DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

ANTHRAX VACCINES: EFFICACY TESTING AND SURROGATE MARKERS OF IMMUNITY WORKSHOP

Tuesday, April 23, 2002

8:25 a.m.

Jay P. Sanford Auditorium
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20814

C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

WELCOME

COL. DANLEY: Good morning. You can see we have some technical difficulties, so I will spend a few moments here while we try to resolve them for our first speaker, Dr. Friedlander.

We have some administrative announcements, but I want to point out to you that for those of you who are not familiar--the microphone is not working?

[Pause.]

COL. DANLEY: I am Colonel Dave Danley. I want to welcome all of you. For those of you not familiar with military rank, we have several services here, as well as the Public Health Service. An Army and an Air Force colonel are the same as a Navy Public Health Service captain, which is different from an Army and an Air Force captain. Army and Air Force captains are the same as Navy lieutenants. Navy lieutenants are different from Army and Air Force lieutenants. So, for the sake of simplicity, let me recommend that we dispense with our titles. Call me Dave.

[Laughter.]

COL. DANLEY: I want to make some

administrative announcements. The smoking area is outside the building in the designated smoking area only. Violators will be shot.

Shuttle vans run until 9:00 and will start at about 1530. That is 3:30 civilian time, p.m. If you need additional shuttle service, please see one of our support staff outside.

Lunch will be served in the foyer at 11:30. Restrooms are also in the foyer, but in a different location. Pagers, beepers, and cell phones, please put them in the vibrate mode or in the off position. Violators will be shot. This is a military base. We take things seriously.

Speakers and panelists, if you have issues, please see Mr. Karl Lackenmeyer during the course of the day.

We do have boxes of slides from the first meeting that we had on anthrax vaccine out in the foyer. You are welcome to take copies of those slides that dealt with potency testing for the vaccine.

But let's get serious here for a moment to start off this meeting. First of all, I want to extend my thanks and gratitude to Admiral Zimble and the staff here at USUHS for letting us use this

excellent facility.

I would also like to thank the cooperation of colleagues at NIAID and the FDA in putting this meeting together. I want to recognize Dr. Kathryn Zoon, Dr. Phil Russell, for their participation in this meeting along with the panelists, guests from industry, the services, our colleagues in Canada and the United Kingdom. This is, indeed, a wide, diversified audience that is going to address your presentations on and hopefully bring to resolution some critical issues required for the licensure of a new or next-generation anthrax vaccine.

I would like to turn the podium over to Dr. Zoon, who is the director of CBER, FDA.

DR. ZOON: Thanks. I will be brief, but I do want to also extend my welcome to everyone and to say how much I appreciate all the organization and cooperation among the cosponsors in order to facilitate in making this meeting happen in such an expeditious fashion, and our host for this meeting here at the Uniformed Services University.

This is an extremely important area for the public health and the protection of the military. The Center for Biologics has been committed to working with all parties to effect the

access and availability of safe and effective anthrax vaccines. So we are very pleased that this meeting could take place to really focus on the objectives of looking at the development of new anthrax vaccines and the type of data that would be necessary with regard to non-clinical and clinical information for the expeditious development and approval of the second generation vaccines.

In looking at this, these products will be extremely important in our armamentarium for public health protection and military protection, and with the colleagues we have in our presence who will participate in these meetings, I think, clearly, this is more than just a U.S. initiative. It is a global initiative to help protect all citizens of the world.

And my sense is, over the next few hours and through the day, we will be looking at sharing data that is currently available, as also discussing what is the information that we will need to gather with respect to having enough information to facilitate the approval of new anthrax vaccines.

Our goal today for CBER is to take the information and to try to develop a guidance

document that will provide clear and consistent communication and expectations for these non-clinical and clinical studies. We hope that in doing so, that we will be able to facilitate the development of these vaccines so that we can process them as quickly as possible.

This workshop will be an important step in achieving this goal, and again, I want to thank all of you for coming, for sharing your thoughts, expertise, and data to further this important program. Thank you very much, and again, welcome.

[Pause.]

PATHOGENESIS OF BACILLUS ANTHRACIS

DR. FRIEDLANDER: Thanks very much. I appreciate the opportunity to talk with you and start this conference off. The events of the last six months have irrevocably changed our lives when it comes specifically to anthrax, but anthrax, as you know, is just one of the organisms and agents that is of concern to both the civilian and the military.

Historically, studies on pathogenesis and vaccine development have gone on concurrently. In fact, we usually develop vaccines empirically and our understanding of pathogenesis and mechanisms of

immunity lags considerably, and that has always been the case and likely always will be the case.

Because of the unique situation with anthrax and similar infections, however, it is imperative that we alter that paradigm because we are going to be unable to test these vaccines in the human population, and, therefore, we need to understand as much as we can, both about the pathogenesis and specifically the mechanisms of immunity in order to develop as much evidence as we can to justify licensure of a vaccine that can likely never be tested for efficacy.

Now, the story starts with Robert Kulp about 135 years ago. This is the life cycle of the anthrax spore. That basically is what he determined, that the spore turns into the bacillus and the bacillus turns into the spore. This was known for the hay bacillus, bacillus subtilis, by Ferdinand Cone [ph.] and it was a milestone in microbiology.

This is what we are faced with today. I think everybody has seen these pictures. It is 135 years to present day from the first identification to this chest x-ray that now we are all familiar with, and it shows the--I will just spend a minute

showing the characteristic findings of a widened mediastinum and pleural effusions with relatively clearly lungs. That is inhalational anthrax.

This is a CT scan showing these enormous lymph nodes and the pleural effusions. That constellation of findings in an acute illness is essentially pathognomonic of this disease. There are very few other things in medicine that cause that finding.

This is the--the center of the disease is in the mediastinum. This is the trachea, the bifurcation of the trachea. It is this node that is the business end of this disease. It is, in fact, a mediastinitis and a hemorrhagic necrotic lymph adenitis involving the mediastinal lymph nodes.

So our job here is to try to understand from that little spore to death caused by a lesion in the mediastinum.

Now, this is what I am going to try to discuss today, something about the organism and pathogenesis, hopefully as it relates to immunity, to keep that in mind. I will then spend a little time--I will spend most of the time on this and then spend a little time on the early approaches to

vaccination, with current and future vaccine efforts, again, just to outline these, and then just mention this, because there will be lots of discussion about this in the--for the rest of the day.

So the organism, I think everybody here is now familiar with this, a gram positive, non-hemolytic, spore forming, non-motile bacillus. There are three known virulence factors, an anti-phagocytic, highly negatively charged capsule around the organism, the lethal toxin, and the edema toxin, and you'll hear more from Steve Leppla shortly about the toxins, which I'll just briefly touch on in terms of their pathologic effects.

This is what the organism looks like. Again, I think, as I have said in some presentations before, we probably know more--the public probably knows more about this disease now than any other disease, including HIV.

Those of you in front can see a nice fat juicy capsule around the organism. This happens to be from a non-human primate. The findings in humans are essentially the same. There is a very high level of bacteremia at death.

This is a scanning electron micrograph of

the spleen and you can see two bacilli here and a crenated red blood cell.

This is what the spore looks like. It is the spore which is, as you know, extraordinarily stable and is the infectious form. The life cycle of the organism is such that it likely requires a mammalian host in order for it to survive and proliferate and amplify, in distinction to the closely related bacilli which undergo cycles of replication within the soil. There is a fine hair-like nap, the exosporium around the spore itself.

The spore, as I said, is the infectious organism. It enters through a break in the skin or the GI tract or through the normal lung. It germinates from the--the spore converts to the bacillus in a macrophage locally or after being transported to a regional lymph node. There is then the local production of toxins, leading to edema and necrosis, spread from the node through the lymphatics, resulting in bacteremia and toxemia and seeding of most organs, most particularly the brain in half the cases.

And death is likely due to lymphatic obstruction, vascular obstruction. You can't see,

I don't think, the--no--some pulmonary hemorrhage and pleural effusions that you saw, and death is thought to be a respiratory death in most cases. There is also clearly a toxemia and the relative importance of the two, it remains really unknown, except in my view, at least, the most important cause of death is, in fact, in the mediastinum, that lesion in the mediastinum.

This just shows from a pathologic perspective, emphasizing the importance of regional hemorrhagic lymph adenitis, particularly in the inhalational form of the disease.

This is a figure from a review by Dixon et al. basically showing the same thing. What I want to point out is, as I said, the first important stage is thought to be uptake and germination within a macrophage and subsequent involvement of regional hemorrhagic lymph adenitis. I will talk more about what goes on inside the macrophage and the consequences of infection in the macrophage and the effect of the toxins on other cells. This is an over-simplification, I think.

In terms of spore germination, there are many physical triggers that are involved in germination. From the perspective of what goes on

in the host, the most important thing is the in vivo site of germination, whether or not a macrophage is, in fact, absolutely required for germination, and what the in vivo germinant is.

That has some implications, obviously, not so much from the perspective of vaccines, but from the perspective of therapeutics and from the--not so much from the point of view of the mechanism of immunity, but also from the development of new vaccines. The critical events in terms of germination from spore to bacillus offer potential new targets for vaccines and therapeutics.

Under a phase microscopy, the spore is refractile. It then becomes non-refractile and swollen and begins to outgrow into the bacillus. This is an initial very susceptible time for the life cycle of the organism, likely before it becomes encapsulated.

In terms of pathogenesis of the organism, once it becomes encapsulated, it is resistant to ingestion by phagocytic cells and essentially proliferates extracellularly without any effective response by the host.

In terms of the spore macrophage interaction, this is thought to be, at least in our

present thinking, one of the most critical events in the early stages of the infection. One of the questions that remains yet unresolved is whether the macrophage environment is an absolute requirement for germination in vivo.

It is, I think, more clear in the lung, which is, of course, the most relevant disease that we are concerned about, inhalational anthrax, that that likely is the case. That is to say, that in order for the spore to be taken up, it may require ingestion by a carrier phagocyte, the alveoli macrophage. Whether or not that is the only mechanism remains yet, I think, to be established because these studies were done with massive numbers of organisms in experimental animals, and under those circumstances, it's clear that the macrophage was the predominant means by which the spore was taken up to the regional lymph node.

Now, older studies actually that might go back before you might imagine, predominately those of Ross, show that the spores are taken up, they are transported to the regional lymph node where germination occurs with free bacilli in about 24 hours. But some germination and killing actually occurs in the lung.

An interesting point is that if there is trauma, you can get germination within the lung itself, not within the node. That may have implications also in terms of some of the cases that have been seen. And by trauma, I mean that in a generic sense. If there is, in my view, at least, if there is likely evidence of ongoing inflammation and exudation in the lung, that may be a trigger for germination by itself.

Recent in vitro studies show variable results of this interaction between the spore and the macrophage, but we all well know that there is a big difference between taking a cell and putting it in culture and exposing it to a spore, that those conditions are at best models for what goes on. But the results show either rapid killing with some persistent live organisms, unimpeded growth, or no growth at all. Those are the current studies that have been ongoing.

As you might imagine, this disease, as I said, goes back to the beginnings. This idea that the macrophage is somehow a key and a very important cell, of course, was discovered more than 100 years ago. This is a drawing, probably not on a slide projector but he probably actually drew it

on the board when he presented this data. This is from Meschnikoff and you can see clearly bacilli that came from spores inside hepatic macrophages of the rat. So it was clear and self-evident that the spores ingested by the reticular and the felial cells and that germination occurs there and it is absolutely critical for infection.

This is a more recent study by the group from the Pasteur which shows colocalization of spores. For those of you who are not color blind, colocalization, I am told, of green and red, making yellow, of a licensed normal marker with the spore, implying that there is phagolysis on fusion.

This is a little out of focus but shows a study from our lab where this is the Sterne bacillus, Sterne strain of anthrax. These are lysosomes marked with horseradish peroxidase. This is an electron micrograph of a macrophage. And you can see a bacillus here which has the horseradish peroxidase surrounding it, indicative of fusion of secondary lysosome.

This is one of the examples. This is from the work of Sue Welkos where we are looking at survival of the bacillus in macrophage cultures over time, and you can see in both primary

macrophages as well as in macrophage cell lines significant killing occurring over a four-hour period. These studies are done in the absence of any antibiotics, which can clearly confound these results, and stand in contrast to studies from the group from Phil Hanner's lab where--I should say the previous study was done with the Ames strain. This is the attenuated Sterne strain. And over the time course of this experiment, there was proliferation of organisms, unimpeded growth.

This is work from Michelle Mock's lab, again showing with the Stern strain, looking at colony-forming units over a three-hour period, that there was no significant inhibition between zero and three hours of total numbers of organisms, no growth and no killing of the Sterne strain.

So three different labs, three different results. It is unclear exactly what goes on in vitro. I think in vivo is self-evident, two things. One, the LD-50 is not 0.5 spores, it is multitudes of that. And so a significant proportion of the inoculum is either killed or never germinates. And two, clearly, germination does go on and the animal succumbs. So these in vitro experiments probably replicate what, in fact,

does go on, that there is some killing and, obviously, there is survival.

This is another cartoon. I am just going to reiterate that once that spore germinates inside a macrophage and is released, it is now encapsulated and resistant to uptake.

I put down here--this is showing the entry of the toxin, and what is indicated here is a non-specific cell target because I think there's been too much emphasis on the macrophage, although it's clearly dear to my heart. It is not the only target. It is the target that we study in vitro because it's most easily studied. But in terms of what's going on in the host, I think it's important not to lose sight of the fact that receptors for the toxins are ubiquitous and likely a multitude of cells may be involved in the deleterious effects of the toxins.

Unfortunately, you cannot see this, but I'll describe in subsequent slides some of the effects, the physiological and pathological effects of the toxins on various host cells that have been studied to date, and they are a limited number of cells, namely cells of the phagocytic cell.

This just shows, to keep in mind the

paradigm that's been established with endotoxin and gram negative sepsis, that one of the central players has been the macrophage with, under normal circumstances, release of factors that are responsible for natural host resistance, but under other circumstances, when there's excessive release, those factors become deleterious to the host. That paradigm has been around now for 40 years.

This is just another view of the sepsis cascade, as it has been called, again, the macrophage being a primary player here, leading eventually to tissue injury, often with endothelial cell damage, and that may well be the case in this disease, as well. But the exact mechanisms that are involved in here remain yet to be determined for this infection.

This is a cartoon or one similar to it that you will see in terms of how the toxin is thought to work, and I'll just mention it briefly, that PA binds to a receptor, eventually captermarizes an edema factor or lethal factor, gets internalized through an acidic/indicidic component into the cytosol.

Now, the effects of lethal

toxins--unfortunately we're not going to see all this, but--have been mainly studied on the macrophage, and I'll just review what is known to date. It's clear that, again, in vitro, that cytolysis occurs, that is the macrophages of many species are lysed with release of all potentially toxic constituents, and that includes the pro-inflammatory mediators, reactive oxygen intermediates, and the lysosomal enzymes, which are clearly toxic and damaging to the host.

The question that again remains unresolved and in the literature is what happens with sublytic concentrations of the lethal toxin. The initial reports were that pro-inflammatory cytokines, TNF alpha or interleuken 1, are released, leading to this sepsis cascade that everyone is familiar with, and that makes sense.

On the other hand, two other laboratories have reported the opposite, in fact, that sublytic concentrations of the lethal toxin block the release of, in this instance, nitric oxide and TNF, induced by LPS and interferon, or in another system by LPS, that the production of TNF, important in host defenses, is blocked, and I'll show you briefly some of the data here. I'll just go

through this quickly.

This is the time course of release of TNF by either LPS or lethal toxin from one of the labs, sublytic concentrations. So the presumption is this leads to inflammation and an over-release of the cytokine mediators leads essentially to the paradigm that we see in sepsis with sublytic concentrations.

Now, other workers have shown the opposite. Here is the release of TNF by, in this instance, LPS and interferon. This is in the absence of any toxin, two different cell lines. And here's what happens with lethal toxin. You see a dramatic blockage of the release of TNF.

And the same results are seen here. These are cells incubated with--we're looking at TNF--incubated with LPS. These are different cell lines. These are the cells incubated with sublytic concentrations of lethal toxin. Under these circumstances, no release, and, in fact, blockage. If you preincubate with lethal toxins, you block the subsequent induced release by LPS.

So the bottom line is that it's thought, I think, at this point in time that the organism, in fact, subverts the macrophage early in the

infection by lethal toxin, preventing it from responding normally as it would with release of cytokines that call in the inflammatory response. In fact, pathologically, one of the hallmarks of this disease is the absence of inflammatory cells. There is no pus in the malignant edema of cutaneous anthrax. There are no neutrophils and there are no macrophages, compared to, say, a staff carbuncle.

Now, in terms of the edema toxin, there are similar effects on human monocytes, that is, a reduction of LPS induced production of TNF. So both toxins in this instance, there's evidence, both the lethal toxin and the edema toxin, block the production of cytokines that are necessary to generate an inflammatory response that would be important in warding off the infection.

So the organism uses essentially both toxins to block the immediate host response of the innate immune phagocytic cells, and, of course, once it's encapsulated, it's resistant to phagocytosis. Whether terminally there is massive release of cellular contents leading to a shock-like state, I think remains to be fully established.

In terms of the--we've heard about the

monocyte and the macrophage. It turns out that there's also inhibition of phagocytosis by the edema toxin. This was studied many years ago. There's also inhibition of LPS priming of the respiratory burst.

And I put down here, as you didn't see in the other slide, but it made it to this slide, again, other cell types. I think there's reason to think that endothelial cells may be involved. There's certainly, as we'll see pathologically, reasons to support the target of the--that the blood vessel may be a target in this infection.

I think I'll skip through some of these. This just shows the inhibition of phagocytosis measured as chemiluminescence by edema factor PA plus EF.

Now, pathologically, I just wanted to end this portion of the discussion by noting that with the release of the full pathologic examination of the cases at Sverdlovsk that just was published finally last year, there were a couple of findings that I think were emphasized in that report, that while present in the older literature were not as noted as significantly and one of them was vasculitis, and vasculitis involving not just the

arteries and the veins but the capillaries, that there was evidence of inflammation in the capillaries in a high percentage of the human cases of inhalation anthrax that occurred in Sverdlovsk.

And significant, and this had been, of course, seen before, as well, there's significant hemorrhage, what was called both high-pressure hemorrhage with really massive release of large amounts of blood, as well as low-pressure hemorrhage involving a diathesis of red blood cells into the tissue, causing in the lung compression, hemorrhagic pleural infusions, and interference with respiratory function, and obviously, in the brain, sometimes causing a subarachnoid hemorrhage.

Now, with the recent cases of inhalational anthrax, again, a couple of other findings in my mind suggest the importance of the vasculitis. Whether or not there's endothelial damage, it's not really been noted--noted pathologically. And some of the cases have had micro-angiopathic hemolytic anemia. Now, micro-angiopathic hemolytic anemia is basically a destruction of the red blood cells, often caused by vasculitis.

Whether or not disseminated intravascular coagulation occurs in conjunction with the

vasculitis is not always easy to determine. Pathologically, it was not present in Sverdlovsk, and although there were signs biochemically in some of the present cases as well as in Sverdlovsk that it did occur. And so it all points to damage of the blood vessels as being another area that I think needs to be looked at. Whether that's toxin mediated or not remains to be established.

Now, let me turn in the last few minutes to a couple points about vaccines. Before I leave, I just want to mention another point is that with all the focus on the toxins, it should be recalled in terms of pathogenesis that we have much to learn. With the new information coming out on the genome sequencing, I think it will be clear that there are going to be other factors that at least contribute to the pathogenesis. We know that some of the potential virulence factors that are present in the other bacilli, in fact, are expressed in anthrax, and how important they are remains to be established.

In terms of vaccines, there are two approaches that have always been taken. One is live attenuated vaccines and acellular in vivo expressed antigens, so-called aggressants. This is

similar to the paradigm that's been seen with all the other vaccines in the development of vaccines for invasive infections.

You know about Pasteur using a mixed culture of attenuated organisms. That subsequently led to the development by Max Sterne of a non-encapsulated toxinogenic strain and the development of a similar live attenuated strain by use in the former Soviet Union in humans. This is a veterinary vaccine that's been used since the 1940s.

The early protein component vaccines are important and interesting and they led eventually to the licensure of the current vaccine. One point I think that's of interest to me is that in the development of these vaccines, the very earliest vaccines that were developed were vaccines that were produced under in vivo conditions.

That is to say that they took tissue extracts, so what you had was in vivo grown organisms with in vivo antigens, all of them, and that's what we're trying to do today, is to find out what antigens are expressed in vivo specifically that may be important in protection as well as in virulence. And such antigens were, in

fact, very protective. They were crude mixtures, obviously, but they were the in vivo expressed antigens in their native configurations.

I'm not going to--you know about the current vaccine which came out of the development that began with these aggressant vaccines.

I'll just spend a minute talking about the approaches to new vaccines. All of the focus at the present time--I shouldn't say all the focus, but most of the focus is on the use of recombinant DNA vaccines. There's obviously an enormous amount of work going on in other areas, including mutants of PA, LF, and EF, an enormous amount of work on adjuvants and delivery systems. Every live attenuated vaccine carrier, I think, just about, has now been--and I heard about another one out in the hall that's going to be done, or has been done already.

The usual other characters, DNA vaccines, other viral replicons, plants, of course, skin delivery, I should mention. And, of course, now the identification of new antigens. There's recent work from the group in Israel and also the group in France showing some efficacy now of spore antigens, as yet undefined.

So there'll be, I think--clearly, this is the first vaccine, the recombinant PA, but we will clearly see a multitude of other expression systems, delivery systems, adjuvants, and new immunogens.

I'll just close with two slides here--no, no, I'm sorry. I have more slides. Humans make antibodies to the toxin components, to the capsule, and to ocellar [ph.] proteins. That's what's known.

In terms of the possible mechanisms of PA-induced protection, there's induction of toxin neutralizing antibodies, that I think Steve will briefly touch on. There's induction of antibodies that inhibit spore germination. This is the work of a group from the former Soviet Union, as well as Sue Welkos. And there's induction of antibodies enhancing spore phagocytosis and increasing the rate of killing, again, the work of Sue Welkos.

I'm going to pass through this. I'm going to briefly just show you the difference between--this is germination over time, pre-immune serum, very rapid, anti-recombinant PA anti-serum, in addition to germination. The exact mechanism for this remains to be established. This is,

again, the work of Sue Welkos.

This shows phagocytosis in monkey immune serum compared to pre-immune serum, increased phagocytosis. This is shown here, as well. This is the Ames strain with immune serum versus normal serum. This is with a PA mutant, where there's no effect of this immune serum. Again, this was somewhat of a surprising event, suggesting that PA may be--or a similar molecule may be present on the spore. But it says something about the potential mechanism of immunity.

This shows a more rapid--this is a number of CFUs per macrophage with immune serum versus non-immune serum, and this is after 60 minutes. There's already evidence of a more rapid killing, although the eventual killing is the same with immune versus pre-immune serum.

And then the last slide shows, again, what we'll talk about. To date, there's evidence that the antibody, the PA measured by ELISA and toxin neutralization correlate with immunity induced by AVA. But similarly, with live attenuated vaccines and a guinea pig model, then antibody to PA correlates with immunity. And it appears--again, this is the work from the group in Israel--that

toxin neutralizing antibody is a better correlative immunity than is an ELISA.

Now, I'll stop here and take any questions you have.

COL. DANLEY: Are there questions?

MS. : I think we're going to hold questions until the end of the discussion.

COL. DANLEY: Okay, great. I have a real quick announcement to make. It's always my pleasure to embarrass people in public, but as many of you know, Dr. Friedlander recently retired from the Army and it's very customary to present to people retiring from the Army things to put on their walls at home. We didn't from our program office have an opportunity to do that and I'd like to take a moment to do that now.

But I'd also like to take a moment to kind of impress on you the accomplishments of Dr. Friedlander and his colleagues at USAMRIID. Suffice it to say, you've seen from the work presented here efforts that he and his colleagues have made over the years in understanding anthrax vaccines, but the two points I want to make are that a lot of the work that was done in your laboratory on antibiotics formed the basis for

treating the individuals who were exposed in the recent terrorism acts.

But more importantly, it's the fact that the support for your work has not always been consistent, that there were lean years, that there were people, myself included, who sometimes gave you a lot of trouble in that process, so that there wasn't a lot of gratitude in that process. And I suppose, as a scientist, you sort of just hang in there and sort of believe that what you're doing is the right thing, and indeed, in this case, it was the right thing.

So I'd like to give you this certificate of appreciation, to Colonel Art Friedlander, for outstanding support and selfless service to the Joint Vaccine Acquisition Program, our program office, and the men and women of the Armed Services. Art, thank you very, very, very much, sir.

[Applause.]

DR. FRIEDLANDER: In the interest of time, I'll shut up.

[Laughter.]

DR. FRIEDLANDER: I was just instructed to introduce an alumnus of USAMRIID. Steve and I have

been working together now for more years than--before, when I had hair and when he had gray hair. Steve is now at--he's been at NIH for how many years now?

DR. LEPPLA: In fact, the program has me affiliated with NIAID, which is not accurate. That may happen in the future, but for the time being, I'm actually at NIH in the National Institute of Dental and Cranial Facial Research of the Dental Institute.

So Art has given you a broad view of the bacillus anthracis pathogenesis and that allows me to focus on aspects specific to the toxin, and I'll make a small number of points which are listed here, basically that there's convincing evidence, genetic and immunological, that the toxin contributes in a major way to virulence during bacillus anthracis infections, and then I'll explain that the cellular interactions of anthrax toxin are very well characterized through work in several labs over the last decade are so.

The physiological effects of the toxin are only partly understood. Art discussed those and pointed out both the gaps in the knowledge and some of the contradictory aspects of the data. And the

major point I'll try to make, based on this other data, is that antibiotic neutralization of toxin can be explained by reference to the known structures of these anthrax toxin proteins.

So just to fill in, what I'll show you is that there's genetic evidence from knocking out toxin genes that each of the toxins plays a role in virulence. Clearly, anti-toxin antibodies are sufficient to protect against infection. In terms of cellular interactions, we have a good understanding of how the toxin gets into cells. The toxin receptor was recently identified. There's evidence about cell type distribution of the receptor, which is relevant to what cells and tissues the toxin will target. And we know how the toxins work once they get inside cells.

Art has indicated in depth what the toxin does in terms of pathogenesis. I'll end, then, speaking about toxin neutralization. We have the structures of all three toxin components and we can use that knowledge to understand how the neutralizing antibodies function.

You know, of course, that the toxin comes in these three large proteins secreted by the bacteria. This is evidenced from Michelle Mock at

the Pasteur Institute, indicating the role of the individual toxin components in virulence. This is in a mouse model, and what you can see is the virulence--this is LD-50 for mice of the Ames, the very well now known Ames strain. Five spores are sufficient to induce a lethal infection in a mouse.

It turns out the capsule is actually perhaps more relevant for infection in mice. I'm sure there will be discussion later about the relative roles of toxin and capsule in mouse models. But clearly, both knocking out toxin production or capsule production has a large effect on the virulence of the organism for mice.

By knocking out individual components of the toxin, it was proven that knocking out edema factor reduces virulence about ten-fold, so it plays a lesser role than the other toxin components. Knocking out PA or LF reduces virulence more than a thousand-fold. So this is genetic evidence, then, that the toxin has a clear, dominant role in pathogenesis.

Anti-toxin antibodies protect against infection. This is why we're here. There's a large volume of experimental data that antibodies to PA are protecting against infection. I can't

attempt to list those. There's a much smaller body of evidence indicating the antibodies to the other toxin components might play a role in protection against infection. So there's evidence that's somewhat indirect because it wasn't done by immunizing with purified toxin components, but at least there's suggestive evidence that antibodies for the other toxin components are protective.

Not mentioned here, because it's unpublished, is work from Darrell Galloway and colleagues using BNA vaccine approaches, indicating that antibodies to LF can, indeed, protect against infection. That's probably the most definitive evidence to date.

This is a little bit of data. This is from the Israeli group, from the paper I just referenced, and here, what they did was to put rabbit serum into guinea pigs, and in fact, this is a post-challenge experiment. So they're giving these antisera 24 hours after intranasal challenge, so the protection is not impressive, but since it is 24 hours post-infection, I think it is clearly significant.

What was shown is that antiserum to PA does protect one animal out of the eight and

prolongs the time of death. Anti-LF at higher doses protects a quarter of the animals and delays time to death, and a mixture is also protective. So this is direct evidence, then, that specific antibodies to toxin are protective in an infection model, and again, this post-challenge model.

So what do we know about the pathways of toxin internalization? You've seen one cartoon. We've redrawn the cartoon, but it's the same information that you saw earlier. We know that PA binds to a cellular receptor. This was recently identified and worked by John Young at Wisconsin to be what he called anthrax toxin receptor. This is, in fact, a variable--one of several transcripts of a molecule called tumor endothelial marker 8, identified just a year ago in Johns Hopkins as a molecule up-regulated on the endothelial cells in colon tumors.

So PA is bound to its receptor. It's activated in an obligatory proteolytic cleavage by furin, a cellular enzyme, small amounts of which cycle to the cell surface. Cleavage allows the fragment to be released into the medium. It has no other role in subsequent steps. The receptor-bound PA-63 aligamarizes and apparently the receptor also

aligamarizes and you get this very tight heptomeric species that can also be produced in vitro and is a very tight complex.

The activated form has a new surface, a newly-exposed surface to which the lethal factor and edema factor can bind. They bind to the same sites. Surely in vivo, you'll have a mixture of LF and EF-bound onto the heptomer. The new evidence from John Collier's lab is that, in fact, there are only three binding sites for LF and EF on the PA heptomer. Originally, we had said there were seven, but there is convincing evidence that it takes two PA-63 molecules to make a binding site for LF and EF.

So you get a complex form. You get endocytosis. Acidification causes a conformational change such that the heptomer inserts in the lipid bilayer to make a protein conducting channel. These enzymes, LF and EF, must unfold to pass through the limine of that channel to reach the cytosol. They must have the ability to refold and become active enzymes, edema factors, and then late cyclase [ph.]. It makes too much cyclic ANP and lethal factor is a protein--I'm sorry, a metalloprotease, which cleaves a number of the MAP

kinase molecules involved in essential signal transduction pathways.

As I mentioned, the receptor for PA was recently identified as TEM 8 in this publication in nature and this is a little bit out of line with our previous results, which indicated that there are receptors for anthrax toxin present on essentially every cell that has been examined. It should be mentioned that most of the cells we look at are tumor cells, the cultured cells, and so it still remains to be seen what the situation in an intact organism is and what cells will preferentially have receptors for the anthrax toxin.

This is from the original description by Kinsler and Vogelstein of the TEM molecules, and TEM 8 is represented here. It has a single extra-cellular domain to which PA binds and a large intra-cellular domain which is potentially able to transmit signals. So this receptor is potentially a signaling molecule so that binding of a ligand, perhaps even PA, to this receptor might have some physiological consequences for a cell.

So again, we know very well what these toxins do inside the cells. The edema factor is an

adeolate cyclase and lethal factor is a metalloprotease and it now cleaves all of the MEKs that have been examined, and as far as is known, no other substrates. MEK 5 appears not to be a known substrate.

But what we haven't discussed is there's reason to consider that there might be additional substrates of lethal factor, and this is largely because we cannot explain the rapid lysis of mouse macrophages by cleavage of MEKs. MEKs occur in all cells, non-macrophaged cells, as well. Those other types of cells do not lyse. It's only mouse macrophages and certainly classes of mouse macrophages which lyse. So we and others, I think, are considering that there may be additional substrates which are relevant.

Toxin roles in pathogenesis, this is largely speculations on my part. As I point out here, Art has pointed to its interaction--to the role of toxin in the interaction of spores with phagocytes. So it's clear that the toxin can inactivate phagocytes from without, either by lysing macrophages or by elevating cyclic ANP levels. You could imagine that a phagocytozed bacteria inside a macrophage could continue to

secrete toxin, and so perhaps that toxin could work from within the macrophage, and then perhaps the lysis of the macrophages is important to release the vegetative cells and establish the bacteremic phase.

Promotion of septicemia, I think there's reason to think that the toxin continues to act. For instance, the evidence I showed you from the post-challenge prophylaxis with antisera indicates the toxin continues to play a role later. Perhaps it's important to continue knocking phagocytes down, but that, again, is speculation.

And destruction of essential tissues and organs, you can clearly kill animals with toxin, but exactly what the targets is not clear, as Art has pointed out. There's new evidence in melanocytes that you can induce apoptosis by lethal toxin, but again, the relevance of that to an infection is not clear.

The established effects of the toxin are that it lyses mouse macrophages. Again, this is probably a peculiarity. As Art mentioned, macrophages have been a focus of attention, but whether they play a central role in pathogenesis in animals is not, I would say, well established, in

part because there are many inbred strains of mice from which the macrophages simply are totally refractile to lethal toxin, and yet those mice can still be killed with lethal toxin injections. Their death is somewhat delayed, but they still are killed.

The other model that's widely used is the rapid lethality in Fisher 344 rats. You inject toxin IV and the rats can die in as little as 38 minutes. But again, other rat strains are much less susceptible to this mode of challenge with toxin. So both of these systems are convenient and important bioassays, but whether they reflect the situation in vivo is not clear.

A more normal situation is probably the death caused in BALB/C mice by toxin injection, which occurs in several days, probably more characteristic of an infection.

Fortunately, we have now the structures of all three of the toxin components and this is helpful for us in understanding how antibodies work. So the crystal structure of anthrax lethal factor was dissolved and reported a few months ago and you see in this structure the end terminal domain, which is very similar to that in edema

factor. This is the structure which interacts with PA to cause internalization of this molecule into cells, and the rest of the molecule performs the catalytic site. It's a metalloprotease. You can see the zinc in the active site. Here it is shown docked with its substrate, the interminal peptide of MAP kinase kinase.

In terms of antibody neutralization, the work I mentioned from Galloway was essentially inducing antibodies to the terminal domain of LF. I might go out on a limb here and speculate that those antibodies are probably going to be more effective in neutralization than antibodies to this domain.

There is I think evidence from diphtheria toxin that antibodies to the catalytic chain are less effective in neutralizing than antibodies to the binding domain. That is perhaps understandable in that an antibody to this region would prevent it from binding to PA. An antibody to this region, in fact, would have to be carried along with the LF into the endosome. The pH would fall, so the antibody would be less-favored environment to maintain its affinity for LF. And then when this catalytic domain unfolds the path to the lipid

bilayer, you could imagine sloughing off in the antibody that was binding to the conformationally determined epitope. So, again, antibodies to this domain may be more relevant for neutralization.

The structure for edema factor was solved and reported just a month or two ago. This is a structure that was solved in complex with its essential cofactor calmodulin. In the picture here, we have subtracted--I should say the crystallographers have subtracted the calmodulin domain, so you only see EF regions, but not too much is known. EF is clearly the less studied of these molecules.

The important one, protective antigen, the structure was solved several years ago. You have the N-terminal domain, which is removed by FURIN cleavage. The domain 2 forms the channel, the bulk of the channel through the lipid bilayer, and domain 4 is especially relevant because it is the receptor binding domain. I didn't mention the new evidence the immunization with just domain 4 can infer protection. So, clearly, this is an important part of the molecule.

What was learned by studies with mouse monoclonal antibodies? Antibodies were made at

USAMRIID in the '80s by Steve Little, and Friedlander, and Leppla and others. The general conclusions I think were that, of the large number of monoclones that were made, only three small site--a small number of those were actually neutralizing antibodies, and they could be sorted into three groups, depended on what they reacted with.

So there is a receptor binding domain in domain 4, which I just referred to, and so these are neutralizing antibodies that neutralize by binding to domain 4 and preventing it from binding to cells.

There is an LF binding region on domain 1, and this is typified by monoclonal antibody 1G3. These antibodies essentially compete with LF for the LF binding site. There is another set where the role is less understood. I especially want to try your attention to this antibody 1G3 because it is a unique molecule in that it will neutralize at less than stoichiometric amounts. So, in cell culture, a tenth of a microgram will neutralize a microgram of PA, and it does that because it only reacts with the activated species, the PA 63. It doesn't waste its time reacting and it does not

react with intact PA. So there is a sparing activity. It is only recognizing the active species. So that is an important antibody. It is one that I hope people will consider for developing as a therapeutic agent.

Just to reiterate, the 1G3 antibody type reacts at the surface, which is exposed by removal of domain 1A. Whereas, 14B7-type antibodies react on domain 4. More specifically, we know that they react with what we call a small loop. We were doing extensive mutagenesis in the small loop of domain 4, and we can show that mutations in the small loop prevent the mutant PA from recognition by 14B7.

And 14B7, the gene has been cloned, and Affinity-improved version of 14B7 has been developed by George Georgio at the University of Texas and shows quite good efficacy in neutralizing toxin in the rat model previously described. So that 14B7 improved variant is a candidate for a therapeutic neutralizing antibody.

So, again, just to reiterate, antibodies to toxin work because there are a number of things going on, on the surface of the cells. You have a number of targets, opportunities for interfering

with toxin action. You can block PA binding to its receptor, you can block the surface on the top of the PA heptomer, to which LF and EF bind. I have not described in detail antibodies to EF and LF. I think those play a smaller role, but they should be better characterized for their potential utility and to understand better the important epitopes on LF and EF that we would like to target.

So, just in conclusion, I can say that the availability of the structures of the three components have led to a description of how the antibodies neutralize the toxin, and this allows us to design serological tests that will be predictive for protective immune response. I think if we understand those neutralizing epitopes, we can look in the antibodies induced by various vaccines and, at least in the laboratory, identify those antisera which contain the right antibodies, the antibodies directed against those neutralizing epitopes.

Thank you for your attention.

[Applause.]

DR. BURNS: Before Art opens this up for questions, I just want to make the announcement that we are transcribing this workshop, so it is going to be important, when you ask a question,

that you use a microphone, and there will be microphones set up down here.

Please indicate who you are and where you are from. Thanks a lot.

DR. FRIEDLANDER: Okay. We'll open this up for discussion. I think it is sort of self-evident that we know a great deal more about toxins. Some of that is because of the interests o of cell biologists and some of it is because it is easier, even though it's not easy, and then what goes on in an animal.

Yes, Drusilla?

DR. BURNS: This is Drusilla Burns from CBER.

The finding that antibodies to PA affect spores is really surprising, and I note that you probably don't know a lot more about it than what you told us, but could you speculate a little bit on how the antibodies may be affecting the spores?

DR. FRIEDLANDER: That is an intriguing question. I don't really have the answer for it. Again, this is, as I mentioned, the work that is done by Sue Wellcos. It followed on some observations that were reported without much data by a group in Russia, and she followed up on that

and basically demonstrated, as I said, one, effects on both germination, as well as on opSimization, and the question then is is, one, is this PA? Is it somehow, I mean, the presumption is this is exposed on the surface. There is an experiment that I mentioned that was done with a colleague from Israel, where a PA-null mutant, an insertion mutant did not show the same effect of opSimization.

Now there are other interpretations of that, though; that is to say, that in the preparation and purification of the spores, it's conceivable that PA being produced is somehow absorbed to the surface even though these are clean spores, wet spores. It's conceivable during the generation of sporulation, when there are vegetative organisms there that are being degraded and lysed, that PA is present and binds to the spore, and that may be the interpretation. I don't know that that's the answer to that. So that would explain also why the PA mutant is noneffective, but it nevertheless is intriguing as to how it affects germination. OpSimization I think is understandable.

DR. ZOON: Kathy Zoon, CBER.

Steve, I have a question. Has anybody looked yet at antibodies to TeM 8 to see if they're neutralizing.

Secondly, and this is to both of you, would you predict that a cocktail of immunoglobulins, with the primary epitopes that have been pointed out to protective antigen lethal factor and other important criteria, might be an approach for developing a therapeutic procedure?

DR. LEPPLA: Very little is known about TeM 8. TeM 8 was only discovered a year ago. There's only two papers published about it. I think the Kinslow lab is looking at questions like the one you raised. A related question is what is the natural ligand of TeM 8. We'd certainly like to know whether there is a normal ligand to TeM 8 and whether PA interaction with TeM 8 would affect the function of the normal ligand.

I didn't mention, in terms of therapeutics, the paper that I showed you from John Young. They did, in fact, express the extracellular domain of TeM 8. In E. coli and in a cell culture model, they showed that that did block toxin action. So I think the extracellular domain as a receptor decoy is a therapeutic that people

are going to be pursuing.

In terms of the cocktail, do you want to respond to that?

DR. FRIEDLANDER: Sure. I would just add one point in reference to the receptor. There have only been limited studies done, and none recent to my knowledge--well, I take it back. There probably are that I don't know about, in terms of the vaccines. What I was getting at was the potential side effects or toxicity of protective antigen by itself. Presumably, there is this receptor. There's some old data in the literature that suggests that there may be some effects of protective antigen by itself. I know that there are some toxicity studies that have been done, and I presume it's been safe, but that's something to keep in mind in terms of this receptor. The TeM 8 receptor for PA by itself, somehow triggering that receptor.

The second point, in terms of a multitude of antibodies, Steve Little did some of the early studies with passive protection with these antibodies, but I don't think there were any cocktails that were studied.

Nevertheless, in other model systems, it

is clear that you can get increases of affinity by a multitude of antibodies, and that's of course the advantage of polyclonal antibodies. Work has been done with botulinum toxin that clearly shows increased effectiveness of a cocktail of monoclonal antibodies. So I think you can anticipate that that would be the case here too.

I think, at least count, every company that has made the human monoclonal antibody is making one. It's up to, I don't know, 12 or something that I know of. I don't know. You probably know more.

MR. SIBER: [Off microphone.] George Siber of Wyeth.

The core of our discussion today is likely to be published on neutralizing antibodies and their measurement. You described three methods: The mouse macrophage for surette[?] and then mouse fality[?]. But when you commented about those, you worried that there may be multiple lethal functions which are not measured by one or the other of these models. What I wanted to know is, is there evidence, in fact, for that? In other words, are there toxin mutants or inactivated toxins that are inactive in one of those models and yet are really

inactive in another?

DR. FRIEDLANDER: I'm not aware that there, but the physiologic effects of the toxins have not been well studied, other than the lethal effect or the edema, and the edema has not been well studied.

So the question as to whether or not there are other effects, if I understood what you are saying in an animal, for example, by an LF mutant, whether LF might have other effects other than its catalytic domain would be hard to know, I mean, it would be unlikely I think. On the other hand, there are multiple functions of proteins, and, I don't know, I haven't thought about that, but it would be hard to know--nobody has demonstrated any effect other than in an animal, but you'd have to see what may be a more subtle effect that you'd be looking for.

DR. HEWLETT: Erik Hewlett, the University of Virginia. Thank you both for your presentations. I have a couple of questions. I will ask them and let you answer, rather than piling the questions up.

The first is that this illness is described as one that is not transmissible from

patient to patient, yet in the phase of bacteremia I presume that this would be behave like a blood-borne pathogen and be transmissible by blood products; is that not the case?

DR. FRIEDLANDER: Absolutely the case.

DR. HEWLETT: Okay.

DR. FRIEDLANDER: I mean absolutely never have seen evidence for that, but I think you can say absolutely.

[Laughter.]

DR. HEWLETT: That's as absolute as you can get.

DR. FRIEDLANDER: Absolute as you can get, right.

DR. HEWLETT: There is obviously an important phase of this infection in which the organisms are residing intracellularly in macrophages or at least passing through. What do we know about, number one, both of you alluded to this a little bit about production of toxin during the intracellular phase versus organisms that are in the bloodstream or resident in the tissues.

Second of all, as is the case now at least in some instances with HIV, are these organisms gaining access to the central nervous system and

other places such as that as free organisms or might they be carried there by macrophages that still have organisms within them?

DR. FRIEDLANDER: Well, there's data from Michelle Mock's lab by looking at gene expression, that the toxin genes are expressed inside the macrophage very quickly. I don't know that there's any data on protein expression. No, no. These were fusion. I think some of these were lack C fusions.

DR. HEWLETT: Of GFD?

DR. FRIEDLANDER: I don't think anybody's done GFD, but there's evidence that it's expressed intracellularly in the macrophage.

DR. HEWLETT: But macrophages are killed fairly quickly by LF coming from the outside or some macrophages are--

DR. FRIEDLANDER: At high concentrations, right. I think the question as to whether, and I alluded to that, whether in other forms of the--I didn't have time to go into it--whether in other forms of the infection, that is, the cutaneous model, whether or not you really need a macrophage I think has not been proven.

In terms of how the organism gets to the

CNS, I have no idea. The speculation that it could come intracellularly is entirely reasonable.

We do know that there are some patients that present with meningitis. In fact, there has been one outbreak, a remarkable outbreak with I think it was food--I can't remember, it may have been handling--where most of the cases in India, I think there were six or seven cases, and five of them had meningitis or something like that. It was extraordinary.

So it is clear that in some instances, that spore gets through really quickly, I mean, the presumption is it is coming through the lung, and seeds the brain, and once that occurs, I think the chances, of course, for survival and the host being able to contain the infection are not very great.

I should also point out that, again, in meningitis, and pathologists may add to this, there is often significant vascular involvement, a direct involvement of the blood vessels.

DR. HEWLETT: Including increased blood-brain barrier permeability?

DR. FRIEDLANDER: I don't know. I mean, that has not been studied.

DR. HEWLETT: The final issue is, in

Michelle Mock's mutant that is nontoxogenic, but still has an LD 50 of only 10 to the 3, what was the pathology and the mode of death in those organisms? I think we focus a lot on toxin. Obviously, with lethal toxin able to kill animals and patients dying, that is the ultimate endpoint that is easy to look at, but how do animals that have only encapsulated organisms die?

DR. FRIEDLANDER: First of all, the observation was made initially by Sue Welkos, where--actually, it was made by some Russians, also, because the Russians made most of the observations, and that is that a PXO1-minus strain kills the mouse. That was what Sue demonstrated.

I don't know that there have been any detailed studies of, and that would be very important to do, of--I don't recall that they were done.

DR. HEWLETT: Thank you.

DR. BURNS: I think, for the sake of time, we are going to need to move on, and I want to thank Art and Steve.

We got a late start today, so we're only going to get a 15-minute break. We're going to start exactly in 15 minutes.

[Recess.]

ANIMAL MODELS

DR. BURNS: Our next session is going to concern animal models. This subject takes on a particular importance for anthrax vaccines because it is very likely that human efficacy trials will not be feasible to conduct, nor would they be ethical to conduct.

In situations like this, the FDA is considering a proposed rule that would allow the use of animal data, data from animal studies, to support the efficacy of vaccines. Now this rule is in the proposed stage. It has not been finalized, so I say everything I am going to say with the caveat that it could change. However it is under final review by OMB. So we are hoping the final rule will be out shortly.

I thought, to introduce the session, it would be important to give you a little education about this proposed rule that we call the animal rule. Now, first, the scope of this rule is that FDA may approve a biological product for which safety has been demonstrated based on efficacy data obtained in adequate and well-controlled animal trials. I think it is important to point out that

the safety data, of course, would have to be in humans. It would be the efficacy data that would be in the animals.

Now this could occur if the product is to be used in the reduction or prevention of serious or life-threatening consequences resulting from exposure to a biological agent. The product would be expected to provide benefits over existing treatment, and human efficacy trials are not feasible or ethical.

Now written as the proposed rule, there are four requirements, and I think we need to keep these in mind as we go through our discussions today. The first requirement is that there is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention by the product.

The second one is there is independent substantiation of the effect in multiple animal species, including species expected to react with a response predictive for humans.

Thirdly, the animal study endpoint is plainly related to the desired benefit in humans, which is generally the enhancement of survival or the prevention of major morbidity.

Finally, the data or information on the kinetics and pharmacodynamics of the product or other relevant data or information in animals and humans allow selection of an effective dose in humans.

Well, in this session, we are going to concentrate on the second requirement, which is there is an independent substantiation of the effect in multiple animal species, including species expected to react with a response predictive for humans. We are going to hear about a number of animal models, including the human.

I think what we need to do is pay particular attention to the following questions: What is the nature of the disease in a particular animal species and does it look like the disease in humans, and does the immune response in the animal resemble the human immune response?

To start out, what we are going to do is hear about the human disease, and Dr. Phillip Pittman, from USAMRIID, will tell us about human pathology and the human immune response.

DR. PITTMAN: Thank you very much. I'd like to thank the organizers for inviting me to talk here today on the subject of human disease

caused by anthrax and the human immune response to the current licensed anthrax vaccine.

The human disease is characterized basically by three forms of disease, which include cutaneous, gastrointestinal and the inhalational form of anthrax. We will also discuss the human response to the licensed anthrax vaccine, which we have been calling for several years AVA, but has been revived now by the name of Biothrax, but I will continue to use the term AVA in this presentation.

We will discuss the background studies that led to a dose reduction, route changed pilot study, which was the basis for Congress funding CDC to do a pivotal study to look at a decrease in dosage and a change in route for administration of AVA, and we will discuss the serologic and specimization studies which was the background to this pilot study.

We will discuss the study itself, and then we will discuss the idea of sustained boosting versus interval boosting of the anthrax vaccine, which was done at Fort Bragg. If there is adequate time, we will go through the analysis of VAERS forms and some future studies.

As you know, the cutaneous form of the disease was fairly common in the recent outbreak. There are also gastrointestinal and the inhalation forms, and the morbidity and mortality associated with these forms are so that the inhalational form is the most morbid. In the most recent outbreak, the mortality rate was 50 percent. You may recall that the old data suggested that the mortality rate approached 90 to 100 percent. So that even with the use of triple antibiotics, the powerful antibiotics that we have today, there was still a 50-percent death rate.

This is an example of cutaneous anthrax. You can notice the classic S scar. Biopsies were taken at these points. By the way, if you take a biopsy, I am told by the pathologists that this is not the best place to do it, but rather to take it close to this area, to the advancing border. That would give more classic findings than where those biopsies were taken.

This is another patient. In this case, the S scar is no longer present. The S scar has fallen off.

This is an infant with cutaneous anthrax. Here we see the classic S scar. This is cream that

was put on the child in order to decrease some of its symptoms.

This is a slide of gastrointestinal anthrax. You may notice the hemorrhage and edema that are fairly prominent. This is a CT scan through the abdomen with IV contrast. I just want to point out here, and you may not be able to see that, that there is edema of the bowel wall, as well as pneumatosis, which is shown here in these areas. These are some of the classic findings of the gastrointestinal form.

Art has already gone through the inhalational form fairly extensively, just to show that, again, the meat of the pathology is in the peribronchial and mediastinal lymph nodes. You saw this slide before. The head is in this direction, the trachea and the bifurcation with this infected lymph node.

This is another view of the same thing. Again, the head is in this direction, the trachea, the bronchi, showing a massive amount of hemorrhage that is characteristic of this disease.

Again, chest X-rays showing mediastinal widening, bilateral hilar adenopathy and pleural effusion. Pleural effusions are seen here, and,

again, the very impressive lymph nodes of this disease.

I will just skip through some of these. Of course, this is the brain. This is the normal brain, and this is the brain of the patient who has succumbed to anthrax, showing the hemorrhagic process that takes place.

There is an effective vaccine that is licensed for the prevention of anthrax, and that vaccine is known as AVA, as we call it, or Biothrax, as it has been renamed. The vaccine is given in a primary dosing scheme of six doses, with three doses being given two weeks apart over four weeks, and three additional doses are given six months apart at six months, twelve months and eighteen months.

We, in our studies of the vaccine, wanted to see if we could improve upon both if we could decrease the number of doses and what we will refer to as the priming doses and also we could decrease the number of later secondary doses from three to two in an effort to get the primary series down to a total of four doses of over 18 months.

Before we get into those studies, I would like to just remind you that Brachman, et al., did

do an efficacy trial in the '50s of a precursor vaccine and that this vaccine did show a 92.5-percent efficacy rate against cutaneous and inhalational anthrax.

Just discussing the background work, two of the dose reduction, route change pilot studies, I will go through briefly some specimization data. These data were collected in a passive mode; that is, patients who showed up to the specimization clinic as a matter of course for--these were at-risk individuals who work in the bio containment laboratories, as well as maintenance workers who have to maintain the facility.

Like any passive study, there are some advantages and disadvantages. The results of the study is in your handout. I should say that apparently these slides did not make your handout, for some reason. I am told by the planners that they will be mailed to you after the conference.

In terms of which adverse events were noticed in the specimization group, there were no differences in the systemic adverse events as reported by either age or ethnic group. However, we did see a significant gender difference, and that is compared to males, females had a higher

incidence of headache, malaise and fever and a few others compare it to males. In terms of local reaction, females had markedly elevated increase incidence of induration erythema and tenderness at the injection site.

We also looked to see if, having received a dose of vaccine and having had a reaction to it, if you were more likely to have a reaction if you received a subsequent dose of the vaccine. What this data showed is that using a logistic regression model, controlling for lot and gender, since we know that those do play a role, we did see that there is a difference, that there is some predictive value to having had prior erythema and induration as a way of predicting whether or not the same reactions would occur to the next injection.

In the odds ratio, there were 13, but again, in this study, most of the injection site reaction were followed by injections in which there was no prior reaction. So that makes this not that great as a predictor.

So we concluded this from the SRP study that despite this being a passive self-reported study with some limitations, that we did notice

some differences in the reaction rate. In terms of gender and in terms of age, we also notice a lot difference. In terms of looking at the serologic response, we did a survey of the specimization clinic looking for individuals.

By the way, the hypothesis was that IgG antibody response of individuals who received a second dose of AVA at intervals greater than two weeks showed so an increase as the interval increases. So, in other words, as the interval between the first and second dose increased from two weeks to three weeks to four weeks, we should see an increase in the seroconversion rate, as well as an increase in the maximum titer at peak. In fact, we did two studies to look at that effect.

We did one study in which we looked two weeks after the second dose of the vaccine, regardless of when the second dose was administered. So this is a constant time from the second dose. We also did a study looking at a constant time from the first dose, and in this particular instance, that was about 49 days. We used an immunocapture ELISA assay to analyze that, and that was previously described in a different report. In this study, we showed that if we

increased the intervals from two, three to four weeks between the first and second doses, this shows the number of individuals. The seroconversion rate was 90 to 100 percent in this case. Geometric mean titer ranged from 450 to 1860. Notice that the geometric mean titer was three to four times as much in the three- and four-week group compared to the two-week group.

The second one, which we look two weeks from the second dose, two, three and four weeks between the first two doses, this column shows the number of people, the geometric mean titer. Again, the geometric mean titer was three to four times higher than the individuals who were three or four weeks late for that second shot, and the seroconversion rate increased from about 50 percent to 100 percent from two weeks to four weeks. So that our hypothesis was verified here.

We decided then, using this data; i.e., knowing that individuals who reported for the second dose at two, three or four weeks, at three or four weeks were higher than those who reported at the second week, and we also used the fact that females had a higher reaction rate than did males. We also knew at that time that in animals, that one

or doses protected the animals, and we know that the anthrax vaccine is the only licensed vaccine for human use and that contains aluminum hydroxide or an aluminum-containing compound that is given subcutaneously. All other vaccines containing aluminum compounds are given IM.

So we decided to look to see if giving the vaccine IM to humans decreased the reaction rate, but yet was as immunogenic as the subcutaneous route. We did that looking at a dose-reduction route change study.

In this study, since no one has studied a single dose before, we decided to look at a single dose of vaccine given either SQ or IM. Two doses of the vaccine given two weeks apart, SQ or IM, and two doses given four weeks apart, SQ IM, and the control group given all six doses over 18 months subcutaneously. We did not do an IM group in this study because the objective at that time was to look at a reduced dose. Some of us, there was a lot of debate because some of us wanted to look at the IM route as well because it could have panned out that IM route could have been safer, and that's all that we--but not as immunogenic, but there were those who felt differently. So, in any event, we

did not do an IM route using all six doses.

One can see here that, again, the schedule of the route and the number of individuals ranged from 22 to 28 and the mean age from 32 to 35. The assay in this case was a validated direct ELISA. We used the peak anti-PA IgG concentration and the seroconversion rate at peak to spore as a positive when needed an IgG concentration of at least 25 micrograms per milliliter or greater or a titer of 1- to 200 or greater. We looked at a random sample of 10 percent of individuals were looked at in a validated toxin neutralization assay. These are the results.

The control group had a very nice response with over 400 micrograms of anti-PA IgG per milliliter. The single-dose groups did not do very well. However, the groups that received two doses two weeks apart did fairly well, reaching about 150 or 200 micrograms per milliliter, and the 0-4 group, as we predicted, did quite well, did as well as three doses over four weeks. So, again, two doses over four weeks, versus three doses over four weeks, and they have the same geometric mean titer at peak. The peak in this case was at six weeks.

PARTICIPANT: [Off microphone.]

[Inaudible.]

DR. PITTMAN: Thank you very much, in case this slide is not very clear.

So that these two routes and schedules were in a known inferiority test were noninferior to the control group.

Now one of the things I like to point out here. Notice before in the background data, the serologic data, we have noticed that the four-week group had about three or four times as much antibody at peak as the two-week group, and that is verified in this particular study. Again, if you compare routes, 0-2 SQ, 04 SQ, three times as much. Similarly, for the IM route at 0-2 and 0-4, it has about four times as much antibody, which confirmed the previous--so this prospective study confirmed the retrospective analyses.

If we look then at the response rate, seroconversion rate, that was 100 percent for the 0 to 4 group, and it was 96 to 100 percent for the 0-2 and the 0-4 groups. Now the single individuals in these two groups did have antibody. They had a small amount of antibody. However, it was not enough to reach the 25 micrograms per milliliter required of this validated test. Nevertheless,

they did all have antibody.

Since they have not reached the 25-micrograms-per-milliliter level, we consider them as nonresponders by this test, by this validated test. This is shown graphically in this slide. Again, the log antibody concentration versus time in weeks. This line represents the 0-to-4 group, the three-dose group. This line represents the 0-4 SQ group, with this line representing the 0-4 IM group.

Now, at peak, again, in a noninferiority test, there is no difference, and that was true for the duration of this study, for the entire four weeks after peak. However, there was a statistically significant difference between Weeks 3 and 5, between to 0-4 groups IM or SQ and the 0 to 4, and that is of course because they did not receive a dose at two weeks, but after that they are all the same.

Also, females had a higher titer, had a higher antibody concentration all along this route, but that did not reach statistical significance. This shows a correlation between the ELISA and the toxin neutralization, that there is a nice correlation there.

I will just be very brief here. This is just to show that IGM is produced in these individuals who are given AVA.

If we turn our attention now to symptoms, there was no difference between IM and SQ in systemic symptoms when the vac--either IM or SQ in systemic symptoms, as we can see here by these P values. However, when we look at the injection site reaction, such as tenderness, subcutaneous nodules, erythema, induration and warm, comparing IM versus SQ, we do see a significant difference in the rate of the reactions.

For subcutaneous nodules, there were none in the IM group. There were no SQ nodules in the IM group. Whereas, in this combined group, there was about 40 percent had subcutaneous nodules. Similarly, for erythema and induration. Even the rate of tenderness, tenderness was a little bit less in the IM group. I am not going to put a lot of value on that.

Now, seeing that the SQ group had such a high reaction rate, we looked at the usual demographics to see if there was a reason for that. When we looked at sex and age, we do not see a difference. However, when we stratified based upon

gender, we did see a tremendous difference. So this slide shows the subcutaneous route stratified by gender. Here we see that for subcutaneous nodules, males had about 24 percent. Whereas, females had 63 percent--so three times the rate of subcutaneous nodules. Similarly, for erythema and even worse for induration.

Now, if we look at the entire six-dose series, these numbers increased to 70 to 80 percent for subcutaneous nodules. I would say, though, that all of these reactions, including subcutaneous nodules last a few--except subcutaneous nodules--last for two to three days, they disappear, and the patient is perfectly well.

The subcutaneous nodule may last for several weeks and occasionally for a few months. We have seen in specimization that the subcutaneous nodules lasted as long as six months. However, the subcutaneous nodule does not cause any symptoms in patients. They just simply know that they are there, and ignore them and go on about their work.

This slide is just to show that there is a correlation between the antibody level and adverse events at the injection site. This was even the case when we included the IM group. So, if we

lumped them all together, we saw a difference. Now, if we knock out this IM group, this difference becomes much more striking than what we see on this slide. The correlation becomes much more striking.

So that this study showed quite conclusively that without any reduction in the immune response or in the immune readiness, since we are in the military, we like to use those kinds of terms, without significant reduction in immune readiness, there is a significant reduction in local adverse events to AVA when the vaccine is administered by the IM route or even when the interval between the first two doses SQ is increased from two weeks to four weeks. The IM route is the route for all other aluminum-containing compounds and that a large pivotal study is required for the FDA to allow a supplement to the licensure for a route in dose-reduction change.

I would say that this study, the pilot study, was funded by JPL, and in our discussion with the FDA back in '95/''96, the plan was to go straight ahead from this pilot study and do a pivotal study. However, the JPL, in its wisdom, decided not to fund the study beyond that point.

However, the Congress did fund FDA to the tune of $20 million per year for five or seven years to do that particular study. We hope that they will vaccinate their first patient soon.

This shows the six-dose schedule. If we look at, again, the log IgG concentration versus time in weeks, and you saw this part of the curve before, if you then give the boost at six months, there is a robust anamnestic response. The antibody decreases over time. You give the next dose at 12 months. There is another great response. It decreases a little bit. Notice that there is a difference in the slope of these two lines, and then at the 18-month dose, there is still a response. Notice that the trough steadily increases, and we think that at some point that a plateau is reached in this trough, and we are doing a study to look at that.

This study gets into the question of whether or not--currently, as the vaccine is licensed, annual boosts are required if an individual remains within an at-risk area. We think that there might be a better way to do that and that the anthrax vaccine, in some conditions, in some circumstances, could be treated just like

all other vaccines, and that is that you prime a person, and then you give interval boosts.

Well, the Fort Bragg study, in essence, kind of gave us some supporting data to suggest that that is possible. In this case, we took individuals who were vaccinated during Desert Shield/Desert Storm for both anthrax and botulinum toxoid. We decided to offer to bring them together to draw blood--well, this was done by informed consent and all--to draw blood and offered them a booster dose of the vaccine, and this is the result of that study.

It turns out that some individuals had one, two or three doses, dependent upon when they received the vaccine during that particular war. Since there was an abrupt end to hostilities, it was felt that there was no need to continue with the vaccination. So that some individuals received one dose, some received two and others received three doses of the vaccine. These are the results from that study. Again, since this was an older study, we used this as titer, and we used the older immunocapture ELISA, not the validated direct ELISA.

I will just go straight to this slide.

Again, this is the reciprocal of the anti-PA IgG concentration, and this is the number of doses given during Desert Storm. Again, these people were given a booster. This is the pre-boost titer, pre-boost titer, pre-boost titer, pre-boost titer, and the post-boost titer. One can see that there is a dramatic increase in the titer before and after. But interestingly, though, many of these individuals did have titer consisting, even after two years after having received either one two or three doses of the vaccine. So that antibodies do persist over a long period of time.

As we can see here, even the group, and we would not think of considering troops immunized if they received only one dose, but even the one-dose group responded in an anamnestic manner.

So the Bragg study did show that antibody persists for up to two years after receiving one, two or three doses, and that one can give these individuals a boost and get a fantastic, robust anamnestic response.

I just want to say one word about the use of anti-AVA plasma. One other useful purpose for individuals who are immunized against AVA is that their plasma can directly be used to help patients

who have serious anthrax disease or, for that matter, not-so-serious anthrax disease, and it might be better to give, if one is considering giving anti-AVA plasma, to give it earlier, rather than after it is too late. Also, it is being collected, as the laboratory reagent.

In an agreement with CDC, NIH and USAMRIID, we are beginning this week to collect plasma that would be available to be used in case of an emergency. We will collect a larger amount that we hope to process and to purify immunoglobulin that will be able to be used. But in the meantime, it is our hope that the plasma can tide us over until the purified immunoglobulin becomes available. This would be used under IV.

So there are still some interesting clinical questions that need to be answered, and I am getting close to the end. Again, the Congress did fund CDC to perform the confirmatory pivotal study looking for a dose-reduction route change. So that the CDC will also look at reducing the number of doses from six to four doses over 18 months and will also look at giving booster doses at various intervals, so that we will hopefully be able to decrease the number of boosters and the

frequency of boosters in these individuals.

As you know, by now, over 500,000 troops have received the vaccine, and one question is whether or not it is safe. Once the CDC's pivotal study confirms the pilot findings so that it is okay to change the hundreds of thousands of troops who have received the SQ to the IM route, we would like to do some study to show that it is safe, although, empirically, I think all of us would agree that there is no reason why it shouldn't be safe, but we would like to provide the FDA with some data to show that that is the case.

One thing that needs to be looked at is why is it that females have such a high reaction rate compared to males when this vaccine is given subcutaneously and not enough work has been done to look at that particular question. Again, we are looking at whether the trough peaks or not.

The question of sustained versus interval boosting is something that needs to be looked at, and, of course, the long-term safety of this vaccine. We are currently doing a study at USAMRIID, in which we will study specimization participants who have received the vaccine up to 30 or more years to see if there is any adverse effect

on them from having received the vaccine.

There are some other interesting titers that need to be looked at as well, epitope mapping, cytokine profiling and determine which, if any, HLA genotypes or haplotypes are responsible for immune response and also for adverse events. Those will be interesting studies to do.

Of course, most interesting for individuals in the military, as well as the civilian population, is the utility of anti-PA plasma in treatment of AVA disease. So we think that there may be a role, but we do need some laboratory and animal evidence to support that.

Thank you very much.

[Applause.]

DR. BURNS: Thank you very much.

Our next speaker is Les Baillie. He is with the Ministry of Defense in the U.K., and he is now currently at the University of Maryland. He is going to tell us about the mouse model of anthrax.

DR. BAILLIE: Thank you very much.

Just to clarify who I am and what I'm doing standing here talking to you guys, my affiliation is really the U.K. Ministry of Defense. I'm on a sabbatical with the University of

Maryland. I've come up here to save the world, and what I'm going to do is talk to you about some work that we've done looking at the mouse model,in terms of a model for looking at evaluating anthrax vaccines and trying to understand some of the issues around the disease itself.

Why use the mouse? Well, the mouse is small and furry, and we can use lots of them. Humans are small and furry, but we're not allowed to use lots of them, so we need to use animal models.

The mouse has been used for over 100 years in anthrax research. It is susceptible to disease by a variety of routes, including the aerosol route. We can use statistically significant numbers, so we can power our experiments. The immune system of the mouse has been well characterized, in terms of the availability of reagents, and look-across studies have been carried out with humans. So that is quite useful.

The mouse response to vaccination with the U.S. and the U.K. vaccines, which are fundamentally the same products in terms of they are made slightly differently by using different starting principles.

As Art has mentioned already, the mouse macrophage has been used extensively to study the effects of the lethal toxin on other agents, and the mouse has been used to generate monoclonal antibodies, which are specific against PA, the primary immunogen of the current vaccine.

Indeed, we have used the mouse model to T-cell map, T-cell epitope map PA, and I might mention that later.

The point is what is known about the mouse model? Now the problem with trying to mine the literature is that everyone has used different mice, they've used different methods of challenge, they've used different anthrax strains, and so they've all got different results. So it is very difficult to cull all of that data and come up with a common perception of the mouse model.

The mouse can be infected by a variety of routes, but the organism cannot cross unbroken skin. So you need to have some form of introduction into the mouse injected and subcutaneous routes have been used as, indeed, has aerosol challenge.

Indeed, the majority of workers have used injected-challenged models. Now the LD 50s for

these different routes of delivery vary for the same organism. The IM LD 50 is not the same as a SQ.

Inbred mice have been used extensively to study the reaction to anthrax and to the animals, and inbred models have their limitations, as will become obvious later. But it is the aerosol route of challenge which is of interest to ourselves, in terms of bioterrorism, and also in terms of the military applications.

The bottom slide gives you some idea of the difference in the infected dose and the different rates of delivery. As you can see, you require 800 times more spores to affect a mouse via the aerosol route.

Again, looking at the limited data available in literature concerning the pathology of the disease in mice, we can see that the inhaled form of anthrax in mouse is very similar to that seen in guinea pigs. Spores are taken out by alveolar macrophages, as Art has described already, and the spores germinate inside the macrophage relatively rapidly. They then go on to cause systemic disease, with organisms being found in the lungs during the later stage, probably as a

consequence of septicemic contamination.

Nothing much really to talk about in terms of, in fact, one of the characteristics of anthrax infection in mice, and in guinea pigs, and in most animal systems is a massive total bacteremia. This is where the toxin issue comes in, in terms of treatment.

Time to death can vary, but is usually three to seven days, depending on the mouse and the kind of strain.

Numerous attempts have been made to develop reproducible aerosol models for the mouse, including studies of our own. A variety of inbred mouse strains have been assessed using the Ames Porton strain, let's call it that. This strain was originally acquired from USAMRIID, and indeed has been sequenced. Indeed, this is the basis of the genome sequence, which we sponsored, and there's a very nice paper coming out soon talking about the strain.

Work is in progress to develop an aerosol challenge model. We are interested in aerosol protection against anthrax, and indeed we have a very active Porton looking at working out a model system which will allow us to challenge

reproducibility mice with an aerosol. Indeed, we have one such study using an outbred strain of mice, a Porton, called the Porton outbred strain. In one study, we can kill these animals with an aerosol, which is nice.

Let's go back to the inbred mouse issue. A lot of this work was done by Sue Welkos of USAMRIID, and they found that you can divide inbred mice up into a group of susceptible, intermediate and resistant. Now what is interesting is that these mice differ, and why do they differ?

Well, as Art alluded to earlier, it appears that the capsule is a much more important phagocytic characteristic than the toxic. You can challenge mice with capsule-positive, but toxin-negative organisms, and they will kill vaccinated animals. You don't see that in primates. It is very unlikely you see that in humans. It is a facet of the mouse.

Saying that, we have selected a susceptible strain of mouse, the A/J mouse, which we have used extensively, and we published on recently two papers in Infection and Immunity last month and this month, describing our work with this mouse model system.

The mouse is given the attenuated strain, lacks C5I80 [ph.], but is complemented efficiently, but it does die reproducibly.

Again, trolling back through the work in terms of the susceptibility to anthrax and the different responses you see with mice, the different vaccine formulations, we have seen that if you give mice only alum-based vaccines, you don't see as good of protection as you see with Ribi. Now Ribi-based vaccine is a TH1-based vaccine, and for some reason, you get better protection in a mass.

You also find that if you use the Vollum 1B strain, which is the original U.K. weapon strain, you can actually protect the mice, but if you use the Porton Ames strain, you cannot. So we are seeing strain-to-strain variation, but we are also seeing variation in the route of delivery of PA to the immune system in terms of protection.

We do know from the primate work carried out at USAMRIID that if you give alum-based vaccines plus PA, you get total protection in monkeys. I would suggest that we are more closely related to the primate than we are to the mouse, but the mouse is useful in terms of at least giving

us some data and giving us an animal model system which allow us to ask big questions about our vaccine candidates.

So what is the utility of the mouse? Well, it should be obvious to a lot of us in the audience that the mouse allows us to do wide-range studies. It allows us to look at different immune formulations that we are interested in. The DNA vaccination work is of interest to a number in the audience, I know. At Porton, we have we looked at using microencapsulation as a delivery system, and again I would point your attention to this month's I&I for review of that work.

We have been using the system to generate monoclonal antibodies, as have Steve and others from USAMRIID, for therapy. Recently, we have actually T-cell-mapped PA in treating haplotypes of mice, and we are hoping that this data will give us some help, in terms of developing better vaccines.

So the mouse as a potency assay, and when I say potency assay, I mean an assay for measuring the amount of biologically active PA in a vaccine. Work carried out at Porton has shown that we get a nice-spaced response curve with recombinant PA, as you can see here. We can protect this model if we

want to against a challenge with the STI strain, which remember that is the Russian human live spore vaccine strain. We can do that with reproducibility. And we have shown that anti-PA antibodies from these animals give passive protection.

So, after that brief gallop, what are our conclusions? At least on the efficacy side, there is no, as yet, validated aerosol challenge model, and this is a key drawback of the mouse model in terms of developing a model system which is going to give us results, which shall directly read across to humans. We need to have an aerosol challenge model.

Other factors, other than the toxin, may contribute towards virulence in mice, and Art has alluded to this already. As I mentioned, the capsule is more important than the toxin in a mouse, but also there's some data from Steve's lab that suggests that there are proteases and other chromosomally encoded factors which are important to virulence in the mouse. Again, I stress in the mouse.

Mice do respond well to protective antigen, and they may have a role to play as this

potency assay, in terms of assaying new lots of vaccine and getting some idea of the immunogenicity of vaccine formulations.

The last one, again, finally, that once more that the A/J mouse is a good model to look at as a potency assay, but work is still needed to be done with it, and it is going to be an efficacy model.

On that, I shall finish. Thank you.

[Applause.]

DR. BURNS: Our next speaker is Gary Zaucha from Walter Reed. He is going to tell us about the pathology of the disease in various animal models.

LTC ZAUCHA: I was billed to talk about guinea pigs, besides rabbits and monkeys, but that is not going to happen. I'm just going to confine my talk to rabbits and rhesus monkeys.

I am currently assigned to Walter Reed, but everything I have to present today is from information I collected while at USAMRIID. This is all aerosol-challenge information.

The animals were obtained from, oh, maybe about 10 or so different protocols. It included LD 50 studies, different vaccine efficacy studies,

correlate immunity studies and even pest transfer study. Most of the challenges were with Ames, but there were also challenges with a number of different, more virulent strains.

I am going to start out with just nonvaccinated control animal data. These rabbits were, like I say, nonvaccinated. About half of them were exposed to Ames, the other half were exposed to different strains from various parts of the world.

In the rabbit, at least, I saw no differences in the pathology dependent on the strain of exposure. The only thing we saw was that the more virulent strains resulted in death within one to two days post-exposure, while the Ames, the average was about two to three days post-exposure.

In the rhesus monkey model, again, the majority of the animals were exposed to the Ames. There was also a fair number of Vollum 1B and just a couple of the more virulent strains.

Lesions between the Ames and Vollum animals were similar. The other two strains, I only had two animals per strain, so you can't really draw much from the numbers, but both of the animals exposed to Namibia developed meningitis and

both of the animals exposed to the Turkish strain had a much more marked hemorrhagic component to the pulmonary lesions.

Now the pathogenesis has been reviewed pretty well already. The lungs serve as a portal of entry, not as a primary focus of infection. The organisms are transported by pulmonary macrophages to mediastinal nodes, where they germinate and proliferate, and eventually enter the systemic circulation through the thoracic duct.

The principal lesions, whether it is in rhesus monkey, rabbit or human, are hemorrhage, edema and necrosis, with a variable, but usually limited, leukocytic infiltration. Most cases develop a septicemic disease, with a high degree of bacteremia and disseminate the lesions. Further on in the talk, I will discuss what I term nonsepticemic disease. It is not absolutely nonsepticemic, but it is different from this type of situation.

Target tissues are primarily lymphoid organs. The mediastinal lymph nodes service the primary focus of infection. Once the disease goes septicemic, the spleen is affected in virtually all cases. There is also high incidence of lesions in

mesenteric nodes.

In the lungs, the primary lesions are edema and also some degree of hemorrhage. While pneumonia is unusual, there is some evidence to indicate that in humans, as well as rhesus monkeys, that in cases that do develop pneumonia, it may be influenced by preexisting pulmonary lesions. In other cases, there's also some evidence that when pneumonia does develop, it can be from secondary hematogenous development, rather than from the primary pulmonary exposure.

Lesions are also common in the GI tract, even with aerosol exposure. It tends to occur in sites that are also rich in lymphoid tissues.

Finally, the brain is a somewhat common site of lesions. This is where there is one difference in the pathology between the species. The rabbits tend to have a noninflammatory CNS lesion. The rhesus monkey, CNS lesion is much more separative, inflammatory, and it is also more common, which is more similar to what we see in humans.

I hope you can make this out. Let me just point out a few things. First, in the lungs, this column is human findings that I obtained from the

literature. I was able to put together about 72 cases of inhalational anthrax from various case reports in humans that had at least sufficient pathology information in the reports. This column is rabbit data generated at USAMRIID and rhesus monkey data from USAMRIID.

By far, across the line, the most significant lesion is pulmonary edema. One difference in humans is that there's approximately about 30 percent of the human cases had natural pneumonia, whereas, rabbits and rhesus monkeys are about half of that.

Lesions in the mediastinum, also very common. They were less so in the rhesus monkey, but this may be influenced by the duration of infection. It was shown by I think Gleiser in earlier studies that rhesus monkeys that tended to live longer through antibiotic interventions tended to develop more hemorrhagic and more pronounced mediastinitis.

Intrathoracic lymph nodes are affected across the board in a high percentage of cases. This line here is CNS lesions in the brain. Human and rhesus monkey are very similar. About 50 percent of inhalational cases develop CNS lesion.

A majority of those, this middle line, 38 percent were inflammatory in humans, while only 14 percent are just basic edema and hemorrhage. It is similar in a rhesus monkey, while the rabbit, only 24 percent had CNS lesions, and all of those in these naive rabbits were just simply hemorrhage and edema without any inflammation.

One thing to note in this table, also, is that the mean survival post-exposure in the rabbit was 2.1 days. In the rhesus monkey, it is 4.8 days. The human is 4.7 days, but this is post-onset of clinical signs. It was basically impossible to determine the exact time of exposure in these human case reports. Colonel Friedlander pointed out I think in Sverdlovsk cases that the incubation period was actually up to about 16 days in people. So you are looking at maybe 20 days post-exposure, as opposed to a very rapid time course in these animal models.

I think the time post-exposure does influence the lesions of the rabbits, with a mean survival of only 2.1 days, had minimum inflammatory changes. Rhesus monkey, with a longer survival period, had an increased incidence of inflammation, CNS signs, pulmonary and hepatic changes. These

changes become more pronounced in animals that have longer survival time.

This is the spleen of a rabbit exposed to Ames. Let me just jump to a higher magnification. This is the white pulp, and there is extensive lymphoid death going on here. Morphologic changes are suggestive of apoptosis, but there is really no definitive study to determine that at this point.

The red pulp is characterized by extensive aggregates of fibrin. I hope you can make out aggregates of bacilli right here. There's also some infiltration by heterophils.

This is the lung of a rabbit, and it shows just the simple edema alveolar spaces filled with this pale eosinophilic fluid. There is really no information going on, and most rabbits the hemorrhage was not really too pronounced.

This is from the lumen of a pulmonary artery in one of these rabbits, just to show the high degree of bacteremia in these animals at death.

This is from a rhesus monkey. Again, the most profound change is filling of the alveolar spaces with this eosinophilic edema fluid. Like most cases, this one is pretty much devoid of any

inflammatory component. There is a fair degree of hemorrhage, though, when compared to the rabbit.

This is another case from a rhesus monkey, which shows primarily pulmonary edema, some hemorrhage. The thing to note is that the bronchus is really normal. There is no primary bronchial lesion. That was determined quite some time ago. All of the activity seems to be going out more in the alveolar spaces.

This high magnification of this same animal does show some infiltration by neutrophils within alveolar spaces, also within alveolar septa. This particular animal has a mild degree of interstitial pneumonia, and the interstitial pattern is suggestive of a hematogenous origin for pneumonia, as opposed to bronch pneumonia. That is more typical of inhalation of other organisms.

Now this is one of the more severe cases of pneumonia in a rhesus monkey. Again, the bronchus is pretty much spared, but the alveoli are just flooded with inflammatory exudate, hemorrhage, and I think there is a higher mag here showing this supportive character of this exudate mixed with large numbers of bacilli and hemorrhage.

This is a mediastinum from a rabbit. This

section here is the mediastinal lymph node--what remains of it. There is some remnants of lymphoid tissue right here, but the rest of this has undergone complete necrosis, depletion of lymphoid elements. There's large amounts of fibrin and hemorrhage. The origin of mediastinitis seems to be spread from lymph node involvement. In this case, you can see lesions extending out into the surrounding mediastinum.

This is higher magnification showing not only large numbers of bacilli, lymphoid depletion, but there is an arterial here that's undergone fibrinoid vascular necrosis, which is pretty common in lymphoid tissues, and I have also seen it in quite a few of the brains.

This is from a rhesus monkey demonstrating extensive tracheal lymph node involvement or bronchial lymph node involvement, while the bronchus itself is, at this point, untouched, which again this reinforces the pathogenesis that the lymph node is the primary focus of infection and other involvement of airways and lung is more secondary.

This is just another lymph node from a rhesus monkey showing severe lymphoid necrosis and

depletion with extension of the lesion into the surrounding mediastinum that you see here and over here.

This is the brain of a rabbit. As I said, the rabbits tend to have just a simple hemorrhagic lesion in the brain without much inflammation. This happens to be cerebellum, the central lesion right here. And at higher magnification, you can see that there is hemorrhage, large numbers of bacilli, but there is really no accompanying inflammatory infiltrate. Now that is in contrast to what's seen in the rhesus monkey and also what's more commonly seen in humans. The meninges here are markedly thickened with hemorrhagic and inflammatory exudate. At higher magnification, you can see the separative nature, large numbers of neutrophils, hemorrhage and also large numbers of bacilli in this meningeal exudate.

This is a section of kidney from a rabbit. This lesion was probably not too important in the overall pathogenesis, but it was very common to see scattered tubules within renal cortex that have undergone degeneration and necrosis with intertubular hemorrhage. This was not readily apparent in the rhesus monkey. So that is one

difference, but it was a relatively minor finding in the rabbit.

This is an adrenal gland from a rabbit. A very common finding was hemorrhages, in this case just multi-focal hemorrhages within the adrenal cortex, but very many of these animals the adrenal gland was really obliterated by hemorrhage. There is the adrenal medulla here. The cortex is running out the capsule here, and that gland, I would have to say, is probably not functioning.

The rhesus monkeys and humans had a similar incidence of hepatitis. This happens to be a rhesus monkey with a focus of hepatocellular necrosis.

As I said, lesions in the GI tract tended to focus on the lymphoid ridge areas. This is the cecal appendix of a rabbit. These, the large lymphoid domes are normal. Out here in the center, there is a lymph follicle that has undergone severe lymphoid depletion and necrosis at a higher magnification. Again, there is typical large numbers of bacilli and mixed with the necrotic cellular debris. Not much in the way of inflammation, though.

This is a sacculus rotundas, which is

similar in structure to the cecal appendix in the rabbit--just another lymphoid area of the GI tract, with a similar finding. At high mag, again, showing just large numbers of bacilli that are characteristic in these lesions.

This is a section of colon from a rhesus monkey, just to show a similar change. There is a lymphoid follicle here that has undergone necrosis depletion. The epithelium is eroded away in this case. At higher magnification, though, in the rhesus monkey, there is a more significant inflammatory component to the lesion, mostly neutrophils.

Finally, this is a section of bone marrow from the rhesus monkey. This is a common lesion, but probably not all significant in the death of an animal, but there were frequently necrosis and depletion of marrow elements.

This table is just to give a little more detail of the influence of survival time on lesion incidents. Now the rabbits--I should say the first half of my talk isn't even in your notebook. This table, I doubt, is in there, but the rabbit data is the same from the first table.

The rhesus monkey data is based on time to

death from Day 3 out to Day 8. As lesions or as time course progresses, there is a gradual increase in the incidence of mediastinal lesions from Day 3 out to Day 6 or Day 7. What happens out here in Day 8 is a little unusual. It is what I term a more nonsepticemic case of anthrax. I will get into it a little bit more later. But in these animals, they may only have just a transient bacteremia. They don't develop the same disseminated lesions. The bacteria seems to see the brain, and they all die of meningitis.

So there is also an increase in incidence in the brain lesion from only 14 percent at Day 3 to 100 percent in the longer survival animals. There is also a shift from a noninflammatory lesion, where none of these animals exhibited any inflammation similar to the rabbit's, but as you increase in time, lesion becomes more inflammatory, more separative.

Now the next set of slides I have are from animals that were afforded protective immunity through vaccination--I believe all vaccinated with AVA. Some were given two full-strength doses, some were given dilutions of AVA. They were challenged with Ames or some with other virulent strains.

The findings in these animals are limited to the lungs. They did not seem to develop any septicemic changes. While these are survivors, they were euthanized at the end of a 28-day observation period. Lesions, in most cases, were minimal to mild, really not clinically significant. These animals were clinically normal. I only examined a single rhesus monkey that happened to die of other causes, after surviving anthrax challenge, and there were no lesions attributable to anthrax in that animal.

So this is the lung of a rabbit immunized with AVA, challenged, euthanized 28 days later. The ones I have photographs of are more dramatic, more severely affected animals. Most animals, the changes are really minimal, but just so you get the point across, there are aggregates of lymphocytes scattered throughout the alveolar areas. Perivascular inflammation is very common. This is a bronchial up here, bronchiolar epithelium. There is quite a significant alveolitis in this animal, thickening of alveolar septa, infiltration by lymphocytes, heterophils, macrophages.

These limpid histiocytic aggregates were relatively common. Macrophages, multinucleic giant

cells and lymphocytes. I did immunohistochemistry on these mildly affected cases and immuno against Bacillus anthracis, and these were generally negative.

Vasculitis was not uncommon. This is the wall of a pulmonary artery. You can subintimal infiltrations of lymphocytes and similar infiltrates in the tunica media and out here in the adventicia.

Now, of all of those animals, I forget how many, maybe 50 to 60 animals I examined, two of these did have what I called a pneumonia. In this animal, it was limited to just the apex of a lung lobe. Here, you see that the normal alveolar architecture has been obliterated by cellular inflammatory infiltrates, large numbers of macrophages. This was the worst of the two animals, and it has a large pyogranuloma, the central core of necrotic granulocytes surrounded by macrophages, fibroplasia, aggregates of lymphocytes out here.

Immunohistochemistry on this animal--first, let me go to a higher mag. One thing I noticed on H&A was macrophages filled with some type of intracytoplasmic foreign debris. With

immunohistochemistry against Bacillus anthracis, it is clear that all of that intra-histiocytic debris is remnants of infection.

A higher magnification from the same animal, most of it is just fragments, but you can see there are discernable bacilli, but these animals were culture negative. What I think is going on is that they did develop a local pulmonary infection following exposure. Never became septicemic. They were able to overcome the infection, but there wasn't a proliferation of the organism in this animal's lungs to cause significant inflammation, and inflammation continues against what I think are nonviable remnants of the organism.

Now that brings me to one other set of animals. These animals, rabbits and monkeys, were vaccinated or provided with immune sera against anthrax, but die from the disease anyway. Some of these animals were given dilutions of AVA. Some of the animals, the rhesus monkeys were given dilutions of AVA or some were given a different experimental PA vaccine. A few animals were given the full dose--well, actually, just two injections--of AVA, exposed to virulent strains of

anthrax and died.

What is seen in these animals is, first of all, an increased survival time. There is a marked increase in the inflammatory component of the lesions, particular in the CNS, and they tend to develop what I term nonsepticemic disease. Now there had to be some degree of septicemia bacteremia somewhere along the line for these animals to get meningitis, but, histologically, these animals really had a very limited bacteremia, and lesions tend to be localized, either to the lungs or the brain.

What I saw in rabbits was these animals developed severe parenteral hemorrhagic pneumonia and mediastinitis, as opposed to nonvaccinates who just developed pulmonary edema.

One thing I noted the pneumonia that developed in these rabbits is similar to what was described in about 25 percent of the Sverdlovsk cases, where they termed it large focal pneumonia. It is also similar to the type of pneumonia seen in resistant species exposed to aerosols of Bacillus anthracis.

This table is just to provide more detail on the--

PARTICIPANT: [Off microphone.]
[Inaudible.]
LTC ZAUCHA: Okay, I'll finish up in a minute.
This is a rabbit, with only partial protection, and you can note the severe inflammatory pleuritis in this animal. There is a marked separative component to the inflammation, large numbers of bacilli. And then immunohistochemistry demonstrate that those bacilli are Bacillus anthracis.
This is the mediastinum of a rabbit that had only partial protection, died of anthrax, and again it's a marked inflammatory component, quite a bit of fibrosis.
This is the lung of a rabbit similarly affected. You can see the severe parenteral hemorrhagic pneumonia. The perivascular lymphatics are markedly dilated, filled with exudate. The bronchials are filled with exudate. Higher magnification showing the inflammatory component within the alveolar spaces, similar exudate within the bronchials. There is a severe vasculitis in these animals.
This is a Giemsa stain of a pulmonary

lymphatic showing the lumen filled with macrophages, lymphocytes, some granulocytes and scattered organisms.

Let me skip over some of this. This is the brain of a rabbit that, again, was immunized, but died of anthrax, and you can note the marked inflammatory component within this, as compared to just a strict hemorrhage and bacteria seen earlier. There is severe perivascular cuffing. There is also the fibrinoid vascular necrosis that I showed earlier and similar findings in the meninges of such rabbits.

This is just a close-up of the exudate. Again, that shows what is really similar to what we saw in the rhesus monkey with separative inflammation, large numbers of bacilli.

So, finally, to separate septicemic versus nonsepticemic anthrax. The septicemic disease, these animals developed severe bacteremia, disseminated to lesions. There is just limited inflammation with or without meningitis. These animals I believe die very rapidly to toxemia-induced cytokine cascade and shock.

This is as opposed to nonsepticemic anthrax, which has a more protracted time course.

The infection is more localized to rhesus monkeys, primarily the brain. Rabbits can be brain or lungs. There is a marked inflammatory component, and death is probably due to a more localized effect, either respiratory failure or CNS depression.

So, to summarize, the pathology appears to be dependent on the balance between host susceptibility or immune status, and the virulence or doses of challenge. Highly susceptible naive rabbits, there is rapid death, septicemic disease, noninflammatory hemorrhagic lesions.

The rhesus monkeys appear to be a little more resistant. They have a longer time course. They still develop, the majority of cases developed septicemic disease. There is an increased inflammatory component and an increased incidence of meningitis, more similar to humans.

Animals given partial protection, they even have a more protracted time course. They tend to develop nonsepticemic disease--lesions localized to the brain, lungs. Bacteremia is very low level or transient. There is a high incidence of meningitis.

Finally, the more resistant host, and it

includes a dog and swine, where they, after aerosol exposure, lesions are strictly confined to the lungs. There is no septicemia at all. And then also fully protected immune animals, they survived with little or no residual changes to the lungs.

That's all.

[Applause.]

DR. BURNS: Our next speaker is Louise Pitt from USAMRIID, and she is going to tell us about the immune response in several animal models.

DR. PITT: Well, good morning. I will be as quick as possible because I know everybody is starving.

The talk this morning is going to be basically in three parts. I am going to give a very brief overview of the animal models that have been used commonly in the laboratory for different vaccine efficacy studies. I will then move on and focus on the rabbit and the nonhuman primate and show some of the vaccine efficacy data that we have obtained in the lab at USAMRIID, and then talk about our approach to developing in vitro correlates.

This is a list of the principal models that have been used in the laboratories for vaccine

efficacy studies during the last century. I won't focus much on the mouse because Les already covered it. Just to point out and emphasize what Les was saying, that the capsule seems to be incredibly important in some of these mouse strains, and when the mice are immunized with the licensed vaccine, although they get a very high anti-PA titer, they are not protected when challenged. However, when the PA is delivered in a different platform, in this case, a bacterial platform, and the mice, the PA, the A/J mice, although get a high titer, again, they are not protected, but in the CBA/J mouse, you can get protection.

In the rat, the rat was used in the 1940s for vaccine efficacy studies. However, the rate appears to be fairly resistant to infection, and immunization doesn't really make much difference, and the rat model is usually used more for toxin challenges than for spore challenges.

Hamsters are very susceptible and have been used extensively in the Russian laboratories. This is their rodent model of choice, and they did recently publish a paper suggesting that in the hamster, they can get breakthrough of vaccines upon challenge. Pat Fellows in our lab did a study

looking at the hamster model, and this table shows that when vaccinated with the licensed vaccine, whether it is two doses or three doses, although they get a very good anti-PA IgG titer, there is no protection at all against a spore challenge.

Now the guinea pig has been used extensively. Ever since it has become available as a laboratory animal, it was the rodent model of choice for anthrax studies, both in the U.K. and in the U.S.

Of course, the guinea pig is susceptible to the spore infection. It seems to be fairly resistant to toxin, but again it has been used extensively to characterize the pathogenesis, as we know it today, to elucidate the role of the toxin. When immunized with a vaccine like our licensed vaccine, which is adjuvanted with aluminum, it gives partial protection to minimal protection, at best.

Now the rabbit, again, historically has been used throughout the century for vaccine efficacy, both in Russia, the U.K., and the U.S. In fact, it was the model of choice prior to the guinea pig becoming available. Rabbits are very susceptible to anthrax. They are sensitive to

toxin, and when immunized with either the licensed vaccine or recombinant PA combined with aluminum, we can get complete protection against both a parenteral challenge and against aerosol challenges.

And we have found that the vaccine efficacy in the rabbit is predictive of what occurs in the macaque.

So we come to the nonhuman primate models, the rhesus macaque, which is accepted as the best model of inhalational anthrax, where there have been extensive studies ranging from the 1940s to the present, where we have shown that both the licensed vaccine and recombinant PA plus aluminum gives complete protection against inhalational anthrax.

I will point out that, although all of the studies carried out at USAMRIID since 1990 to present have used the rhesus macaque, that a large majority of the studies that were carried out in the '50s and '60s was done with cynomolgus monkeys. In fact, they were used interchangeably for the anthrax study.

So, to move on to looking at the guinea pig, the rabbit, and the rhesus macaque, this is a

comparison of the LD 50 studies. This is a study that was carried out at USAMRIID under the same conditions, using the same spores, using the same aerosol conditions, with a mass median aerosol diameter of one micron, which means it's a single spore aerosol, and this is to show that looking at these three animal models, under similar conditions of exposure, that the LD 50 is very similar for these three animal models.

This is just a table showing you some of the efficacy data we have in the rabbit model. This is against the licensed vaccine. Again, complete protection against both aerosol and subcutaneous challenge.

This is a table showing, again, some of the efficacy data we have with the rhesus macaque. Again, with the licensed vaccine showing the protection, even out to 100 weeks, following two doses of the vaccine, against a very significant aerosol challenge.

This next study is looking at recombinant PA compared to the licensed vaccine in the three animal models: the guinea pig, the rabbit, and the macaque. Again, it was two doses of vaccine, and the animals were challenged with the Ames.

This shows you that in the guinea pig vaccinated with the licensed vaccine, you get poor, minimal protection, 20 percent. Whereas, you get 90 to 100 percent in the rabbit and the rhesus. And then looking at the different doses of 55 and .5 recombinant PA with aluminum, you get extremely good protection in both the rabbit and the rhesus. You start to see a dose titration down in the .5 microgram in the rabbit, but in the guinea pig there is a fairly flat line. There is no obvious difference between the groups, regardless of the dose of PA.

This the anti-PA IgG response from that study, showing a titration effect. The 5 and the 50 micrograms really gave a similar response in this study. What is of interest is the animals were challenged at the 16-week time point, and you can see host challenge, the rise in anti-PA IgG titer. Of interest here is that it is an inverted response, that the lower dose that you got by immunization, the .5 micrograms gave you the least immune response prior to challenge, but upon challenge, it gives the highest post-exposure response.

This is the anti-PA IgG response in rhesus

macaques, again, showing you that titration, as the dose drops, you get a drop in the immune response. This is another study in rhesus, comparing recombinant PA with AVA, again, showing the immune response. And the animals in this study were challenged at 112 days, and this is to show you that in the rhesus macaque, as well, there is a fairly decent response to PA, post-challenge, which is obvious at three to five days post-challenge.

I put this slide in here to show, and to emphasize, that guinea pigs, when immunized with an aluminum-adjuvanted vaccine, you do not get protection against a spore challenge. However, when PA is presented with a different adjuvant, in this case, MPL, you can get complete protection or excellent protection in the guinea pig model.

Now, moving on quickly to our approach to in vitro correlative immunity that we developed in the rabbit inhalational anthrax model. Our approach to doing this, because we get such excellent protection with full doses of the licensed vaccine, our approach was to dilute the vaccine down so as that we would have some nonsurvivors in the study, so that we would be able to compare the response of the nonsurvivors to the

response of the survivors and come up with a correlate.

So the study design was very simple. We diluted down the vaccine, gave two doses. We bled the animals prior to challenge, and in this case we focused on the humeral immune response, looking at anti-PA IgG and the toxin-neutralizing antibodies.

As you can see, as we diluted down the vaccine, we got a fairly nice titration in survival in the animals. We can also show a titration in the anti-PA IgG response, both at six weeks, which is at peak, the titer--that's two weeks after the second dose--and at ten weeks, which is the time of challenge, and the TNA gives a similar pattern.

This is just showing you each individual graph, with concentration of anti-PA IgG versus the dilution they received, and the open circles are the dead animals, and the closed diamonds are the animals that survived. Statistically, this is extremely significant.

We then repeated this study with a second dose of the licensed vaccine and came up with the same conclusions, and this study has, in fact, been published recently.

We then went on, of course, to look at

recombinant PA plus alhydrogel. This is the work of Steve Little doing a similar study to see if recombinant PA also would give the same correlate of immunity. The design is fairly similar. In this study, this is a one-dose, rather than the two-dose that we gave of the licensed vaccine. The animals challenged at week four, that's four weeks after the one dose that they got.

This is the results to date, showing that as you titrate down the dose of the recombinant PA, you get a nice titration in the number of survivors, and you also get a good titration in the amount of antibody, and there is indeed a very strong correlation between the levels of anti-PA IgG and survival. This is just the individual animal's graph to show you the pattern.

So, in summary, in terms of the in vitro correlate in the rabbit model of inhalational anthrax, we feel that we have shown that antibody levels to PA, both at the peak and at time of challenge, have shown to be significant predictors of survival. At this point, with recombinant PA plus alhydrogel vaccine, right now we have shown that one dose of vaccine correlates with protection, and studies are ongoing right now with

the two doses of the recombinant PA to verify that will be the same.

We did look at the nonhuman primates and looking at doing a dilution study there too. This was just a pilot study with very small numbers of animals to see if we would get the same pattern in the nonhuman primate, as we had done in the rabbit. As I said, this was a very small study. Insufficient animals to actually come to any statistical conclusions, but we did get, we chose doses of the licensed vaccine from 1 in 12.5 to 1 in 100. We gave them two doses and challenged six weeks later.

We did get a nice titration in the IgG response, the TNA, and we also looked at lymphocyte proliferation indices. We also got a titration survival, but these results are inconclusive because we did not have a large number of animals to come to a statistical significance.

We then went on to say we have shown that antibody to PA is a correlate and can predict survival, but how good is it in the passive transfer? Can the antibody actually passively protect against the inhalational anthrax?

So, first of all, we made some

convalescent sera, and we made some immune sera, both against the licensed vaccine in the immune sera and against recombinant PA.

In the first study, we did intradermal challenges, using the spores Vollum 1B and the anti-sera was introduced intraperitoneally. We started off with the convalescent sera. This, again, was just a pilot study to see what we would get. We had three animals that received the convalescent sera and one control. As you can see, all survived the challenge, while the control died.

We then went on and looked at the immune sera that was raised with the licensed vaccine and again got similar results. This was, again, with an intradermal challenge against Vollum 1B.

We then went on to look at subcutaneous challenge of passive transfer. These studies were done under contract with Battelle. The design was similar, except the challenge was subcutaneously with Ames spores this time, and the challenge dose was 100 LD 50.

We initially looked at the immune sera from animals that had been immunized with the licensed vaccine. As you can see, the line in blue is the animal that died; whereas, all of the others

survived. The animal that died was one that got only one dose of the anti-sera at time zero.

We then went on and looked at the immune sera raised with the rPA. So this is anti-sera to rPA, again, against a sub-Q challenge, 100 LD 50. The lines in blue and green are the animals that died; whereas, all of the others survived.

We then went on to do a study against an aerosol challenge, which is what we were wanting to do all of the time. The challenge dose was 205 LD 50s with Ames. This is a study that has just been completed. So I don't have any of the IgG data levels of antibody on board, but the results were very good. We killed 10 controls, and all of our test animals survived against a 205 LD 50-challenge.

So, in conclusion here, we used the rabbits and the rhesus macaque as our chosen animal models to look at vaccine efficacy. We have based this decision on the pathology of the disease, as we know it today, and the response to vaccination. And we have demonstrated that anti-PA antibodies do correlate with protection of inhalational anthrax, and we have demonstrated that the anti-PA antibodies can, in fact, protect.

In conclusion, I would like to thank everyone who has contributed to this work. I would especially like to thank Steve Little, Bruce Ivins and Pat Fellows for all of their work and dedication over the years that have made most of this work possible.

Thank you.

[Applause.]

DR. BURNS: Okay. I know we're a little late for lunch, but I think it's important to take a little bit of time for discussion. Again, could I remind you, if you have a question or a comment, please use the microphone and identify yourself.

PARTICIPANT: [Off microphone.] [Inaudible.]

DR. ALVING: Carl Alving, WRAIR.

As Colonel Pittman pointed out, I don't believe there are any other vaccines in which aluminum salts are used subcutaneously. Why were they originally used for the anthrax vaccine?

[Laughter.]

MR. ALVING: That's really directed towards the regulatory people here probably.

DR. ROBBINS: I can only give you a negative answer. I tried very hard to find out

what--

DR. BURNS: Can you tell us who you are.

DR. ROBBINS: Robbins of the National Institutes of Health.

I tried very hard to find out why that immunization schedule was used by George Wright and Milt Cusis [ph.] many years ago, but there is no reference, and there is no explanation for it, nor is there any explanation why they used it subcutaneously and intramuscularly.

I did want to say one thing about your presentation, and that is it confirms what has been done with diphtheria and tetanus toxin for many years in humans of all ages, that increasing the interval, increases the amount of antibody. But what is not commonly appreciated is that there are several studies, including one done at the Massachusetts Public Health Laboratories years ago to say that reimmunization with nonadjuvanted aqueous solutions of toxoid makes more antibody than when the adjuvant is used a second time.

I suspect what happened is they were trying to induce antibodies as quickly as possible with that schedule, and it turned out to be incorrect. But it might be worthwhile to take a

look to see if altering the use of adjuvants and dosage might be able to give you high levels of antibody quicker.

DR. FRIEDLANDER: Art Friedlander, USAMRIID.

I would just like to add a couple of comments in reference to the question about the dosage schedule and the route. As best I can tell, and there is no one around yet who can really answer the question. There are statements that are made in the literature that say that it is based upon animal experiments. Now there are other vaccines that were also given subcutaneously at that time.

The other point I think to keep in mind, when we look back and think why were these people so ignorant or at least we think they were, is that when you look at the immune response, in terms of the titers, the point that John says is apparent; that is to say, yes, it is true that the titer will increase the longer the dose, but during that first six-week period, you are much better off to have a 0-2-4 schedule than a 0-4 schedule.

So, while we think this was a long immunization schedule, if you wanted to induce

rapid immunity with such a vaccine, this may have been the best way to do it.

DR. SIBER: George Siber, Wyeth.

I wanted to just probe Dr. Pitt a little bit more on the choice of animal model. Obviously, rabbits and macaques are somewhat cumbersome as a workhorse animal model for routine use. My understanding of the reason why mouse might not be optimal is the capsule as a virulence factor. However, if you use nonencapsulated strains, you end up having a model where the main virulence factor would be PA. If that is what the vaccine is, that's what the model needs to address.

So my question is are there other reasons why mice could not be made into an adequate model here?

DR. PITT: I don't believe so. We have gone back more closely and looked at the different strains of animal models, not so much for vaccine efficacy yet, but in terms of using it as a screening model for antibiotics. We have, in fact, developed aerosol model for four different strains of mice recently and are using it right now, as we speak, as a model to screen antibiotics, in fact, using a virulent strain, not the Sterne strain. We

are using Ames, and it works very well.

So I believe if we start looking much more closely at the different strains of mouse, as Les was suggesting, that you might very well come up with a mouse model that would be adequate for screening, but I believe you still need to go to a more relevant model to make sure that you haven't missed something I guess would be the way to say that.

DR. BURNS: Could I follow up on that and just ask this panel of experts what is your consensus on what the best animal models are? If you were going to use one to get the efficacy data that you needed for humans, would they be, as Louise has suggested, a nonhuman primate and then, secondly, rabbit? Do you have any other thoughts on that?

DR. BAILLIE: I think I agree with Louise. I think that we need to have a nonhuman primate in there somewhere and probably the rhesus macaque. As things done at the moment, the rabbit seems to be the best model, in terms of looking at aerosol challenge and in terms of breed across to human. So they would be my choice models, but it's not up to me.

DR. DANLEY: Dave Danley, with the Joint Vaccine Acquisition Program.

I was interested in a comment that you made that cynomolgus monkeys were also used back in the '50s and '60s. Now, with the shortage of rhesus that we've got, what is the perception about going back to a more available nonhuman primate model so that we can get the work done potentially faster?

DR. PITT: Well, as I mentioned, the cynomolgus was used very extensively in the '50s and '60s. In fact, all Brachman studies were done with the cynomolgus monkey. I think it would just need to have some minor development to look at the pathology and to determine what the LD 50 is compared to using the new strains, et cetera, but I believe a lot of the old pathology was done on the cynomolgus monkey as well. So they were used interchangeably. So it's a possibility, for sure.

DR. ROBBINS: Robbins, NIH.

The purpose of a vaccine is to prevent a disease. Therefore, looking at the disease process can be distractive, distractive from the purpose of trying to predict whether a vaccine will be effective. I think there is an overwhelming amount

of evidence that serum IgG PA antibody with neutralizing activity will prevent the disease. There are limitations in human experience, but the AVA vaccine, which only induces PA antibody to any degree, has not had a breakthrough even though it's been used in high-risk populations for over a third of a century.

It's an AVA vaccine. So, therefore, attention should be directed at the very best way of reliably predicting the ability of a vaccine to elicit PA antibody. What is missing, I think, is some sort of consistency in evaluating these vaccines in animals. I don't think there's two studies in which the amount of antigen, the route of antigen, the vaccine strain, the challenge strain, have ever been used in the same way. It's very confusing to draw conclusions from this.

Just remember that the control assay for AVA was protection against lethal challenge in guinea pigs that have now been discounted. I think we should spend more attention to characterizing the protein antigen as a physical chemical entity and by reliably measuring its ability to induce antibodies.

DR. BURNS: Any comments? Anybody else?

PARTICIPANT: [Off microphone.] I want to respond to [inaudible].

[Laughter.]

PARTICIPANT: There have been 68 monkeys that have been vaccinated with recombinant PA, essentially the same recombinant PA, and 64 of them have survived challenge.

There are now, you have heard, studies with recombinant PA being tested in the rabbit model. Again, well-characterized product. So, while it is true that AVA has its problems, in terms of differences in lots, I think we do now have a database with recombinant PA well characterized, in terms of the amount and its physical characteristics, that will give us the answer, and is giving us the answer, as to what titers, for example, are going to be predictive of survival.

DR. TAUB: Floyd Taub, LifeTime Pharmaceuticals.

In other models, broad spectrum of immune stimulants or some co-stimulatory molecules have been used to enhance immune response in total, and in some cases, antibody response. I was wondering if there's any experience how those types of agents

work in the models that you have been describing this morning, whether one or another has shown results with those broad-spectrum immuno stimulants or co-stimulatory-type strategies using the vector models?

DR. PITT: Are you talking about things like CPG?

DR. TAUB: CPG. We use one called beta LT as being general stimulants. The B-7 or other co-stimulatory molecules might have been tested with some other plasmids.

DR. PITT: I don't know of any studies. Do you, Les?

I know CPG has been looked at, yes.

DR. BAILLIE: We have done some preliminary work with DNA vaccines, looking at optimizing the PA to enhance CPG motifs, but that is all preliminary work. I don't know anyone out there that has done it yet. That is not to say people aren't thinking about it.

DR. BURNS: I think we're going to have to stop there if we want to eat lunch today.

Lunch is just right out here. I am told it is just right out here, and you will see it right away.

Try to get--let's make it 1:10, okay?

[Whereupon, at 12:11 p.m., the proceedings were adjourned, to continue at 1:10 p.m., the same day.]

A F T E R N O O N S E S S I O N

1:12 p.m.

DEVELOPMENT OF SURROGATE MARKERS:

POSSIBLE STRATEGIES

DR. MEADE: Welcome to the afternoon session. We have a lot to cover this afternoon, so we want to go ahead and get started.

I am Bruce Meade, with the FDA Center of Biologics, and will be moderating the first of the Afternoon Session, which is entitled, "Development of Surrogate Markers: Possible Strategies."

In this morning's session, the topic was to review what is known currently and this afternoon is to take a little different strategy, and that is to discuss what we need to do now to move forward with this field. Again, our goal is not to be focusing on the details of any methodology, as to really define what data we need to move forward.

Again, you should note that there is one change in order. The last two speakers, Dr. Hallis and Dr. Phipps, will be changing order, since Dr. Phipps' and Dr. Quinn's talks are coordinated.

Again, I think I will just mention some of the complexities. Some of the goals is to be

working on next-generation vaccines. Many of it will probably be recombinant PA vaccines, and some of the data you will be hearing about this afternoon and some of the approaches will involve some of the AVA and CAMR products, which are nonpurified vaccine. So it's one complexity to be tuned into.

Again, for the afternoon session, I will just go through the issues that we asked the speakers to discuss, which are listed here, and we asked them to discuss the following:

We asked them to describe studies that either are being conducted or should be conducted in animals and the data that should be collected in these studies; we asked them to describe the clinical studies that are being conducted or should be conducted and the data collected from those studies; and then, three, to discuss how the data from the animal and human studies could be utilized to develop a surrogate marker of human protection; then we also asked them to describe how the data could be used to determine a human-immunizing dosing schedule; and, finally to be prepared to discuss some of the pitfalls or limitations to the approach being discussed.

Without anything else, I will have the first speaker of the session, who is Dr. Drusilla Burns from CBER.

DR. BURNS: Thanks, Bruce.

To start out, I'd like to go back to the proposed animal rule that I discussed a little bit earlier this morning, and I want to go over the requirements, again, that I went over this morning and see where we stand on these in relation to anthrax vaccines and new anthrax vaccines that might utilize this rule, as far as efficacy data is concerned.

The first requirement in this proposed rule, and again let me emphasize this is only a proposed rule. This isn't the final rule, so it could change.

[Pause to repair Dr. Burns' microphone.]

DR. BURNS: I think that we do know that the organism is, that the spores are taken up by macrophages. The organism then germinates, grows to high levels in the bloodstream and produces a lot of the toxin. There is some evidence to suggest that neutralization of the toxin would go a long way to prevent the disease, but I think we need a little bit more work to pin that down

exactly.

Secondly, the second requirement is that there is independent substantiation of the effect in multiple animal species. We heard a lot about animal models this morning, and I am looking forward to the discussion in the panel session on what the appropriate animal models would be or do we have appropriate models to move forward using this rule.

Thirdly, the animal study endpoint must be plainly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity. In the case of anthrax, I think what we have to or are concerned about, if it is used as an agent of bioterrorism or a biological warfare agent, it will probably be dispersed in the air. Therefore, I would imagine what we'd be interested in looking at in the animal model is a challenge with aerosolized spores and survival of the animals that are vaccinated.

Finally, the last requirement, and I think this is perhaps the most difficult one to get our hands around, and this is really the subject of this next session, the data or information on the kinetics and pharmacodynamics of the product or

other relevant data or information in animals and humans allows the selection of an infective dose in humans.

Now this rule was written very broadly to cover drugs and biologics. If it were written simply to cover anthrax vaccines, I think it would say the data or information on the immune response elicited by the vaccine allows selection of an effective dose in humans.

So the question really is what is the protective immune response and how do we show what that immune response is?

So I have come up with a possible strategy for doing this, and this is only one possible approach, and there are several approaches, and you are going to hear about some of them this afternoon. I really am just putting this forward as a basis to start discussion more than anything else.

First, we would need to evaluate efficacy in appropriate animal models, and we need to determine the type of immune response, and the magnitude of that response that is protective in animals.

Then we need to translate that immune

response, the animal immune response, to that of the human response. In that way, we can estimate the magnitude of the immune response that would protect humans and, finally, evaluate immunogenicity in humans to determine the number of people that would respond in such a way that they would be protected. That way you could come up with efficacy.

So how might we go about this, really? And what I am going to do is take the situation or take the simplest case, and that is that neutralizing antibodies to PA are protective. Now that is, I think, an assumption. There is some good data to suggest that is going to be the case, but I don't think we have really pinned that down, and we'd have to do that in the experiments that we designed.

So, since I am starting with that assumption, I think you also have to be careful in designing these experiments that you have to take samples such that you could look at a variety of immune responses, just in case antibodies aren't the correlate, you could look at other immune responses to see if they are the correlate.

So I'd start with immunization studies,

and determine antibody levels in the animal that protect against aerosol challenge. This would be done relatively simply. I would give different doses of vaccine and look at the antibody response, and I should get a dose response curve, as is shown here.

And then after challenge of the animals, you could monitor survival and death. If it actually does correlate, if antibodies do correlate with survival, then you should get a level of antibodies above which all of the animals survive, and that would be the animal correlate of protection.

Once you have that, you need to compare the quality of animal antibodies to that of human antibodies. So, if we are looking at neutralizing antibodies, how could we do that? Well, one possibility is to just look at the amount of animal antibody needed to neutralize a certain quantity of the toxin and compare that to human antibodies. That way we can translate the animal antibodies into human antibodies, and then using the above information, estimate the quantity of human antibodies that are necessary for protection.

A second way of getting a correlate of

protection and ultimately getting at the surrogate marker protection would be passive immunization studies. I think these are actually going to be a very good way to look at whether antibodies are indeed the correlate because you are looking at antibodies alone and not the rest of the immune system.

So passively immunize with animals with human antibodies and determine the level that protects the animals from challenge. In that way, you could estimate the magnitude of the human antibody response that would provide protection in humans.

Now I think passive immunization studies would give us a maximum level of antibody that is needed for protection; that is, the maximum amount of antibody that has to be there at the time of challenge.

It would be interesting to compare the results from the passive immunization studies with those of the active immunization studies. If it takes more antibodies to protect in the passive immunization studies than it does in the active immunization studies, then the possibility exists that memory or boosting plays a role.

Therefore, we need to look at that, need to if, indeed, you get a booster response upon challenge, and you don't have to have the antibodies there at the very time of challenge, but they come up very rapidly after and help protect, then we'd need to evaluate the kinetics of the boost response in animals and compare those kinetics to the kinetics of the booster response in humans that we see in the clinic just to make sure that the kinetics of the response in the animals is similar to that of humans.

So I would just conclude by saying I think that--oh, I'm sorry. We need to do the human studies, of course, and that would be to determine antibody levels attained after immunization with the vaccine in humans and the proportion of individuals that respond to the vaccine.

We probably also want to do assessments of the rate of antibody decline over time. With these data, we would be able to estimate the efficacy of the product in humans simply by determining the percent responders that we have, and we could also get an idea of the duration of efficacy by looking at the rate of antibody decline over time.

So I would suggest that three types of

studies are needed: Active immunization studies in animals, passive immunization studies in animals and, finally, human immunogenicity studies. I think, with these data, we would be able to get a handle on whether neutralizing antibodies to PA are indeed a surrogate marker protection in humans.

Thank you.

[Applause.]

DR. MEADE: Thanks, Drusilla. I think we will hold questions to the end of the session.

Our next speaker is Dr. Conrad Quinn, who is now based on at the CDC, and he will be describing some studies done at CDC and their approach to developing correlates of protection for anthrax vaccine.

DR. QUINN: Good afternoon, everybody. It is a pleasure to be here this afternoon to tell you about the CDC Anthrax Vaccine Research Program and its integrated study in determining correlates of protection in macaques and surrogate markers of protection in humans, if all works out.

Before I start, I'd like to introduce my colleague, Brian Plikaytis, from the Biostatistics and Information Management Branch at CDC, National Center for Infectious Disease. Brian's group will

play an integral part in the design and the analysis of the correlates of protection studies which I will tell you about this afternoon.

In 2000, the Institute of Medicine instituted a committee to prepare a report on a congressional mandate on the safety and efficacy of AVA in humans. AVA is currently the only licensed anthrax vaccine available in the U.S., as you all know.

This committee concluded that AVA, as licensed, is effective in humans protecting against anthrax. It is reasonably safe when used according to the label, but studies are needed to establish a quantitative correlation of protection levels and antibodies in animals and humans after immunization and correlates of protection in animal models can be used to test the efficacy of AVA, as well as of new vaccines. So we are trying to set the stage here not only for an analysis of AVA, but other second- and third-generation anthrax vaccines that are in development at different stages.

As we have heard this morning, and as we know probably as a collective anthrax research interest, there are a variety of key hurdles in anthrax vaccine research and implementation. In

the context of AVA, its ability to prevent inhalational anthrax in humans is unknown. Although the Brachman study of the 1960s used inhalational anthrax cases as the denominator, the numbers were actually too small to come to a firm conclusion about inhalation protection.

Surrogate markers for protection in animals and humans remain undefined, despite the extensive work that has been done over the last 50 or even more years. A clear correlate, perhaps with the exception of the rabbit model and the excellent data we heard this morning, a clear correlate still remains to be defined for humans.

The role of PA versus the other antigens and AVA are still to be defined. We know the PA is the central protective component, but work done using lethal factor and the DNA vaccines that Darrell Galloway has implemented indicate that lethal factor may also have a protective role in this vaccine. We know that AVA vaccines do not always respond to lethal factor, but nonetheless it is a component of the vaccine and merits investigation at some level.

The duration of immunity following AVA vaccination is unknown at this time. And, finally,

but not exclusively, the schedule and route of administration may be suboptimal for AVA. As we head from Phil Pittman this morning, the pilot study done at USAMRIID indicates that we may be able to reduce the number of doses and to change the rate of administration without affecting antigenicity.

The USAMRIID study which was conducted between 1996 and 1999 and the report which published or made available in 2000 demonstrated this. The Pittman, et al., showed that the peak antibody levels, using intramuscular doses at zero and four weeks, were not inferior to the 0-to-4-week sub-Q regimen at the 6-month level or post the third jab.

Unfortunately, again, as Phil told us this morning, there was insufficient statistical power to support a label change at that time. This led the U.S. House and Senate to recognize that additional studies needed to be done, and this Senate recommendation, congressional recommendation, forms the basis of the CDC Anthrax Vaccine Research Program, and the objectives were quite specific.

The study should determine the risk

factors for adverse events using AVA; it should determine immunological correlates of protection on vaccine efficacy in humans; and we should optimize the vaccination schedule and its administration in humans to assure efficacy; and that this should be a collaborative project between the Department of Health and Human Services and the Department of Defense.

So the CDC Anthrax Vaccine Research Program, which I am here to tell you about, falls into three major categories:

An AVA human clinical trial which is multi-center, double-blinded, randomized and placebo controlled. Our target is to enroll over 1,500 participants. The endpoints of the study will be based on noninferiority of the immune response, and I will tell you more about that in the next few slides. Our target is to reduce the dose and change the rate of administration, and there are also additional substudies, including an analysis of progesterone across AVA recipients and the HLA typing. There is also an immune correlates of protection study which I will also tell you about.

The immune correlates of protection study

in humans is integrated with our primate study, which involves AVA dose ranging and challenge in rhesus macaques. Our objective here is to study the effect of dose on survival versus challenge time post-vaccination and to determine, within the limits of the study, what is the duration of protection in macaques.

This is integrated with an in vitro study of samples generated from both Part A and Part B, as we call them, to determine the immune correlates of protection in primates and to try and use this information to determine surrogate markers of protection in humans.

This is the design of the human study. It falls into six groups. Here we have the licensed regimen of 0-4 and then 6 months, 12 months and then boosters. Here we have the comparator by the IM route and subsequent routes, where we drop one dose, the two-week, two doses, three doses, and here we have our control groups with IM and sub-Q administration of saline.

The study primary endpoints are to demonstrate noninferiority of the anti-PA IgG antibody levels by ELISA. This will be expressed by the geometric mean concentration between study

groups and also to demonstrate, as a co-primary endpoint, a four-fold elevation in anti-PA titer that is not inferior to the licensed regimen.

These quantitative analyses of antibody will be corroborated by using a functional assay, the toxin neutralization assay, which was developed at USAMRIID, and we have adopted it for this study. The endpoints for this assay will be expressed as the effective serum dilution, giving 50-percent neutralization, and this is similar to the output that was generated in the Pittman study and allows us to bridge to that study. We will also be reporting the IgG neutralization concentration and titers.

The relevance of these endpoints, as we have heard this morning probably in more detail than I need to go into here, is that PA is the central protective component. Anti-PA antibody has a precedence as the protection correlate, and the toxin neutralization is a measure of function such that we can compare the functional capability of changing the route from a sub-Q to IM. We would hope to see no inferiority in terms of functional antibody generation.

The ELISA that we used for determining our

endpoints are based on standard curve in triplicate and our test serum in duplicate. The assay is specific and sensitive, with a diagnostic specificity of 95 percent, diagnostic sensitivity of 98 percent, and these are our ability to detect false negatives and true positives, respectively. Its sensitivity, in terms of detection, its minimum detectable concentration is .06 micrograms per ml. Its reliable detection limit, as calculated from the 95-percent confidence intervals around the bottom of the curve, is .09 micrograms per ml, and we have adopted this as our lower limit of quantitation.

The reactivity threshold, based on analysis of nearly 300 negative controlled serum from the [?] Hanes[?] collection, indicates that 3 micrograms per ml is the reactivity threshold and that this is the upper 95-percent confidence interval of our negative control group.

The goodness of fit used here for comparative purposes, realize that this is not a linear relationship, is .99, and the range of quantitation of the standard curve is, effectively, the whole standard curve from .06 to 1.7 micrograms per ml anti-PA IgG.

In terms of its reproducibility, the assay is precise with an intra-assay precision of less than 10 percent and interpreted precision of less than 20 percent. Its accuracy in terms of how effectively it returns QC sera of known concentration are given here for the 3QC that we use.

The Toxin Neutralization Assay, again, is based on a 7-point standard curve in triplicate. These numbers, the data here represents where we were in our validation process at the end of March, and these numbers are slightly updated now, certainly, in terms of the N number tested.

But, effectively, we again, similar to the ELISA, we have a standard curve, which is sigmoid, and we have a 4-parameter logistic model to it. Its goodness of fit R-squared is .99. Our effective concentration range between these three points is 1.7 to .43. Its reproducibility here is very good, its precision, and its intra-assay precision and interpreted precision are very good for biological assays. Although not as sensitive as the ELISA, it does have very comfortable lower limits of detection and lower limits of quantitation. So those are our endpoints for the

clinical study.

To summarize the clinical part then, our objectives are dose reduction, a change of rate of administration. There are six study groups, and our target recruitment is 1,560 participants. It is a 42-month duration.

Our primary endpoints are based on anti-PA IgG responses, and we have our interim analysis at seven months after the end of the enrollment procedure, which effectively will be about 16 months into the study itself.

The substudy groups, which I only briefly mentioned, progesterone analysis and HLA typing, are done by our participating study sites. The correlates of protection is being done at CDC and in collaboration with contractors at the Emory Vaccine Center, Emory University and the Battelle Memorial Institute. This is integrated with a nonhuman primate study, which I am going to tell you more about now.

This primate study, NHP, the nonhuman primates, the rationale is that AVA efficacy in humans cannot be directly tested. The rhesus macaque, as we head again this morning, is accepted as a good representative model for inhalational

anthrax in primates, and we hope to be able to apply the "Animal Rule." We realize that it is not yet approved or implemented, but we hope by the time the study is completed or we come to do our analysis, that we will at least be able to use it to some extent for extrapolating to humans. Obviously, for identifying correlates in macaques, we will have that information readily.

The objectives of the primate study are to identify the correlates at 12, 24 and 36 months into the vaccination regimen. We want to extrapolate these correlate markers for protection in humans, and we hope to use this information, again, if the "Animal Rule" is available, to support proposed changes in AVA administration and dosing, particularly when it comes to the booster doses.

The methods that we are employing are to modulate the primate immune response by using dose variation, dilutions of the human dose of AVA. Starting with the human, 1 in 5, 1 in 10, 1 in 20, and 1 in 40 dilutions, and using saline controls.

We are giving them three intramuscular injections at 0, 4, and 26 weeks, and we are going to challenge them at 6, 24, and 36 months after

their third vaccination.

We are going to use the combined information here from challenge and immunological profiling to build our model of predicting survival in the macaque. And then we anticipate applying this relationship to the human clinical study, in which we will be taking parallel samples to predict protective status of human vaccinees.

The direct relationship between the two studies are shown here. Where the regimens parallel each other, are the target primary series in humans of 0, 4 and 6 months IM.

The timing of the challenges in the macaques will parallel vaccination points of boosters in the human study groups, and the timing of the blood draws in both cohorts, the human cohort and the primate cohort correspond.

So our first assumption, our first precedent for the study is that we can modulate the immune response in macaques using different dilutions of AVA. This slide shows that using the anti-PA IgG response, this is indeed the case. In fact, it's a rather textbook example of immunomodulation based on humoral responses at this time.

Here we have the controls in yellow along the bottom, and at the top in blue we have the human dose responses. They are jobbed at 0 weeks, 2 weeks and at 26 weeks, and we have a peak here at 8 weeks, which then drops not exactly to background, but to very low levels, and then responds very, very quickly after the third vaccination.

The kinetics of this response is very similar between all groups. We get a graded response. The others are hidden in here. But we can see human, 1 in 5, 1 in 20, 1 in 40 and 1 in controls.

This is important because selection of the dilution series was not straightforward. We based this on discussion with our colleagues at Fort Detrick and other experts in the macaque field, including our colleagues at Battelle, and we felt that it was necessary to try and get a distribution of survival and death in all of the test groups selected, rather than all survival or all death, but of course we have our human dosing here for the top end of the spectrum.

We also felt it necessary to try and give them sufficient vaccine that we would be able to

measure our immune response, so that we would have something to build our model with.

If we look at the TNA responses in the same groups over the same time point, we see a very similar profile, 0, 2 and 26. Again, a peak at about 8 weeks, receding to background, and then a very rapid response after the third jab, and a very high response.

If we go back here to the ELISA, we can see we're getting 1.3 milligrams per ml. These are the geometric mean concentrations of the groups. There is distribution within each group, but the geometric mean concentration here was over 1 milligram per ml.

So, although we can demonstrate some level of immune modulation in the macaques based on their antibody responses, the actual survival is unknown, but we would predict that over time, after their vaccinations, we get a grading of survival, and this is what we hope to establish.

To make maximal use of this gradation and mixed response, we have built a very flexible model, which we built on expert advice and external consultancy, that we believe gives us a level of flexibility to maximize our statistical power and

the number of animals that we use, while minimizing the number of animals that we need to use. Our target is to have 105 macaques on study by the end of the program.

So last year we vaccinated five groups: Undiluted human, 1 in 5, 10, 20 and 40.

We have just recently begun our first challenge schedule, starting with the 1 in 20 groups, and we have allowed ourselves three scenarios to be data driven and dependent on outcome.

In scenario, where survival is low, we then have a series of precision points and actions which allow us to maximize the use of our animals.

Similarly, in scenario two, where the response is intermediate, which is actually the target response, 50/50 distribution, we also have a series of precision- and data-driven processes to take us through the end of the study. We will be using macaques that are on study at Battelle and also under vaccine study and immune profiling at the Emory Vaccine Center in Atlanta for this.

Scenario three is based on good survival at all levels, and this determines how many animals get carried forward and when they will be

challenged.

To build our model of efficacy, we need to have immune response analyses and generate the variables that will be put into the model together with survival.

We are coming up to some changes in the slides that are not in the handouts. If anybody wants them, just e-mail me. They were important, but we made them late.

Our output variables, in terms of measuring the humoral immune response are that we will determine the nature of the anti-PA response across all of the study groups and post-challenge in the macaques. We will monitor changes in the nature of that response over time and post-challenge. We will look at the neutralization efficacy of the humoral response and the contribution of other responses, other than PA, to neutralization and bacterial clearance. We do this because we recognize that although PA is probably the primary protective component and immunogen in the vaccine, other protein antigens in that complex mixture may contribute, at some level, be it positively or negatively, to the effect of AVA.

We are also going to look at the cellular

immune response. This, again, is through our collaborators and contractors. The output variables here will be cytokine profiles for The cells and macrophage activation during vaccination. We want to know when protection is acquired, at least in the context of CD4 memory cell priming and immune competence.

When is B-cell memory established? How long do these B cells circulate and survive? When do they differentiate and when are they put down into the bone marrow, and how long do they last when they are there?

We also want to look at the relevance of the Th bias, which we think will inform vaccine design, and also the performance of the vaccine regimens by comparing between groups.

So we hope to use this information or we intend to use this information to build a model of predicting the immune status of humans and macaques, starting with the macaques and then extrapolating.

We are not tying ourselves down to any one approach, and we intend to use a variety of approaches, both established mechanisms, such as logistical discriminant analysis, cluster analysis,

but other exploratory techniques, and Brian is much more informed about this than me, and he will take all of the questions on the statistics.

We'll use logistic discriminant analysis as our placeholder and as our example. This model will list a series of assay endpoints or variables to survival, and we will use this information to construct a discriminant function from the results using a formula such as this, where we will calculate an immunologic score for the vaccinees.

We will select this information to give us the greatest discriminating power. We will then correlate the immunologic score for all subjects and plug it into the model, such as the discriminant cutoff, and placed to either maximize sensitivity or specificity. In this study, we want to optimize sensitivity, and we will set that to 95 percent.

Our model will look something like this, where we have our population that were vaccinated and survived challenge, our population that failed challenge, and this is our discriminant cutoff. We will hope to use our immunologic score to predict where a person falls along this axis.

What the model will actually look like

when it is built is that we will have a probability of survival against the immunological score for individuals. In the ideal world, where, and if we take, for example, three of our dilution groups, the 1 in 10, 1 in 20, and 1 in 40, six months after vaccination, we would ideally like to see a distribution like this, where we have a whole bunch of animals. Zero is death, one is survival. There is nothing in between.

A whole bunch of animals at the low vaccination group all dead; in the middle vaccination group, a nice distribution between survival and killing; and in the higher vaccination concentrations, everybody survives. That's what we'd love to see. It gives us a nice step function so anybody we plug into this model, if their immunologic score falls over here, we know they are protected. That is the ideal.

In reality, though, what we anticipate, and what we are actually finding it will look like, is something like this, where we have distributions of death and survival across the groups with, again, the ones getting the least vaccine clustered down here, and the ones getting the most vaccine clustered up here, and that allows us to build a

sigmoid curve, where we have, in this example, I have split them into quartiles, where we have percentage per quartile, allowing us to predict, probably in more realistic terms, where an individual might fall as a part of the group.

So how does this correlate to the human study? While we assume that the nonhuman primate immunogenicity and survival curve can be used to predict protection in humans, we are very aware of the differences in the immune systems between the macaques and the humans, as far as is published, and that is just something we have to deal with.

We then intend to apply our discriminant function to the Immune Correlates Protection clinical trial data from humans and see where they fit on this model and examine how vaccinated and unvaccinated individuals can be scored. We will then hope to use this information where they fit in the model, what is their predictive survival, to convey information of when does protection start and to how long does it last.

So, to summarize our NHP study, we know at this point, one year into the study, that vaccine dose-dilutions can elicit variations, at least in the humoral immune response; we have started

challenging with bacillus anthracis at Battelle; and we are correlating the primary out of that immune response with our serological data. It is too preliminary to present at this meeting, so I won't say any more about it; we are 14 months into this study, and we have a 42-month study duration.

So, just to finish off by giving an overview of what we consider to be a very integrated study, we have our human clinical trial and our macaque dose-ranging and immunogenicity study. Both of these studies will give us humoral and cellular immune profiles in macaques and humans.

We will use the macaque study to determine survival against virulent challenge. We will use the combination of cellular and humoral immune profiles and virulent challenge to build our immune competency model and hopefully identify immune correlates of protection.

We then hope to apply the "Animal Rule" to correlate the human responses with the monkey responses and identify human surrogate markers of protection. That should effectively close the communication loop back to our human clinical trial and monkey study.

I would just like to finish by identifying the key players in this at CDC: Brad Perkins, who is the principal investigator; Nina Marano, who is the project coordinator; Dave Ashford and Jairam Lingappa, who are the technical leads for the macaque study; our colleagues at NIP; George Carlone, who has been a terrific consultant in this whole process; Brian and Tom, who are the statisticians; John Stamper, data manager; Jennifer de Pietra, technical assistant; and the lab team who does the serology.

Our collaborators and contractors external: The Emory Vaccine Center and Emory University in Georgia, Professor Rafi Ahmed and Bob Mittler; Battelle Memorial Institute, we have Dave Robinson, Jim Estep, Bob Hunt, Andrew Phipps, and initially we had Herb Bresler. Also, subcontractors at OSU and CAMR.

Finally, but not least, our collaborators in the government and the primary study sites--I see some of the PIs are here today--AVIP, USAMRIID, NIH, FDA, our study coordinator sites, BioPort for the vaccine, and TRI are our CROs, contract research organizations.

So that is it. I would be to try and take

any questions you may have.

[Applause.]

DR. MEADE: Thanks for that excellent presentation.

I think we will move next to our next speaker. Again, we have changed the order a little bit. The next speaker will be Andrew Phipps, who will describe the work being done on correlates of protection at Battelle Laboratories.

DR. PHIPPS: I'd like to thank the organizers for inviting me to speak. Without wasting any more time, I will get started.

I'd like to begin by talking about our rationale behind the study. The rhesus macaque is an accepted in vivo test system for modeling human immunologic responses following vaccination, and the several different speakers talked about this morning, it is also an accepted model for looking at the pathogenesis following inhalational exposure to Bacillus anthracis spores, and therefore was chosen to be studied in our Part B, nonhuman primate trial study.

Our overall experimental objective was to characterize the cellular and humoral immune responses at the molecular, cellular and whole-body

levels in individuals vaccinated with the anthrax vaccine absorbed. That was AVA by BioPort Corporation. It is also to look at those same parameters in our cohort of rhesus macaques.

This cartoon depicts a very simplified version of the study design, where we have vaccinations that are parallel between the human trial study and the primate trial study, with challenges occurring at various points--12, 30 or 42 months following vaccination, and those correlate with booster doses in the human cohort.

We also have coordination between the blood draws between the two groups. As Conrad mentioned, this is infinitely more complicated by the decision tree and the fact that we have various dosing regimes and routes of administration. However, I'd like to just point out that we do have integration between the two studies, and that allows us to compare our parameters across the human trial study and the primate trial study.

Conrad also showed this slide previously, that we needed to modulate the immune response in our rhesus macaques, and after much discussion decided on using this dilution scheme of the vaccine with the idea of hitting approximately

50-percent survival in the 1 to 20 vaccine dilution group, with having greater survival in the 1 to 10 and slightly less survival at the 1 to 40, and that those would shift at 30 and 42 months such that we could evaluate our parameters in relationship to survival. I won't spend any more time talking about this, as Conrad has already covered it.

I would like to spend most of the time talking about the immunologic markers that were chosen and how we plan to evaluate those and our rationale behind them. As we go through those, I'll discuss them briefly and then go back and talk about the methodologies and the rationale.

We are looking at patterns of cytokine mRNA synthesis and cytokine secretion by T cells following protective antigen stimulation in vitro. We are also looking at proliferative responses by protective antigen-specific T cells in vitro.

We are looking at anti-PA, anti-LF and anti-EF immunoglobulin profiles, toxin-neutralizing and opsono-phagocytic antibody activity.

More specifically, for our cytokine response profiles, we are making a determination of mRNA and/or protein levels of the TH-1 cytokines, gamma interferon and IL-2; of the TH-2 cytokines,

IL-4 and IL-6 in humans; and nonhuman primate PBMCs following stimulation with PA.

We are also making a determination of mRNA and/or protein levels of IL-1 beta and TNF alpha cytokines that are characteristic of macrophage activation. Our rationale for choosing those cytokines is that the TH-2 cytokine production is critical for the formation of immunity to extracellular pathogens and toxins. The TH-1 cytokine production is critical for the formation of immunity to intracellular pathogens, and that macrophage activation is often required for effective license of intracellular bacterial pathogens.

As was mentioned earlier today, the adjuvant can play a big role in modulating the TH-1 versus TH-2 response, and therefore we are also gaining information about that type of behavior of aluminum hydroxide in both rhesus macaques and humans.

We are looking at T-cell proliferation, and we are doing that by tritiated thymidine uptake of PA-stimulated cells in both AVA-vaccinated subjects and NHPs, as compared to our placebo or naive control groups.

In vitro proliferative response is giving us an indirect measure of the increasing frequency of PA-specific T helper cells in vivo. The proliferative response is independent of functionality. When I say that, we look at proliferation as a measure of DNA synthesis, and that doesn't relate necessarily back to the functionality of those T helper cells, but because we have profiled the cytokine response, we can look at that in relationship to the TH-1 versus TH-2.

As Conrad mentioned, modulation of the T-cell proliferation also occurred with our vaccine dilutions that we chose.

Is there a pointer?

At time zero, we had--this is a log simulation index and the time in weeks. At time zero everyone was below what we would consider to be a positive cutoff. Following the first immunization, you can see that we had modulation of--and this is a geometric mean of the vaccine dilution group, modulation of the stimulation index, and following the second immunization at four weeks we have the undiluted at the top, the 1:5, 1:10, 1:20, 1:40, and then we have the saline control group at the bottom. This is a little bit

misleading in that I have connected these two points along here. This would be following the third immunization at six months, and in actuality, we most likely would have seen a decline in stimulation index following with a return with the third immunization. But I went ahead and connected these, although this is probably not the case because there's no--currently there are no time points reflecting this period.

Following the third immunization, we saw a merger of all of the vaccine dilution groups such that there really is no statistically significant difference probably between the vaccine dilution groups and that this has held steady out to the point that I've shown on this figure.

We're also looking at total antibody ELISA as an assessment of the levels of antibodies or IgG (IgG subclasses 1-4, IgA, IgE, and IgM) to the AVA components PA, EF, and LF by ELISA, and we're also making an assessment of avidity by looking at high-avidity antibodies by ELISA and assignment of avidity indices to serum samples.

We chose to do this because antigen-specific immunoglobulin plays a critical role in the protective immune response to

pathogenic organisms and toxins following vaccination. And as we heard earlier today, we're very interested in the anti-PA, IgG response along with defining subclasses, and we're also looking at IgA, E, and M in addition to antibodies against EF and LF.

We know that affinity maturation and isotype switching occurs following repeated immunizations, and changes in the avidity of antibody attachment may also play a role in protection.

It's necessary to look at the functionality of that antibody. It's not enough to have it recognize the antigen in a format of an ELISA or Western blot, but we need to know or determine its ability to neutralize the activity of PA. So we're looking at relative magnitude, nature, and toxin-neutralizing efficacy of antibody responses to both PA83 and PA63 conformers. And it was pointed out this morning by Steve Leppla in his diagrams that there are multiple points along the pathway of intoxication where antibody can play a role. And by looking at the ability of antibody to act both on PA83 and PA63, we can determine where in this pathway antibody may be important in

neutralizing the effects of the binary toxin system.

We're also able to dissect the neutralizing activity of serum antibody responses to EF and LF utilizing assays that measure individual enzymatic activities of the EF and LF or antibodies that can neutralize the adenylate cyclase and MEK-1 endopeptidase.

We need to do this because AVA antiserum to neutralize anthrax lethal toxin at different stages in the intoxication process is important to understanding how the immune response relates back to protection, and that toxin neutralization has been demonstrated to correlate with protection in both rodent and rabbit models of anthrax.

We're also looking at the ability of antibody to--or its involvement in opsonophagocytosis, so we're making a measurement of opsonophagocytic antibodies using differentiated tissue culture or tumor cell lines as effector cells. We're looking at fluorescently labeled vegetative cells, and we're also looking at PA-coated fluorescent microparticles in conjunction with the differentiated cells. And I think there's some words missing on the slide there.

We need to evaluate the ability of anti-AVA antiserum to promote PA- or other antigen-dependent clearance of capsulated Bacillus anthracis. And, again, this links back to a complete understanding of the immune response and how that's related to protection.

I won't spend much time talking about this, as Conrad covered it in detail, but we are working with the CDC to develop models that would allow us to construct a discriminate function from the results of these parameters such that Conrad--excuse me here. Conrad mentioned that we're building an immunogenicity score using a combination of parameters of variables with coefficients up to the number of variables in the model, and from that we can come up with a discrimination cutoff where we look at survive challenge and failed challenge versus the immunologic score.

That brings me to the end of my presentation. I'd like to acknowledge the Centers for Disease Control and Prevention, specifically Dr. Bradley Perkins, Dr. Nina Marano, Dr. David Ashford, Dr. Jairam Lingappa, Dr. George Carlone, and Dr. Conrad Quinn.

I'd also like to acknowledge the individuals who have worked with me at Battelle Memorial Institute on this study: Dr. Carol Sabourin, Dr. April Brys, Jim Estep, Robert Hunt, and Roy Barnewall. Then I'd also like to mention those individuals who have worked also on this project as subcontractors to Battelle Memorial Institute: Dr. Lawrence Mathes and Dr. Kate Hayes at the Ohio State University; and Dr. Andrew Robinson, Dr. Nigel Silman, Ms. Moya Burrage, and also Dr. Matt Wictome at the Centre for Applied Microbiology and Research.

Thank you very much.

[Applause.]

DR. MEADE: Thank you, and I noticed we are--I should thank the speakers. Now we're back to being on schedule. So in addition to excellent presentations, we're back on schedule, so thanks very much.

Again, the last speaker for this session is Dr. Bassam Hallis from CAMR, who will talk about the work being done at CAMR on correlates. And, again, I think he is the one in his group who has come the farthest, so we really appreciate their efforts. I think we'll have an opportunity to

learn about the other vaccines being used from their group. So thank you very much.

DR. HALLIS: Thank you. I'd like to thank the organizers for giving me the opportunity to come and talk to you about some of the work that we've been doing in the U.K. at CAMR to try and understand the U.K. anthrax vaccine.

The U.K. anthrax vaccine has been licensed and produced at CAMR and been available for human use since the early 1960s. The vaccine is given in 0.5 ml doses given intramuscularly, and these are given--three doses are given within three weeks intervals at 0, 3, and 6 weeks, and these are followed by a six-month dose given after the third one. Finally, boosters are given annually.

As part of the commitment really at CAMR for continued production of the anthrax vaccine and in order to answer a number of regulatory issues, we started a program of research to try and understand the composition of the anthrax vaccine. We wanted to know what's in the vaccine and--what's in the vaccine, as well as--this is the first phase for work we're doing now trying to understand the composition of the vaccine. Once that is completed, what we aim to do, use that information,

again, from here to try and understand which of these components that are present in the vaccine are contributing to protective efficacy of the vaccine. We also wanted to know can correlates of protection be measured in our vaccine and which of these components that are present in vaccine, again, are contributing to the reactogenicity of it.

As we answer these three questions, we wanted to move on and see can any information and data generated from these pieces of work inform us into the development on either second-generation or third-generation vaccines.

So the first thing we wanted to do, in order to start answering this question and look into the composition of our vaccine, we went through a number of small activities starting with developing an extraction method to allow the proteins to be available and analysis to take place. The next stage was to develop a number of specific reagents which we then use in order to develop specific assays that we employ in order to look at the composition of the vaccine. And in the notes that were handed out this morning from the meeting last December, we have detailed method of

the assays. The principal assays and their application are in the notes, so I won't get into that.

As we developed these assays in order to answer this question, which is the composition of AVP, really more relevant to this meeting we then went and modified all the assays in a way so rather than look directly and measure for the component, so we could use these specific assays as we would modify them in order to move on from the composition and really try and understand and answer these questions here. So a number of these small activities, going to the first one is the reagent provision, and, again, I won't get into much detail on this, but we developed a number of expression systems that allows the production, expression, and purification of the three toxin components.

We also acquired a clearance given to us from the Institute of Pasteur that allowed us to produce the S-Layer proteins, both SAP and EA1.

The standard chromatography technique that's been published really for a number of years were used to purify these components, and then we use this purified antigen to develop and product

high-titer polyclonal antibodies produced in rabbis and guinea pigs, and we also produced other additional reagent that are required, and I'll point them out in a little while, for really the performance of the functional assays that we have.

So the variations were developed and a number of assays were developed. To start with, sensitive and quantitative in vitro assays, both immunological and functional assays were developed. These allowed us to quantify the immunoreactive toxin components and the S-Layer proteins and the functional assays allows us to measure the functionality of each of these toxin components individually, and, again, the two lethal toxins.

The assays have been applied to support the continued anthrax vaccine manufacture in the U.K., and now we're moving on to investigate the immune response of these components in vaccinees.

I'm going to show just very quick examples of applying these assays directly to really define the composition of the vaccine. And here the first one is applying the direct ELISA--the antigen ELISA to monitor and measure the amount of PA and LF in five recently produced batches of AVP in the U.K., and from these data we find that the amount of PA

per intramuscular dose varies between 0.6 to 1.1 microgram. These are per dose. As in the case for LF, we're talking about a third of that actually was present for LF. There is roughly about 0.2 to about 0.4 microgram per dose of LF.

The next set of assays we applied to monitor the composition of the vaccine is the cell lysis assay. This is a standard macrophage cell lysis assay, which what we used for--in a typical example here we have--this is here just purified PA mixed with LF to form lethal toxin, and we add each of our vaccine samples like in the case here to see how much lethal toxin present and how much actively lethal toxin we have in the vaccine. That sample then is spiked with known amount of either PA or LF in order to try and distinguish if we don't have activity, whether due to one or both of the components.

What I want to do from now really, point out that although this assay is being used as it's presented now in the next few assays, to directly measure the functionality of the toxin component. All these assays have been modified to allow us to monitor the ability of antibodies to neutralize the functionality of these various components and the

toxins as well, and I'll come into that in a little while.

The next set of assays applying the endopeptidase assay to monitor and measure whether the LF that's present in our vaccine, whether it is active or not, whether it can maintain its endopeptidase activity or not, and here we have an example of two different batches showing maintaining their endopeptidase activity.

The other five assays is to monitor and measure the adenylate cyclase activity of EF in extracted vaccine, and, again, here really we're looking at adenylate cyclase activity of two different batches. And in a while I'll move on to show how these assays have been modified to assess the antibodies in vaccinees.

So with regard to the composition of the vaccine, what we have done, we've applied a number of immunological and functional assays in order to characterize our vaccine and support manufacture.

We then went on as well as this, and actually we started, initiated applying these assays in the QC really in hoping that include these assays eventually as part of the batch release of the licensed product--as part of the

batch release of the product. Sorry.

And, finally, the assays formats, as I said, have been modified now to allow us to assess the immune response in vaccinees--in vaccinees certainly for the diagnosis of infection, but as well as that in animal models.

So the first type of assays that we went on and formatted are the directed antibody assays. These assays have actually been developed, reformatted to measure a range of immunoglobulins from total IgG and IgG subclasses, IgM, IgA, and IgE, against all the five principal components. These are the three toxin components and the two S-Layer proteins in vaccinees and clinical anthrax cases and in animal studies, as well as the assays that other people alluded to, is actually to look--we're looking at IgG avidity in vaccinees and applying standard TNA assays to see what the ability of antibodies to neutralize lethal toxin.

Here I've got an example of using the ELISA to measure antibody titer. This is whole IgG against PA and LF and EF here in a rabbit that's been vaccinated with a U.K. vaccine.

This is another example of applying these assays to monitor the antibody response against PA

and LF in this case in a cutaneous anthrax case in the U.K., and this is actually just applying it a few weeks after--a couple of weeks after symptoms and all the way to a couple of months, and, again, going back really a few months later.

Immune responses in a U.K. vaccine have also been monitored using these antibody ELISAs, and in here really looking--just to give you an example, looking at an antibody response, this is an anti-PA IgG and this is anti-EF IgG in a vaccinated person, as well as monitoring whole IgG against PA, LF, and EF. As I mentioned, we've got the various IgG subclasses, and in here we have this assay showing an anti-PA IgG-1 and anti-PA IgG-4 in two vaccinated individuals, and third on the bottom is the negative control.

As well as measuring and monitoring the total antibody response between subclasses of IgG, we just apply a standard avidity assay to look at avidity, and this is really ability of the antibodies to still--to bind in the presence of different concentrations of thiocyanate, really standard antibody avidity assay.

As well as these, and you've seen some of these examples a number of times today of using

turning around, modifying the macrophage cell lysis assay to use--to turn it into a TNA assay to really assess the ability of antisera to neutralize the lethal toxin in macrophage cell lysis assay.

So having done all these and applied them, we're moving on now to say the correlates of protection as a general slide really probably can be defined as "a biological response determined by laboratory analysis or by clinical measure, that is predictive of clinical protection." So one could determine immune responses in terms of measuring Ig or CMI, and compare the immune responses in relation to protection afforded by vaccine in Phase III clinical trials and maybe in animal models if Phase III clinical trials are not possible, like in these cases.

In this case here, what do we know from human work? We've heard a lot really this morning and this afternoon between what know in both human and animal. But with regard to human work, we know that effective licensed anthrax vaccines contain or produce either PA, LF, and EF, and other cell components. So we know really certainly that effective licensed vaccines produce or contain all of these PA, LF, EF, and other components, mainly

the S-Layer proteins, Sap, and EA1.

We know certainly that vaccines induce antibody, antibody response to these components to all these three, certainly least to these five components.

We know that antibodies to these antigens are present in convalescent sera as well.

With regard to what know from animal work, we know that PA alone can protect in animal models, and we know that anti-PA antibodies are associated with protection.

I have to actually say that we don't really know enough as yet to say that there is a direct measure on correlation between the protection and really specific anti-PA titer or certainly not in human.

Components other than PA certainly contribute to protection from a number of work, and you heard about the work that's being done in Galloway's group.

And, finally, it's likely that different animal models are likely to identify different correlates of protection.

What we're proposing to do in the U.K. is actually U.K. clinical study to run a proposed

U.S.-U.K. bridging study actually to that of the CDC AVRP study. And the idea is to run a U.K. clinical study by doing only in human but not using NHP challenge.

We intend to carry on using U.K. vaccination schedule using the same route, intramuscular route, and using the same schedule that is applied now.

We want to actually try and determine immune responses, again, in terms of Ig and CMI, using the AVRP assays, and by this stage these assays have been fully characterized, actually fully validated, and actually will give us a good linkage really, bridging between the two vaccines. By monitoring total Ig responses to PA, LF, and EF, by measuring avidity, neutralization and opsonic antibodies, and Dru and Conrad actually talked in detail about the various assays.

We want to compare immune responses between the AVA and the AVP and see how these two vaccines really compare. And we wanted to try and look at correlates of protection determined by comparison to AVRP NHP immune responses at the stage they become available.

So, in summary, really what we've got,

we've got a range of immunological and functional assays that are currently being used to characterize the U.K. vaccine and really trying to define the composition of that vaccine. We'll also apply--modify these assays to allow us to move from measuring directly the actual components in the vaccine to really assess the immune response in vaccinees and for diagnosis against these various components. And correlates of protection for the U.K anthrax vaccine will be determined through a planning immune response study in vaccinees, bridging to the CDC AVRP study. And, finally, the assays could be applied to really hopefully a lot of next-generation formats of anthrax vaccine as they become available.

I'd just like to really finish by acknowledging the U.K. Department of Health and the Medical Supplies Agency, part of the Ministry of Defense, for supporting all this work and various colleagues at CAMR and all throughout CAMR between Research, Manufacturing, and QC Divisions, and a number of collaborators for their generous gifts for various mutants and clones and reagents.

Thank you.

[Applause.]

x DR. MEADE: Good. Well, again, thanks for these excellent presentations. Amazingly, if you'll notice the clock, right on time. So we can open up for some discussion. I will remind everyone to introduce themselves and give their name and affiliation when they ask a question. Feel free to start the discussion.

DR. ROBBINS: My name is Robbins at the NIH. I have a question for Dr. Quinn and a question for Dr. Hallis.

Dr. Quinn, could you comment about the effect of reducing the aluminum content as well as the antigen content when you dilute the vaccine? Because, as you know, the concentration of aluminum within an injection has an important effect upon the immunogenicity.

Dr. Hallis, I'm under the impression from Ternbill's (ph) work that the U.K. vaccine only induces antibodies to LF after prolonged immunization and not in most cases to the EF, and at least for the human vaccine, it's very hard to show antibodies to anything but PA even after prolonged immunization. I was a little surprised by your comments.

DR. QUINN: Shall I answer question one

first?

We thought long and hard about balancing the aluminum content and adjusting it, as you say, because it does have--aluminum on its own, in its own way will have immune-modulating effects. But after much discussion with our collaborators and those who have to actually perform the assays, we decided that the risk of introducing more variables was greater than the risk of keeping the--adjusting the aluminum.

DR. ROBBINS: But if you were to use purified PA, you would have to keep the aluminum constant with various dosage of protein, so the two results may not be comparable.

DR. QUINN: That is absolutely correct. Again, it was something that we have--the amount of discussion and scrutiny that the study has been under since it started has brought these points to the fore several times. With the recombinant PA vaccine, you have that opportunity to mix and match as you see fit and within your study design. With AVA, which is pre-manufactured and purchased, we don't always have that opportunity. But I take your point, and we have discussed that.

DR. HALLIS: In our experience, certainly

the antibody response to LF appears to be that at an early stage in vaccinees, certainly in a number clinical cases where we have sera samples even from early stages, we also see a measurable and a really good, high antibody titer against LF as well.

DR. ROBBINS: At least in Ternbill's articles, which is the only one that I know that are published, there was little LF and hardly any EF produced by the American vaccine. Now, the English vaccine does not produce EF, only in a few cases after prolonged immunization, the EF, and the LF is a variable response. It really looks like it's the same vaccine as ours. They're PA vaccines.

MR. : Actually, I think you'll find that the U.K. vaccine does stimulate LF antibodies, and I have a good number of [inaudible - off microphone].

DR. HALLIS: Certainly that--

DR. ROBBINS: But it's not published.

MR. : [inaudible].

DR. HALLIS: We have actually studied the composition of a huge number of vaccine batches, and they all consistently contain LF in the amount I showed really up to certainly 0.5 of a microgram,

if not more than that as well. And most of them contain EF, but to a much lower amount.

MR. : I'd just like to compliment the investigators at the CDC for the design of the monkey and human studies, because I think they'll yield an enormous amount of valuable immunologic information relating the macaque response to the human.

I wondered, though, whether you've considered what Drusilla suggested, which is direct passive protection experiments of your monkeys with human serum maybe obtained by pheresis to draw that link very directly.

DR. QUINN: We have indeed considered that, and there are so many things that we would like to do, but we had to draw the line based on our resources and our capabilities and our funding. And that study we hope will be done, but not as part of the AVRP but as part of the immunoglobulin for therapy study. We would hope to do exactly that, and that study is in the planning and implementation stages, and I think Phil Pittman alluded this morning that recruitment for plasmapheresis is ready to start. So we would hope that will be part of that study.

MR. : Will you be pheresing the same volunteers that you have in the study you've described?

DR. QUINN: Phil, do we have an answer for that?

DR. PITTMAN: Negative. These will be individuals who have been immunized (?) and have received the anthrax vaccine as part of our (?) immunization program.

MR. : Another comment is that I think you have an opportunity--and maybe, again, you're doing this to evaluate the anamnestic response in real time. And although that's not the primary thing that we're asking for, it certainly would be nice to know if anamnestic responses might contribute to protection in people whose titer has fallen off. Are you drawing samples at early time points with your booster doses to look for neutralizing activity and how quickly it comes up?

DR. QUINN: Yes, we are indeed. Bryan, did you want to comment on the timing of those doses--or draws?

As part of the booster kinetic studies we're taking samples, I believe--and I stand to be corrected--at three days, five days, seven days, 14

and 30 days after each--after the six-month and after the 18-month boosts, and we will study the onset and the magnitude and duration of our kinetics response.

DR.. BABCOCK: Six-month dose, 30-month dose, and 42-month dose.

DR. QUINN: Six months, 30 and 42. Janiine is the PA at the Walter Reed Army Institute of Research on one of the human study arms. Again, Janiine?

DR. BABCOCK: There will be a range, but basically all the people will be randomized--

DR. MEADE: You probably should speak in the microphone.

DR. BABCOCK: Basically the people will be randomized into three groups, Groups A, B, and C. Group A will give their kinetic sample in the first--days 3 to--I think it's days 3 to 8. B will give it after the six-month dose from 8 to 11 or something. And then it's 11 to 14. Then the groups switch after the 30-month dose, and they switch again. So we're getting a continuous range so there will be a continuous range of samples from 3 to 15 days afterwards and then again at 30 days.

DR. QUINN: Thanks, Janiine.

A point here is that we are not taking discrete time points and maximizing the number of those time points. Our objective is to build the full curve of the response, so we're taking multiple readings over multiple days.

DR. BURNS: I just wanted to interject a quick question as a follow-up to George's on the passive immunization studies. You are talking about using sera from individuals who've been immunized with AVA. Have you given consideration to using sera from individuals immunized with recombinant PA so you don't have a more complex sera but, rather, it's antibodies to a single protein that might give you information for the next-generation vaccine.

DR. QUINN: That would be nice to do, but within the context of the immunoglobulin trial, it's, again, not feasible because the licensed product is AVA. But what we are finding--and we've heard it several times this morning--is that the predominant antibody response in AVA sera--anti-AVA sera is PA directed. We have very few lethal factor responders. We have no edema factor responders. And when we correlate the IgG neutralizing concentration in the TNA to the IgG

concentration in the ELISA, we get a very, very good concentration coming back. So it's something that would be nice to do, but we're not set up to do it yet.

MS. : On your proposed studies with the cytokine profiling, there was--is this not on? There. Okay. I just wasn't close enough. Your proposed studies with the cytokine profiling, there's a group that I've come in contact with recently that are doing real-time PCR on a number of cytokines, and they've been doing these on clinical samples for quite some time with rheumatoid arthritis patients. And I think that in terms of the experience my lab has had in real time and also looking at micro arrays, the system that they have is really nicely standardized and would probably be very useful in your system.

I don't know what you've already made arrangements on that, but I can give you more information on it.

But then the other thing I wanted to mention is in terms of the adenylate cyclase activity that's associated with--you were the one that mentioned that, weren't you? I'm not--I suddenly can't remember who it was.

DR. PHIPPS: Yes, we both did.

MS. : It was you, yes. In our micro array studies, we've also picked up a number of other genes that are regulating adenylate cyclase, and that might be something interesting for you to look at, too.

DR. PHIPPS: Thank you very much for the comment.

DR. ALVING: Carl Alving, WRAIR. I just wanted to weigh in on the aluminum question that John Robbins raised before. It's my understanding that the aluminum has to absorb the antigen, and so if you just put aluminum in without absorbing the antigen, then you might not have--I mean, you may--you might not take advantage of the depo effect of the aluminum.

If you put more aluminum in with the same amount of antigen, when you diluted the antigen out, you might get greater absorption.

So I would say that to take advantage of the depo effect, it's perfectly appropriate to simply dilute the aluminum along with the antigen as well, because I don't think the aluminum is--it may have effects independently of the depo effect, but you're going to alter the depo effect

dramatically if you change the aluminum.

MR. : Carl, if you reduce the aluminum, you reduce the response. If you reduce the aluminum, you reduce the response to the antigen.

DR. QUINN: Could I interject? That was actually one of our objectives in the primate study, to reduce the immune response. And after much discussion--and this point did come up several times--we thought that it was a higher priority to maintain the antigen-adjuvant ratio rather than to balance the amount of adjuvant we weren't giving. So, yes, we are trying to modulate the immune response here, and it was one of the objectives.

DR. ALVING: Just to add to that, if you increased the aluminum but didn't increase the antigen, you--

MR. : You get a higher response.

DR. ALVING: You do?

MR. : Yes.

MR. GOLDING: I'm Basil Golding with the FDA. I'm very curious about the functional activity you notice in your IVP regarding EF and LF in your assays, and I have two questions related to

that. One is: I would assume that--and, you know, I haven't seen people injected with the IVP so I don't know. I would assume that there is some reaction, some local reaction if you have the toxins in your vaccine. And my question is how much of a local reaction do you get and how do you know how much EF and LF you want to be in there in order to make this a safe vaccine.

But the second question is more theoretical, and that is, if you have EF and LF and you have a local reaction, I would assume that that causes inflammation at least of cytokines and would influence the immune response probably in a positive way. And I don't know how much--you know, you're talking about batch-to-batch control, and that may turn out to be important also in terms of efficacy of the vaccine.

DR. HALLIS: With regard to your question on how much we want to have LF and EF, this is another story. What we're looking now, not optimize the components in terms of composition. We're looking to see what's in the vaccine. The vaccine certainly contains the three components. We believe the way the vaccine is actually--or the components precipitated on the aluminum does not

allow the toxins to be formed, and that's what is stopping side effects and toxicity from the vaccine.

MR. GOLDING: And in terms of local reaction, you think that--so you don't think that there's any inflammation related to it because the formulation prevents any effect, is what you're saying.

DR. HALLIS: Yeah.

MS. POLONIS: Hi, Vicky Polonis--

MR. GOLDING: Excuse me, a minor question. Don't you use formalin?

DR. HALLIS: No, our vaccine does not contain formalin.

MS. POLONIS: Vicky Polonis from the Henry Jackson Foundation. In terms of Dr. Burns' suggestions for comparison of immunogenicity in animal models versus human vaccinees, I wondered, has anyone done one-year epitope mapping studies using PA, for example, the interminal region thought to elicit the neutralizing antibodies using technologies like the Geisen (ph) pep-scanning method? Because it would be interesting to note if the pattern and magnitude of epitope reactivity in human sera versus animal sera in any of the animal

models is similar or different. And is it thought that linear epitopes do play a role in neutralization? Or is it known to be confirmational antibody dependent? Can someone address that?

DR. QUINN: Your answer is on the way down the steps.

DR. BAILLIE: Yes, I must say that we actually epitope-mapped PA in a variety of mouse models, and we are keen to look at the human immune response by taking T-cells from immunized individuals and seeing if they respond to the same epitopes. I'd be really keen to look at these epitopes in terms of whether we see different responses in different individuals based around their T-cell responses. So, yes, there are plans to do it.

I also know people have been looking at confirmational epitopes and there's a lot of work at USAMRIID trying to work out where (?) binds and (?) bond.

In terms of the third part of the question, there's a lot of interest in the main four in terms of the antibodies that bind to it and work going on to devise antibodies which would bind

to that region to neutralize.

DR. MEADE: Does anyone else on the panel wish to add to that?

DR. QUINN: As part of the CDC study, we'll also be doing CD mapping with Raffi Ahmed (ph) who's a world-renowned leader in this area of HIV research. So there's a lot of people doing this now.

DR. MEADE: I guess I have one additional question. I think studies were proposed looking at opsonophagocytosis. Is there any evidence for the particular types of antibodies that would be sort of phago-(?) --opsonophagocytic? Any evidence, for example, that PA would contribute in that way or do any other--the character and nature of the antibodies?

DR. QUINN: Who was the question to, Bruce?

DR. MEADE: I'm asking if anybody who's--if there's any evidence, any data yet coming from any of the studies yet that would speak to that.

DR. QUINN: Not from the CDC study at this stage. Art alluded to this sort of effect this morning.

[Inaudible comment off microphone.]

DR. MEADE: Okay. Well, good. If there are no more questions, I think we're on time. Again, I think thank the speakers for a very excellent presentation.

[Applause.]

DR. MEADE: We're to gather back here at 3 o'clock for our panel discussion. I think we've set the stage for hopefully a very interesting discussion beginning at 3:00.

[Recess.]

PANEL DISCUSSION: HOW DO WE DEMONSTRATE EFFICACY OF ANTHRAX VACCINE?

DR. McINNES: Thank you very much. We're going to move into the final session this afternoon, which is a panel discussion, and we have four panel members who I would like to introduce to you: Dr. Emil Gotschlich on the left-hand side; Dr. Arthur Friedlander, who you heard this morning; Dr. Erik Hewlett, University of Virginia; and Dr. George Siber from Wyeth. All four have had many years of experience in a variety of vaccines and being called upon many times to think about difficult and challenging problems and propose interesting solutions to them.

And in thinking about this panel and how we might structure it, Drusilla and I had talked a little bit about how the day would go and how we should end up, and it's very clear that she would like to have independent input from the committee and from the panel on some of these questions, and she really would very much like to have input from all of the participants here in the room who have thoughts about these topics.

We're going to do our best to get a microphone to use should you indicate that you would like to speak. It is rather a vertical room, and when you stand up you have the feeling you are going to fall forward. So I do understand. If you'd like a microphone brought to you, we will do the best we can to accommodate that.

The first question that is posed to the panel is: Which animal models or models best approximate the human disease and the human immune response? And we certainly heard some of that this morning, and I'm going to pass that to the panel, whether there are comments, and perhaps, Dr. Friedlander, I could ask you to respond first on this.

DR. FRIEDLANDER: I think my feelings on

this subject are already in practice, that is, I've made my decisions, and the evidence for that is the approach that we've taken over the last ten years now in terms of studying anthrax, this particular infectious disease. And while some of it was clearly just, frankly, intuitive, some of it was based upon observations in the literature. And that is, as you've heard, that at least pathologically, not immunologically, the primate, the non-human primate most closely approximates the pathologic findings that are found in the human population. And that was basically the consensus of opinion of a previous generation of pathologists.

And I think it's been substantiated in terms of the unfortunate opportunity to have looked at some of the human pathology again. But that's just from the perspective of the pathology, and that's the non-human primate.

In terms of the immune response, which is really the other side of the coin that you're looking at when you're trying to understand the mechanism of immunity, there I think the question could be put out to the broader community. I'm not an immunologist, but intuitively, one feels that

we're closer to a non-human primate than to a mouse or a guinea pig or a rabbit. And at least in my discussions with other primatologists, I think that's a reasonable assumption, and someone would have to make a case otherwise.

Now, one question that does come up is which non-human primate, and that was addressed a little bit in some of the questions about the non-availability of the rhesus macaque. I raise that point because we know for other infections that there clearly are differences in terms of different species of non-human primates. Whether that's the case or not, I really don't know. As Louise mentioned, years ago the cynomologous monkey was used to a large extent in many studies.

So to end my answer, I think the best model to date is a non-human primate. I would say that, if anything, humans appear on the basis of pathology to perhaps be somewhat more resistant than the rhesus macaque--more resistant--and that one can, in fact, garner--develop a spectrum of sensitivity to the disease, where the rabbit is more sensitive, dies more quickly than a non-human primate than the human, I believe.

In terms of other models, again, we

have--I think one could make--we stayed away from the mouse for the reasons that you've heard, but I think the point that was made, that someone made, I think it was a reasonable one, and I happen to agree. If it's a PA-based vaccine, one could make a case for the mouse as a screening as opposed to rabbit. We chose the rabbit, again, because it had been used in the past, because it is predictive of the vaccine-induced immunity in the macaque. So that's the reason that we came down with the rabbit and the non-human primate.

DR. McINNES: Dr. Siber, do you have any comments?

DR. SIBER: Well, mostly I'm reflecting what I've heard today, but I would just say this: I think what we're trying to do is ask the question of what the nature of the immunity is that will protect, and we want to mimic as close as possibly the human situation in the absence of humans as opposed to a release test for a vaccine, which is very different.

And, therefore, I guess what we've heard is the aerosol challenge is the most difficult to protect against. It has the greatest mortality and morbidity and is the most likely threat to us. And

so I think the model has to be an aerosol model, and we also know, I think--or I think we know that of all the models we've looked at, the primates are the closest to us in terms of the physiology of the toxin working, although we haven't seen a lot of specific data that many of the animals different that much in that regard. But certainly macaques would be a good choice for that.

So I would vote with primates.

DR. McINNES: Dr. Hewlett?

DR. HEWLETT: Thank you. I have a couple of questions that I'd like to pose along with making an answer. The first is in the context of thinking about the animal model and the guidelines that Drusilla provided for us in the proposed rule, there wasn't mention of feasibility in terms of acquisition, availability of animals. As part of that, there was a suggestion that more than one animal model could or should be used, if that's possible. And I wonder about the consideration of looking at, in light of what Dr. Friedlander said, the relationship between several of the representative models, the rabbit and the non-human primate and the human, to the extent that we have data in the human, to use a validation--use the

non-human primate to the extent that we can and need to, but then in the context that George Siber just brought up, for control testing and release, to be able to fall back on a rabbit or some animal that is not quite so hard to come by and not so problematic in terms of acceptance of its use.

Now, the other part that I think is important, we don't have the criterion up here. The other thing that Drusilla mentioned was reasonably well understood pathophysiologic mechanism of the toxicity of the substance to be protected against. And we have come down to the fact that we're talking about the toxins, EF and LF and PA. I'm still concerned about the capsule and what the capsule might be doing in some animals, and I don't think that we know about humans.

But in light of that, we haven't talked very much--I'm not convinced that we know a lot about the pathophysiology. We have made some assumptions in the past based on reports in the literature on release of cytokines, a story that makes reasonable sense. But I'm not sure--we certainly haven't seen those data today, and I'm not convinced as to what the sequence of events is and what role the cytokines play.

I know that that can make a big difference from one animal system to another in making the comparisons. If we're just talking about up-front protecting against PA binding and binding of LF or EF to PA, I think that's a lot easier. But the downstream pathophysiology is also important, and I think if we just focus on PA, we're going to be neglecting that.

So I do agree that the non-human primate is very important and probably the best to be used, but I would like to have a backup, some correlations with another animal that could be used more easily in the long term.

DR. McINNES: So the derivation of a work horse animal for all the studies with the nice correlation to the non-human primate and then to humans. We do have work horse.

Emil, any comment?

DR. GOTSCHLICH: I have two comments. One is that I must make a disclaimer. I am a member of an IOM committee that is reviewing the CDC program which you have heard presented this afternoon by Dr. Quinn and also by--I'm afraid I already forgot the name of the gentleman from Battelle. And, therefore, anything that I say this afternoon about

those two programs is my personal opinion and not the opinion of the IOM. Louise, are you satisfied? Very good.

I think that anything important about the animal models has already been said. The data that was presented by Dr. Louise Pitt was, I think, very convincing about the applicability of the rabbit and the non-human primate model. The only thing that may not have been mentioned yet this afternoon and needs to be mentioned by somebody like myself who is not yet used to the fact that money is absolutely no object is that one should keep in mind that rabbits are a hell of a lot cheaper than monkeys.

DR. FRIEDLANDER: Can I just add a comment to what Erik said?

DR. McINNES: Yes, go ahead.

DR. FRIEDLANDER: I would hope that the presentation I gave this morning, if anything, said we know much less than what know about this disease. We know about the toxin because it's easy--it's easy--it's easy to do in vitro experiments. It's very, very hard to do in vivo experiments. And, you know, this toxin is not very potent in a primate in terms of lethality. We're

talking milligram quantities to kill a primate. That's a lot of toxin. That's not to say the toxin is not important in its pathogenesis. But I think there's more that we don't know than what we do know, and I think we're very, very fortunate, though, for a PA-based vaccine, extraordinarily fortunate, to have a functional assay. If we didn't, we'd be in more trouble. I think that offers us really a hope that we can actually pull this off.

DR. McINNES: Thank you.

Are there comments from the floor regarding the animal model best approximating human disease? Yes, please, sir?

MR. : Are non-human primates susceptible to infection with toxin-negative?

DR. FRIEDLANDER: You know, we've been talking about doing that experiment for a long time. I've been thinking of that for a long time. I don't know the answer to that.

The presumption--I don't know any data about it. The presumption is that it's going to be attenuated and essentially avirulent, as it is in the guinea pig.

DR. McINNES: Dr. Alving?

DR. ALVING: Carl Alving from WRAIR. I would say that the question as it's phrased is perhaps--may be changed a little bit. Instead of saying which animal models best approximate the human disease, the animal model is not necessarily supposed to precisely reflect the human disease for certain types of regulatory actions that might be taken.

For example, if you were simply to change from IM to--from sub-cu to IM or to change the number of doses, it appears to me that the animal model should merely reflect the antibody titer and not--you wouldn't need to know all of the pathophysiology and all of the other issues. However, if you're going to change the vaccine radically in some way to go to a different antigen or attack a different part of the disease process, then it might be more appropriate.

But I think we are already assuming--correct me if I'm wrong--that the present anthrax vaccine actually works and that it is protective. So we already have a protective vaccine, so we already have the best animal model, which is the human. And so the human is inducing antibodies, and so it seems to me that simply for

small changes in the vaccine that could change the regulatory issues involved, simply a rabbit would be good enough or a non-human primate.

DR. McINNES: And you're speaking specifically about AVA now?

DR. ALVING: Yes, only about AVA. Now, for other vaccines, that may also hold true, but we're talking about AVA here.

DR. McINNES: All right. Any other comments? Yes, please, sir?

MR. GIRI: My name is Lallan Giri, and I'm from BioPort Corporation. I think in a situation like this, it's always a good idea to have some input from the vaccine manufacturer, and that's why I thought I would make this attempt. I think one of the panel members has already echoed it, and I would like to say that definitely it's no secret now that many manufacturers have been forced out of the vaccine manufacturing business as a result of the cost of development and manufacturing and cost of compliance. So I certainly sincerely hope that as time goes along, we will learn enough from the comparative study of the rabbit as well as rhesus macaque, the non-human primate, and it will be not too long before we can definitely make a switch to

a less expensive animal model, yet a model that can definitely assure the efficacy, safety, and the potency of the anthrax vaccine.

Thank you very much.

DR. McINNES: Thank you.

Yes, please?

MR. BALADY: Mike Balady, JPO. Dr. Siber, I agreed with your comment concerning the aerosol being the most stringent case, but I think that in the climate we have today, with the general public having concerns about not just the aerosol but including the cutaneous form, we need to address that here, too, in the forum.

How are we going to do that with our animal models? How should that be addressed?

DR. SIBER: I guess my question would be--and I would ask the experts--whether the amount of antibody that will protect against aerosol would be expected with great confidence to also protect against cutaneous and GI challenges.

What I've heard, I think I've heard, is certainly that those are gentler challenges, if that's the right term, and that you would certainly expect that. But maybe you need to do an experiment or two to nail it down and convince

yourself of that.

DR. FRIEDLANDER: If I may--

DR. McINNES: Dr. Friedlander?

DR. FRIEDLANDER: Just a comment. I think the overwhelming concern here is still--remains inhalational anthrax. Cutaneous anthrax is readily identified now--I mean, that's not to deny that it's a concern.

But in regard to the other point, I think it's fair--there's not any data except in the guinea pig--

MR. BALADY: But you have to relate it back to the animal rule. Remember what we went through earlier. The animal rule says it has to be as good as the current vaccine, and you have to have the indications that the current vaccine has. So when this work has to be done, I think the expectation from the agency, and including the public, will be that you will have shown experimentation with any new vaccine that will equal the current vaccine. And the indication is for cutaneous.

So, I mean, you can't--

DR. FRIEDLANDER: Say what--

MR. BALADY: The indication is for

anthrax--

DR. FRIEDLANDER: No, it's not.

MR. BALADY: It's not for aerosol.

DR. FRIEDLANDER: It's for anthrax.

MR. BALADY: For anthrax. Well--

DR. FRIEDLANDER: Exposure to anthrax spores.

MR. BALADY: Well, it includes the cutaneous form.

DR. FRIEDLANDER: Not just cutaneous.

MR. BALADY: It includes the cutaneous form.

DR. FRIEDLANDER: Yes.

MR. BALADY: Therefore, the expectation is that this new vaccine, whatever it would be, should have that indication also, and it hasn't been addressed in these discussions.

I agree with you that the aerosol is the most important.

DR. FRIEDLANDER: Okay. I would just say in reference to what we know about the guinea pig, yes, that it's certainly more difficult to protect against an aerosol challenge. On the other hand, remember, this disease is a disease that occurs in the mediastinum, at least we think it does. And so

where the vaccine works, of course, is not so clear. I mean, how the vaccine works is not so clear, this one or any other, yet. But it's closer to a systemic infection or, if you will, an inoculation in the mediastinum as opposed to the skin than a pneumonia, which at least pathologically it seems that way. So any vaccine--you'd have to demonstrate it, but any vaccine that protects against aerosol and mediastinitis you would--that would protect against cutaneous disease.

DR. McINNES: Dr. Robbins?

DR. ROBBINS: If a person is exposed--

DR. McINNES: Microphone, please.

DR. ROBBINS: Excuse me. If an individual is exposed, or an animal, really, is exposed to an inhalation of anthrax, we presume from the animal experiments he will not be protected by vaccine alone. The animal work shows that if you are exposed within a day the vaccine has no effect. No effect.

Now, my interpretation of that is if you don't kill the inoculum of an organism and you allow it to grow, you haven't got a vaccine. So worrying about what the organism does in the

mediastinum and in the lymph nodes is a non sequitur if you're studying how to predict the vaccine is going to work.

The best information is, in animals, that antibodies to PA alone will protect, and in humans, the information is limited. The only good clinical study we have shows that it protects against cutaneous anthrax 92 percent efficacy and it was 5 and 0 against inhalation. Not enough for statistical significance, but no breakthroughs.

So that if the purpose is to design a program to predict whether a new anthrax vaccine will work composed of PA, what you want to do is make a reliable measure of how much PA antibody that vaccine makes, presumably after a full course of immunization and a defined period. Animal models may be important for therapy, but if you have the disease, you haven't got a vaccine. The vaccine is designed to prevent the disease. It prevents it by serum antibody. Is anyone here advocating having a new vaccine for anthrax that doesn't make at least as much antibody as AVA?

DR. FRIEDLANDER: Well, I'm not quite sure of the point you're making, John. Clearly, antibody is the mechanism of protection. How

exactly it works I think remains to be determined. There's evidence that, in fact, it does not prevent infection but that the organism replicates upon challenge in an experimental animal. It does not, therefore, prevent uptake, nor does it prevent outgrowth. The--

DR. ROBBINS: It prevents anthrax.

DR. FRIEDLANDER: It prevents the disease, right. And this is limited data. That's all. So, yeah, I certainly agree that antibody is the way to go. I have no illusions whatsoever that you will ever license a vaccine that induces an inferior mean response to that which occurs with the current licensed product.

DR. McINNES: Good. Two other pieces that came from this discussion, before we move on--I will get to you--is the clear understanding that we do need to know more about pathogenesis, but not at the expense of waiting to push the vaccine, because that is an urgent need right now; and perhaps to try to characterize--finish the studies characterizing cynomologous to allow for additional access to primates which will be needed for some of these studies.

Yes, one more comment; then we'll move to

the next question.

MR. : Well, if antibody does not prevent at least the initial phase of in vivo replication of the organism, then obviously it is protecting the organism or certain parts of it to the extent that the immune system, either innate or specific in amount of response. So have there been any studies of passively immunized animals who are challenged in the presence of specific immune lesions if you have a macrophage-depleted animal, if you have an animal with anti-TNF, to focus in on what immune mechanisms are allowed to clear infection if they're preserved long enough by immune status or by antibody to come into function?

DR. McINNES: Does anybody from the panel wish to comment?

DR. FRIEDLANDER: No such experiment has been done other than for in an intoxication model where there was an experiment done with depletion of macrophages. But no such experiments have been done. I suspect they'll be coming on down the road.

DR. McINNES: Dr. Gotschlich?

DR. GOTSCHLICH: I actually would like to go back to the question that Dr. Robbins raised and

actually for once respectfully disagree with him.

I do not think that the standard for the future vaccine is the amount of anti-PA antibody that the current AVA vaccine produces. It is really actually astronomical. And in people--there is no other vaccine in which we produce over a milligram of antibody.

I think that the future of the PA vaccine will rest in figuring out what actual amounts of antibodies are required and trying to achieve this rather than trying to achieve what the AVA currently does.

DR. McINNES: Yes, ma'am?

MS. WIMER-MACKIN: Yes, Susan Wimer-Mackin with LigoCyte Pharmaceuticals. I'm fairly new to the field, so I certainly don't know everything about this. But it's always seemed strange to me that all the protection has been correlated with merely survival of the disease. Obviously, in humans that's not necessarily going to be acceptable and maybe comments on--should we be looking at morbidity in these animals?

DR. FRIEDLANDER: Point number one is survival. Number two is the ability to turn the television on. And I don't mean to be--

[Laughter.]

DR. FRIEDLANDER: I don't mean to be superficial about that. I mean, we're talking about a disease that otherwise is invariably fatal if untreated and unrecognized. So I don't--the answer to your question is yes, certainly. They're very difficult experiments to do. The experiments were not--the experiments that we've done to date other than for the last series were not really designed to address that question specifically.

We do know that the animals in general--you know, they were not moribund. They were not lying in their cages. But they were not designed to measure the physical and psychological activity of the animals. They could so be designed. But I think our first step is to show that we've got--in any new vaccine that we've got significant survival, certainly. If we can get there, then I think we're well on our way.

DR. McINNES: Yes, Erik?

DR. HEWLETT: Let me make one final point. I want to echo George Siber's compliment to Dr. Quinn and his colleagues in the study that they have designed. I think this particular question is going to be very well served by the results from

that very elaborate trial in terms of the relationships for correlation and a lot of the mechanisms along the way.

DR. McINNES: All right. Thank you. We're going to move to the second question, which has five subparts to it. So, moving right along, what types of studies will be needed to identify correlates of protection and to validate a surrogate marker of protection for anthrax vaccines in humans? And correlates about which we are very certain.

Comment on the need for active and/or passive immunization studies in animals and how such studies might be designed.

Now, today we heard several talks that talked about the role of active immunization and passive immunization studies, relationships of human studies to animal studies, and the specific design I don't think we had much detail about, but I wondered if the panel would like to comment on, first of all, the need for these studies, the relevance, and what they might contribute, and whether you had any insights on design issues that might be taken into consideration.

DR. GOTSCHLICH: I think that actually the

passive immunization studies have not received proper discussion this afternoon except by Drusilla Burns herself. And, essentially, everything that I'm going to say really repeats what she said, but in slightly different words.

I think a passive immunization study has the great virtue of making absolutely certain that what you're doing is you're using human antibody without any complications, without any immunological memory, and determining the amount of antibody that is required to produce the protection.

It may very well be, as she pointed out, a higher level than you may see in an active immunization status, but that isn't really quite the point. The point is it will give you a level that you need to know. You need to know it for a number of reasons. You need to know it, first of all, because you want to know it as an upper limit. You need to know it also very much for the challenge of how to deal with post-exposure vaccination, because you need to know how much antibody at least has to be there before you can remove the antibiotics.

So I think it really does require a higher

priority than it has received this afternoon.

DR. HEWLETT: I think the issue of passive immunization also is very important, and I wonder about--I agree with George, the issue of possible challenge of animals with human serum. Drusilla mentioned in her criteria again evaluation of the quality of the antibody response. And I was trying to think what that means other than neutralization per unit of antibody, per unit of ELISA antibody. But the other way that quality can be evaluated is exactly in the in vivo setting.

I know George has experience with passive immunization in pertussis studies in the past, and I wondered whether in that context you ever used the human sera that were being given to humans in animals to see whether--what the effect was. You might comment on that.

DR. SIBER: Yes, Erik's referring to studies with a pertussis immunoglobulin which we investigated, and, yes, they were extensively looked at in the aerosol challenge model of mice and shown to produce protection in that, and that published, I think, in I&I. And that's very useful.

Maybe I can make a comment on the question

also, which is I guess the active experiments--and Louise Pitt showed us very elegant examples of how they can be used in an aerosol challenge model to establish what levels are associated with protection and what levels are not, and perhaps some intermediate levels. So you generate the kind of curves, the S-shaped curves of antibody level versus protection.

It would seem to me that the prime purpose of this whole experiment as we're talking about is to establish levels that we can extend to humans, and I think the first step is to get IgG class antibody concentrations in macaques from active immunization that are associated with protection and Coxson neutralization titers or concentrations associated with protection, and look at later to dose of the challenge to do that.

Then the trick is how do you draw the link to humans who are being immunized with the same vaccine, and I believe that's where we absolutely have to do the passive experiment, to take the human antibody, put it into macaques, and see if the amount the macaques need of human circulating antibody is hopefully similar or identical--make it simple--as their own. If it is, I think you then

have a very strong link to conclude that humans with that amount of antibody would also be protected.

I think that the other things that are being done are very interesting scientifically, like affinity measurements in support of neut(?) activity or subclass or class and so forth and we'll learn a lot. But I think they are secondary players to the primary ones of neut(?) titers in IgG.

The reason I feel strongly about IgG is that ultimately a lot of work has to be done, and I think in general it's easier for different labs to reproduce an IgG ELISA than it is a functional assay, although I must say the CDC data on variability of their neut(?) is incredible. It knocks your socks off if they're that precise. But I don't think every lab can do that.

DR. McINNES: Yes, Art?

DR. FRIEDLANDER: There are a couple of points I guess I'd make.

One, I alluded to this before, and that is, I think we have--we're very fortunate to have a functional assay, and the way we've looked at this is--or one of the ways we've looked at this is, at

least in vitro, we don't think that the FC receptor--FC portion of the immunoglobulin molecule has anything to do with the toxin neutralization.

What that does is it allows you to compare across species the functionality of the immunoglobulin, which otherwise is almost impossible to do, one, because of primate--and I don't know what the current status is, but it's still probably not very good in terms of immunoglobulin classes. That's something somebody ought to find somebody to do. And then what does that mean in terms of total IgG of whatever isotope, in a rabbit, in a cyno, in a macaque, in a human, in a mouse?

So what we've sort of tried to establish over the years is a functional ratio between toxin neutralization and quantitative IgG, for example, or whatever class. But it's the functional assay.

So what the approach, I think, is potentially most useful--and we don't know the answer to this yet--is how does an equivalent toxin-neutralizing antibody level from human, macaque, rabbit, mouse, guinea pig function in a given species of animal, in a passive protection model.

If they function at an equivalent level, that is, ten toxin-neutralizing units of human, rabbit, guinea pig, functions in a guinea pig the same way, or a rabbit, whatever, or a mouse, then that says a couple of things. It says the FC portion has nothing to do with how it's working, which tells you something about how it's working because if it's opsonic, it may well be working that way. And, two, it gives you now a direct path to humans to take human toxin-neutralizing antibody and show that it functionally in a second animal works the same way as a primate.

Now you can do this passive protection study and say I get a certain level of antibody and it protects against X number of spores. If you can demonstrate cross-species equivalence, then I think you're home free.

DR. McINNES: Comments from the floor on this point about the need for active and/or passive immunization studies in animals and any thoughts on how they might be designed?

DR. FRIEDLANDER: One other thing I wanted to say, and I'd be interested to hear what other people say, too. This business about active--the equivalent protection you get using active versus

passive immunization, once you establish what level of circulating antibody protects against a given challenge by active immunization, if that's equivalent to what you see with passive immunization, that says one thing. If it's not equivalent, it says quite another thing. And there is some anecdotal evidence, anecdotal in the sense that some primates were protected when we could just about barely measure any antibody, and at levels that you would think would not protect at all.

DR. McINNES: In an active immunization.

DR. FRIEDLANDER: Yes, in an active--animals were protected actively who had circulating antibody levels that were just barely protective, which suggests again, as someone pointed out--and that's what we've always sort of thought, is that the anamnestic response--this is an acute infection, but, still, that the anamnestic response contributes to some of the immunity. And that raises, again, the question I think Emil was mentioning, or implied perhaps, is that the level that you see with passive protection--and you don't know that until you do the experiment, but the level that you see with passive protection may be

much higher than what you really need.

And so that opens the question as to how do you approach that, and I think the first thing is to do the experiments and see whether--what the relationship is between levels conferred by active versus passive protection.

DR. McINNES: All right. Thank you.

Yes, please?

MR. KENNEY: Rick Kenney with IOMAI. I appreciate the utility of the qualitative nature and comparison with the passive protection experiments. I've done a lot of this type of experiments in monkeys and have looked at the different models with other systems. But I get troubled when we start talking about quantitative comparisons because the cross-species differences and the pathophysiology may be fairly important, and I was wondering if the panel could comment on that. The way--the different way that the monkeys will respond to a toxin challenge or to a spore challenge may be quite different in a quantitative sense than the way that the humans will.

DR. McINNES: We'll see if something emerges on that point.

Please go ahead.

MR. ADAMOVICZ: Yes, Jeff Adamovicz from USAMRIID. My question was related and, in fact, what I wanted to do was get Dr. Friedlander to expand on his comments related to the passive antibody studies, specifically in light of, say, the assumption that perhaps PA is somehow associated with the spore and that, for instance, anti-PA antibody is important in clearing the spore, not necessarily in preventing intoxication.

In that case, you could imagine that the FC portion would be important, and then, in fact, you would assume, you would have to be very careful in the animal model that you chose to do these passive studies principally for the reasons that were just mentioned, the differences in the pathophysiology.

Can you address that?

DR. FRIEDLANDER: I think you have to do the experiment and ask that question, and, two, you could compare--there's be two fragments, for example, to the intact immunoglobulin, and that will, in fact, help understand how the antibody is protective. I mean, if it functions equivalently--I mean, if it were just toxin neutralization--well, I'm not so sure that the FC

portion might not have anything to do with it. It might. Still, but it might help understand exactly how the antibody's working.

I mean, I think the compelling thought is it's working by neutralizing the toxin. But it's also important, I think, to do that, to ask the question--as I said before, to try to answer the question as to the species functionality. Because if it's the case that they're equivalent, it makes it, I think, a much more compelling argument than to be able to measure antibody in humans and be more confident that what you're measuring is predictive of protection, because you're using this in another species. It's a heterologous system still. And there may be subtle differences even though they both protect, monkey serum and rabbit serum protect in a guinea pig or a rabbit, it still--there still may be subtle differences. And it would be much--it would be nice if it turned out not to be the case, that they were equiv--the same level of protective in a rabbit, whether it was rabbit, human, guinea pig, gave equivalent protection.

DR. SIBER: On that same point, I think we shouldn't expect too much of these correlates in

terms of their levels of precision, i.e., plus or minus twofold. I think that's just--we don't have data at that level. And the fact of the matter is that even with antibody systems where we do require FC function, the animal experiments of protective levels often come out--the passive ones--rather similar to what we estimate as human protective levels. The example I'm thinking of Hemophilus Type B where Dr. Robbins estimated, and Dr. Schneerson, a level of 0.15 microgram as being the amount of passive antibody that's necessary in gammaglobulinemic children to protect them from HiB. And in the infant rat experiments, when we used human antibodies to protect them, obviously we're requiring FC function for bactericidal activity. We came up with essentially the same value.

I think it's going to turn out somewhat similar for pneumo where we're within two- to four-fold in animal passive protection and humans. So I think it's--there is FC function. I'm sure there's complexities and subtle differences, but it may be close enough for what we're trying to achieve.

DR. McINNES: Yes, sir?

MR. : Perhaps one possibility for addressing the question is to finish the answer to this part (b), part (c)--part (a), excuse me, is how would the studies be designed, and perhaps George has already done these, so perhaps could speak to it. Do you challenge the animal and then add the serum to them, in other words, to shorten the time frame for an immune response to the antibodies? Or is it something that you give to the animal first and then--

DR. SIBER: You're asking the passive immunization study?

MR. : Yes, yes.

DR. SIBER: Well, just off the top of my head, without having given it a lot of thought, I guess if you had enough plasma or immunoglobulin purified from the donors--or from the subjects who were actively immunized, what you would like to do is achieve in macaques levels of antibody around what you expect, have already estimated as the protective level by neutralization with the human serum and ask whether you see a similar protection on a challenge, let's say, a day later. You don't want to have a long interval between passive immunization and a challenge for a number of

reasons. One is that antibody will start to wane, and also because if there is an immune response to that antibody, you will start to see accelerated decay, typically after about a week in passive experiments. So you want to do the challenge fairly shortly after the passive immunization.

MR. : Why wouldn't it be the other way around? Because in the clinic, the person that's been exposed to the bacteria has been exposed to the bacteria for a number of hours or days, and you're going to--

DR. McINNES: So you're talking about a post-exposure scenario.

MR. : But isn't that something that's the end result of this, is to have a therapy like that? So you're just talking about potency then?

DR. SIBER: Right.

MR. : Well, then, why not say in vitro? What is wrong with the quantitation that you would gain from an in vitro assay that's different from what you would do in vivo? Why wouldn't the quantitation in an in vitro assay be much more successful--

DR. SIBER: Well, that's what your neut

already is. Your neut is already an in vitro comparison of neutralizing activity of the toxin in vitro. And I think what we're asking for is something a little bit more closer to the real-life situation of what's going on in vivo. And obviously we chose monkey because of the reason that was suggested from someone up there, that how do you know that the hosts are similar and don't have different sensitivities to the toxin. Well, the best we can do on that is to pick something that's physiologically as close as we know.

MR. : But you are introducing the immune response of that animal to the whole neutralization of that antibody, and that complicates the quantitation, I would think, from animal to animal.

DR. SIBER: Not in the space of the experiment--of a passive experiment. It shouldn't last more than a few days. There won't be an immune response to the foreign antibody in that time.

MR. : You give the organism enough time to actually mount an infection, or are you just killing it so fast that it's really not a good test?

MR. : You want to kill it immediately.

MR. : But is that a fair test of the antibody response.

MR. : If you don't kill it immediately, you get anthrax. You're not testing immune response. You're testing the level of antibody that can kill [inaudible].

MR. : I think we're very focused on only one limb of the immune response, the antibody, because for years we've had good tools to measure antibody. If you look at Listeria, tularemia, other infections which start as infections within macrophages, if you prime an animal--at least a mouse with BCG and activate macrophages via the TH1 mechanisms, they clear that infection with no antibody.

I'm just curious whether in the case of anthrax it's been investigated whether in vitro gamma interferon prime macrophage can control rather than be permissive for the replication of the organism, or whether potentially CPGs or gamma interferon or BCG can stop the replication by taking advantage of the TH1 cytokines in activating macrophages.

I'm not sure that the protection that Dr. Pitt shows after antibody levels have waned, when she did her challenges, is purely an anamnestic response. It may also be a reflection that a TH1 response can activate macrophages and clear the infection at a very early phase.

DR. FRIEDLANDER: I think we don't know the answer to that completely. I would say this is an aluminum adjuvant that provides protection at two years. I don't think there is--and other people can address this question here. Again, I'm not an immunologist. It does not induce a good TH1 response. You do not protect against tuberculosis or Listeria with an aluminum adjuvant. That in itself, in addition to the passive protection, I think argues to my mind--and please stand up and punch holes in it--that this is an antibody-mediated vaccine.

MR. : That could be why the MPL adjuvant provides perhaps better protection in some cases, by--

DR. FRIEDLANDER: I'm not saying that you couldn't generate--the other thing about MPL is it induces an extraordinary antibody response. An extraordinary antibody response above what aluminum

hydroxide does.

But in reference to non-specific stimulants, there are people there who have been studying CPG. As you well know, BCG and about everything else you can put in, including albumin, can protect against not just facultative intracellular organisms but against extracellular organisms. So that in itself is not evidence if you get protection with other non-specific stimulants.

But there is an experiment that has been done with CPG.

DR. McINNES: I'm going to move on to the second question, which is to comment on how correlates of protection derived from animal studies might be translated into a surrogate marker of protection in humans. It's my impression that we've actually really covered this. Does the panel agree? Are there any other points you would like to raise on point (b)? Emil?

DR. GOTSCHLICH: No.

DR. McINNES: Okay.

[Laughter.]

DR. McINNES: I will move to the floor. Any participants who feel they--yes, please?

MS. : [inaudible].

DR. McINNES: The microphone.

MS. : I am very new to this field, but I am a microbiologist, and I wonder if the panel or the CDC group and other knowledgeable people in the audience could respond to the--it's not coming through? Can you hear me now?--to the issue of the defined inoculum. So what I'm hearing is a lot of very good scientists proposing animal studies where the inoculum in terms of the number of spores that are delivered in these animal models is frightfully well designed in terms of the number of LD50 units. And we're talking now about correlating these animal studies and their immunological parameters to protection in humans where we're envisaging in a worst-case scenario a bioterrorist event.

I'm just wondering if people have contemplated that the dosage that needs to be given in these animal studies has got to correlate to the wide variety of spores that might be encountered actually in a bioterrorist outbreak. Again, ten LD50 units sounds like a lot, but has someone thought about the number--and, again, this harkens back to the dreadful discussions that have gone on

in the newspapers about the terrible failure to predict, in fact, susceptibility to the disease, the statements, again, that it would take 8,000 spores and that people--

DR. GOTSCHLICH: I'm happy to see that you have moved on to point (c) because perhaps that way I can catch my 6 o'clock Metroliner.

[Laughter.]

DR. GOTSCHLICH: I think the issue of the spore--of the challenge or the dose that should be used for challenge is a very, very important one. But I think the issue there is one that actually I don't know very much about, but that our military colleagues and people who have concerned themselves with this issue on a large--for many years should really respond to.

What is the likely exposure in a military situation? I think that for us to aim at the extremely unusual circumstance that occurred recently in the bioterrorist attack as the most likely, most probable challenge, and the kind of thing that we need to be able to design a vaccine to prevent may be trying to shoot too high. I would think that it would be useful to know what the military thinks in terms of what the usual

challenge is that they might encounter.

DR. FRIEDLANDER: There is a document--Colonel Danley, is there not?

COLONEL DANLEY: Yes, sir.

[Laughter.]

DR. HEWLETT: I would like to add to that question, see if we can get any further along with this. It seems to me in the studies that were discussed, we were somewhere in the vicinity of 200 LD50s. I recall numbers above and below that. And I wonder if in the setting of the animal challenge studies in which there was a level of protection that was effective against that number of LD50s, what happens if you double that or triple that? Is there a relationship between the level of immune response that has been elicited and the level of challenge organism against which there is protection?

DR. McINNES: Good point. Anyone wish to comment on that?

DR. FRIEDLANDER: That is a good point. There was--there have not been very many studies done to answer that question. There was a study done in the guinea pig by somebody sitting in the audience that looked at--this is in the guinea pig

model, which is a little different, but it wasn't a strict linear, so there was a vaccine inoculation--if I get this wrong--Bruce, do you want to address this? I think it was 10, 100, 1,000 LD50s, and the differences were not that dramatic. It was easier to protect against 10--Bruce, fill in the numbers.

MR. IVINS: Bruce Ivins, USAMRIID. Yes, I think that probably the guinea pig isn't the better--isn't the best model, but in the monkeys we found that, you know, if you're protected at 100 LD50s, you're protected at 1,000 LD50s, too, and it's not that, you know, there's some sigmoidal curve, and that protection is protection. And you either are or you aren't protected, and so it's not, well, if you're, you know, 100 or 200, then, you know, you're only half as protected, or 400 and so forth.

So I think we usually use, oh, approximately in studies now about 100 LD50s, and it would be my supposition that if we get, you know, 90 to 100 percent protection with a particular vaccine in a rabbit or a macaque, 100 LD50 challenge dose, we'd probably get virtually the same thing with 1,000 LD50s, too.

So in the guinea pig, we see some drop-off in protection as challenge goes up. I don't think we're going to see that with the macaques because two years, as Dr. Friedlander said, two years, 1,000 LD50s, animals which have been--macaques which have been given doses of vaccine at zero and two weeks, seven out of eight were completely protected.

DR. GOTSCHLICH: Could I make another comment, please?

DR. McINNES: Yes, please.

DR. GOTSCHLICH: I was very pleased to hear that Colonel Danley does know what the most likely challenge dose is that we need to know in order to design a vaccine for protection of the military. And I would be very happy for him to contribute this knowledge to us and tell us what it is because, otherwise, we really can't design a military vaccine.

COLONEL DANLEY: You're going to put me on the spot, huh?

DR. GOTSCHLICH: Right.

COLONEL DANLEY: Well, in light of what's happened with the bioterrorist threat--and I don't know much about the characteristics of the anthrax

that were in the letters except what I read in the newspaper--I'm getting the feeling that someone has discovered a way to make anthrax less like an infectious disease and more like a toxic chemical, which is to say something that's easily dispersed that exposure is a rate times time or a dose times time phenomenon, which means that given in a building, if you're there for eight hours a day and you have a small amount of organisms in the air, and you get exposed to 1,000 LD50s, is that the same as getting 1,000 LD50s in one fell swoop?

Now, in listening to the nature of the discussions about immunity or protection against anthrax, I'm kind of reminded of chemical warfare agents. And right now we had--there were some studies that defined the LD50 for nerve gas, and I think John Wade's here in the room, who did excellent work in that area. We're going back and relooking at that number, and we're relooking at that number because, it turns out, if you get exposed to small amounts of nerve agent over a longer period of time, it's not the same as getting exposed to a bolus of agent.

So I can give you a number, if I could remember it, on which to protect--800 LD50s,

something like that. But does that really mean the same if you're getting it in one big bolus versus something over an eight-hour period of time where you body has a chance to clear the organisms? I don't know. And we are doing studies along that line in infectious diseases, like we are in the defense side. So I'm not sure that a single number is going to give you the answer you want.

Now, quite frankly, I look at vaccines as a part of a system, and we will vaccinate our forces to give them an optimal level of protection. I firmly believe that if our forces are exposed to anthrax, they will be put on antibiotics to give them additional protection, because it's not a feet-up and feet-down situation when you're dealing with our forces. In fact, chronically ill individuals are a greater drain on the resources that we have than individuals who die.

So it's a very complicated issue, and not one that I'm really knowledgeable enough to speak to, because right now in the Department of Defense we're looking at that issue of what does it mean to be hit with a biological attack, and what does it mean in terms of our ability to function in a theater of operation.

Does that help?

DR. McINNES: Yes. Thank you very much for sharing that. I think it has raised the issue then of a continuous exposure over a longer period of time than perhaps we had heretofore thought about in challenge situations, and that really does bring another facet to the thinking.

Anyone wish to comment? Yes?

DR. HEWLETT: Obviously, I'm not an anthrax expert at all. I come to this, as do many of--some of the other people in the room, from the field of working on pertussis, in which the circumstances are rather the opposite of what we're dealing with here. There is pertussis in the population, and it's easier to do some of the studies on humans in the population that are getting pertussis and look at protection than it is in animal systems because there aren't any very good ones for the study of--that's analogous to the disease process.

In thinking through the circumstances that we're facing here of the Brachman study in which there weren't quite enough cases to tell for sure about the protection with this vaccine and the circumstances that we're facing at the present

time, I don't want to be presumptuous because I know in terms of preparedness that many of you have already thought of this; but in thinking about the pertussis antibody decay curves and some of the data we've been looking at today, obviously there are many--the members of the military are getting anthrax vaccine. They're getting boosted continuously, and probably you have already generated a population antibody decay curve so that you can--if the unfortunate circumstance occurs in which there is a challenge like this, you will be able to tell something in retrospect even about the protectedness, the ability of this vaccine to protect simply by looking at those data and getting an estimate of what it is that has been--the level of challenge that has occurred.

DR. McINNES: Dr. Robbins?

DR. ROBBINS: When Dr. Grady worked on AVA, they did experiments in cattle and another animal species where they tried to measure how long the vaccine-induced protection would last. And I'm getting a little old now, but it's start waning after two years, quite reliably. I think after two and a half years it goes down to very low levels. I think the animals were given four injections of

vaccine.

The reason I have been thinking about this is what Emil said. It is surprising how much antibody this vaccine makes compared to the others. If you take a look at mice who were injected with just a tenth of a human dose of--a comparable human dose of PA, they had precipitating antibody in their serum. And, in fact, antibodies to PA were detected as recent as 20 years ago by just doing immuno-diffusion analysis because it precipitated so easily.

I don't know if we have any data on humans that were vaccinated and then were kept in contact with anthrax through their occupation or perhaps working in areas where there's action to find out how long after vaccination that antibody level remained protective. Really, all we could say is that where people were exposed and were vaccinated, they never got disease. We know it works. The animals would suggest that when the levels go down that perhaps the antibody is no longer at a sufficient level to protect.

If you--I don't like to look at the disease because my point is if you have a disease, you haven't got a vaccine. But it is remarkable

how much bacteria and how much antigen there are in patients who are sick. Remarkable. I mean, you can almost use pleural fluid as a source of antigen for making a vaccine. That's how much is there.

I think we may need a lot of antibody for this pathogen, and this pathogen is really not like any other pathogen that invades us, maybe with the exception of tetanus. It infects anything. It infects all mammals. It infects even some--

DR. FRIEDLANDER: Well, there are others that infect--

DR. ROBBINS: But mostly--nothing--I mean, meningococcus only infects humans.

DR. FRIEDLANDER: This is a zoonotic disease. The other zoonotic diseases also infect a wide spectrum of--

DR. ROBBINS: But in humans, human pathogens.

DR. FRIEDLANDER: Tularemia, brucella.

DR. McINNES: We're going to move on--

DR. SIBER: Pam, may I just say one other thing?

DR. McINNES: Yes.

DR. SIBER: As a practical matter to take away, I just wonder whether it would--it doesn't

make a lot of sense to use the kind of model that CDC is using in the current evaluation, the macaque model, and do a careful dose-ranging study with a quick exposure, and even go as high as the levels that Emil says we may not be able to protect again, like what you might get from sniffing an envelope, just to understand what the dose response curve looks like. And you may find it's quite flat, as you were suggesting, Art, and then you would take a lot of assurance away from that that you've protected against a very wide range of doses.

Again, experimentally, we can express the issue of a single massive exposure versus a continuous ongoing expose in the macaque model also, and that would be nice to know. But those are side experiments to complement the information that's coming from that experiment.

DR. FRIEDLANDER: Just one point, and maybe it didn't come across in some of the presentations. This vaccine is protective against probably 1,000 LD50s in two doses. That's a full human dose.

DR. McINNES: Moving on to point number four, what additional studies might be needed to demonstrate efficacy in a post-exposure scenario

versus a pre-exposure scenario? So pre-exposure being a proposed prophylactic regimen as is used in the military and might be used for some high-risk populations, for example, postal workers, versus a post-exposure scenario where exposure to organisms, presumably placed on antibiotic therapy, what do we want to know about use of the vaccine in that scenario in order for you to withdraw the antibiotic therapy? So I move to the panel for some thoughts about pre-exposure versus post-exposure and what source of levels of conflict we might want to know about the behavior of the vaccine in both of those.

DR. FRIEDLANDER: I don't mean to dominate this, but I'm the only one who's worked with anthrax, right? You never cultured the organism? I have, Bruce, right. Okay.

[Laughter.]

DR. FRIEDLANDER: This is also on the table now and being discussed, and that creates, I think, a different set of circumstances. The issues there are slightly different, related but slightly different. You're talking here about trying to--I would assume--develop an immune response as rapidly as you could. This zero-four

regimen then may be off the table? It's something you need to--that needs to be--with the current vaccine or one similar to it.

Obviously, the design of that experiment needs to be fleshed out, but it would be asking the question how long do you have to be on antibiotics after you've been immunized in a post-exposure mode. And there is some historical data to suggest that period may be quite short. But that also is a function of the inoculum, very much so there. So I think that's one of the parameters that needs to be put on the table. Rapidity of onset is now probably the prime factor in such a design of a vaccine, and, you know, there are various ways of thinking about designing the experiment.

DR. HEWLETT: You're talking about active immunization, but it seems to me if you believe the magnitude--the contribution of toxin to this disease process, there should be at least some consideration given to passive immunization. Where does that stand in this whole thing?

DR. FRIEDLANDER: Again, I think that's been alluded to. There's a program underway to develop an IV/IG using the immunized service members to develop a product and evaluate it and so

on.

There are two scenarios, therapeutic scenarios post-exposure, one that--or three, I guess, that active/passive immunization plus antibiotics--think about that one, but for sure--and versus active antibiotic versus passive antibiotic, and so on and so forth.

DR. McINNES: Any comments from the floor? Yes, please?

DR. BABCOCK: I'm Janiine Babcock from WRAIR. In December, I had the pleasure of being invited to the CDC to participate in a colloquium, and I was part of the post-exposure prophylaxis group. And several of the physicians who were there--well, our task was to propose what studies we felt needed to be a national priority, and our group was supposed to work on post-exposure prophylaxis. And at that time we outlined a basically fairly extensive five-arm animal study that addressed the concern that the physicians in this group had about the persistence of viable spores beyond the 30-day or the 60-day window that antibiotics were being proposed at that time. And at that time the question was were we going to offer vaccine to the postal workers and the people

in the Hart Building.

We mocked out, I think, a very good set of studies where basically monkeys were going to be--would be challenged. They would be started on a vaccine regimen. They would also be given antibiotic doses. We proposed different LD50 levels to change the amount of spores. And then we proposed that the monkeys be sacrificed at various times out, because I think there are a few studies where even out to 100 days, I think, in one animal there have been viable spores found, but the animal was fine and was well at the time of euthanasia and necropsy, but nobody really knows.

We do know that the spores can stay dormant. We don't know--they certainly don't synchronize when they germinate, and we have no idea how far that goes out and what is the pathologic significance post-exposure.

Unfortunately, this plan was put aside because it was felt to be impractical because there are no monkeys to do the study. And it was discarded as not feasible or possible.

DR. McINNES: Dr. Robbins?

DR. ROBBINS: I realize that the Department of Defense has an important central role

in this program, but if we're going to immunize, we have babies, young children, and they're not being mentioned, and the problems that they pose. I think in consideration of future studies of vaccines, attention should be drawn to that.

DR. BABCOCK: I'm also a pediatrician, as was the other physician in my panel, and we also drew up proposed pediatric studies with a modified dose and a regimen, and those have been proposed through the CDC, and I believe they're going to be funded through NIH and hopefully actually might work through the anthrax vaccine research program.

DR. McINNES: Thank you very much.

Any other comments? Yes?

DR. SIBER: I'm still confused about the issue of whether anamnestic response is likely to be important so that one can interpret the waning immunity levels that I guess are seen with this antibody.

Art Friedlander mentioned data from Sverdlovsk where the mean time to presentation, as I remember, in humans was 16 days. And then there's another piece of data we heard from the clinical review that the mean time from presentation to death in humans is 4.7 days, as I

remember.

Now, do we know whether in Sverdlovsk that 16-day period was all post-exposure, or were there ongoing exposures? Was there incubation going on? Were there events going on where a low level of antibody might have basically inhibited the process? Do we know anything about that?

DR. FRIEDLANDER: I'm glad you asked that question. I'll answer quickly so Emil can go.

[Laughter.]

DR. FRIEDLANDER: Oh, he's already missed the train? All right. Now I'll relax.

A lot has been said about Sverdlovsk, but unless somebody's got information that we don't have, I think you can disregard a good part of that. First of all, these people lied to us for ten years. Secondly, there's no data in that report. There's no data that's believable. We don't know whether they got antibiotics. We don't know whether they got anaserm (ph), at what time. There were little hints that they did at some point in time. The details are just not there.

This idea that there is a prolonged incubation period I think is suspect--except under the circumstance in which intervention has

occurred; that is to say--and we don't have a lot of data--except for one statement in a comment to a published article--not in the article. The only evidence of prolonged incubation period in inhalational anthrax is with animals that have been treated with antibiotics. In our--that is, to suppress the spores that are going to germinate in the first week, whatever it is.

There's one statement of an incubation period of 98 days. No primary data whatsoever. The Sverdlovsk data does say that the incubation period is, whatever it is, you know, 16 days or something, but we don't know what happened to those people. The primate data suggests that is not the case, the rhesus macaque primate data.

So I don't know the answer to that, but I'm very suspicious of any of the data that has come forth to date about Sverdlovsk other than the pathology. There's information there about survivors. No basis, zero basis that these people has anthrax. No clinical--no hard data, no culture, no pathology, no radiology. Interviews. That's nine cases in a city of, I don't know, a million people who said they had anthrax.

DR. McINNES: To close the loop on the

anamnestic issue, I think Drusilla had proposed as part of the strategy assessing memory in animals and evaluating booster kinetics. And so I presume the panel endorses that approach for the evaluation.

All right. The train, Emil. We're moving on to (e). If antibodies to protective antigen do not correlate with protection, what other approaches might be taken?

DR. SIBER: Could you rephrase that and say "even if protective antigen correlates with protection"?

DR. McINNES: I think that's a different question. It's sort of a depressing question, Drusilla, that protective antigen will be--but if not, what other approaches might be taken?

DR. SIBER: To prevent Art from answering this question, I'm going to try.

It seems to me that the other virulence factor we were told about was capsule, and we also heard about situations where people who are partly immune don't seem to get the septicemic form of the disease but, rather, get a more chronic disease, which looks an awful lot like encapsulated bacterial infectious disease. The meninges, you

get meningitis. And so one wonders whether a capsule or conjugate or some sort of cancer-based vaccines could be sort of the complement to the toxoid or toxin vaccine and also give you sort of a safety net in case you have waning immunity, because that likely would have some of the features that we know and love about conjugates, which is to provide that anamnestic response and protection after exposure. And you get that very early. That's really an area that deserves investigation, not to hold up the initial vaccine in any way.

DR. McINNES: All right. Any other comments?

DR. HEWLETT: I do have a question. The issue that was brought up about the activated macrophage and whether that works, Art dismissed that on the basis of the fact that that's not how the vaccine is working, and that may be the case.

But I'm interested in whether anyone has information on whether or not activated macrophage by one form or another does, in fact, not tolerate the germination or the survival or proliferation of organisms intracellularly.

DR. McINNES: Anyone from the floor wish to comment on that, add anything to it?

DR. GOTSCHLICH: I'll only say the following: As far as I remember, this has been done, but I can't quote you chapter and verse. It kills activated macrophages just as well as the other ones. But I can't quote you chapter and verse.

DR. FRIEDLANDER: There is an experiment--Bruce, why don't you make a comment about CPG? This is in vivo, and there's ongoing work with in vitro.

MR. IVINS: We've looked at the ability of CBG oligonucleotides to offer either non-specific or specific protection against spore challenge in--let's see, we've got mice, guinea pigs, we're going to do rabbits, and monkeys have been done.

We find non-specific protection in mice. We find some augmentation of specific protection in guinea pigs. That is in combination with vaccine, either the human--the currently licensed human vaccine or with recombinant PA aluminum hydroxide vaccine. And these studies, incidentally, are taken--or have been done in collaboration with Dr. Dennis Kleinman. In rhesus macaques, the oligos, the CPG oligos, we haven't done any challenge experiments, but they enhance the antibody titers

to PA and the titers stay higher for a longer period of time. And we're about to go into rabbits this summer.

DR. McINNES: Thank you. I would--

DR. FRIEDLANDER: Can I ask a question? Again, to the immunologists here, because we've sort of struggled with this question a few years ago. If it's not antibody, is there any test of cell-mediated immunity or any other immunity other than antibody that one could conceive of as being a quantitative test to use as a correlate of immunity? Other than antibody level. To license a vaccine.

DR. McINNES: Your challenge has--yes, Emil?

DR. GOTSCHLICH: I really think that's a wonderful question, but it's totally inappropriate for what we know about this disease. There's--

DR. FRIEDLANDER: I agree.

[Laughter.]

DR. GOTSCHLICH: Then don't ask it.

DR. FRIEDLANDER: No, no, but there are a lot of people--

DR. McINNES: He was being provocative.

DR. GOTSCHLICH: Actually, I think it's an

important issue that should be briefly discussed. People get very, very misled with this TH1, TH2 adjuvants, et cetera, into believing that TH1 means cell-mediated immunity. It doesn't mean that at all. It simply means it's a different response to the antigen.

It does, in fact, have a higher probability of raising cytotoxic T-lymphocytes, but they have nothing to do with this disease as far as we know. This is a disease where the immunity is clearly antibody-mediated, and there is no evidence from any of the--as a matter of fact, it's notable that nobody's even mentioned lymphocyte transfer experiments today. There is no evidence that any of the lymphocyte transfer experiments have worked.

DR. McINNES: A nice clean ending.

I want to thank Drs. Gotschlich, Friedlander, Hewlett, and Siber very much for their thoughtful--

[Applause.]

DR. McINNES: Thank you very much. And I'll pass the meeting back to Colonel Danley.

COLONEL DANLEY: Before we leave, I want to thank all the participants for their excellent presentations. I want to thank Dr. VanDeVerg, Dr.

Burns, Dr. Goldstein for organizing the meeting. I want to thank the CAMR contractors and the SAIC contractors for organizing the meeting. But most of all, I want to thank you and I want to say something about you, the audience here, in terms of the following statement: We need a better vaccine. In this audience right now, there is a remarkable diversity of individuals from different organizations, and all of you have heard the phrase "we need a better vaccine."

For the people giving that vaccine, such as Colonels Randolph and Grabenstein, as part of the anthrax vaccine program, their better vaccine is a vaccine that has no side effects. They're not worried so much right now about protecting forces. What they're fighting every day are the complaints that the current vaccine is unsafe. So their better vaccine has no side effects.

But the manufacturers who are here, your better vaccine might be a vaccine that's easier to produce, has a better return on investment, most importantly, something that doesn't slime your company's name that reflects poorly upon the people that are working to try to make a better vaccine.

For the FDA, a better vaccine is one that

has the data that says this vaccine is clearly better than that vaccine, and I think that's what the FDA was trying to find out today. What should that data look like?

And for the scientists who are here that really form the basis for all of our work, I'm afraid that a better vaccine is the one that I invented, as in "My vaccine is better than your vaccine."

But, clearly, our nation is asking for a better vaccine, and that word "better vaccine" encompasses all of our areas of expertise.

I think this meeting has been very, very successful in defining what that better vaccine is, but I would remind you that the enemy in this room right now we wear on our wrist. It is time. We don't have all the time in the world. One of two things will happen: either someone will discover that anthrax is a great way to terrorize a nation and our nation will be looking for that better vaccine; or someone will decide that anthrax is not a good way to terrorize and we'll never see it again, and the efforts that we're putting out will be lost to the next problem that our nation has to face. The funding will decline, the interest will

wane, and the problems won't be solved.

So there is a sense of urgency that we have to take away from this meeting to accomplish that goal of making a better vaccine.

I thank you all for participating.

[Applause.]

[Whereupon, at 4:33 p.m., the meeting was adjourned.]

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