase that is from Hong Kong and all of the other internals are from the PR8 virus. That is

not all of them. I mean Tom Braciale for years has worked with A/Japan but others, some of the more recent studies also have used some of the more recent H3N2 viruses as well.

Anyway to go back to the point that I am trying to make in terms of these sets of studies and as I said a lot of people have worked on these for years. The early studies were done by Gordon Ada and Gapp and a lot of those studies clearly showed that there could be heterosubtypic immunity by the T cells. Throughout the years as knockout mice came into vogue in a sense, particularly Peter Dougherty's lab and I can list a lot of the people, some of those in the room whether it is Maryna Eichelberger, Jackie Katz, David Topham, you know, I could go through a whole variety, Gabrielle Bell, Steve Turner, Ralph Trip; you know I could really go through a whole range of different people who have worked on these sort of studies who have also found some protection in terms of what I have listed here in terms of protection.

In more recent years I think and some of these overlapped with some of the things that Peter has done, Suzanne Epstein has also done things in terms of immunoglobulin mice knockouts as well as gamma delta T cell knockout mice and a variety of these things showing that gamma delta T cells to some extent also play a role but in

some of the other antibody knockout mice clearly they show a role in terms of protection for CD8 as well as CD4 T cells.

So, there has been a whole list of different things and one other study I want to mention that comes to mind in terms of Peter Dougherty's group and John Stambiss I think has done some with the avian influenza where they have shown some protection as well that is alleviated by T cells. In these studies in terms of this depletion also there has been depletion where they have used antibodies and knocked out the CD8 T cells or CD4 T cells with antibodies and shown the effect that that has as well on whether they muted things and in most of these cases there is a modest level of protection, at least I would say one log. Often there are several, you know, tens of percentages or in some cases even more of mortality reduction, sometimes 100 percent mortality reduction in terms of these protections. I think the strongest data and I think that it is a little bit in contrast in my own mind to some of the other things and the most recent one is by Dick Dutton, some of those studies that he has done in terms of these things and I think he has shown some of this, and this is using a cold-adapted virus as an immunization and shown that there is homo and heterosubtypic immunity as well.

In terms of trying to get down to specificities as well as just showing these things as I have described already that there are these hierarchies; so, we could even go back to some extent back to the old studies and say, "Okay, well, we know what these specificities were because that is what you get when you do these responses," but there have also been immunizations to internal proteins.

Some of the early ones if you will, there was a Merck study. John Donnelly used DNA to nuclear protein and they were able to show protection in that particular study.

Sue Epstein has also done studies where they used a nuclear protein as well as the M1 and also used prime boost studies along with the VRC with Gary Nabel's group and shown that they can get protection as well from these internal proteins and it was described earlier that the antibodies to the NP do not protect in any way.

So, there are immunization protocols as well in terms of the vaccinia viruses as well. My own experience with the vaccinia viruses, I will be very frank with you, we started with these things very early on back in the mid-eighties and when we started to look at a lot of these things we saw very little reduction in this, okay, from the internal proteins about a log or something to that effect.

What really confused us and we stopped even trying to look at protection studies in those particular

cases was that we were using a control against a vesicular stomatitis virus protein that was expressed and we also saw almost a log reduction in that which confused us and said that we can't really make a conclusion based on some of those things.

So, some of it is mild but I think the preponderance of the evidence you know in terms of what people have found is a great deal of protection. There also have been bulk T cell transfers.

Some of these were done really in the very early days almost 30 years ago by Gordon Ada and Gapp and some of these things where they transferred T cells and they clearly got protection in those studies as well as there are also other studies of transferring T cell lines. Some of the earliest ones were by Lynn Inasconas but Tom Braciale and Aaron Leukaker also did transfers. They had two specificities for clones that were transferred, one that was specific and one that was cross reactive. In all of those cases there were, well I should say that we are up to 3 logs I think the reduction in virus titers that were done in those and the more recent ones from that are in some TCR transgenic mice, okay, Moskofitis and Graham Price also was involved with some of the later studies that he did in terms of looking for protection, okay, using a T cell clone that was specific for the nuclear protein 366

from NT60 actually.

That cell they could get clearly things and I am going to come back to that study because there were things. There were some other comments that I wanted to make about that particular study, but there clearly was shown that you could get protection from lower doses of virus in that. Graham's studies in that particularly showed that he could use those cell lines as well to generate mutants of virus the escaped mutants of the influenza virus as well.

So, what are some of the specificities if you will that correlate with anti-influenza protection, okay? To my knowledge and somebody could correct me if I have missed something here but to my knowledge all of the immunodominant specificities tested can be protective. There are only two exceptions to this, and this is why I put this subtype here, and both of them can be protective, okay? But the first one of these is that David Woodland showed that the PA224 when it was immunized as a peptide could, he observed some detrimental effects upon challenge so that what he saw was not as much virus reduction and he saw later virus titers that were higher later in things so that there was not the virus titer reduction thing.

The reason I used this as well as an exception is because later Peter Dougherty did some studies where they used a lipo probe peptide of the PA224, okay. This is an

acidic polymerase peptide and was able to show clear protection from that. So, it is not as if that peptide or that determinant in this particular case is always detrimental or anything else for that.

The second one I list here is immunopathology and that is because in the Moscovitis studies okay, when they did the transfers of the NP366, the nuclear protein specific T cells what they got in that particular case on high virus dose challenges when it was relatively high doses, they saw more immunopathology and a quicker time to death. So, you can see some of those things, but I have never observed at least yet in the few that have been looked at anything where there was an immunodominant specificity that didn't show some type of protection.

Another point I want to make about these things is in terms of multiple function repertoire diversity in terms of what you have for specific T cells or higher avidity T cells may provide optimal memory generation and/or protection but has not been rigorously demonstrated. I don't think in terms of the influenza system that this has been shown clearly, okay, that if you have more multiple function, in other words if you are testing for TNF alpha as well as IL2 as well as gamma interferon are those triple as well as cytotoxic function? If they have more functions like that are they more effective in terms

of the response and I don't think that has been clearly shown yet for influenza or if you have higher avidity T cells. You could imagine in all of these cases that you would expect that to be the case but I am not sure that that is clear in this thing and there are some studies I know of that will come out probably relatively soon in terms of repertoire diversity.

The last thing in terms of this part that I want to discuss is the precursor frequency associated with protection. I don't believe it has also been examined. In other words we quantitate these responses nowadays mainly by tetramer positive responses or gamma interferon positive responses and they are usually quantitated as a percent of CD8 T cells that are present and it is not clear to me at least in terms of that or in terms of quantitating back what is the lower level of the precursor frequency or how many T cells do you have to have in order to get some type of protection and I am going to go off in a quick tangent here that actually I had planned on doing this, and I think this addresses some of what Peter did because I also saw that in terms of cell-mediated immunity but it relates to this, but I also saw that we weren't in this thing.

It is just one slide here. It is that I think there are innate immune issues that I think need to be looked at in terms of is there innate memory okay to

influenza; what is its role in heterosubtypic immunity? In other words T cells have to see the dendritic cells. Okay, they have to, antigen has to be presented on macrophage dendritic cells in order to stimulate that response, okay? And there has been some suggestion that there is some memory within innate immunity.

So, I think there needs to be perhaps some looking at this and what are these responses and another is are there aspects of the innate response together with measurements of adaptive immunity that would give a better idea or correlates of immunity such as natural killer cells or cytokines or chemokine levels falling back early on or something along those lines, and one other one, and this is really more to emphasize the fact of innate immunity issues is that all of the mouse strains that we have been really studying are all MX negative, okay, and in fact MX plays an enormous role in terms of a natural immunity to things, perhaps even a bigger role than what in terms of log titer reductions or how much virus you have to use to infect the mouse than the CD8 T cells or CD4 T cells or something like this.

So, there may be several aspects of innate immunity okay, that we haven't really looked at that could be enhanced in some ways.

DR. COUCH: Don't go away. We are cutting into

lunch hour, but yes, and Tom Braciale I don't know if you have a comment but there are two or three of you that this is pure cytotoxicity and pure T cell immunity. So, now is the time to bring up your questions and comments.

PARTICIPANT: I actually wanted to discuss retrotypic T cells and we have immunized mice with X31. We can protect against TRH and that is clearly not antibody mediated. Nevertheless when we immunize mice with vaccines we get 40 percent of all CD8 T cells to be specific for pure virus and 40 percent by gamma tetramer. We can see no protection or just marginal protection. So, I don't think frequency plays a role. So, you can't really top 40 percent. I think it is quality and probably location.

DR. BENNINK: I don't really have a comment on that. I really think that location obviously is a very important thing and I think that from the discussions before I think there is a timing event as well probably not in the studies that you are talking about but in terms of, yes, in terms of how long and where these T cells are and David may address that after.

PARTICIPANT: I want to ask you a general question about heterosubtypic response in T cells because we know that in restat(?) strain the homology among HA protein is extremely high. For example in HA Indonesia, whatever it is about 90, 95 percent and it is still about

40 percent comparing H1, H2, H3, H5. How do you think these markers, how much seasonal immune response impacts the response to avian when there is a 40, 50 percent homology and how much we can talk about heterosubtypic response in T cells when the homology between H5 duck in Indonesia or Vietnam is 95 percent?

DR. BENNINK: But it just depends on you know in terms of what I was trying to describe some in terms of the specificity of the T cells. Some of these are going to depend on the individuals because it depends on what their MHCs are. It depends on how well those antigens are processed. Some of it depends on if you are talking about and I am not clear in terms of what you were discussing. Some of these in terms of immunizations okay I would predict and I think this is true that the cold adapted viruses are going to give you much better immunization capabilities for vaccines than if you are just talking about subtype specific vaccines.

PARTICIPANT: I was mainly talking about pandemic vaccine and work in humans in pandemics through vaccinations and we tend to say that we are all naive against H5 but we also know that for T cell conformation is less important than antibody and that the homology with seasonal hemagglutinin is extremely high. Do we care about how much of the impact of the seasonal immunity has on

theoretical T cell responses against avian flu?

DR. BENNINK: I think you care if it really is going to have an effect and do I really think it has an effect? I think that the last study you showed in here I think Sue is going to talk on some of the heterosubtypic immunity and I think I am going to delay until that. There are some cases in terms of the Ohio case that an argument can be made and there are some aspects of that that suggest that yes but I think it is modest.

DR. COUCH: Last one.

PARTICIPANT: Last one, again. So, a couple of comments. One, in some of the clinical studies we are doing with the pandemic vaccines we are starting to see a correlation in people who get annual or regular annual flu vaccines responding better to the pandemic strain. So, I don't know if there is enough data there yet to make the point strongly enough but it is going in that direction.

The comment about having high frequencies of CTL circulating, again, location is important. We know from the mice, some of the mouse studies that in mice that can't maintain cells in the lungs that in spite of very high numbers and high frequencies in the spleen, lymph nodes, blood, etc., they are not as well protected and then a comment about the innate response in terms of delay in the secondary we recently have done some mathematical modeling

of immune responses to flu and the one thing that jumped out from those models was that the dendritic cells controlled the tempo of the response whether it was a primary or a secondary response. So, anything that affected their behavior or maturation had the most impact on the outcome of the infection. So, that early aspect of innate immunity I think is key to understanding both vaccine responses and secondary protection.

DR. COUCH: This topic will surely come up again and I have to comment. One of my reactions is that T cell immunologists are T cell immunologists but they love influenza because of that protein, that antigen, those definitions and the manipulations and influenza is benefitting from that synergy, and our final speaker before we have lunch is none other than Harry Greenberg who is going to discuss some human responses to vaccine.

Agenda Item: Cell mediated immune responses in humans following natural infection and vaccination

DR. GREENBERG: Thanks. We are a bit late, and I find myself standing between all of you which not enviable. I am going to try to go as quickly as possible.

Let me say just two things to start out with. If you are looking for correlates of immunity it is best to set up your structure where you have a randomized placebo controlled trial. You can't do that with natural infection

because you can't predict who is going to get infected.

If you use vaccines to set up your correlates unfortunately our glass in this case is half empty. Our vaccines are relatively effective. So, it is very hard to do correlates of immunity when your intervention works all the time and so you need very large studies to find the place where your intervention doesn't work if you are really looking for a correlate of immunity and then finally if you are looking for things other than simple immunologic measures such as HAI or neut it is very hard to do those on very large patient bases. So, the system is not ideal.

That said, what I am going to do today is just go over with you very quickly some studies that I and colleagues have done at Stanford really looking simply at the immune response and the cellular immune response and what I would like to say here is I am really not even an immunologist. I am a virologist, but cellular immune response I would like to think of it, B cells or cells and so cellular immune response includes B cell responses and T cell responses and I would simply, one message as an aside is we have tended over the last 60 years because it is very easy to measure the endpoint or the effector or at least one of, the most important effector, the immunoglobulin molecule of B cells but B cell responses can be measured in many other ways and that might be a place

that we should train our guns.

So, what we have done here is take advantage of the fact that we currently have two vaccines that are licensed for interventions in humans, a live attenuated and the inactivated vaccine and what we did is we have used these vaccines within their indication which is healthy young children and healthy adults and compared and contrasted and the experimental design here was based on the fact that both of these vaccines have been judged by a variety of people including the registration process in the United States to be safe and efficacious. They both work and I would say as a big generalization in healthy children and healthy adults they work more or less over a large number of studies done by Belshe, Murphy, etc., more or less equally.

In very young children recent studies have said that maybe the live attenuated might actually work better especially in the area of heterotypic immunity but in most of the people that we are studying we only study them within the licensed indication and in the time frame I studied the live attenuated could not be used under its license in very young. So, I don't have a lot of data on that.

So, our idea was here are two vaccines that are very different. They both work sort of the same and let us

study the immune response which perhaps is different and we will get some idea of correlates of immunity.

The experimental design is seen here basically vaccinate control group, either live attenuated or inactivated and then you draw blood at zero, at 10 days in the first year of study and 38 days and analyze the cellular and humoral immune response.

A big difference and one methodologic difference that I will point out to you in humans and all of these studies in humans which are harder to study than mice, way harder; so, not the least of which is you only can get peripheral blood.

If we could take out the spleens of our humans or better yet respiratory lymph nodes we would really know a lot more but we don't have that ability.

One of the problems of studying cellular responses is that the T cells and B cells are not on the same time interval and just I will simply say that methodologically this is really a big issue.

If you want to study B cell response, effector cell response to immunization more or less you have to draw blood on day 7. If you want to study a T cell response day 7 is not an ideal time to draw blood especially in immuno naive.

So, in our 2 years as you see in the second year

of study we drew it on day 7 because I won the fight with my T cell colleagues and I got to draw the blood when I wanted and so we did a whole bunch of assays and most of these are familiar to you. We did flow-based assays to measure cellular immunity and basically these were gamma interferon and some tetramer assays which I won't show you and for B cells we simply did a simple ELISPOT assay and then added a memory assay using the Rafiamed(?) cocktail to stimulate memory B cells to become effector cells and so this is the general T cell assay, a flow-based assay and it involves incubating PBMCs with flu for a long period of time, 17-hour incubation which for us was basically critical. Shorter times do not work as well and then looking for gamma interferon, CD4, CD8; we, also, did a bunch of NK cell stuff which I am not going to talk about today and then also looked to some degree at more characterization other than simply the number of CD4, CD8 T cells and that was the expression of activation markers like CD38 or their expression of markers, differentiation markers, CD27 or cytotoxicity markers like porphyrin(?).

So, the next two slides are a summary of a boat load of data and I don't want to go through all of these except to say that as you might expect despite the fact that these two vaccines work the same, that is they are relatively equally efficacious in most studies in the

populations I am looking at they really stimulated on the T cell side, they were quite different.

So, if you simply look in children, okay, the inactivated vaccine actually in children and adults we were unable to see a quantitative change in the number of T cells after vaccination in our assay either in children and adults whereas the live attenuated as you can see here we could see in children clearly a rise in the number of T cells.

So, they changed the number of T cells and it varied. Neither vaccine were we able in our assays to see any effect on CD8 T cells in adults, okay? I mean those are our findings.

On the other hand both vaccines in fact did have an effect on the T cells and if you look down here the two vaccines had an effect on the percentage of cells that were CD27 positive or for that matter the cells that expressed CD38.

So, in summary just a very quick summary influenza vaccine induces both quantitative and/or phenotypic changes in flu-specific T cells and I would say simply here that in humans simply counting the number of T cells may not be the total story. I think that is coming out. It is certainly coming out in the HIV world and the problem that all of you are facing is exactly what are you

going to look at in T cells. There is an awful lot of things to look at. It is very easy to see changes. I was surprised to see that we can find reproducible changes in the phenotype of T cells after let us say TIV immunization with no changes in them quantitatively and so it varies on the age of the vaccinee, the type of vaccine and the marker that you are measuring.

Now, I am going quickly because I can hear the stomachs growling. So, next I am going to go to B cells and this is the assay we used. Again, you can't get that many cells from especially younger children. So, we did ELISPOTs in a single well using two conjugates. So, we could look at IgA secreting cells and IgG secreting cells and this is the memory B cell assay and it is pretty standard and relatively simple to do, and probably could be done in large numbers if you wanted to.

The first thing I will say is that the vaccines interestingly differ, the giving the vaccines. So, one thing we did because we studied people over 2 years, we could look simply and this is not a cell-based assay but we looked at baseline flu specific serum antibody level before vaccination in the second year of our study. These are vaccinees who, adult vaccinees who were not vaccinated in the year previously, adult vaccinees who got the inactivated vaccine in the year previously and adult

vaccinees who got the live attenuated in the year previously and as most of you would have predicted the adult vaccinees who got the inactivated vaccine in the year previously had higher levels in let us say, and this is actually true for all three but in H3N2, but what most of you would not have predicted or at least I didn't predict, and I am sort of amazed and we could talk about this, the adult vaccinees who got the live attenuated in the year previously had statistically lower levels of HAI than the adults who got no vaccine.

So, having received live attenuated the year previously reduced on average the HAI titer a year later in the vaccinees despite the fact that all or data would say these two vaccines are equally efficacious.

If you look at simply effector cells, so for those of you who don't know this, the humans and I guess mice as well although I haven't done this experiment in mice an amazing thing has happened when you either parenterally or infect somebody as far as B cells go and that is about 7 days almost like clockwork after your antigen immunization you get a true rush of plasma blasts in your circulations that is immense.

So, this is antibody secreting cells in the blood here around 9 days. This is the first year of study after immunization. If you were to do the same assay, well, we

have it here times zero there are basically no antibody secreting cells in the blood.

So, you go for flu. If I take any of you here in the audience now and I assume none of you have had a flu vaccine for at least, if any of you got your flu vaccine within the last 7 days you are very late in getting it. So, I am assuming you haven't gotten it. Basically I would have to take a liter of your blood to find an antibody secreting cell to flu. If I gave you an immunization and drew your blood 7 days later you could have up to 1000 antibody secreting cells per 10 to the 6th cells.

So, in fact this experiment of nature if you want to study the specificity of B cells in humans 7 days after infection you can put your hands on one helluva lot of actually potentially clonally separatable human B cells. What I want to show here is that for the live attenuated and inactivated they actually look pretty similar as far as IgA secreting cells in the periphery in adults but as you might expect IgG secreting cells are greatly enhanced after TIV.

If you go into children and these are clearly all immune children, these are children who had some exposure to flu but certainly not as much as the adults actually the antibody secreting cells both IgA and IgG are roughly comparable.

So, the separation of TIV being far more immunogenic in the IgG window increases as time goes on and people have more and more either vaccination and/or infection.

So, this is just a little bit more data showing you the plasma blast level appearing in the peripheral blood and these are inactivated, live attenuated vaccine, inactivated vaccine. We pooled data over 2 years which probably isn't statistically fair but it is interesting and what you should see here is one the peak after inactivated vaccine looks like it is sharper and that makes sense to me because you are sort of synchronizing. You are giving a bolus of infection whereas live attenuated it is a broader and slower peak and that is probably because you are having antigen exposure over a number of days.

The difference between IgA and IgG level remains constant over time with the inactivated basically except for this first day the IgA and IgG levels are almost identical in the live attenuated, so somewhat different kinetics and timing.

An interesting finding that we made and this was reproducible over a 2-year period for those of you who are the regulators in the audience you will know that one of the gnarly facts of the live attenuated vaccine is that in healthy adults it is crummy at inducing a humoral immune

response. We just heard and I believe deep in my heart that antibody to flu is the critical determinant of protection. The live attenuated vaccine is a good protector even better in children than in young and healthy adults but it works fine in young healthy adults.

At the same time sometimes you have to die trying to find a humoral immune response to that and so that has always been a conundrum since I believe in antibody but I couldn't show or frequently couldn't show much of an immune response.

If you characterize a humoral immune response as an antibody secreting cell response as opposed to an increase in HAI or neut the live attenuated and the inactivated vaccines basically are very similar.

So, at least at one level there is a disconnect between the humoral immune response as measured by HAI and neut in the circulation of adults and the humoral immune response as measured by the effluence of antibody secreting cell plasma blasts at day 7, the latter being a more efficient way to say in the words of Al Kapician the vaccine was not water. Something happened to B cells. The live attenuated vaccine does something to B cells in healthy adults. Even when you don't see a humoral immune response you see more plasma blasts in the circulation and that is interesting and might have some regulatory point of

view.

I am getting to the end here. You can also use these assays to look at memory and while you saw that prior year immunization seemed to have an effect on antibody levels in vaccinees prior year immunization has no effect on the number of memory cells in people a year later, and I don't know the true significance of that.

Both the live attenuated and the inactivated are capable of stimulating memory cells. The inactivated clearly is a better stimulator of memory cells 30 days after immunization. As you can see here the inactivated much bigger increases than live attenuated. Both of them, however, are statistically significant.

At the same time if memory is judged by rather than assay by protective efficacy which is really what memory is supposed to be all about if you vaccinate are the people, do they have enough memory their vaccination to be protected? They are similar.

So, this would say that there is a lot more memory IgG cells at least 30 days after TIV than LAIV but both of those people when groups of people generally healthy adults when they see a new virus are protected.

So, how am I doing? Do I have a minute or two more? So, I have given you all these points. So, you know what, two last slides here. All I am saying is we are now,

one of the issues that using more multiparameter assays to study immune response, so, here we are doing a relatively small number of assays. We are measuring gamma interferon. We are looking at CD4. We are looking at CD8. We may be measuring CD38. We may be measuring NK cells. We may be measuring you know 10, 15 multiparameter assays. People at the NIH and in Gary Nabel's place you know were getting to, you can measure 100 immune parameters and how do you begin to look at all that data and sort of say, "What is the correlate?" Of course, you can as Brian said, you do enough experiments and you can find data to support anything. You measure enough immune parameters and you can find something that correlates with anything.

In any case we are trying to get a handle on the modest number of immune parameters that we have been looking for and here what we have done and I don't really understand statistically how it is done but luckily that is why we have statisticians, but basically because we have 2 years of work we analyze correlates in year 1 and then use that to generate a hypothesis that we could test in year 2, and so just to give you an example of that we said, "What are the predictors of change in CD4 and CD8 in response to vaccination?" and for CD4 and I will show you again for CD8 what we found is the pre-vaccine level of CD4 specific anti-flu immunity numbers or the CD4 level to predict

changes in CD8 in both cases the best predictor, the most robust predictor of change in titer was the CD4 level prior to immunization. It sort of makes sense but it certainly for CD8s wasn't CD8 numbers.

So, basically baseline levels the more things you can measure and measure accurately the more playing around you can do with trying to identify correlates and I would simply say that we had several talks about mice. Mice are incredibly powerful, you know, with the exception of rare genetic abnormalities you can't walk around and knock out genes of humans and so mice are incredibly valuable in looking at affecter mechanisms, looking at contribution.

They have, also, taught us how to measure many things. All of the things that Jack was talking about and that Tom talks about being able to do in mice, one is beginning to be able to do in humans, and I would say that while I think I would doubt that we are going to find an assay that correlates way better than neutralization, there is much more to understand about what that means especially with heterotypic immunity, what exactly is making some type of, well, for example, why do people who get the live attenuated vaccine, little children, maybe have more heterotypic immunity than people who get the same antigen injected in their arm.

That is not going to come out of doing

neutralization assays until you drop. It is going to come out of some way of looking at cloning what the B cells are making and then understanding how antigen might be presented.

So, I will stop there.

DR. COUCH: Okay, now, don't go away. I decided sometime ago that I was going to limit, restrict your lunch hour to an hour, not an hour and one-half. So, that leaves us about 3 minutes by my watch, and you opened up so many topics I am not sure we can even begin to approach it in that period of time, but let us take the 3 minutes anyway.

Janet McElhaney?

DR. MC ELHANEY: There has been identified a population of T cells that are both CD4 and CD8 positive that increase with aging. They are virtually absent in people under age 30. Have you looked at the expression of both of these markers and seen what is happening in this cell population?

DR. GREENBERG: I think they probably are in our data. I should simply say because I didn't really do proper acknowledgements, this work is done on a grant that involves a lot of people but Ann Arvin and I are the two co-directors of this grant, but Chosun He is the senior research associate who has done most of that. If he has looked at that he hasn't told me about it.

DR. GOODMAN: This is sort of a simple question, but has anybody like the, maybe it is the dynamics. Rather than the baseline level in the hemagglutinin you have it is how rapidly you respond to a virus that is important in a natural infection. Have you or anybody gone along and taken like a group of patients like you have who are immune and maybe where you show some have antigen secreting cells but not high levels of antibody and then taken them and even tested dynamics of the actual antibody response, if you come back at them either with hemagglutinin or with an infection?

DR. GREENBERG: We have not. You know, these are very hard studies to do. So, at least in theory if you could bleed everybody at the exact same day, day 7 after immunization you might get some sort of dynamic number because you were then comparing sort of rate of rise of plasma blasts but the fact is you know if you have done human studies at day 7 it means somebody comes in on day 6; some come in on day 7; some come in on day 8.

So, to generate enough data so that if you are doing dynamic measurements you can really compare it is hard. So, we haven't done it. It is a reasonable question, but we haven't done it.

PARTICIPANT: In most cases when we measure titers of antibodies they are dominated by one or two

different functions, an assay that is HAI or when you do your ASC assays where is the function coming to?

DR. GREENBERG: That is a great question. Our antibody secreting cell assay is simple. So, we measured antibody secreting cells to whole purified. So, we limited it to H3N2 just first to study and we measured, we compared antibody-secreting cells to the vaccine versus antibody-secreting cells to purified H3N2 virus that was in the vaccine, and basically our results were highly similar. You wouldn't have expected that. You would have expected having, so, in one assay we have all the proteins of one virus and in the other assay we have hemagglutinin and neuraminidase and all the contaminating flu proteins that are in the vaccine that were supposed to be purified HA and NA but are not in the other. We are measuring just total numbers and by and large they are the same. The actual look of the ELISPOT is much cleaner when you use the vaccine than when you use purified virus in our hands.

So, we used the homologous vaccine

PARTICIPANT: One other question regarding IgA because we hear a lot about that and I am starting to wonder whether this is an important assay that needs a little bit more pursuing. Does it matter whether IgAs are monomeric or dimeric, secreted and so forth? Maybe some other people in the room can --

DR. GREENBERG: I think it probably does. Brian may have. So, the best data I know about this is data with rotavirus actually although Brian has lots of great data about flu, but it turns out that acutely after infection the measurement of antibody, IgA antibody in the serum and the measure of IgA antibody in the intestine are correlated well. The longer you go after your infection the correlation breaks down and I am assuming that is because exactly this. Acutely after infection you have mucosally derived IgA secreting cells. So, your serum actually has a fair amount of dimeric in it. Things home back to the intestine and you revert back to the situation where most of the IgA you see in the serum is monomeric.

So, as far as understanding effector mechanisms long term Brian probably has better data on whether nasal wash versus serum IgA has any, you know what is the relationship with those two numbers. I have not done that.

DR. COUCH: You have to remember these are peripheral blood lymphocytes he is looking at. You want to look at the ones that are at the mucosa for production.

DR. GREENBERG: We have tried to get that for rotavirus by saying that we can count in peripheral blood IgA secreting cells that have mucosal homing phenotypes and that has not been as, it sounds great, but it hasn't been as perfect as it sounds.

DR. COUCH: The reason he said that is Brian did some studies on serum in relation to secretions.

Last comment if you have got it, as brief as possible and then lunch for the growling stomachs.

PARTICIPANT: It is basically the antibody that winds up in the lumen of the respiratory tract that is the active. Monomeric IgA in the serum will behave like monomeric IgG and will follow the same rules. Dimeric antibody that is present in submucosal plasma cells that get subject to excretion across a concentration gradient is the antibody that is most important in protection for the IgA.

DR. COUCH: A few housekeeping comments from Dr. Weir before lunch.

DR. WEIR: First of all I think we should thank Bob, all of the speakers, Jesse, David, everyone this morning that did such a great job.

(Applause.)

DR. WEIR: The second thing is as you notice on your agenda lunch you are on your own, but there are some cheat sheets on the outside table that tell you some local restaurants, places to go. I think the good news about this location is none of you should have any problem finding someplace to eat. If you don't like what is on there take off in any direction and you will find something.

The other thing is considering the time I hate to overrule Bob, but why don't we start back at one-fifteen rather than one. That will put us a little late but not bad.

(Thereupon, at 12:03 p.m., a recess was taken until 1:15 p.m., the same day.)

AFTERNOON SESSION 1:15 PM

Agenda Item: Session 2: Immune responses to avian influenza infections and vaccines for novel influenza viruses in humans -Moderator: Jacqueline Katz, PhD

DR. KATZ: I would like to welcome you to this afternoon's session on immune responses to avian influenza infection and vaccines for novel influenza viruses in humans.

I am Jackie Katz from the Influenza Division of the CDC and I will be moderating this session.

Just one announcement before we start and that is there is going to be a panel discussion at the end of the meeting tomorrow in the afternoon and the panelists are going to include the four moderators of the sessions and then some additional invited panelists and your name is listed on that last page and the organizers have requested that those individuals stay after the end of this session today at the very end, so around five-fifteen or five-thirty and for a brief discussion and then also if anybody has any ideas that they want to address particular suggestions for the panel discussion tomorrow please provide those to the organizers at the table outside.

So, I am going to start the session off by introducing Dr. Nancy Cox who is going to give the plenary talk giving us an overview of avian influenza virus

infection in humans.

Agenda Item: Plenary Talk: overview of avian influenza A viruses in humans (including virus heterogeneity)

DR. COX: Thanks, Jackie. So, instead of talking about the immune response I am going to be talking about the beast that causes the immune response or at least one member of the family of beasts.

This slide, I think is very nice because it shows with green dots where outbreaks of avian influenza have occurred in birds either in wild birds or in poultry.

Now, of course, there are some areas where we know that there has been fairly active outbreaks in birds but we don't see very many green spots. These are the reports that have come in to OIE and FAO and then we have shown in yellow circles and blue triangles, yellow squares and then finally in purple diamonds the human cases and the human cases most recently in 2007, are shown by the purple diamonds and they have been primarily along the Nile Delta in Egypt and also in Indonesia but there have been some additional cases as well.

The most recent cases have been reported in China near Nanking in Jangsu(?) Province and the report had already been delivered to the WHO last week about a 24-year-old male who developed symptoms on the twenty-fourth

of November, was hospitalized shortly thereafter and then died a few days later, and today we also found out that his father who took care of him, his 52-year-old father who took care of him while he was very gravely ill has also been diagnosed with H5N1 infection.

We have been in contact with our colleagues in China and there really doesn't seem to be anything unusual about that virus and of course we have seen limited human to human transmission in family clusters before.

So, this just shows the case of this avian influenza, H5N1, of course, between 2003 and 2007, and you can see that we have had fewer cases in 2007 than 2006 which was a very active year. We can also see that the case fatality rate has remained around 60 percent throughout the course of time of these infections.

Now, the nomenclature was getting very, very complicated for the H5N1 viruses and each set of investigators had used their own nomenclature. So, FAO, WHO, it was actually an WHO initiative, WHO, FAO and OIE experts got together and came up with a nomenclature that would be a unified nomenclature and hopefully everyone will settle on this. It is posted on the WHO web site, and it will actually make it much easier for us to understand what we are talking about when we refer to these different subsets and subsets of viruses or clades and sub clades.

Now, if we look just in the past 3 years we have had nine different clades and various sub clades circulating in birds, and of course a number of these have infected humans and we really do need to keep a handle on what is going on in birds because as we have seen in the past it is unpredictable which of these viruses will be passed on to humans, and you can see there is a lot of genetic divergence just in the hemagglutinin which of course is the primary target for the immune response and if we count the viruses that circulated in 1997 in Hong Kong we have a total of 10 clades.

So, this WHO system was really meant to unify the classification of isolates and remove stigmatizing that goes along with calling a virus by its geographic reference, and it can be all inclusive because we can actually follow not only the HA but the NA and the internal genes as well, and it also assists us in selecting new H5 vaccine candidates and the web site where this information is posted is listed there, and there will be a publication coming out shortly.

So, if we look in more detail this is a simplified tree and you can actually see that within Clade 2 there are actually sub clades, 2.1.1, 2.1 to .2 and so on. So, it actually becomes quite complex but at least now we have the ability to name these different groups of

viruses in a sequential way and to follow the evolution.

Now, we have shown here in yellow the viruses that have been used to make candidate vaccine strains. So, we have viruses throughout the tree and this just represents the groups that have infected humans and the humanized lists are shown in blue.

This is just a larger view so you can see how very diverse Clade 2 is and Clade 2.3. So, these are all Clade 2 viruses, 2.1, 2.2 and 2.3.

So, Clade 2.2 is really very diverse as you saw from the previous slide as well and here again we have the humanized list in blue. We have not yet selected one of the Egyptian isolates shown here as a vaccine candidate.

Now, it was very important to know whether the phylogeny would be reflected in the serologic reactions and so this table represents the HI hemagglutination inhibition reaction of influenza H5 viruses.

These are all wild-type viruses and the ones in red are the ones that have been selected as vaccine candidates as representative of Clade 1, 2.1 and 2.2 and 2.3 and you can see that the reactions that you get, the high reactions you get with the homologous viruses as they are grouped by clade and sub clade really does correspond to their positions on the tree.

So, there is a serologic correlate to the genetic

divergence that we see and that is very important of course. I think I will skip through these fairly quickly but this just allows you to see that we have chosen within Clade 2.3 a number of different viruses for vaccine candidates. The same is true for Clade 2.2 although we haven't yet selected an Egyptian virus and of course we have the Clade 1 viruses that have been used already in trials in the 2.1 virus represented by Indonesia 5.

Now, I attempted to find an NA tree or actually to get one that corresponded to the WHO/OIE/FAO HA tree but I couldn't manage to get it through the security of the CDC security system on my computer. So, here is the bottom line. The phylogeny follows the same pattern as HA, and I think that is important to note especially for our NA neuraminidase aficionados.

We have heard quite a bit about outbreaks in Saudi Arabia recently. This is particularly important because of the Hage(?) coming up. We have also heard about outbreaks in birds. This slide shows where the outbreaks have occurred in poultry in orange and wild birds in the lighter color and also there has been a lot of activity in Indonesia and in Pakistan recently as well.

So, we know that this is the time of year when activity really starts heating up in the bird populations. So, we can certainly expect to see more human cases.

We have been very fortunate to receive quite a number of H5N1 viruses isolated from birds in Vietnam during the period 2001 to 2007 and our very preliminary findings are that there are 10 different, at least 10 different virus genotypes detected in the poultry. There have been multiple introductions of H5N1 and of course we know the borders are very porous. It is not surprising and that now viruses with Clade 2.3 HA predominate and have replaced, essentially replaced Clade 1 viruses except for a small area of the Mekong Delta and the H5N1 viruses in Vietnam poultry have the internal genes from Clade 1 viruses. So, perhaps there is an evolutionary advantage for Clade 2.3 HA and Clade 1 internal genes. We really don't know for sure.

So, there really is a very active continuous evolution through point mutations and reassortment in birds and of course viruses isolated from infected humans reflect the viruses in birds.

I think all of you are very familiar with reverse genetics. This is just to remind you that this has become a very robust technique, and we can actually complete the production of a high-yield avirulent vaccine virus in about 9 days. That is about the best we can do.

Of course, the work has to be done in BSL3 and we are looking for 6:2 reassortants and sort of the critical

issues are that if the vaccine viruses are going to be used in humans that requires that vaccine certified Vero cells or other certified cells are used in the reverse genetics process in the transfection, and of course that the reverse genetics technology technique is protected by patents in many but not all countries particularly developed countries.

So, there are WHO guidelines and most of you know the criteria for bringing the viruses out of BSL3. We look at pathogenicity in ferrets. Sometimes pathotyping is done in mice and certainly it must be done in chickens.

So, now we have all of these vaccine reverse genetics modified reassortants on PR8 backbone. Some of them have been distributed fairly widely. Others are still waiting some sort of regulatory approval and then there are additional reassortants in preparation.

So, we are really trying to beat the clock from the first time that person-to-person transmission is detected because we would have to go into large-scale production either in eggs or cell culture and cell culture vaccines have been approved in very few countries. So, there really is a very steep hill to climb.

We have been focusing quite a bit of attention on receptor binding by the HA both before and especially now that Jane Stevens has joined the influenza division and it

has been known that the HA receptor specificity changed from the avian to the human specificity prior to the pandemics and that the receptor specificity really has great effect on host range, tissue tropism and transmissibility.

There has been an evolution in the technology used to look at HA receptor specificity and now glycan arrays are the method of choice.

So, these slides are compliments of Rubin Donis and Jane Stevens and what these experiments are really trying to get at is precisely what the receptor specificity is and there are really a lot of different glycans and so the testing has gone to glass slides with covalently linked glycans. This is a reference where the technology was really described in detail and you can actually use recombinant baculovirus derived HA or whole virus and get essentially the same results and of course these experiments are done in collaboration with Jim Paulson at the Scripps.

This slide is one of James' really nice slides. So, you can get at a whole variety of things. You can look at antigenic drift and receptor specificity using clinical isolates looking at egg versus cell, propagated virus. You can look at host range, and you can look at a variety of subtypes for risk assessment and you get very interesting

data because you can actually see that in H5 viruses; this is actually fairly important, among the H5N1 viruses that have been isolated from humans you can see some weak but variable affinity for human receptors which are shown here in blue and this particular virus you can see that there is some binding to the human specific receptors.

I am also going to talk about amantadine resistance though it doesn't, antiviral resistance although it doesn't really affect immune correlates because it is very important for public health, and I wanted to be sure that you all know that there is a lot of heterogeneity among the H5N1 viruses. So, for the adamantanes we have resistance represented by a number of different mutations in the different groups and subgroups and vastly different percentages of resistance among the different groups.

Switching to neuraminidase inhibitors we really don't know the genetic markers of resistance especially for H5N1 viruses. There are strain and drug-specific resistance markers. We know that for sure, but we have yet to define them all and there also has recently been discovered a vulnerability in the design of existing neuraminidase inhibitors and this was determined by the N1 crystal structure and the mutations have occurred in the 150 loop that has conferred resistance.

So, the neuraminidase inhibition assay is the

current assay of choice. So, you are really looking at neuraminidase activity. We are using the NAStar kit and have validated that. Lorisa Gubereva has done a really marvelous job since she joined us and others around the world are doing a lot of work in this regard.

So, just to quickly go through the data there have been some new mutations observed for resistance to neuraminidase inhibitors. I will just say one word about virus sharing. The story is not over and so there have been a number of meetings and there is a lot of controversy about virus sharing right now, and without those viruses we really can't do the proper risk analysis in vaccine development.

So, we can see that genetic variation is great among H5N1 viruses. There are multiple clades and sub clades of HA along with multiple reassortants. Antigenic variation corresponds with genetic variation and HA. We really haven't looked at antigenic variation in the neuraminidase. Variation is observed in receptor binding specificity and of course changes in receptor binding sites certainly often affect antigenicity and there is variation in antiviral susceptibility patterns among the different clades and sub clades.

I have many, many people to acknowledge within the WHO collaborating center and within the agricultural

community and I want to especially thank Rubin Donis, Jane Stevens, Larisa Gubareva, Sasha Klimov and Jackie Katz for their contributions.

Are there any questions?

(Applause.)

PARTICIPANT: Nancy, that is a beautiful summary. I don't want to introduce more complexity into a very complex situation, but I am going to do so. That is to ask you in terms of your strain comparison, your clade comparisons, etc., do you have adequate data on the difference in passage history as these different viruses are isolated as the different strains are isolated? In other words, how much of this might be due to simply host selection by using various hosts and also the number of passages in a given host?

DR. COX: That is a very good question. We sequence both directly from original clinical specimens and from isolates when we can, and we find very good correspondence. We are doing all of our virus isolation in eggs at the CDC right now because we find we get a better yield than we do in MDCK cells at least in our hands. So, we really do, for the HA which we concentrate most on and NA secondly we do see good correspondence between what is in the specimen and what comes out of eggs.

PARTICIPANT: The other thing I would like to

mention, and I will go into this more tomorrow if I get the opportunity but we discovered a long, long time ago that the PR8 in Ann Arbor was not the same as the PR8 in New York or the one in London, so that you have intrastrain variability, antigenic variability also that you can detect in the simplest of tests.

DR. COX: Sure, yes, those viruses have had extensive laboratory passage and do have differences in all kinds of properties.

Peter?

DR. WRIGHT: Peter Wright. In birds you see little evolution and variation in influenza strains and I am wondering what this tells us about H5 in birds. It seems to be rapidly evolving in a way that I don't know that I would have predicted with other avian viruses.

DR. COX: That is a very good question because of course the dogma had been that avian influenza viruses are very stable and you don't see the degree of antigenic variation that you do among human viruses but the viruses that were examined at that time were viruses primarily from wild birds and waterfowl where basically there are certain subtypes that you see more frequently in certain species and so I think that there was a lot more stability and far fewer isolates then but I think that the reason we are seeing, part of the reason we are seeing so much divergence

here is that we have these viruses that appear to be incredibly genetically robust and evolutionarily robust. They can infect many different species. Each time they move from species to species even though it is from one species of bird to another species of bird we think there are selective pressures that are exerted and so in addition there is vaccination.

Now, we don't know what effect vaccination might have on evolution and certainly vaccination wasn't started until probably 2004. So, we don't really think vaccination has a great deal to do with it but rather the spread of the viruses and the movement back and forth from bird species to bird species.

DR. SHAW: Alan Shaw. The surveillance system has captured three hundred and some odd cases of influenza severe disease and two hundred and some odd cases of death. What do we know about the overall population exposure in Indonesia for example to H5? I mean there is probably a lot going on there that you just never see.

DR. COX: I think that it is highly likely that there are many more ducks that haven't been captured simply because surveillance doesn't occur in many of the outlying regions in developing countries and I think that we don't, we haven't had an opportunity to do the kind of serology that was done in 1997. So, a lot is being done at the

moment. I expect that there will be sort of a burst of publications over the next 2 years but I think that some of the serologic studies are really complicated by the timing of the collection of serum and so on and so forth. So, we know that there are mild or there have been mild or asymptomatic convections based on the 1997 work and also subsequent work but we really don't have as yet large population-based serologic studies to really know what is going on but there are some countries that are attempting to do that.

PARTICIPANT: Nancy, the limited numbers of human-to-human transmissions in family clusters that have occurred if they have occurred in different clades or sub clades do you see any sequence changes that are seen in more than one of these family clusters?

DR. COX: No. Actually we have looked very carefully especially the Carol(?) cluster where we thought there was likely possibly human-to-human-to-human transmission, we looked for changes and have also looked very carefully in some of the other transmissions from mother to daughter and so on, and we really have not been able to see changes that we think are associated with passage in humans.

PARTICIPANT: My question is a very simple one and it is trying to get straight in my mind. I don't do

these things, but are the viruses truly evolving in the way we think of human viruses having evolved or is information evolving; we are learning more and the more you capture and the more varieties you see and that sort of thing or is this thing really sequentially moving in time?

DR. COX: No, it is not sequentially moving in time because there are discrete geographic distributions of the viruses. So clade 2.1 viruses are primarily in Indonesia. So, you have instead of you know when you do a long-term evolutionary tree of H3N2 viruses for example you see that the changes that are fixed are maintained and you just have a very long skinny tree basically.

PARTICIPANT: Are viruses disappearing?

DR. COX: Clade 1 viruses are still there in Southern Vietnam in the Mekong Delta but certainly the range of the Clade 1 viruses is much less than before. So, it may disappear, yes.

PARTICIPANT: I think a good while ago I asked you and Rob Webster, is this like HIV, that is a clade to A, B and C, and he suggested yes, and I think you are saying the same thing.

DR. COX: I am saying the same thing. I am saying that you may see some displacement but basically you have got these quite stable clades that are now dividing into clades and sub clades.

DR. KATZ: Thank you very much.

Agenda Item: Immune responses in poultry workers

DR. KATZ: I am going to be talking about immune responses in poultry workers and by way of introduction I am also going to be including some of our early data looking at the serological response to individuals that were actually culture confirmed infected with H5N1 viruses as well as I am also going to try to touch on what we know about serological responses to H9N2 and will be discussing some studies that have been published on the H7 subtypes.

Clearly all of these viruses have caused mild to severe illness in humans although H5 has often been of greater focus especially in recent years.

So, I am going to begin with some lessons learned from investigating the 1997 outbreak in Hong Kong where you will recall a single index case in May 1997 followed by a cluster in November and December, a total of 18 human cases and six deaths and that this outbreak ended in both poultry and humans when all of the poultry were culled from Hong Kong.

So, at that time we had been working with a microneutralization assay in our lab to look at antibody responses in human sera and when we compared the traditional avian red blood cell based assay for hemagglutination inhibition with this microneutralization

assay in looking at the antibody responses in culture-confirmed cases it became very clear to us that in fact this neutralization assay was more sensitive and could detect antibodies to higher titers than could the traditional HI and so most of our studies at least from CDC are based on this assay. So, I am just going to really remind you what it is briefly. Twofold serial dilutions of sera are made. In our lab we start, we call the first dilution 1:20. So, a 1:80 titer is the first three wells showing inhibition and then virus is added to the diluted sera, 100 tissue culture infectious doses, incubated for an hour and then a suspension of MDCK cells is added and the plates are incubated overnight.

The next day the cell monolayer is fixed with acetone and an ELISA is run to detect viral influenza A in P antigen using a monoclonal antibody with an ELISA-based read out, and when we used this assay to look at the serum antibody responses in individuals that had been confirmed to be infected in the 1997 Hong Kong outbreak we found that with the exception of two individuals, this individual here who didn't make any sort of immune response and this individual which was a young female that we found out subsequently we just couldn't detect her neutralizing antibody response with the Hong Kong 156 virus we used in this assay but when we used a different virus we could

detect a response.

So, these responses are all to the prototype Hong Kong 156 virus, and so you can see that roughly about 14 days or more after symptom onset the majority of individuals are making a robust serum antibody response and we measured, I think we had the opportunity to look about 7 months out here in one individual and found that the antibody titer was still roughly within about twofold of what it was within the first 25 days but again that was a very isolated time point that we were able to address.

So, we established at that time a cut off of a titer of 1:80 as being seropositive for H5N1 antibody and the reason we did that was that we were performing many sera epidemiological investigations where we had only a single serum sample and that was taken at a certain time point after the supposed exposure of an individual, and so we had to establish a criteria for seropositivity, and when we did our specificity and sensitivity analysis we also found that we occasionally in unexposed individuals would get titers down in this area of the curve anywhere from 10 to 40.

So, we felt a more stringent cut off was seropositivity of 80 or more and we decided that we needed to see such a response in two independent assays. Of course, if we had well-timed paired sera then

seroconversion would be indicative of infection and to enhance the specificity we found that a second assay in our hands at CDC we used a Western blot detecting antibody at a dilution of 1:100 using baculovirus expressed recombinant H5HA protein.

Our collaborators, Dr. Wilena Lim at the Hong Kong Department of Health used a single radial homolysis as performed by John Wood and his colleagues as a conservatory assay but all of our seropositivity was based on obtaining first a neutralizing antibody titer of 1:80 or more and then having a confirmatory positive in a second assay, and this is the result of the study led by Carolyn Bridges in poultry workers right at the time of the culling operation at the end of 1997.

At that time Rob Webster and Ken Shortridge also were doing surveillance in the poultry, in the live bird markets in Hong Kong and they determined that about 20 percent of chickens were in fact positive for H5. So, there was probably a fairly high level of exposure that poultry workers in this situation had experienced.

In about 1500 poultry workers that were enrolled we found that 10 percent of them measure our criteria for seropositivity and in contrast a smaller number, about 300 government workers that had just experienced exposure to infected birds during the culling operation, so in a fairly

short one-to-two-week window we found a seroprevalence of 3 percent and in fact in one individual we did see a seroconversion.

So, telling us that we could pick up in most cases asymptomatic infections of course, this is a retrospective study and we were not able to link any level of disease or infection to the seropositivity rate. However, when the epidemiologic analysis was performed we found that our criteria for a seropositivity of a titer of 1:80 did in fact significantly correlate with risk factors where individuals who butchered poultry and had exposure to birds that had greater than a 10 percent die off were significantly associated with seropositivity, and the more types of exposure they had their risk increased.

At the time H9N2 viruses were also circulating in the live bird markets in Hong Kong and roughly again according to Rob Webster and Ken Shortridge roughly 4 percent of chickens were also positive for H9N2 at the time, and we know that there were multiple distinct sublineages of H9N2. The so-called G9 or Y280 lineage had circulated in Hong Kong and Southern China for some years and then there was also a G1 lineage and in 1999 two cases of human infection in children with H9N2 was documented and these were individuals that were infected with the G1-like viruses, and one of the two children did have a serological

response and by the neutralizing antibody assay we determined that that child had a titer of 1:640. That was a 4-year-old child and the other child was a 1-year-old child who did not make an immune response.

So, we also went back to a subset of the poultry workers we had tested for H5N1 seropositivity and looked at the seropositivity now for two subgroups of the H9N2 viruses and I should say that both for H5 and for H9 antibody we looked at a non-exposed group or supposedly non-exposed group and these were blood donors from Hong Kong, and this was sera that was collected earlier in 1997, and before the large outbreak of H5N1 in poultry in the bird market and here we found the baseline reactivity of about 1 percent in over 170 individuals, and we saw a similar reactivity for H5 which I forgot to mention, but when we went back to the poultry workers and we tested about 250 of them randomly selected from the larger subset, we found again that only a small percentage seemed to be positive for G1 but an extremely high level of seropositivity was detected with a G9 virus and this was when we used our microneutralization cut off plus Western blot.

So, we really felt that there was something unusual going on here and we went back and tested with a reassortant virus that was an H9N7; so, now no longer had

the N2 antibody because we thought that might have been playing a role in this high degree of seropositivity, but when we went back and reanalyzed we found almost the same percentage of individuals were still showing neutralizing antibody suggesting that we were looking at a level of antibody to the HA which is primarily what is detected in the neutralizing antibody assay.

So, we decided that we needed to do a further specificity testing and when we did that we reduced the seropositivity down to about 23 percent which is still rather high and the way we did that further specificity testing is to actually absorb the serum with human influenza strains and this is a fairly arduous process where you purify the virus and you use about 100 micrograms of total virus and absorb out followed by an ultracentrifugation and additional absorption out with red blood cells to remove any residual virus and then at the end of that the sera is re-evaluated in the neutralization assay and this is an example of what we found with an individual, a 50-year-old male who had high seropositivity to the H9N2 virus. If we did an absorption within a relevant influenza B virus we still saw a substantial titer but we could remove the response with either an early 1968 H3N2 virus or an H2N2 virus and in contrast if we try to do the same with the confirmed case, serum from the confirmed

case we couldn't absorb the serum out with these; we couldn't absorb the reactivity out with these viruses, and a somewhat similar phenomenon; so, these results suggest that there is some level of cross reactivity occurring with the H9N2 virus with earlier strains of human viruses, and this is taken from some work of Maria Zambon and Ian Stevenson where when they looked in vaccinated individuals they found that pre-existing antibodies, so prior to H9N2 vaccination they saw actually an age-related distribution of the response which seemed to cluster between the years. It seemed to be peaking in the years that were consistent with the H2N2 era, but not exclusively because we have also seen some early H3N2 era reactivity. So, it sort of remains for this to really be followed up and my point here is that when we are looking at antibody responses to novel strains there is some level of cross reactivity occurring potentially at the level of the HA across subtypes and that is something that we need to better understand.

Moving forward to the more recent experiences with H5N1 viruses as Nancy has clearly demonstrated there are multiple clades and sub clades circulating that have dispersed into distinct geographic regions and I can't show you the data but we have done a fair amount of work with our Indonesian colleagues in 2004 and early 2005, assessing sera from again the culture or PCR confirmed H5N1 infected

individuals and in general we found the same kinetics of response that we saw in 1997, that the majority of individuals if we got serum out long enough if the individuals survived such that sera could be obtained 14 or more days postinfection, then a majority of individuals made a good serum antibody response that ranged from at titer of 1:80 to a titer of 1:1280 and in one individual there we had the opportunity to go back 5 months later and did see again that the same amount of, the similar serologic response was retained.

So, more recently Ian and Maria Zambon have developed the horse red blood cell hemagglutination inhibition assay, and using horse red blood cells because they have a predominance of sialic acid in the two-three linkage to galactose the preferred receptor for avian influenza viruses and this was shown in their studies to enhance the detection of antibodies to avian influenza viruses of the H5 and this is just a demonstration of the similarity in the response. This is a postinfection response where the microneutralization titer was 1:20. If they used traditional turkey red blood cells they got almost no HI antibody but if they used the horse red blood cells then they would achieve a fairly similar level of HI titer comparable to the microneutralization titer and so in many labs now the horse HI is being either, in our lab we

are trying to compare it directly with the microneutralization assay and in some labs as Nancy mentioned that are now doing more extensive serologic investigations they are using the horse red blood cell as a primary screening tool, and we showed using sera collected from the first part of the NIAID supported Vietnam 1203 clinical study and these sera were obtained thanks to Linda Lambert and her colleagues and we did a direct comparison in our lab with horse HI assay or our microneutralization assay and found a pretty good correlation of the responses here with a correlation coefficient of almost .9, and we found very similar results when we did a similar analysis with antibody from infected individuals. The correlation was exactly the same.

So, we have been involved in a number of studies with international partners looking for the evidence of asymptomatic or milder infection with H5N1 viruses and we have really not seen any substantial evidence. In Korea potentially Dr. Chun Kang is wrapping up a manuscript now I believe where she has looked at over 2500 poultry workers and found about nine positives and within that is included I think one or more individuals that seroconverted but again that is a fairly small number of individuals with that evidence.

We have ongoing studies with colleagues in

Cambodia, Russia, India and recently completed a study in Nigeria which was led by Mark Hetz and Justin Ortiz from our epidemiology group and there they looked at poultry workers that were involved in the culling operations during the first outbreaks of H5N1 in Nigeria, 320 individuals with no evidence of using our microneut assay and a horse HI assay, so no evidence of seropositivity in that group and Surinda Vong has also published a first study where they went back to look at residents in a village. So, these would be the backyard farmers where H5N1 infection did occur in several humans and again found no evidence for mild or asymptomatic infection.

However, in another study that is ongoing at the present time with our colleague, Surinda Vong we have found some evidence of infection and this is sera collected again going back to villages that had experienced human infection with H5N1 and out of a total of about 600 individuals 7 individuals were positive where the sera was collected 1 to 2 months out after the exposure and so you can see some of these are quite robust microneutralization positive.

However, when follow-up sera were collected 10 to 11 months later several of these had dropped below what we would call our level of positivity.

So, I think it is very important in doing these studies that sera are collected in a timely manner and this

may be an example of a more rapid decline in antibody because we were looking at a mild or asymptomatic illness.

So, in the last few minutes I just want to turn to the H7 viruses. Multiple subtypes of both low pathogenicity and high pathogenicity H7 subtype viruses have infected humans. We had H7N2 virus isolated from an adult male with respiratory symptoms from New York in 2003, and we were lucky enough to obtain acute and convalescent sera. So, this was obviously a North American lineage low pathogenicity virus. It was characterized to be very similar to the viruses that are circulating in the live bird markets in the Northeast and we found in the acute sera as you would expect there was a baseline level of antibody but in the convalescent serum sample collected 5 months later we found a titer of 1:80 and this individual was Western blot positive also.

Earlier the previous year we had also investigated 80 poultry workers that were involved in culling operations during the 2002 outbreak of H7N2 low-pathogenicity H7N2 in turkeys in Virginia and one of these 80 individuals was found to meet our criteria for having a positive neutralizing antibody response. He, also, had an H7 IgM response that I will show you in a moment and this individual did in fact when we went back to the epidemiologic records report a temporally related

respiratory illness. He had had no prior exposure to poultry, but he did have a history of hunting including birds.

So, again, for us this was the first demonstration because this study was done in late 2002, it was the first demonstration of H7N2 antibody detection by neutralization assay. So, we performed an absorption with an H3N2 virus and we chose the H3N2 virus because in structural studies the H3 and the H7 hemagglutinin are more closely related than the H7 and the H1.

So, we felt the H3 would be a representative current contemporary strain and you can see in this individual that we failed to absorb out the antibody response but only when we used the homologous or H7N2 virus, the turkey Virginia virus could we remove the response, and we got a similar response.

I should mention that the first serum was collected 21 days after the start of the culling operation and then we went back again having found this serum positive, we went back 7 months later and so by now the IgM titer had disappeared.

Different results have been obtained in different H7 outbreaks and the one here is of course one where most number of individuals were infected in the Netherlands in 2003, where there were over 80 cases of conjunctivitis or

conjunctivitis in ILI or a few cases of ILI alone but as far as I understand there has been no serum neutralizing antibody detected and one study by Mira et al that used a horse HI assay and a fairly low cut-off value determined that there was a high seropositivity both in the individuals directly in contact with the poultry and then in household contacts of those individuals although the authors themselves say that this data must be interpreted with caution because of the low seropositivity and I would also like to add the lower amount of virus that was used in this HI assay.

Finally moving back to North America in 2004 there was an outbreak of what turned into highly pathogenic H7N3 in British Columbia. There were 650 federal workers, but not all of those were exposed and in fact two individuals were culture confirmed. One had conjunctivitis and coryza and a low pathogenicity H7N3 was isolated from this individual. A second had conjunctivitis and headache and the high path strain was isolated although these two viruses differed in their hemagglutinin I believe only by one amino acid in the cleavage site, but again, neither of these individuals made an antibody response in convalescent sera; it couldn't be detected by neutralization, Western blot or horse HI and similarly in a follow-up study of 167 cullers, the farmers and family members were investigated

and again they were all negative for serum antibody.

One other example where antibody has been detected to H7 viruses is in the study from the Italian group and includes Maria Zambon's work, the study by Puzelli et al where they looked over a number of years for outbreaks, two outbreaks of low pathogenicity H7N1, 0 out of 126 individuals tested positive. To high pathogenicity H7N1 outbreaks again 0 out of a larger number but where they did find a small handful of positives was in two low pathogenicity H7N3 outbreaks in 2002 and 2003 where they met the criteria for being microneutralization antibody positive with a confirmation by another assay.

So, just to conclude it seems for the H5N1 viruses that we can clearly associate neutralizing antibody titers of greater than 1:80 in appropriately timed sera and if we use this criteria for assessing the extent of infection in poultry workers in Hong Kong in 1997, we also saw a significant epidemiologic association with this titer with a more intense exposure in the poultry workers.

However, taking all of the studies into account the seroprevalence for anti-H5 appears to be low in populations exposed to infected birds. However, as Nancy mentioned there are quite a number of studies in China, in Vietnam. Many countries have serum, large quantities of serum banked up and they are only now just in the testing

process. So, I think we really need to have a better understanding and the other limitation might be that in mild or asymptomatic infections we may not be seeing a very long-lived response.

H7 viruses appear to pose more of a problem. Serum neutralizing antibody to H7 viruses is often not detected even though individuals are confirmed to be infected with the H7 virus by isolation or PCR. So, what is going on there? Is it just that our assays are not detecting H7 viruses appropriately or in many cases H7s are only causing conjunctivitis and is it that by that route of infection we are not getting a consistent serum antibody response?

Again, for the H5 it appears that there is a good correlation with the horse HI neutralization assay and I think that remains to be an open question with the H7 viruses and maybe Maria can shed some light onto that in her presentation later. I will leave it there.

Thank you.

(Applause.)

DR. KATZ: Oh, I just need to acknowledge my colleagues in my own team in the branch and then our many international partners that have contributed to this work.

Maybe there is time for one question if anyone has a question or not.

Kanta, thank you.

DR. SUBBARAO: So, you have talked about how you established the criterion of 1:80 based on the number of sera that you had available in 1997, from H5 infected individuals. How would you propose to go forward with subtypes that we haven't seen that many infections with? What is a good algorithm? How do you think we should do this?

DR. KATZ: I mean we have been using the same algorithm and that might set the bar too high and that has been a concern but I think the key thing that needs to be done for any new subtype and any new variant within a subtype is you need to do specificity analysis in a population in as many sera as you can get from a well-aged, a broad age range of individuals and look what the baseline is in terms of your serological endpoint titers.

So, it needs to be done for each assay. It needs to be done essentially for each virus as you move forward to do live seroprevalence studies because we have seen and I think John Wood is going to talk a little bit more about this tomorrow, we have seen differences in specificity even with some of the different H5N1 viruses.

DR. SUBBARAO: So, given that microneutralization is very virus specific would ELISA be something we should be looking at harder?

DR. KATZ: In our experience at least the traditional indirect ELISA is not adequately sensitive in adults. You get a very broad response and you need to have very stringent baseline sera to again establish a cut off and we never report that data in general because we don't feel comfortable with it especially IgG. IgM is a little clearer and it is a little clearer in children where you can get cleaner results in individuals that are clearly not infected with H5 for example.

DR. COUCH: You caught my attention a little bit late with that H7 comment. The serology for the Mockering(?) infection was not of value in the Netherlands outbreak and do you have trouble getting antibody responses in animal models like ferrets or chickens with H7?

DR. KATZ: No. You can, well, in most of those models people are infecting them intranasally but a majority of human infections with H7 viruses have been conjunctival.

DR. COUCH: It was in the Netherlands outbreak also where the serologic responses not there either?

DR. KATZ: By neutralization they weren't and perhaps Maria can speak more to that.

DR. COUCH: They were by HI?

DR. KATZ: They were by the horse HI, but they used a very low cut off and they used a reduced amount of

virus to demonstrate it. So, it is a bit questionable.

DR. ALFONSO: Thank you for your talk. I am Claudia Alfonso, WHO, Geneva. I am a veterinarian. I am an animal doctor and I just want to clarify that avian influenza is a disease of poultry but it very, very rarely causes disease in wild birds. There are actually almost non-existent outbreaks of avian influenza in wild birds. I have a question regarding the cut off of the microneutralization assay, why it was chosen a positive cut off of 1:80 rather than looking at what would be the cut off of, the negative cut off and then anything above that would be considered.

DR.KATZ: In essence that is what we have done. I mean we used two approaches. We looked and said, "What are infected individuals making in 14 or more days; what is their antibody response 14 or more days out?" and the lower limit there was 1:80.

If we looked at unexposed control individuals over a broad age range in our assay we can find titers anywhere from 1:10 to 1:40. So, really we set that titer at the next level up, twofold up of 1:80 as being the cut off. So, we took both things into consideration essentially.

Okay, I think we had better move on.

Our next speaker is Dr. Maria Zambon and she is going to be telling us about some of the vaccine clinical

trials conducted in Europe using non-replicating avian influenza virus vaccines.

Thank you.

Agenda Item: Immune responses to non-replicating avian influenza vaccines in clinical trials conducted in Europe.

DR. ZAMBON: Good afternoon, everyone. It is a pleasure to be here with you. I would like to thank the organizers for inviting me to speak to you.

Clearly pandemic vaccine studies are designed to try to answer some key questions including what would be the nature of the dose and the regime of vaccines that we could give to provide optimum protection in a pandemic; what indeed would cause the protection that we might be looking for in anything that we could measure serologically be and what kind of longevity of response might we expect and as we heard from a number of the various speakers there are really quite a number of problems in trying to address these questions. We have heard many speakers talk about the issues of immunogenicity assessment.

Now, you will notice on the slide I have actually put a picture of the man on the moon and I did this actually not in response to the earlier picture we had of the Matterhorn representing I think an aspiration of what we are trying to do or the difficulty of trying to make

pandemic vaccines but rather to remind me to speak about the landscape for funding for pandemic influenza vaccine studies particularly in Europe. The fact that we are dealing with the lunar landscape and perhaps those of us in that landscape having comparison of lunatics might actually not be lost on some people in the audience, but the reason for saying that is that it has been a rather barren landscape until fairly recently, deserted and full of unexpected pitfalls, shifting sands of alliances and consortia and opportunistic rather than strategically targeted to try to address questions.

So, although there is a substantial body of work that has been done in the last 7 to 10 years on this I think it is important to say that it isn't always easy to try to pull together different aspects of the work that has been done for some of the reasons to do with lack of standardization.

Now, the types of vaccine that are in use in Europe are inactivated vaccines in general and clearly there are a number of different sorts of inactivated vaccine, whole virus, split virus and subunit vaccine.

When we had the last pandemic these vaccines represented the majority if not all of the vaccines in use at the time. In Europe currently these represent the bulk of the vaccines which are used. Over 90 percent, probably

95 percent of vaccines in use in Europe are split virus vaccines and indeed most of the studies that I am going to speak about have been conducted using these sorts of vaccines.

Some whole virus vaccines are still manufactured but they represent the minority. I am also going to say something about adjuvants that are used. Clearly vaccines in general the use of adjuvants to improve immunogenicity is a substantial research agenda in its own right. There are many examples of them. The two that are licensed for use in Europe are alum mineral soils or emulsions such as MF59 based on squalene. Others are experimental adjuvants and some vaccine studies have used experimental adjuvants but the majority of vaccine studies I am going to speak about have actually used licensed adjuvants with good reason. I think it makes more sense in a commercial development program to use something that is already licensed.

Methodology we have heard quite a lot about, different sorts of methodology for evaluation of immunogenicity. I don't want to say too much more about this because I can just add to the comments about the difficulties of standardization but I would make one point. These are essentially bioassays and more than that they are dependent in general on key biological interactions at the

receptor level. So, part of a research agenda in terms of thinking about more, better and different assays really ought to be to ask the question how do we find more objective measures of antibody antigen interaction which we can use in a systematic and organized fashion to substitute for bioassays that in many respects have served well over the last 40 years.

Criteria for evaluation of pandemic vaccines, partly why we are here today is to discuss these criteria. I don't really propose to go through them in detail other than to say that in discussing pandemic vaccine studies from Europe I will be talking about the way in which they match European guidelines for licensure, and there could be a whole argument about how useful those guidelines actually are but they at least represent a, if you like fixed goalpost against which the immunogenicity of vaccine studies is actually evaluated and they are similar but perhaps not quite as stringent as the existing current US FDA guidelines.

Jackie has already mentioned some of these points I am going to make in the next couple of slides but the earlier study that we carried out in 1998, using a surrogate for 1997 H5 vaccines showed us very clearly that when we used standard tests we underestimated post vaccination responses where we knew from evaluation of

other methodology antibodies were actually being produced. So, that was the first clue I think that existing methodologies were not going to be good enough and we would need to think harder about how to detect antibodies post vaccine.

In many ways listening to the remarks about H7 I am reminded of these early vaccine studies where we knew that antibody was there or would very likely be there. We just hadn't figured out how to measure it.

Clearly there are a number of variables to talk about in trying to pull together massive vaccine studies. Importantly the subtype that we look at I think there are some lessons in common from different subtypes but also some things which are different. The types of vaccine that are used I have already mentioned the substrate and the route of delivery. Before I sort of go into a substantial number of trials I just want to remind you of some very early data from 1977 which makes an important point about antigen dose and in fact we have almost had to rediscover it 20 years later.

In an unprimed population given H1N1 whole virus vaccine in order to achieve what we might consider to be an appropriate serological response we really need to be giving high doses of antigen and in a primed population that is much less or if we give a two-dose schedule that

also requires much less hemagglutinin. We also learned from studies in and around this time that whole virus vaccines tended to give rather better immune responses than subunit vaccines and in a way we sort of rediscovered some of that as points all over again most recently with H5N1.

Although I have already discussed in some aspects this H5N3 study I want to make a point here which I will come back to later following some recent data of ours. Individuals given H5N3 subunit vaccine with or without adjuvant really didn't make a particularly good response if they were given subunit vaccine without an adjuvant. In fact they would not have met licensing criteria as applied to these vaccines. The adjuvanted vaccine however clearly did provide a reasonable immune response suitable to meet licensing criteria after a second shot but I ask you to remember that point when I come back to some very recent data later on.

When we gave similar vaccines as part of a separate trial to look at mucosal delivery we gave it in a trivalent formulation. We found that the H5 vaccine responses were as we would have expected from a monovalent formulation proving the point that at least we could if we needed to include H5 with seasonal flu vaccines and not see any particular inhibition of response either to seasonal flu vaccine antigens or of the H5.

Now, the pace of vaccine studies has escalated in the last couple of years I think in response to the threat from H5N1 and this is just a summary of what I know to be the ongoing vaccine studies worldwide. I mean I may well have missed some from this slide which actually appeared in Nature I think earlier this year, my point being that there are a lot of different things going on involving a lot of different companies and different formulations and rather than trying to go through each one of them what I tried to do is to rank them and pick out what we think look like the conclusions from a body of work and the kind of general direction of the information.

The early trials, part of the NIH initiative indicated very clearly that if you used a split vaccine as an adjuvant you would really need high doses of hemagglutinin to achieve what you thought or what could be considered to be reasonable immune responses and as time has gone on with various different studies declaring over the last year or so particularly data being presented from companies at WHO regular meetings it has become clear that antigen-sparing can be achieved particularly using whole virus vaccines or subunit vaccines with powerful adjuvants and indeed one recent study described at WHO whole virus vaccine with alum indicates that you can achieve that sort of regulatory barrier if you will with a single dose of 6

micrograms.

So, some trends emerging about the possibility of antigen sparing and some conclusions reflecting probably also what had been seen in 1977. Whole virus vaccines were a little bit more immunogenic than subunit vaccines and indeed if we move to a different subtype our work with H9N2 did suggest that lower doses of antigen we did see rather better responses with whole virus than with subunit vaccines and we saw the modest or rather the Hehme group, the GSK Hehme group in 2002 saw some modest adjuvanting activity with whole virus vaccines using alum indicating again the possibility of some further antigen sparing there and our recent studies with H, that should say H7N1 indicate quite clearly that alum does have an advantage, not a huge advantage but an advantage when looking at subunit vaccine made in the Percy(?) six cell environment but interestingly given the conversations we have just had about H7 antibody responses in general the responses that we saw from this H7N1 vaccine trial were poor even by early experience with H5. With reasonable doses of hemagglutinin without alum we really did see some very poor antibody titers and so I think with H7 particularly when we are coming back to thinking about non-H5 vaccines we are almost back to square one of actually understanding how to measure immune responses. So, what are the conclusions in regard to

dose and adjuvant? We need a high antigen dose without adjuvant. I think we have proven that several times in different ways. Alum adjuvant has a modest effect. It is not always predictable and seems to be somewhat dependent on antigen type.

More powerful adjuvants such as MF59 and AS, unlicensed but squalene-based adjuvant from GSK do show significant antigen sparing and these do not affect, so far do not appear to be affected by trivalent formulation. Whole virus vaccines may be more immunogenic with or without adjuvant but the caveats in regard to trying to sort out and summarize these data are the standardization of the vaccines themselves and the immunogenicity measures.

What about age-related responses? Pediatric studies are in progress in the European Union. So, we don't actually have any data on those at present but we do have some data in regard to vaccination of the elderly and this question of pre-existing antibody.

Jackie has already mentioned some of the data that we found when we looked at our HI in two vaccine studies. When we looked at individuals who were over 35 years of age that is who would have had pre-existing exposure to H2N2 we did find rather better responses to vaccine whether these were whole virus or whether they were subunit and perhaps of interest we did notice that we

didn't see much of a dose response although we didn't see much of a boosting response when individuals became positive after the first vaccine. We didn't see much additional benefit from a second dose and in younger individuals we saw that whole virus vaccines were a little bit better perhaps than subunit vaccines.

If we turn to H5 it has already been mentioned that there is some evidence of a pre-existing H5 antibody in the population.

Certainly when we have undertaken age-related seroprevalence studies of the United Kingdom population where we don't expect to find H5 antibody we do find around about 10 to 15 percent of the population positive in the older age groups which have a test which we choose to look at and interestingly data presented earlier this year at WHO in a Phase II study of H5N1 subunit vaccine with alum adjuvant conducted in elderly in France if you looked, if you took the elderly individuals enrolled about 16 percent of them had a detectable antibody at baseline to H5 and if you looked at the way in which those individuals responded to vaccine the individuals with a pre-existing antibody did not seem to derive much benefit from a second shot of vaccine and indeed responded quite well from the first shot of vaccine.

So, pre-existing vaccination anti-H5 antibodies

were seen in about 16 percent of that elderly population in France. Two doses were needed to optimize the immune response in the population with undetectable antibodies but the elderly with pre-existing antibodies didn't seem to derive much benefit from a second dose.

The pre-existing antibody that we found, we did find some and the H9N2 antibody study seemed to correlate best with exposure to H2N2 and I would echo Jackie's comments.

I do think observations of this sort are if you like hypothesis generating even if not hypothesis testing and there are some hypotheses which could be tested here about heterosubtypic antibody and its usefulness.

If you look at a population that has definitely been primed taking H2N2 vaccine we see that the way that older individuals who have definitely been exposed to H2N2 behave in a similar way to those that we suspect had been primed heterosubtypically.

Moving now to the question of diversity of immune response in our first studies we vaccinated with H5N3 but clearly as events unfolded with H5N1 it was important to ask how well would vaccinees who were vaccinated with H5N3, how well would their sera protect against later and developing strains and this is work done in collaboration Jackie and her group at CDC where if you

recall we vaccinated with adjuvanted and non-adjuvanted H5N3 and what we could clearly see is that the vaccinees who had received adjuvanted vaccine had a much better, much greater response against drifted strains probably to do with the height of the antibody response.

More recently and of course that was vaccine using squalene-based adjuvants, more recently we have asked similar sorts of questions with vaccinees who received reverse genetic virus subunit with alum and what we see there is the kind of cross reactivity which you would predict from animal model data from ferret antisera and you see clearly that vaccinees receiving the vaccine strain do show reactivity against more drifted strains but obviously reduced in titer.

One interesting observation here is that if you do this work with wild-type strains as compared with reverse genetic strains you do appear to pick out higher antibody titers and potentially show evidence of rather better cross reactivity another methodological point to be considered in regard to the use of wild-type strains versus reverse genetic strains in the methodology but I don't want you to take from that that I think that the evaluation of these sorts of vaccine studies can only be done or should only be done with wild-type strains.

What I mean by this is that if we looked at the

neutralizing antibody titers against that same neut 14 and its homologous wild-type strain we see a sort of a shift of the actual data, scatter points towards detection, more sensitive detection with wild-type strains and we have seen this with several H5N1 strains. So, I just include two here for example, the Vietnam 1194 and the Turkey/Turkey 05 strain and I think this relates to the way that we measure the expression of our protein in highly pathogenic strains where you have a very fast reputation kinetic.

So the broad response to diverse strains we do see cross neutralization when we use several different vaccine types. That is both with the squalene-type adjuvant and indeed with the alum adjuvants and the kinds of cross reactions that we see are in line with the animal data. We suspect that the height of the antibody response is important and we don't see, I should just say it in regard to the most recent data I showed you with the H5 strains, it doesn't seem to be a particular advantage with alum adjuvant in terms of a cross reactivity that is used and the cross protection may be, cross neutralization I should say may be improved with adjuvants such as MF59 and AS but this may reflect the higher antibody titers that are used, are generated.

Now, what about a final few minutes on boosting experiments. Here is an example from the Australian CSL

vaccine study using plain alum adjuvanted subunit vaccine and you see with the 7.5 micrograms at 42 days following the second dose you see a really rather modest immune response in line with what you would predict from other studies. By 6 months that has declined to close to zero but not quite zero, a little bit detectable and coming in with the third dose at 6 months you do see a reasonable boosting antibody response.

So, that is I think the sort of response that one expects for homologous boosting and is in line with what we saw when we did more or less the same sort of thing with H5N3 where we went back after 16 months and boosted individuals who had received the H5N3 vaccine and we did see boosting even in the individuals who hadn't received adjuvant.

Now, we wanted to ask what would happen if we went back to those individuals who had received H5N3 in 1999. They were given a wild type vaccine which was H5N3 and a subunit vaccine. We wanted to ask what would happen if we went back and boosted them in 2007 with a reverse genetic vaccine.

Now, plainly we had a small study to start with. So, it was a question of whom could be found to be revaccinated but we did end up with about 15 per group and we haven't attempted to segregate them according to the

original dose that they received but if they received an unadjuvanted vaccine in the first instance they received in the second, they were grouped but they received an adjuvanted MF59 vaccine the second time around and we analyzed them separately and the control group here are a newly recruited naive group to H5 vaccine.

We were rather surprised with the scale of the antibodies that we actually detected. At 8 days following vaccination you can barely detect anything in individuals that are unprimed. In individuals that had received H5N3 vaccine 8 years previously we saw a very vigorous immune response even at 8 days which would actually have met licensing criteria. Having said that this is a very small number of individuals which didn't actually increase very much over the course of the next few weeks including with a second vaccination day 21 although the results that we saw with the unprimed were really as we would have seen previously.

If we try to put the scale of these responses together with what we have seen with different kinds of boosting these are the sorts of, I wanted to do this to give you the kind of scaling of these rather impressive boosting responses that we saw

So, this was the Australian homologous boosting. He are John Treanor's responses with recombinant

hemagglutinin given at 90 micrograms to those who received the initial 90 microgram dose 7 years later.

Here are those individuals who received plain subunit vaccine which would not have met any licensing criteria boosted 8 years later with an adjuvanted vaccine and here are those who received an adjuvanted vaccine and then boosted with adjuvanted vaccine much later.

So, part of what we are here to discuss and has already been alluded to is is serum antibody necessary for protection. We have had some questions about or people have alluded to data that have been demonstrated with H5N1 in the murine model where limited antibody or no antibody doesn't correlate survival with from lethal challenge and we have also seen this most recently with our H7N1 lethal challenge in the murine model where indeed again challenge with a highly pathogenic virus in the absence of antibody does actually lead to survival following vaccination.

So, you know there remain some questions about what levels of serum antibody, what are we measuring in terms of protection and I give you that example of the booster study where any criteria that we might have applied for licensing would not have been met; yet we clearly achieved a boosted response.

So, to just finish the serological assays have, there are quite a few serological assays. We don't

presently use SRH as we find it does need to be optimized for recent H5N1. Western blot is useful for confirmation and further important data may accrue from analysis, careful analysis of different forms of hemagglutinin you can detect particularly in native virus and we agree ELISA is problematic. We don't find this particularly useful.

Our experience with the baculovirus expression of hemagglutinin is in fact that if you are going to get anything meaningful out of it in terms of specific antibody response you need to use HA1 rather than the full length of hemagglutinin.

So, my key messages based on our European Union experience is that antigen sparing is possible. Cross protection against diverse viruses within a subtype is likely.

Immunity can be maintained if you want to call it that after 5 years post-vaccine even if antibodies decline and I think there are some important questions about the effect of pre-existing heterosubtypic antibody for want of a better word and I definitely think we should move from hypothesis generating which is what a lot of this observational data is to hypothesis testing.

The lunar landscape is a little bit less lonely these days in Europe and I would particularly like to acknowledge work from many, many different people in

particularly the flu lab at HPA, very dear colleagues at NIBSC, in particular John Wood and Diane Major, our clinical colleagues at University of Leicester without whom much of the clinical work for these studies would not have taken place, our colleagues in University of Bergen, Lars Haaheim, Becky Cox and many of the European vaccine manufacturers who have been extraordinarily generous with their time, resources and collaborations to help make some of this data happen.

Thank you.

(Applause.)

DR. KATZ: We have time for one or two questions.

PARTICIPANT: I would like to look back a little bit. I remember at the time that the subtypes for influenza A were being established at a meeting in Geneva, that if I recall correctly some cross reactivity among H5s and H1s was shown with monoclonal antibody out of Winston's group. Is that confounding some of these analyses particularly of your slow response to adjuvant? This is perhaps not necessarily directed at you, Maria but others as well.

DR. ZANBON: I don't think so. I mean it is a fair point because if we were going to pick on any kind of cross reactivity for H5 we would probably pick on H1 as being an important indicator but I don't believe that the data that we have there, particularly the boosted response actually

represents H1 although it is a good point and one which we haven't really pursued and I thank you for reminding me of that.

PARTICIPANT: You have to define your population with respect to the whole arena of H1. That is the problem.

DR. KATZ; Okay, one more question.

PARTICIPANT: Just to push your hypothesis-generating figure around the H9N2 pre-existing immunity perhaps a little further it does look like there is a normal distribution around the H2N2 birth cohorts and I am interested in what might be the explanation for older birth cohorts who also would have lived through that pandemic experience why they would not have pre-existing immunity as well and whether that may be invoking some kind of original antigenic sin hypothesis

DR. ZANBON: You know one hesitates to construct enormously how can I say, elaborate hypotheses based on very small data sets. I don't think that one wants to really say what we measure as pre-existing H9 antibody is definitely due to H2, but it is something that does need to be sorted out, and I agree it is a logical fallacy in the actual data that we have.

Jackie's point earlier about when you move into working on a different subtype one of the things you really have to establish is looking at population-based data in an

age-stratified way so that you have got a really good representation of what is in the population.

That particular study that we had was really based on 18 to 50 year olds I think with relatively few people. So, we weren't properly examining that.

DR. KATZ: I think due to time we will move on to the last speaker before we take a coffee break.

The next speaker is David Cho who is going to be talking about immune responses to non-replicating avian influenza vaccines in clinical trials in the US.

Agenda Item: Immune responses to non-replicating avian influenza vaccines in clinical trials conducted in the USA

DR. CHO: My name is David Cho. I am a Program Officer within the Influenza Group at NIAID with within the Division of Microbiology and Infectious Disease and I was given the task of talking about clinical trials for pandemic flu within the United States and specifically of the trials that we have been involved with within our group at NIAID.

So, the outline of the talk in general is to give a summary of the series of clinical trials evaluating the inactivated pandemic influenza vaccines and so we have been involved with 12 trials right now that have been completed or are in progress with H5N1 and then I have a slide or two

on H9N2 trials that we are working on and a planned H7N7 trial.

I am not going to be talking about the live vaccine because I think that is going to be the subject of a talk later on and if I have time later on I have a slide or two just talking about new influenza vaccine technologies that are under development.

I think Dr. Fauci did a very nice job this morning talking overall about our influenza research program within our group, but we do have a concentration of the vaccines and of course on clinical trials which is a topic today and just to reiterate again the outbreaks that occurred for bird flu back, well, it was pointed out a decade ago but also recently in the last couple of years prompted NIH to start looking at doing some more development of vaccines for H5N1 and so we started to work with our partners and at that time what we needed to do was gain experience with technical and logistic issues such as generating vaccine reference viruses with reverse genetics, support the companies who produced the vaccines and also standardize and qualify assays and provide reagents.

These are all topics that we are talking about today, also, and in addition of course to rapidly implementing the controlled clinical trials of various

populations we want safety and immunogenicity looking at all populations, adults, elderly and children and rapidly provide trial results to the global community.

So, this was our goal, and at that time just quickly going over this slide we had a contract with St. Jude's and on animal influenza surveillance but we also used this to help us with producing some vaccine using reverse genetics to generate some vaccine reference viruses suitable for vaccine production such as the Vietnam avian now 1203 and just an announcement that we have started to have some contrasts that we use to develop our vaccines for H5N1 and some of the obstacles that we realized from the beginning were mentioned before, just gaining the experience for technical and logistic issues and so generating the reference virus using reverse genetics, selecting age and exemption issues. We needed more reference reagents for standardization, also, working with obtaining the vaccine from the manufacturers with licensed products and to evaluate the safety and immunogenicity of H5N1 in well-controlled studies in different populations.

Also, there were no internationally, at that time no internationally recognized standards for use in HAI or microneut assay validation studies. We knew that avian RBCs or the turkey and chicken red blood cells have limited sensitivity for H5N1 viruses and so the horse RBCs improved

assay sensitivities for H5N1.

Some of the caveats, there was no defined correlation of any H5 antibody assays with protective clinical outcomes and lab to lab variability in assays limits our comparisons between the studies.

So, within our institute we had a contract out with Southern Research Institute and they served as our central laboratory for performing the serological tests for H5N1 clinical trials and we used the horse RBCs for H5N1 and we had serological assays, SOPs for HAI development report filed to the IND and I believe I heard recently that our microneut, I know the microneut SOPs and then the developmental report I believe have been filed also.

Just to mention this, we are very conscious of wanting to have reproducibility, consistency. We wanted to have robust assays in place and so far from what we have seen we have seen good correlation and comparability between the HAI and the microneut assays.

The trial that you have probably all heard much about we worked with Sanofi Pasteur to develop an H5N1 vaccine and we completed a series of clinical trials to evaluate the vaccine safety and immunogenicity. So, we looked at adults and we looked at elderly and within the adults and elderly we had four different groups of 7.5, 15, 45 and 90 micrograms for HA per vaccine dose and we also

had a placebo. We, also, looked at children aged 2 to 9 years and had a 45-microgram HA dose also in a placebo group there, too.

We, also, had did studies in two or three doses of H5N1 vaccine or placebo IM injection approximately 1 month apart and our endpoints were to look at safety which was to look at the vaccine reactions and also look at the antibody responses which in our case we were looking at the hemagglutinin inhibition assay and microneut assay, and the results were published within the New England Journal of Medicine back in March 2006. Treanor et al published this and you can look at that paper for the specific information but in general a summary of what we found was that the vaccine was found to be safe, well tolerated at all the dose levels in all the age groups and then the antibody responses were dose dependent.

So, the higher the dose the higher the titers. The titers were similar across all the age groups and the third dose, after the third dose boosted titers back to post-dose 2 levels.

The assays as I mentioned were similar in trend and in the results. So, I mentioned at that time we had the hemagglutinin and inhibition assay qualified. The microneut assay was also used at that time and we did see long-term consistency.

Just a kind of a general overview of the results from that trial, like I said, you can go to the paper for more specific results or specific information there. In general there wasn't a significant difference between the different age groups that we saw within the 45-microgram group and then within the 90-microgram group we haven't seen significant differences to date.

This vaccine went forward to FDA, and it was approved. So, it was again the first US vaccine for humans against H5N1 approved earlier this year, April 2007, and so we had more questions we wanted to ask obviously and so one of the questions that we wanted to ask was can the intradermal administration of the H5N1 vaccine improve the immunogenicity. So, we did a comparison between the intradermal versus intramuscular routes and we looked at healthy adults that received two doses approximately 1 month apart.

The intradermal group received 3 mgs or 9 micrograms and then the intramuscular group received 15 micrograms or 45 micrograms and the results so far are that we have seen that it has been well tolerated but that there hasn't been a very clear advantage of the intradermal route at dosages that we evaluated, the 3 and the 9 micrograms. The third dose. At the third dose at the 7-month mark the antibody titers seemed to decline but boost back to at

least as high as 1 month post-dose 2 level.

So, we wanted to look at a higher intradermal dose trial and that trial is ongoing at the moment. So, results are pending.

The other question that came up was can a clade 3 vaccine prime for a clade 1 vaccine response. So, if you remember back from 1997, 1998, those cases that received the clade or were exposed to clade 3 virus we had 37 subjects in this re-vaccination study who received the two doses of the recombinant H5HA vaccine, the clade 3 vaccine back in 1998 and 1999, and these 37 subjects were now given a single 90-microgram dose of the Sanofi H5 vaccine in 2007

The results from that, from the 37 subjects, we found that the antibody responses in the prime subjects compared against the H5 vaccine naive subjects were that they exceeded those who were unprimed and they exceeded those in the original 1998-99 study and they exceeded those who received the two times 90 mid-doses of vaccine. The responses we are not exactly sure but the responses could be due to generation of long-lived memory CD4 cells or memory B cells. I think we can have more of a discussion and more experts out there to be able to help us to answer this question. The new clade 2 H5N1 vaccines will provide more opportunities to assess immunological priming. So, this is in production now.

So, we had a nice summary earlier of some of the type of adjuvant trials going on in Europe and not all of these trials are within the US but the ones highlighted in blue are the ones that we are particularly working with, but I thought it was a good a summary to show you what is going on basically within the field of trials with inactivated H5N1 vaccine using an alum aluminum adjuvant base.

So, there are numerous trials completed or ongoing or planned. The published trials, Sanofi Pasteur has a published trial that they have looked at, two doses of vaccine at three different dose levels using the alum. So, far they have seen it has been well tolerated and the adjuvant resulted in no significant increase in the immunogenicity so far.

Sinovac has also done a trial using two doses of whole viral vaccine at different dose levels with alum. It has been well tolerated also and their two times ten microgram dose vaccine seemed to give the highest response.

Those studies have been completed but preliminary results have been reported and are still ongoing and involved some of the ones listed here, and we are working particularly with Novartis with multiple doses, the 7.5, 15 and 30 microgram doses plus or minus alum, in addition with working with Sanofi also on their alum adjuvanted vaccine

in the adults and within the elderly population.

So, in summary it seems to be that the safety profile is that these vaccines are well tolerated in the adults and in the elderly. The picture in alum adjuvants so far does not appear to significantly enhance the immune response to H5N1 but as you can see we have ongoing studies going on.

So, there are also quite a few trials with other adjuvants as you have heard of and several trials have been completed or are ongoing and one of the trials that we are working with particularly is with Novartis in the UK with and without their MF59 adjuvant and the trial that we are working on so far we have seen that we can go down to as low as the 7 point microgram dose so far and the safety profile also shows that this is reported to be well tolerated within the adults and these studies need to, are obviously ongoing and I listed GSK as another group that is looking at it I know that several other companies are also looking at this, too.

We, also, have been working with Baxter to look at their inactivated whole viral H5N1 vaccine and so we have a Phase I/II trial to evaluate the dose related safety and immunogenicity within the adult population.

This is a two-dose trials, approximately one month apart and at several dose levels, the 3.75, 7.5, 15

and 45. This was unadjuvanted or a pre-absorbed with alum mixture and so far from the safety results we have found that it has been well tolerated and the immunogenicity is, the results are pending at the moment. We hope to have it in the early quarter of 2008.

We have many upcoming trials that we are looking at, too, to try to get a better picture of what is happening with H5N1 or what the vaccine possibilities are for different clades and so the previous studies for clade 1 strains of H5N1 I have mentioned but we are looking at clade 2 and as has been shown to you several times there have been multiple clades, the clades 2.1, 2.2 and 2.3 and so we are targeting trials within each of these subclades to be able to look at what is happening within the population.

We are looking at the A/Indonesia/05, the reassortant A/Indonesia/05 for clade 2.1 and so that clinical trial is planned for the late part of this year and the other clades, 2.2 and 2.3 we hope to get this going soon. There is vaccine production ongoing at the moment. You can see who we are working with and the trials are planned for sometime next year.

We,also, have been looking at other avian strains such as the H9N2 and the inactivated H9N2 vaccine plus adjuvant was evaluated and we worked with Novartis. This is

inactivated H9N2 subunit vaccine and we did this with and without MF59 adjuvant and this was done within 96 healthy adults and at two doses of the different dose levels, so 3.75, 7.5, 15 and 30.

Again, the safety profile showed that this was well tolerated. The antibody titers and the frequency of the responses were higher in all doses with MF59 than any dose without adjuvant and the single 3.75 microgram dose induced an antibody titer that reached the bench mark many considered or we considered to be predictive of protection at the time. So, the 3.75 microgram dose seemed to have type of effect there, and the results you can see the full study in this publication listed.

So, one of the trials we have planned is this H7N7 vaccine trial. We want to look at the subunit vaccine that has been produced by Sanofi Pasteur and we hope to look at this Phase I trial to evaluate against a dose-related safety and immunogenicity within adults, healthy adults and the planned dose levels are 7.5, 15 and 45 micrograms, two different doses approximately 1 month apart.

Before I move on to this part here I just wanted to give you a summary basically of currently where all of those trials were within our institute and that we are looking at multiple different areas of different vaccine

possibilities.

So, I wanted to move on a little bit to a different area here, some of the major challenges of the vaccine development and availability and I don't really need to go into too much more of the detail but obviously we need to look at different other vaccine production aspects and then also accelerating the development of the modern vaccine itself here as well as different technologies and new targets for antigens.

So, just to kind of end on this note of looking at different new platforms, too, the reason why I just bring this up is that there is a lot of interest in a lot of vaccine candidates looking at broad spectrum possibilities.

Several of us I think attended a WHO meeting last week where we saw a lot of potentially great candidates out there that are going to come across questions down the road here of how to proceed with them and a lot of talk of correlates of protection and correlates of immunity came up in that meeting.

So, you can see that with all these new technologies that we really need to explore those questions in depth.

So, the progress of our H5N1 vaccine development program within our institute has been so far we have

successfully used reverse genetics for the vaccine reference virus production and we are trying to develop assays, reagents, strain libraries to standardize everything there and efforts are under way to try to decrease the lab to lab variability and so that is why it was important for us for example to work closely with SRI during our vaccine trials to try to get it standardized as much as possible there and of course we need expanded manufacturing capabilities. That is definitely needed and continued development and evaluation of the multiple approaches that I had mentioned at the end and throughout the talk, the adjuvants, different substrates and the delivery devices or routes and probably you have seen this slide many times. This is my last slide I think it is a prerequisite if you work for NIAID to have this on your slide but definitely it is true though. We definitely need to approach this as whatever we can find for pandemic and solutions we can find for pandemic will definitely help us with our seasonal vaccine evaluation.

So, I think that is it.

(Applause.)

DR. KATZ: Any questions for David?

PARTICIPANT: A lot of our efforts are geared towards H5 or maybe even H7 and a little bit against H9, but isn't it equally possible especially now that H5 is

going down in human infections that we get surprised by an H13 or an H8 or that H2 comes back and are you doing anything to make some vaccines for all of them?

DR. CHO: There is definitely a possibility that any of those strains could come out. I don't think I would be telling the truth if I didn't say that there was a possibility. Currently we don't have anything specifically planned in terms of trials but we have had discussions about what we would need to do if something like that would come up. So, there has been a lot of internal talk about what we might need to do for that but currently in terms of some of the production aspects we don't have anything currently right now ongoing.

DR. WRIGHT: Peter Wright. I want to ask a broader question as to whether the poor immunogenicity in general of certainly unadjuvanted vaccines is a result of the strain, a result of the assays or a result of this being a novel immunogen and what you may be able to learn from looking in young children who are indeed undergoing their first exposure to influenza with vaccination.

DR. CHO: A very good question, a loaded question. Definitely I think that we need to find out more information about exactly what is happening with the adjuvant itself. What these trials were in place were to get something out in the manner, in an expedited manner and

we looked just completely at just like the AJIs and at the neuts and now we are starting to be able to expand and start looking at some of these other possibilities like the adjuvanted vaccines that you mentioned.

I think that I have a portfolio where we do a lot of vaccine development trying to look at adjuvants and there is a lot of groups that are trying to look at that question of what exactly is happening and I personally don't know if I can give you an answer other than I think all of those things that you mentioned are possible. It could be any of those things really or a combination of things that actually could lend to that.

PARTICIPANT: How much cross reactivity do you see in the antibody response to the vaccines that you have tested so far against clade 1 in terms of testing against clade 2 or the various subclades?

DR. CHO: I don't know if I have the specific answer for the cross reactivity because I wasn't involved necessarily in all the trials. I am looking at some colleagues to see if there is any answer for the cross reactivity.

Sorry.

DR. TREANOR: You know at least with the unadjuvanted clade 1 vaccine there is relatively little cross reactivity to clade 2 in the limited number of

samples that were tested.

When we looked at people who had gotten clade 3 and followed by clade 1 still most of the response was directed towards clade 1 but their response is a little bit more broad. One thing that we didn't see was that people who had been originally vaccinated with the Hong Kong when they are re-vaccinated with the Vietnam we don't see a twisting of the response predominantly towards the Hong Kong. So, in that sense we didn't see anything that looked like original antigen in that sort of small sample set.

PARTICIPANT: My second question is how long does the antibody response that you see that boost with the third dose, how long does that last?

DR. CHO: I might need help again.

DR. TREANOR: To my knowledge the only data that exists is at the 1-month time point after the third boost. We saw no evidence of antibody to Hong Kong in people who had received the vaccine 8 years previously. So, by that point there was no detectable Hong Kong antibody.

PARTICIPANT: In your portfolio of work looking particularly with the adjuvanted vaccines do you have studies underway or planned for the quality of the immune response as well as the quantity of the immune response?

DR. CHO: Specifically no because the portfolio that we have, we encourage, our portfolio is based off of

the ideas that come from our community and what they are looking at and right now not specifically looking at those areas although there has been a lot of discussion to try to target those areas because that is really kind of the next step to look to see exactly what has happened with those adjuvants. So, it is an area that we have been just having a lot of discussion about that we are trying to open up possibilities for.

PARTICIPANT: I am going to follow up on John's point. I don't think he has actually seen this data yet, at least not the analysis but we did memory B cell ELISPOTs a la AMED(?) on those re-vaccinated subjects and in those that responded to H5 vaccination very well in terms of increased frequency of B cells they also tended to respond to H1 at least the currently circulating strain of H1. So, again there is some evidence that there might be some level of cross reactivity but that again, remember that doesn't measure protective antibody. That is not functional. It is more like an ELISA. So, I am not quite sure what that means yet.

DR. KATZ: Okay, thank you, David.

I think we are going to take a 15-minute break now and then return for the final three speakers in the session.

(Brief recess.)

DR. KATZ: Let us get started. All right, we are going to continue on in this session now with Ruth Karron who is going to be telling us about immune responses to live attenuated A/Ann Arbor cold adapted avian influenza virus vaccines that are in clinical trial.

Thanks, Ruth.

Agenda Item: Evaluation of immune responses in clinical trials of live attenuated A/AA ca avian influenza virus vaccines

DR. KARRON: Thank you, Jackie and thanks to the organizers for inviting me. It was very good for me to hear Jackie's and Maria's talks earlier today because it made me realize that there are a few points I probably need to highlight as we move forward.

So, what I wanted to tell you about today is the experience that we have had over the last few years with live attenuated A/Ann Arbor vaccines that we have been evaluating at Hopkins.

These are vaccines that were developed at the NIH by Kanta Subbarao and her colleagues and under a CRADA with Medimmune as well. We have evaluated two H5N1 viruses, A/Vietnam 2004 and A/Hong Kong 2003, one H9N2 G9 virus, Hong Kong 97 and one H7N3 virus, the British Columbia virus.

The H7N3 virus we just evaluated this fall and we

don't yet have our immunogenicity data completed. So, I won't be discussing that but I will be discussing today the two H5N1s and the H9N2.

So, just to give you a sense of how these trials are done they are currently inpatient trials. So, they are open label trials that are done in an isolation facility. We have done them over the last 3 non-influenza seasons in our region in 2005, 2006 and 2007. We admit people to our unit 2 days before vaccination. We emphasize to them the importance of remaining on the unit until discharge at the time of enrollment, at the time of admission, at the time of vaccination and throughout the study.

Vaccine was administered to these individuals by nose drops or by nasal spray. People were examined daily. Nasal washes were obtained daily for viral culture and for RT-PCR until the time of discharge and our criterion for discharge included being RT-PCR negative for vaccine virus.

Tamiflu was available for significant illness or in the event of early departure. So, in case you are interested this is what our isolation unit looks like. We also had requirements for our clinical staff which is that they had to have gotten seasonal influenza vaccine within the past 6 months either live attenuated or inactivated vaccine. They needed to wear gowns, gloves and masks on the unit during and after vaccination and if they developed any

fever or any respiratory viral symptoms they were to be started on Oseltamivir pending the results of an influenza PCR from their nasal swab.

I wanted to say something about the assays that we did. We did hemagglutination inhibition assays. We used horse red blood cells for the Vietnam 2004 and for the H9 virus; we used turkey cells for the Hong Kong 2003, having to do with the difference in receptor binding of these viruses. We did microneutralization assays which I will tell you about in a second. These were essentially derived from procedures used at the CDC but with some modifications that I will describe.

We are, also, in the process of doing ELISPOTs to measure IgA and IgG antibody secreting cells and those studies are really in process and we might be able to say a bit about them.

So,just a comment on the microneut assay. As I said it was really based on the CDC assay. The most important difference between the CDC assay and the assay that we do is that we did use the Ann Arbor and since that is a temperature-sensitive virus our incubation was at 32 degrees instead of 37. Otherwise it is actually quite similar and the readout is the same.

So, first just to talk about the replication of the Vietnam 2004 virus what you can see is that the

replication was highly restricted in adults. We were only able to recover virus from two individuals. This is a cultivatable virus, one subject on day one and one subject on day four, both at very low titers. We did have a large number of people particularly in that second dose group who were PCR positive. They were really PCR positive for the most part on day one and although we don't know this with absolute certainty this could very well represent input virus.

We saw actually very similar results with the Hong Kong 2003, again very limited viral replication and some PCR positivity as I mentioned.

These are the results of our assessment of immunogenicity both looking at HI responses and microneut responses and as you can see and as you might have predicted from the level of replication these responses were really uniformly quite poor with only a couple of individuals responding in each group.

Thinking then about the H9N2 virus this was a vaccine that also was quite restricted in replication in individuals and one comment that I wanted to make about the H9N2 and again taking off on Jackie and Maria's point we enrolled individuals who were born after 1968, and we did that deliberately because we were hoping not to have individuals with prior exposure to H2N2. When we initiated

our studies we were not screening individuals because we thought that enrolling those younger individuals would be sufficient.

However, we found to our surprise that about 30 percent of individuals were H9 antibody positive of these young individuals. So, in subsequent cohorts that we enrolled we actually screened them and the data that I am showing you here are from HI seronegative individuals.

So, we had about 50 subjects in all and I should say again not only seronegative but seronegative individuals who received two doses of vaccine. So, those are the people shown here.

So, again, these seronegative individuals vaccine virus was highly restricted in replication. Really the data don't look particularly different from what you just saw with the H5.

However, the antibody data do look quite different. Here you can see that of 24 individuals who received two doses of vaccine 22 had a four-fold rise in antibody titer after the second dose of vaccine. Nineteen had a four-fold or greater rise in microneut antibody titer.

This slide just shows you the distribution of antibody titers. You can see that most individuals had a titer of about 1:16 with some distribution on either side.

This slide just shows the correlation between the HI and the microneutralizing antibody titers following H9N2 and what you can see is that these really correlated very well as shown.

So, what I have to say about these vaccine viruses is that they were well tolerated. I didn't show you those data but you can take it perhaps on faith or I have those slides if you are interested and highly restricted in replication, that two doses of the H9N2 induced four-fold rises in H1 titer in 92 percent of subjects and microneut titers in 79 percent. In contrast to doses of the H5 viruses either one was really poorly immunogenic and induced HI responses in a very small subset of individuals.

So, I think that one important conclusion is that the antibody responses to these Ann Arbor viruses containing avian hemagglutinin and neuraminidase vary depending upon the surface glycoproteins included in the vaccine and can't be predicted based upon detected viral replication.

For recipients of the H9N2 vaccine there was a strong correlation between HI and microneutralizing antibody responses with HI detecting a slightly greater number of responses and finally since the insertion of the avian hemagglutinin and neuraminidase genes appears to further attenuate Ann Arbor cold adapted viruses and we

have now observed this with H5, H9 and H7 viruses we feel that consideration could be given to cautious outpatient assessment of individual strains outside of the influenza season following initial inpatient assessment for characterization of vaccine virus shedding, and I will leave you with that. This is the work of a large number of individuals as shown on the slide, people from Hopkins, from NIAID and Medimmune.

Thank you very much.

(Applause.)

DR. COMPANS: Dick Compans. Although you have shown that there isn't evidence of virus shedding is it possible that the genome segments are persisting in a way that could reassort with a super infecting virus? Could you consider an experiment where you actually super infect with a seasonal influenza and look at the possible presence of reassorting?

DR. KARRON: It would be a hard thing to do.

PARTICIPANT: I was going to ask one question. Have you looked at the mucosal antibody responses with H9?

DR. KARRON: In process.

PARTICIPANT: I don't know if I missed it but the antibody secreting cells especially with H5, what did they look like?

DR. KARRON: Kanta, do you want to comment? We

didn't look at it with H9. We started this with H5s and we are in the process of looking for H7s and with H5s it was largely the same as the serum, so, disappointing.

Bob?

PARTICIPANT: Your last conclusion was you thought you could move these to outpatient studies which sounds perfectly reasonable. I think the other thing I might conclude would be that these are overly attenuated and that you need to do something to make them less attenuated. I wondered what the thoughts were generating those viruses.

DR. KARRON; I am going to let Kanta comment on the generation of those viruses.

DR. SUBBARAO: We absolutely agree. We have got viruses that are over attenuated and from all the experimental data and clinical trials data on the H1N1 and H3N2 cold adapted viruses it suggests that the avian HAs and NAs are over attenuating or further attenuating this. So, we are looking at a couple of different possibilities. We are now currently trying to make chimeras with the transmembrane of cytoplasmic domain for the H2 hemagglutinin and N2 neuraminidase swapping them out to see potentially an interaction of that part of the HA and NA with the internal protein and we are also passaging these viruses in human airway epithelial cells and in ferrets to

identify where adaptive mutations might be occurring.

The fact is I don't think it is as simple as an alpha 2,3, alpha 2,6 receptor specificity because the Hong Kong 2003 virus actually has both and did not replicate to a higher degree than the Vietnam virus did and the G9H9N2 also has an alpha 2,6 preference. So, I don't think it is as simple as the alpha 2,3 versus alpha 2,6 but those are some of the things that we are testing. The problem is that we don't have a predictive model to evaluate preclinically before going into clinical trials. So, you know all we can look for is we can make sure that the viruses don't lose any of their phenotypes that we want to see in them but we can't evaluate for enhanced replication without going into clinical trials.

PARTICIPANT: I may have missed it because I was out of the room but certainly once you start changing the backbone you get into while it is scientifically interesting it is complicated from a regulatory standpoint.

What about just upping the dose? Have those been done? That seems to be a simpler first strategy.

Did I miss that?

DR. KARRON: Yes, I mean initially with the Vietnam 2004 we tested 10 to the 6.7 and 10 to the 7.5 and after that it becomes probably prohibitive in terms of dose.

PARTICIPANT: Prohibitive?

DR. KARRON: From a manufacturing perspective and also even you know the other thing is that at least with the was it H3N2, Brian, at a higher dose you see non-specific febrile responses when you give 10 to the 8.

PARTICIPANT: Those are with other viruses.

DR. KARRON: Humans, right.

PARTICIPANT: Cold adapted.

PARTICIPANT: No, no, I know cold adapted but those are human hemagglutinins.

PARTICIPANT: That is right.

PARTICIPANT: I just wonder if you or Kanta would like to comment on the ferret model as a replicative model for influenza, a preclinical model?

DR. SUBBARAO: I will be talking about that tomorrow. So, stay tuned.

PARTICIPANT: I think I heard a hint that the H7 also did not from the data you have so far, did not replicate well.

DR. KARRON: No, the H7 appears to replicate somewhat better. We don't have our data fully analyzed yet, but certainly better than the H5 and it appears even a little bit better than the H9.

PARTICIPANT: Ruth, do you want to just describe what an H1N1 or H3N2 CA virus you would expect from

replication of these viruses to show so that people have an idea of what these titers, and what does a titer of less than one mean, you know what I mean, just so that people understand that this is a very substantial reduction in the frequency of responses?

DR. KARRON: And you are talking about in a naive host really. So, really the correct comparator is to naive children, right of say of an H1N1 or something like that. So, then I think you would expect to see replication somewhere between 2-1/2 and 3-1/2 logs in young children for several days. Usually it comes up fairly early with these viruses, so let us say days 1 to 4 or days 1 to 5 although young children can actually shed virus out for a week or so.

PARTICIPANT: And also it grows to about 10 to the 3.

DR. KARRON: That is what I said.

PARTICIPANT: Okay. One other thing is that when you look on these vaccines there are two properties. One is attenuation and one is infectivity and here what we are seeing they oftentimes go hand in hand. Here clearly we are having an alteration in infectivity as well as in those individuals who are infected in the level of replication. So, both of these parameters seem to be modified with these avian recombinants.

DR. COUCH: I think it is a fairly obvious comment that you need better predictive factors which Kanta alluded to in your animal models, the preclinical before you go to that clinical and if you have got H7N3 that got a unique neuraminidase and you are getting much more replication you are beginning to get there with the variation you need to as Wendy says, validate the clinical model using the human studies rather than the other way around the way we usually think, and if you go with H2N2 with the Ann Arbor parent itself I heard that Ron thinks and I agree with that now you are going to probably get good replication like we are used to seeing with H1N1 and H3N2. That is all you need now to have the animal guide you.

DR. KATZ: We will move on to the next talk. Next we will be hearing from Laszlo Palkonyay and he is going to be talking about immune responses to non-replicating avian influenza virus vaccines that are in trials in other parts of the world that we have not heard about yet.

Agenda item: Immune responses to non-replicating avian influenza vaccines in clinical trials conducted in the rest of the world

DR. PALKONYAY: Thank you very much. As you might realize I changed a little bit of title. It was the rest of the world or something like this and I came from the WHO

which is an organization serving 193 member states and from Liechtenstein to Luxembourg and all of them so I used the other term, and I have another excuse to do this because I go into Europe. This was Maria's territory and unfortunately Austria, Bohemia and what have you and Hungary is in Europe. So, I am very sorry for this but I had to change it, and the scope of the presentation and this scope is not necessarily a series of teams like that, rather leitmotifs which will be used during the presentation and I feel so much encouraged by history that there are many references to history here today and I just would like to add one thing. I always felt uncomfortable with the statement that hepatitis B vaccine was the first human recombinant vaccine. I think it is the influenza vaccine, the classical reassortment methodology is really a recombinant product before the juschinanglo(?) nucleases were even discovered.

So, that said, having said that whole virion vaccine concept will be used. We heard many, many quotations today about this concept way back from the mitzerantes(?) and undulation concept in general with special reference to alum partly because in this type of studies mainly alum was the adjuvant which was used and potentially it was a prospect.

The historical perspective the clients with

pandemic prototype vaccines was a study influenced by undulation through epidemiological situation.

In 1976 we had the pandemics which did not occur and thereafter there was the pandemic with the reappearance of the H5N1 vaccine after more than a 20-year period which gave an interesting experimental possibility for clinical trials.

So, here is the first 1976-1977 trial experience which was a big effort. Then it was quietness until the shock in Hong Kong in 1997. Fortunately at that time it was really adjudicated(?) at least in this area and a lot of work started after this. If I remember well the first one was a recombinant product used in buffalo viral system and of course exploratory H5 works and H2N2 was a model for pandemic prototype vaccines.

Since 2004, we are in a Phase III pandemic earlier stage and since that we had a plethora of clinical trials with emphasis mainly on H5N1 strains.

This is just a notion, actually a repeated notion. It was talked today already, the possibilities and the potential advantage of whole virion approach in unprimed individuals. I don't go into it because it was very nicely presented by Maria before and what was the clinical evidence accumulated before the 2004 outbreak regarding pandemic prototype vaccines? There was a message

that whole virion vaccines are found more immunogenic in population from 1976 to 1977 and from the early H5 trials it turned to be a situation that for a pandemic model works in two doses of at least 15 micrograms hemagglutinin needed but in case of H5 vaccines may be more than 30 micrograms and this is a combination of the two peak experience and there were initial evidences from H2N2 and exploratory H5 trials that dose sparing might be possible both from whole virion or split prototype vaccine, MF 59 data for split vaccines and alum data for whole virion vaccines H2N2.

It was very interesting to see what was the situation before 2004. This is a modified quotation from one of John Wood's articles and basically what was the issue which was in the focus of research. There was a need to find consensus on the type of vaccine, whole virion, subunit, split, adjuvanted or live attenuated, dosing and with adjuvationautic(?) vitamins to stimulate protective immune response and we can appreciate the fast development of the field when it is mentioned that we have to gain via exponential genetics technology when it is really flourishing, this area. Let all issues relating equity of vaccine supply, in fact, we talked about the issues. I would say this issue is probably resolved. Since that approval for licensing we are experiencing international

harmonization in this area and one should ask, suggested to develop their own pandemic plans. This is a given today.

This was the situation up to the end of 2006, limited information on clinical trials and very, very limited information and in 2007, February, there was really a mushrooming of results. Maria was referring to this meeting and you can see two web links below which actually you can look more details from this meeting and at the end you will have all of these web links. I will provide a few more web links at the end of the presentation.

Basically at that time it was claimed that more than 10 countries are developing prototype pandemic vaccines against H5N1. At least seven adjuvanted vaccines induced immune response that meet international criteria for influenza vaccine licensing. These are of course mainly the inactivated products.

There were developments in the adjuvant field and very significant dose sparing first reported and the first sign of some in a sense potentially protective response against strains of H5N1 virus as related at different times in a variety of geographical locations obviously closely monitored.

The presentation focuses here really at the two ends of this diagram as 99 percent of the presently produced influenza vaccines belong to the split or the

subunit groups which from the clinical point of view probably they are almost interchangeable and very, very small part of the market is covered by live attenuated vaccines or whole virion vaccines but they do exist and they are used in doses more than a million a year in doses that are marketed.

I have to focus on only influenza vaccine production technologies and obviously I don't think I have to spend too much time on this slide with the audience but maybe at this one. These are the available H5 vaccine platforms and what is really highlighted, there are two technologies. It doesn't mean any potential or theoretical advantages. It simply is a reflection of the number of persons who participated in the clinical trials. So, these two are where we have the most information and this is just a little reminder for inactivated vaccine approval process. This is really just a cross section of the criteria for the adult and basically in the seasonal vaccine there is only one criterion that should be fulfilled for a passing mark. It is recommended for prototype H5N1 type novel vaccines that all these should be met. So, this is what the criteria are used by the industry when they are evaluating their vaccines.

Of course the data are not comparable for the reasons which were discussed many times before today. So,

it is the first group. Again, we are back with the whole virion concept. This is the list of the vaccines which were in clinical trials and obviously many of them belong to the presentation scope today. One of them is the Baxter vaccine. The vaccine is produced by four members of the Japanese consortium which are different vaccines but they are developed in harmonization and together Denka Seiken, Keta Sauto and Karka Kuchen; that is more difficult than my name, Karka Chuken, yes, sorry and the next only was a Hungary product and the Sinovac product and the others of course belong to different territories.

Here is one example, the cell-derived inactivated whole virion wild type H5N1 vaccine is very unique, is wild-type vaccine. So, it is an inactivated wild-type isolate. Here it is from the Vietnam strain. There is a two-dose schedule. The studies were carried out in Asia. That is why I mention it here and the results were presented partly a week ago at the WHO meeting which was concentrating on broad spectrum and long-lasting immune response influenza vaccines and it seemed to be that the non-adjuvanted formulation was more immunogenic and cross neutralization was measured or demonstrated against clade 1, 2 and 3 viruses and cross protection studies were carried out in animals.

This is the next one, Chinese vaccine. This is

from the Phase I trial. So, the numbers are relatively low. However, basically with a 10-microgram hemolofinin(?) content alumentum(?) hydroxide adjuvanted whole virion vaccine all three EMEA criteria were met and actually these were the actual results. As far as I remember they used turkey blood cells. We heard from Jackie's presentation that the issue what type of blood cells are used in the hemagglutination inhibition test is an issue.

The next one is the Omnivest group which is again an unmargeriented(?) egg-based whole virion vaccine which is a marketed vaccine in Hungary. At this point more than 650 patients participated in three trials which includes the elderly up to the age of 83. This was mentioned already by Maria. It is a one-dose schedule approach with 5 micrograms hemagglutinin content and all three EMEA criteria were met with one dose approaching the others and the elderly group according to the presentation last week and according to the, and in the Phase I study which is published also, is compatible with the statement.

Cross neutralization both with hemagglutination inhibition and microneutralization test was detected with H5N1 strains from different phylogenetic clades and to just go further the following strains were tested, from clade 2.2 a senchute(?) reverse genetic strain from another clade 2.2 group, actually it is a classical reassortant strain

and from the CDC another reverse genetic strain clade 2.3. I hope the spelling is good because I feel that writing down the nomenclature of influenza vaccine isolates or bad links, long, long bad links is something adult prone like influenza propagation. So, I hope I am correct with this quotation, with the names. At least I tried to be correct, and this is just a segment of the results because these are non-published and I didn't want to go beyond just to get into segment. Basically the plus signs mean passing this particle at hemagglutination inhibition test. It is very interesting that we used chicken blood cells for the testing which is supposed to be less sensitive and this is really a cross immunization for positivity and the little summary statement about alum adjuvanted whole virion vaccines. I think we can state for this group of companies that some alum adjuvanted whole virion vaccines were highly immunogenic and showed significant cross neutralization with H5N1 strains of different phylogenetic clades and here I am speaking about five, six or maybe seven companies now. So, these are not isolated cases. There is a big cover for that. No alum free control arms except the Baxter study but the Baxter vaccine is very, very different than any other vaccines being applied by the isolated inactivated product that I would not consider it as a generally acceptable control, I mean stating that it was tested.

The issue here is of course is the alum really needed or not. Inactivated subunit vaccines, it is a list of vaccines. Some of them, the studies are finished. Some of them are already registered by regulators. Others are ongoing and the situation is the same for inactivated split H5 vaccines.

I don't spend too much time on this. I think this is a fair conclusion that alum adjuvation(?) up to now has provided only modest or not antigen sparing effect. There is this initial clinical data with split and non-adjuvanted vaccines.

A generalized conclusion in WHO style, safety and immunogenicity, we can state that vaccines reported to the WHO at four meetings during the last 2 years held at WHO were described as safe and well validated in the age groups studied.

Vaccine immunogenicity was demonstrated to vary based on type of vaccine, dose and the presence of adjuvants and the next meeting for the discussion of the progress of clinical trials with novel H5 or similar vaccines will be held early in the first quarter of next year and here is the fullest conclusion about the situation, the whole virion concept and the novel adjuvants. When I speak about novel adjuvants I call them, I really mean under this particular context as oil and

water immersions because the successful adjuvants with split or subunit vaccines belong to this group, MF 59, AS or AF 03, etc. There are potentially all the other, similar other adjuvants and then you see the two highlighted things, whole virion vaccine which has the most promise based on all the data were never tested according to my understanding with these novel adjuvants. It would be interesting and we can also say as I mentioned earlier that whole virion vaccine was not tested without alum with the notable exception of this mentioned Baxter situation.

So, it is a situation where I think from the scientific point of view it would be interesting to test that. However, we have to face the situation that most of the companies who are going into the split novel adjuvation approach, they have their licensed product which is a split product. So, they are probably not much interested to go into the whole virion direction and the other company which tested alum adjuvanted whole virion vaccine probably is not interested to go into the research without alum because the actual licensed seasonal product is an alum adjuvanted influenza vaccine, but from the scientific point of view it would be interesting.

I just would like to mention that in Canada there is a whole virion vaccine which is still licensed according to the committee on regulations and it was in use until

late 1997. So, that would be a theoretical candidate going into this direction.

Some selected electronic publications as I promised at the beginning from the initiative for vaccine research at the WHO. It was already mentioned the clinical meeting. We are heading for the next one in 2008. The first one and the second one the summaries were already published in peer-reviewed journal. We are hoping that the third one will be also published but at the moment it is accessible from the Internet.

It is accompanied with the tables on the clinical trials of pandemic influenza prototype vaccines with many details about serological data and about the trials. So, it is worthwhile to check and we will be updating this probably after the next meeting in February.

I think today covered all of the potential vaccines except maybe the Russian live attenuated influenza vaccine which really complements the US Medimmune NIH type of work with live attenuated influenza vaccines for pandemic preparedness and this was also published and it is very interesting because here all of the presentations are there. Whenever we have WHO meetings sometimes certain presented data are withheld by the presenters but this is a unique situation. Somehow it happened that nobody withheld any presentations. So, it is really complete. So, you can

find the complete list of presentations. It is not like a selected list of presentations like in other cases and the last one I mentioned the IP. It was an issue especially a few years ago. I think probably many issues are resolved since that. There is a document accessible from our web site which deals with intellectual property related to pandemic influenza vaccine productions.

Thank you very much.

(Applause.)

DR. KATZ: Any questions?

DR. WRIGHT: Peter Wright. Just for historical perspective the recognition that the whole virus vaccines were more reactigenic came out of the experience with the swine influenza vaccine. It was much more marked and evident in children than in adults, but it included more febrile reactions and actually with whole influenza B virus seizures associated with administration of vaccine.

So, caution will have to be used with whole virus vaccines as and if they move into children.

DR. PALKONYAY: I certainly agree with the historical notation and a lot of history going on in this at this meeting. I would like to say two comments. It is not contrary, just complementing what you just said. According to the GSK data which were published about H2N2 whole virion products adjuvanted with a combination of

aluminum phosphate and hydroxide that besides local reaction there was no difference. If I go back early nineties from my earlier experience as regulator I have seen face-to-face comparison between subunit and whole virion vaccines for regulatory introduction and in not very small children and basically there is no difference seen. Some people would suggest that for the higher antigenicity at least partially the higher endotoxin content of these older less sophisticated vaccines maybe it has a role. So, with caution but it could be explored.

PARTICIPANT: Talking as a regulator is there good scientific evidence and consensus that cross reactive antibodies would be protective?

DR. PALKONYAY: Only animal data and definitely there are cross protection. These are just cross immunity. I mean it would be difficult to challenge with H5N1 humans.

DR. COUCH: I guess the whole virus I want to comment a little bit back from Peter. No question about the fact of what happened to children in the swine flu and the greatest reactigenicity was in the Merck vaccine but the Merck vaccine had a huge amount of hemagglutinin in it as well. You see great immune responses. Merrill(?) National had not quite as much but still was more reactigenic than the split products. That was very clear. It was not so clear in 1968, I get this from my pediatric colleagues with

the purified whole virus vaccine that those were excessively reactigenic in children of various ages. Most of those were very small trials, so that I would be echoing Peter's comments saying that if you want to move whole virus into children the data we have available say that you need to be cautious but the data don't say that they are excluded I don't think.

DR. PALKONYAY: Exactly and this vaccine mentioned this is as I mentioned a whole virion vaccine which is adjuvanted routinely with alum and the indication is 3 years and up and it is used more than a decade with yearly more than 1.2 million doses distributed. I mean this is an area which needs of course caution but there are possibilities.

DR. KATZ: Laszlo, I had one question. You showed a couple of clinical trials where the immunogenicity was conducted by an H1 using turkey or chicken red blood cells and some of the results seemed that there was quite a good vigorous response which is somewhat surprising based on other things we have heard today.

DR. PALKONYAY: This was the reason I mentioned that because this is what they claim and actually the turkey is published and the other one is direct information. So, these are confirmed information.

DR. KATZ: So, were there any efforts to do some

sort of neutralizing antibody study on the same sera or to share the sera to other labs that could perform a neutralization assay?

DR. PALKONYAY: I can see that probably John could respond to this because I know there are some connections.

DR. KATZ: Okay, John?

PARTICIPANT: The sera from the Hungarian trial they were actually shared with Maria Zambon's lab who tested for neutralizing antibody, because they contributed towards the new international standard, they had good levels of antibody.

DR. KATZ: I think we will move to Fred Hayden and Fred is going to talk to us about the WHO research initiative for H5N1 infection in humans.

Agenda item: H5N1 Infection of Humans

DR. HAYDEN: Thank you, Jackie, and good afternoon. I would like to thank my WHO colleagues and the other organizers for the opportunity of being with you this afternoon. They needed to make a change in schedule to accommodate me and I appreciate that very much.

So, unlike the majority of the previous speakers I am not going to share primary data with you but rather try to discuss several specific research initiatives which have been recently launched at WHO and I hope that this

particularly in the context of the discussion that will be happening in the breakout sessions on Wednesday will lead to the possibility for some future collaborative work particularly feeding back to the Southeast Asia Clinical Influenza Research Network and the person who will be occupying the position of influenza research coordinator at WHO.

So, these are the three areas that I would like to briefly touch on in the next 10 minutes or so. We convened a consultation on clinical aspects of human H5 infections in March of this year in order to pull in experience from the individuals, the clinicians who were actually taking care of these patients in the field and this was in part to try to understand about changes in the disease and best management practices and one of the reasons for this that in fact there is no current H5 database that really captures clinical and treatment outcomes information.

Currently WHO does have a database but it really has only basic demographic risk factor and exposure information and ultimate outcomes of the patients in terms of survival or mortality, and because of this we felt that there was a need for an integrated database for in part timed risk assessments and to look at then changes in disease presentation whether this is changing with the

evolution of the viruses and of course this relates to more rapid recognition, examine the prognostic features of H5 infection and hopefully come up with more effective treatments as well as assess the safety and tolerability of a number of the other treatments which are being used empirically in these patients.

So, in the context of the Turkey meeting we had discussions about the need for an integrated database and concluded that that would be an important future activity for WHO, and indeed has been agreed to by senior management and this is part of an ongoing discussion internally.

So, the proposed database will try to capture both retrospectively information from the patients that have been recognized to date, but also importantly in terms of the risk assessment side in particular look prospectively at new cases and as timely a fashion as possible in order to protect both the clinicians who would like to publish their own data in the field but also the confidentiality of the patients. There will be no release of individual patient data but what we will plan to do is to provide regular updates on the WHO web site or through the weekly epidemiological record, but again this is an issue that is currently in discussion. We will be having a meeting with our regional advisers, in fact, later this week and I hope that that will provide an opportunity for

further discussion but your input on this would be welcome.

As an aside then we did add information when we presented our updated advice on H5 clinical management which is summarized on this particular web site. We added two other documents to the web site. One is a clinical case summary form and another is a supplementary case summary form which gives more detailed virology for clinicians and other individuals in public health to share individual patient data back with us at WHO.

So, there is a mechanism in place already for capturing this information.

The second research initiative that I would like to spend a little bit more time on is the Southeast Asia Influenza Clinical Research Network.

This particular network was founded really in 2005 and had its first network-wide meeting in 2006 to try to foster investigation not only on H5 and other novel influenza viruses but also to advance really our understanding and management of human influenza irrespective of the viral etiology and again this grew out of the recognition that although there was a lot of information regarding surveillance of influenza particularly H4 disease, there was a paucity of information regarding what was happening in terms of disease pathogenesis, diagnostics and improved case management.

So, this is a true international multilateral collaboration involving clinical centers in Southeast Asia and also four key international partners including the National Institute of Allergy and Infectious Diseases, Oxford University; the Wellcome Trust as well as the World Health Organization.

The principles are summarized on the web site which I showed you on the previous slide but just to briefly comment these are to develop knowledge on influenza pathogenesis, therapeutics diagnostics and prevention through protocol-based studies most of which will be hospital based but some also in the outpatient setting.

There is a strong emphasis within the network on building the capacity for independent research both at the individual and investigator level but also for the institutions that are members of the network. Of course, part of this will be compliance with international standards for clinical research so that the study data that are generated can be used in fact for regulatory purposes in the future. The network is committed to prompt sharing of data and isolates and genetic sequences, of course with the approval of the relevant national authorities and we have striven to also create publication guidelines that are inclusive particularly focusing on the investigators in the

affected region.

So this is the current depiction of the actual clinical sites. There are as you can see five in Vietnam, four currently in Thailand and two in Indonesia both in Jakarta. We are in the process of adding a third site in Central Java in Indonesia as well. The clinical center at NIH is also a collaborating clinical site obviously for studies of severe human influenza due to seasonal viruses.

Now, one proof of the existence of the network of course is our grant program, and this gives you some sense of the complexity as one tries to move forward with an international collaborative effort like this but looking from the ground roots upwards you will note that we have what we call country coordinating groups for each of the three participating countries, Vietnam, Indonesia and Thailand and then these groups, these working groups are with the primary investigators at the different institutions, and their support staff can meet on a regular basis to examine the status of particular protocols but also to come up with new ideas in terms of future research. Specific studies are generated, then ultimately through a protocol team that has advice from a variety of advisory committees where there is expertise both within and outside the network and then protocol implementation is overseen by a trial operations committee.

This whole activity in terms of the day-to-day endeavors is supported by the network coordinating center which is located at the Oxford University Clinical Research Unit at the Hospital for Tropical Diseases in Ho Chi Minh City and the overall activities and strategic direction are decided upon by the Network Steering Committee which includes representatives from each of the participating countries as well as the international partners.

Just to give you some examples of the kinds of studies that are in progress or under discussion right now first on the antiviral side which is where we started initially because of the need for improved clinical management and you have seen the figures about the current case fatality rates in confirmed H5 disease, we focused heavily in this area but I would just hasten to add that these are just one set of studies. There are over a dozen now that are in different phases of either implementation or development which include other aspects of influenza.

The first interaction study in an Asian population looking at oseltamivir with probenecid loading has been completed and I look forward to seeing the publication of the results of that study in the near future.

This large multicenter study of dose comparison of standard to higher dose oseltamivir therapy in either H5

disease or severe human influenza was launched in July of this year and is gradually enrolling patients currently. Of course, this is dependent on the influenza seasons in the affected countries.

We are trying though as quickly as we can to try to move toward implementation of a parenteral neuraminidase inhibitor study specifically in avian influenza patients because as you are well aware there are a number of accumulating cases currently in Indonesia and that will be a priority for the network and again with the effort to try to improve drug delivery and hopefully antiviral effects in clinical outcomes.

As I mentioned this is just one facet of these trials. I have tried to summarize here for you some of the immunology studies that may be of greater interest to this particular audience.

Within the context of the oseltamivir treatment study there are measurements not only of viral loads but also of innate immune response markers in virus specific T and B cell responses.

Among the survivors then of H5 within this study but also the previous survivors there are plans for long-term follow-up studies not only clinically in terms of their functional status, pulmonary function testing chest CTs but also looking again at virus specific antibody T and

memory B cell responses. Some of these patients are now up to about 4 years in terms of their survival from initial infection and in fact some of this work is already in progress in Vietnam at the National Institute for Hygiene there and also the Hospital for Tropical Diseases.

As part of this effort there have already been studies done again in Vietnam to look for the avidity of antibody responses and then selection of clones for development of human neutralizing monoclonals and you have already seen initial publications regarding the effectiveness of some of these in relevant animal models and there will be further work to look at cross reactive antibody and T cell responses to both avian and human viruses.

In Vietnam, also, there is a plan for a community cohort study looking at pre-season antibody T and B cell responses in relation to the subsequent risk of influenza infection and illness so that this is just some sense for you of the kinds of studies that the network is either undertaking currently or plans to in the future.

So, this gets at the issue of how do new protocols come into being and this may again be of interest to those who are potentially interested in collaborating with individuals in the network but basically the concept development starts with one of the investigators at one of

the existing institutions. A draft concept is developed which is then initially reviewed by the Network Coordinating Center. Assuming that it meets certain standards in terms of both scientific rigor, interest of the network and feasibility it is then moved up to the Network Steering Committee for initial review and after approval then a more detailed protocol is developed with the help of a protocol team as I mentioned before and then this final protocol will undergo both internal review by the Trials Operation Committee of the Data Safety and Monitoring Board Ethical Review Committees of course and then ultimately it is implemented with the help of the Network Coordinating Center.

So, the final initiative then is this new post of influenza research coordinator. This was advertised originally in the summer of this year and it comes about from a discussion between the Wellcome Trust representing a group of non-commercial research funders and the World Health Organization to try to get a better understanding of what kinds of investigation are being done currently with regard to flu and where the gaps are and what could be done in order to fill those gaps.

A formal interview process has been completed and I hope it will be announced relatively soon of the individual who will be taking this post funded through the

Wellcome Trust but then seconded to WHO to oversee this activity but just to summarize this person will be helping to develop a central inventory of research activities related to human influenza. The focus will really be on three areas, vaccines, drug therapies obviously including antivirals and population science, both surveillance epidemiology but also modeling kinds of studies.

They will coordinate a series of road mapping exercises to identify key gaps in knowledge and then liaise with the partners that have been identified internationally to develop a cohesive research agenda. The person will also serve to facilitate interactions between major non-commercial biomedical research partners, governments and various foundations, NGOs and then assist these funding agencies in their activities and implementation of various studies.

So, I will stop there and leave you with this particular web site where you can read more details regarding the Southeast Asia Network and thank you for your attention.

(Applause.)

DR. KATZ: Are there any questions for Fred for that very interesting overview?

Fred, could you tell us the studies that you listed with parenteral neuraminidase inhibitors and the

standard versus high dose, are any of those, are the standard and high doses ongoing?

DR. HAYDEN: The oseltamivir study was initiated in terms of first patient enrollments in July of this year and is gradually enrolling patients since. We anticipate that most of the patients of course will be those with more severe seasonal influenza leading to hospitalization. I should comment that in most of the centers this is a protocol that involves both children as well as adults and it incorporates in terms of the measurements not only sort of standard efficacy outcome measures but again a variety of immunologic markers to try to get a better understanding of disease pathogenesis as well.

Naturally you could understand that trying to gain access to H5 patients is a very unpredictable business and it is not clear how many patients we will actually be able to enroll but I know that we have enrolled at least three H5 confirmed patients in that protocol to date.

DR. KATZ: Suzanne?

PARTICIPANT: Because of the unique resources being in that part of the world is it possible to do enough surveillance that you think you could tell if something were preventing H5N1, you some particular characteristic; could you compare the cases that do occur even though they

are very few in number to the rest of the population?

DR. HAYDEN: I think given the very rare rate of infection right now despite the extent of exposure that would be a very challenging undertaking but I know that there are plans again within Vietnam to try to look at some case and family based studies presuming that you get family contacts and household members who have been exposed both to case patients but also to the same environment to try to look again retrospectively there at what may predict the likelihood of developing infection and disease, that there again will be a number of genetic studies undertaken, whole genome mapping as well as trying to look at some of the immune markers that may be relevant there but this is an area again where suggestions from this group would be I think very welcome in terms of specific kinds of things to look at and if there are particular laboratories that have for example assays that would really foster that effort I think that the individuals in the Southeast Asia Clinical Research Network would like to hear about it.

DR. KATZ: One last question. I realize the focus is Southeast Asia but is there any discussion to extend it to areas where clade 2.2 might be infecting humans?

DR. HAYDEN: That is an important question that really hasn't been addressed by the group within Southeast Asia because we are still obviously very early days in

trying to just make sure that we can do the studies that we have committed to well and there is an enormous amount of capacity building as you can imagine in an effort like this where most of the centers that are collaborating have not really done a clinical investigation before certainly not at an internationally recognized standard but I think that would be an important consideration in terms of trying to develop similar kinds of networks in other regions in the future.

DR. KATZ: Okay, one more question.

PARTICIPANT: Are there any and I don't know if this is really to Fred or to Jackie but is there any information to be gained from the vaccination of poultry that would either inform our human vaccination program or help us to understand why this virus is different?

DR. HAYDEN: I could clearly say that this is a question for Jackie.

DR. KATZ: I don't know that I have an answer to that. I think the vaccinations that have gone on in poultry in some part of Asia have now been shown to perhaps not be optimal and particularly there are some problem species there. I don't know how exactly we would relate that to the human situation. Is that your question? Yes, they are having trouble, too. I guess that is all we can say.

DR. HAYDEN: Maybe the take-home message is that

bad vaccines in birds might predict bad vaccines in humans as well.

DR. KATZ: But there are other reasons. I mean there are poorly matched vaccines still being used. So, there are many other issues, lack of standardization. So, there are many other issues that are involved with inadequate vaccination in poultry I think.

Okay, thank you.

So, I think we will finish the session there, and I would just like to thank all of the speakers again.

(Applause.)

DR. KATZ: And I believe that is the close of today and just remind the panels who will be on the spot tomorrow that there is going to be a brief meeting for them right now and then I believe we start tomorrow at 8 a.m.

So, we will see you then.

(Thereupon at 4:55 p.m., a recess was taken until 8 a.m., the following day, December 11, 2007.)