FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

OPEN MEETING OF:

THE VACCINES AND

RELATED BIOLOGICS PRODUCTS

ADVISORY COMMITTEE

June 7, 1994

Holiday Inn
8777 Georgia Avenue
Silver Spring MD

Proceedings By:

CASET Associates, Ltd.
3927 Old Lee Highway
Fairfax, Virginia 22030
703-352-0091

PARTICIPANTS

EXECUTIVE SECRETARY NANCY CHERRY, Scientific Advisors & Consultants Staff, CBER, FDA(HFM-21) Rockville MD 20852-1448

COMMITTEE MANAGEMENT ASSISTANT STEPHANIE MILWIT, Scientific Advisors & Consultants Staff, CBER FDA(HFM-21) Rockville, MD

COMMITTEE MEMBERS
STANLEY LEMON, MD CHAIRMAN, Prof. of Medicine, Microbiology & Immunology, University of North Carolina, Chapel Hill

MARY GLODE, MD, Prof, Pediatrics, Children's Hospital of Denver, U. Colorado School of Medicine, Denver, CO 80218

ALISON O'BRIEN, PhD, Professor of Microbiology, Uniformed Services Univ. of Health Sciences, Bethesda, MD 20814-4799

RAYMOND ROOS, MD, Professor of Neurology, Dept of Neurology /MC 2030, Univ. of Chicago, Chicago, IL 60637

TEMPORARY VOTING MEMBERS
CLAIRE BROOME, MD, Acting Deputy Director, CDC, 1600 Clifton Road, N.E., Building 1, Room 2000, D-14, Atlanta, GA 30333

THEODORE EICKHOFF, MD, Professor of Medicine, Division of Infectious Diseases, Univ. of Colorado Health Sciences Ctr, Mall Container B-168, 4200 E. Ninth St, Denver, CO 80262

PATRICIA FERRIERI, MD, Professor of Pediatrics, Laboratory of Medicine and Pathology, University of Minnesota, Box 296, Delaware Street, S.E., Minneapolis, Minnesota 55455

RICHARD JOHNSTON, MD, Med. Dir, March of Dimes Birth Defects Foundation, 275 Mamaroneck Ave, White Plains, NY 10605

CBER CONSULTANT
DR. DAVID CARZON, Department of Pediatrics, Division of Infectious Diseases, Vanderbilt University Medical School, 1161 21st Avenue, South, Nashville, Tennessee 37232-2581

GUESTS AND SPEAKERS
DR RAYMOND DATTWYLER, Div of Allergy, Rhumatology & Clinical Immunology, SUNY School of Med., Stonybrook, NY 121794-8161

DR DAVID DENNIS, Bacterial Zoonoses Br, Div. of Vector-Borne Infectious Diseases, CDC, Box 2087, Fort Collins, CO 80522

DR. ALLEN STEERE, New England Medical Center, Box 401, 750 Washington Street, Boston, Massachusetts 02111

TABLE OF CONTENTS

	Page
Call to Order and Administrative Remarks	1
Open Public Hearing	6
SESSION I: VACCINES FOR THE PREVENTION OF LYME DISEASE	
- Introduction - Dr. Margaret Miltrane	7
- Epidemiology of Lyme Disease - Dr. David Dennis	16
- Clinical Overview of Lyme Disease - Dr. David Dattwyler	32
- Immune Response to OSPA in Natural Infection - Dr. Allen Steere	58
- Presentation by SmithKline Beecham	84
- Presentation by Connaught	93
- Presentation by Medimmune	109
- Concluding Remarks and Questions - Dr. Margaret Mitrane	128
Committee Discussion	134

P R O C E E D I N G S

(8:13 a.m.)

Agenda Item: Call to Order and Administrative Remarks.

DR. LEMON: I would like to call this meeting to order. This is a meeting of the Vaccines and Related Biological Products Advisory Committee of the Food and Drug Administration.

I am Dr. Stan Lemon from the University of North Carolina, and I will be chairing the meeting today.

I would like to start by turning the microphone over to the executive secretary, Nancy Cherry.

MS. CHERRY: I would like to welcome everyone here to this meeting of the vaccines advisory committee also. And if anyone has anything they wish to tell the committee, if they would see me, I will see that the message gets to the appropriate committee member.

We are going to have open session until approximately 2:00 o'clock this afternoon, but of course, that is subject to change, depending on discussions and the open public hearing sessions.

I wanted to mention that our committee, although it has not met for a few months, has been very busy. Two of our members, or former members, have served as liaisons to other groups.

Dr. Eickhoff represented this committee on March 15th at the meeting of the National Vaccine Advisory Committee's Ad Hoc subcommittee on childhood vaccines. And Dr. Mimi Glode has accepted the position of liaison to the National Vaccine Advisory Committee's Subcommittee on Future Vaccines and, as such, she attended their first meeting last week.

I have no other administrative comments at this time, so I think Dr. Lemon wants to take over.

DR. LEMON: Thank you, Nancy. I did want to ask Jack Gertzog if he wants to make a statement.

DR. GERTZOG: Thank you, Dr. Lemon. I am Jack Gertzog. I direct the Centers for Advisory Committee Program, and I have one brief announcement.

Each year, the commissioner recognizes exceptional meritorious service on behalf of FDA, and the public whom it serves, with its personal award, known as the Commissioner's Special Citation.

The award consists of a certificate, an engraved plaque, and the Harvey W. Wiley medal.

There are a large number of nominations for this award, but only a few are selected. It is with much pleasure and gratitude on behalf of the commissioner, all of us at FDA, and the public, that I present the Commissioner's Special Citation to the immediate past chair of this

advisory committee, Richard B. Johnston, Jr., senior vice president and medical director for the March of Dimes Birth Defect Foundation.

The citation reads: For exceptional performance and accomplishments in strengthening the Food and Drug Administration's role in the approval of new biologicals and protection of the nation's health.

Dr. Johnston.

(Applause.)

DR. GERTZOG: Congratulations, sir.

DR. JOHNSTON: It has been a privilege. It is a first-rate group.

DR. LEMON: Thank you, Jack. Now we have a statement of conflicts of interest to be read by Ms. Cherry.

MS. CHERRY: The following announcement addresses the issue of conflict of interest, with regard to the open portion of this meeting, and is made a part of the record to preclude even the appearance of such at this meeting.

Based on the agenda made available, and all reported financial interests as of this date, it has been determined that all interested firms regulated by the Center for Biologics Evaluation and Research, which have been reported by the participating members and consultants, present no potential for an appearance of a conflict of interest at this meeting, with the following exceptions:

Dr. Lemon has reported that he consults with Connaught on related matters. Therefore, the agency has granted him a full waiver for the discussion on Lyme disease, and there is no other restriction of his participation after this disclosure.

Dr. Eickoff, a temporary voting member at this meeting, has disclosed that he consults with SmithKline Beecham on unrelated matters. Based on FDA's waiver criteria, Dr. Eickoff is authorized to participate after disclosure of these interests.

A copy of this waiver statement is available under the Freedom of Information Act by written request.

Pursuant to the authority granted under the VRPAK(?) charter of the director of the FDA Center for Biologics Evaluation and Research, has appointed the following individuals as voting members for the meeting of June 7th, 1994:

Dr. Claire Broome, Dr. Theodore Eickoff, Dr. Richard B. Johnston, and Dr. Patricia Ferrieri.

With regard to FDA's invited guest speakers, the agency has determined that, because the services of these guest speakers are considered potential for a thorough discussion of the issues, any reported interests of the guest speakers will be made a part of the public record for this meeting, to allow participants to objectively evaluate

their presentations:

David Dennis, M.D., an employee of the U.S. Public Health Service at CDC has disclosed that:

A, he was an invited member for the first meeting in May of an independent oversight committee of vaccinations for SmithKline Beecham Pharmaceuticals;

B, that he has accepted an invitation to meet with Connaught Laboratories to provide expert information on epidemiology and diagnosis of Lyme disease; and

C, that his employer, the Centers for Disease Control's National Center on Infectious Diseases, is participating in a cooperative research and development agreement, or CRADA, with SmithKline Beecham Animal Health.

Raymond Dattwyler, M.D., has disclosed that he is employed at the State University of New York at Stonybrook, and as such, he has worked on grants from CDC, NHD, NIAID, New York State, as well as the fact that they are currently studying strain-related variability in B. burgdorferi antigens, immune reactions to antigens, and various treatments for B. burgdorferi Infection. Some antigens under study are potential vaccine candidates.

Allen Steere, M.D., has disclosed that he has consulting arrangements with Connaught, SmithKline Beecham and Medimmune.

In the event that the discussions involve any

products or firms not already on the agenda, for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement, and their exclusion will be noted for the record.

With respect to all participants, if any products or sponsor should be discussed, we ask that, in the interest of fairness, that they address any current or previous financial involvement with any firm whose products they may wish to comment on.

Agenda Item: Open Public Hearing.

DR. LEMON: At this time, we have scheduled an open public hearing. If there are any members of the public who wish to make a statement at this time to the committee or to the FDA, they are welcome to do so.

Such individuals have been asked in advance to make notice of this. As far as I understand, nobody has requested time, but if anyone present wants to make a statement, now is the time to do it.

If not, then I think we should proceed with the agenda today, which is a full agenda. As we have heard already, we will be discussing Lyme Disease and vaccines to prevent it today, as the primary focus.

I think we are in the exciting position of having three different vaccine manufacturers involved in the

development of vaccines for the prevention of this disease.

We will start today's discussion with an introduction of the issues by Dr. Margaret Mitrane of the FDA.

Agenda Item: Vaccines for the Prevention of Lyme Disease. Introduction.

DR. MITRANE: On behalf of the Center for Biologics, I would like to welcome everyone to the Vaccines for the Prevention of Lyme Disease session of today's vaccine advisory committee meeting.

The purpose of this section is to discuss relevant clinical issues pertaining to phase III trials with Lyme Disease vaccines. My introduction will highlight various aspects of Lyme Disease, which will be expanded upon by our guest speakers and the companies participating in this session.

Lyme Disease is a multi-system disorder caused by the spirochete Borrelia burgdorferi. It is the most common arthropod-borne infection in the United States. The arthropod vector for Lyme Disease is the Ixodes tick.

Cases of Lyme Disease have been reported in nearly all states. Most cases occurred in endemic areas. The northeast, mid-Atlantic, north central, and the Pacific coastal regions, are the endemic areas in the United States.

In 1975, Allen Steere first described Lyme Disease

as a clinical entity, manifested by oligoarticular arthritis. Seven years later, the causative bacteria was isolated by burgdorferi.

Borrelia are microaerophilic, gram negative bacteria, phylogenetically grouped with treponema and leptospira. Borrelia can be cultured in Barbour-Stenner-Kelly medium.

Slow growth in culture makes it difficult to isolate Borrelia burgdorferi from blood, cerebral spinal fluid or synovial fluid. Isolation of Borrelia from skin biopsies of erythema migrans has been more successful, with yields as high as 85 to 95 percent.

Borrelia burgdorferi have cytoplasmic and outer membranes, between which is peptidoglycan. Flagella are inserted at the ends of the spirochete.

Borrelia burgdorferi have three major outer membrane surface lipoproteins which are, 31-32 kilodalton OspA, 34-36 kilodalton OspB, and 21-22 kilodalton OspCs.

Immune response to OspA and OspB develops late in the course of infection. Early in the disease, the immune response is directed against the 41 kilodalton flagellar antigen.

Lyme Disease can be divided into three clinical stages: Stage 1 - Early localized infection; Stage 2 - Early disseminated infection which occurs in the first weeks

to months of disease; Stage 3 - Late persistent infection, which occurs in the first month to years into the disease.

A patient infected with Borrelia may manifest the infection in various ways. A patient may have isolated infection, may proceed through all stages of disease, or may present with stage 2 or 3 disease, without having had any symptomatic earlier stage disease.

An individual infected with Borrelia may also be completely asymptomatic.

Erythema migrans is the pathognomonic skin lesion that occurs at the site of the tick bite. It has a classic annular appearance with an erythematous border and central clearing.

The rash is warm to touch and half of patients experience burning or pyritis.

Erythema migrans occurs in 60 to 80 percent of patients. Some patients who do not recall the rash, may have had an asymptomatic lesion in an inconspicuous location.

Here is an example of the classic erythema migrans rash, with an erythematous outer border and central clearing. This patient also has a secondary smaller erythemal migrans lesion.

Untreated lesions usually resolve after several weeks. Treated lesions usually resolve within several days.

In stage 2 of Lyme Disease, additional cutaneous manifestations may occur. Patients may have secondary erythema migrans lesions, diffuse urticaria, malar rash, or non-specific small evanescent red lesions.

Early neurologic manifestations occur in 15 to 20 percent of untreated patients, and may manifest as meningitis, encephalitis, cranial nerve palsy -- most frequently involving the seventh cranial nerve -- and peripheral radiculoneuropathy.

Cardiac manifestations occur in four to eight percent of patients. The most common abnormality is fluctuating high grade atrioventricular block, either winky block or complete heart block.

The duration of Lyme carditis is usually brief -- from three days to six weeks. Mild, asymptomatic mild carditis, or pericarditis, may also occur.

Musculoskeletal manifestations in stage 2 are transient and migratory, and involved both articular and periarticular structures.

Pain without swelling, of small and large joints, occurs at only one or a few sites at a time.

Neurologic manifestations in stage three include peripheral neuropathy and sub-acute encephalopathy, and become evident late in the first year, or after the first year of disease.

The arthritis associated with Lyme Disease was found to develop, on the average, six months after disease onset and is an intermittent inflammatory, mono or oligoarticular arthritis, involving large joints, especially the knee.

Ten percent of untreated patients with joint involvement develop chronic Lyme arthritis, which is defined as joint inflammation lasting longer than one year.

Chronic Lyme arthritis has been associated with an increased frequency of the HLA DR2 or 4 allele.

Dr. Steere has identified a subset of chronic Lyme arthritis patients, who are HLA DR4 positive, have antibody reactivity to OspA or B, and are unresponsive to antibiotic therapy.

An aberrant immune response to Borrelia may play a role in the pathogenesis of arthritis in this subset of patients.

In conjunction with the clinical picture, serologic tests are used for the diagnosis of Lyme Disease. The indirect immunofluorescence Assay, enzyme linked immunosorbent assay, and western blot have been used.

The ELISA is the most widely used assay to support a diagnosis of Lyme Disease. An IgM response usually develops by two to four weeks after the onset of erythema migrans, and the IgG response is seen by four to eight

weeks.

Serodiagnosis of Lyme Disease is complicated by cross-reactivity of spirochetal antigens with other antigens, delayed development of humoral antibody response, dampening effect of early antibiotic therapy, variability of immune response in various subjects, inability to predict stages of Lyme disease, and lack of standardization.

A retrospective and prospective analysis was conducted by Dressler, to develop acceptable criteria for positive western blots.

In a retrospective analysis of 225 case and control subjects, the best discriminatory ability of test criteria was obtained by requiring 2 of 8 most common IgM bands in early Lyme disease, or 5 of 10 most frequent IgG bands after the first weeks of infection.

When these criteria were applied in a prospective study of all 237 patients seen in a Lyme Disease clinic during a one-year period, and in 74 patients with either erythema migrans or summer flu-like illnesses, IgM, by western blot, had a sensitivity of 32 percent, and a specificity of 100 percent. The specificity for IgM by ELISA, was 94 percent.

IgG, by western blot, had a sensitivity of 83 percent, and a specificity of 95 percent. The specificity for IgG by ELISA was 72 percent.

Therefore, western blot can be used to increase the specificity of serologic testing in Lyme Disease.

Polymerase Chain Reaction has been successfully used to detect Borrelia burgdorferi DNA in cerebrospinal fluid, urine, joint fluids, skin, and serum of Lyme Disease patients.

In a study by Nocton, three separate regions of Borrelia burgdorferi genome was targeted by four sets of primers and probes.

Borrelia was detected in the synovial fluid of 75 of 88 patients with Lyme Disease, and in none of 64 control patients.

Seven of ten chronic Lyme arthritis patients, treated with multiple courses of antibiotics, had negative PCR test results.

This suggests that the arthritis in these seven individuals is not due to the persistence of spirochetes.

The ability of recombinant OspA to induce protective immunity, has been demonstrated in multiple animal models. Mice immunized with recombinant OspA were protected against challenge from Borrelia that were delivered by syringe or tick.

On the other hand, mice immunized with the 41 kilodalton flagella protein are not protected against challenge from Borrelia.

Yang developed a mouse model of Lyme Disease which allows analysis of mice with mild, moderate, and severe pathologies, after inoculation with Borrelia burgdorferi.

Infected C3H HEJ mice developed severe arthritis and severe cardiac abnormalities, while infected BALB/C mice developed mild arthritis.

Higher levels of Borrelia burgdorferi DNA were detected by PCR in the tissues of infected C3H HEJ mice, than in the tissues of BALB/C mice.

The genetic regulation of severe pathology was analyzed by infecting the offspring of a cross between C3H, HEJ and BALB/C mice.

The F1 mice developed severe arthritis and contained high levels of Borrelia DNA in the heart and ankle, similar to the C3H HEJ parent.

These findings indicate that susceptibility to severe arthritis is a dominant trait and suggest that it may correlate with high levels of persisting spirochetes.

I would like to conclude my introduction with FDA's questions to the advisory committee. We ask that the committee consider these questions while they listen to the presentations this morning.

Number one. Is the CDC case definition for Lyme disease appropriate for a pivotal efficacy trial. Please comment on laboratory assays to support the diagnosis of the

disease -- that is, culture, western blot and polymerase chain reaction.

Two. Lyme disease has a wide range of clinical manifestations which occur in the acute and chronic phases of infection by Borrelia burgdorferi. Please comment on appropriate primary and secondary end points that provide specificity in diagnosis of the disease for a pivotal efficacy trial with an OspA vaccine.

Three. How should the safety of OspA vaccines be evaluated, especially as it relates to individuals with HLA DR2 or 4 haplotype.

Four. How long should immunized individuals be followed to attain adequate safety and efficacy data.

Five. How could the safety and efficacy in children be assessed.

Six. What other studies could be performed to answer additional safety and efficacy questions with the OspA vaccine. For example, how should the use of the vaccine be evaluated in seropositive individuals and in those with a history of Lyme Disease. Thank you.

DR. LEMON: : Thank you, Dr. Mitrane. As always, the FDA has given us a set of challenging questions to address here. Are there any questions for Dr. Mitrane from members of the committee before we go on.

If not, then maybe we should proceed with the next

speaker. I would like to thank Dr. Mitrane for sticking within her allotted time. I encourage people to do the same. We are, of course, ahead of schedule, but I would like to remain so, so that we have more time for discussion when the time comes.

We are luck to have a number of experts in Lyme Disease to help us address these difficult questions posed by the FDA. And I would like to ask Dr. David Dennis to begin these presentations with a discussion of the epidemiology of Lyme Disease.

Agenda Item: Epidemiology of Lyme Disease.

DR. DENNIS: Thank you and good morning. I am going to address some broad epidemiological issues and try to relate them to the vaccine studies that are in place.

As I represent the Centers for Disease Control, which is the nation's prevention agency, it is quite obvious that our roles are in national surveillance, epidemiologic studies, and research leading to prevention and control strategies.

This bar diagram shows the reported numbers of cases to the Centers for Disease Control by states over time. This describes the curve of increasing reports showing that Lyme Disease is a rapidly emerging disease in the United States. There have been more than 55 thousand cases now reported totally, from the nation, about 9,000

cases a year in the last three or four years when there has been a uniform national surveillance system in place, about a 20-fold increase from the less than 500 cases reported by 11 states in 1982.

The distribution of the reports of cases by state in the nation -- this is for 1993 where there were about 9,000 cases reported, you can see that 45 states reported cases, but the vast majority of cases -- in fact, over 90 percent of cases -- occurred in the localized areas of the northeast, the upper north central, and the Pacific coast.

And those states that are in green are states in which we have identified enzootic cycles of the parasite, Borrelia burgdorferi, in situations where humans are exposed to infection.

Lyme Disease is a tick-borne zoonosis, Borrelia burgdorferi, in sensu latu -- latu is the agent. The vector ticks are of the Ixodes risctanis complex. This is a picture of the ixodes scapularis, the principal vector in the northeast, in the north central United States, and similar to the vector on the Pacific coast, Ixodes Pacificus. It is a three-host tick, involving a two-year life cycle.

Rodents -- in particular, mice -- this is the peramiscus lucopis, the white-footed mouse -- served as the reservoir host of the Borrelia burgdorferi in nature. Other

wild rodents also served as reservoir hosts.

The deer, however, does not serve as a reservoir host of the parasite, but it does serve as a maintenance host of the ticks, because it is a principal site of mating and feeding of the adult ticks that need to take a blood meal in order to lay eggs.

It is the deer, the introduction of the deer, to new geographic areas that allows the introduction and the establishment and maintenance of populations of the ticks that transmit Lyme Disease in the United States.

This is a typical environment in which intense enzootic cycles occur in the United States, exposing most people to risk. This is a suburban area in which homes have been placed into deciduous woodlots. These are succession forests that have ample saplings for feeding of the deer. They have a deciduous leaf litter that is a favorable environment for the ticks.

You can see stone walls, other places, that the rodent reservoir can use as nesting sites.

In addition to a pararesidential exposure, of course, there are high risk occupational and recreational exposures throughout the areas in which Lyme Disease is endemic.

The force of infection showed by these magenta arrows in nature, is from rodents to the immature stages --

larvae and nymphs. There is transtadial transmission, but not transovarial transmission. And the nymph is the primary source of infection for humans. The nymphs also transstadially transmit their infection to adult ticks, which less frequently, are a source of infection for humans.

If we look at the graphs of the distribution by dates of onset, or months of onset, of cases with erythema migrans, you can see in the lower chart there, that there is a very marked seasonal incidence in May, June, July, August time period.

That is the time period in which the nymphal stage of the tick is most active, supporting the observations of the sufficiency of nymphal ticks transmitting infection to humans.

Lyme Disease is a disease of equal effect upon males and females. There is, however, a very pronounced bimodal distribution with high risk, and high rates of infection in young children and older adults.

The distribution of Lyme Disease throughout the world is driven by the distribution of Ixodes ticks of the ricinus complex. And you can see here their distribution is limited to the northern hemispheres.

The disease is endemic across Europe -- Russian -- to Korean peninsula, to Japan, and to northeastern China. In the United States, it is a limited foci in Canada and

widely distributed in the northeast and, to a lesser degree, in the north central and the western coastal states.

We mapped the distribution of the principal vectors of Lyme Disease in the United States several years ago. Ixodes damini now has been renamed -- reverted to its original name, Ixodes scapularis.

These are the distributions of the known populations of Ixodes scapularis in the northeast and the north central area, and Ixodes Pacificus on the west coast. Red is where there are established populations. Almost always there is also enzootic cycling of Borrelia burgdorferi identified in those counties. Yellow is where the tick has been reported, but not yet have there been established cycles of the parasite, Borrelia burgdorferi.

If we look how this distribution relates to the frequency of occurrence by rates of disease in states, it follows quite well that the seven states with the highest rates -- and we are only talking about rates of about 80 to 50 per 100,000 -- are clustered in those areas where we saw that the tick population occurs.

The point of this slide is that these seven states account for more than 80 percent of all cases and yet, you can see the rates are really not very high. It is not a disease of very high frequency of occurrence.

It is also a disease that is very focally

distributed, even within states. These are the counties that, in 1992, had rates greater than 30 per 100,000. And you can see that there are only 13 counties -- some of them with very limited populations and rather unstable rates -- that had rates per 100,000 or greater.

And there are just a few states as well that have rates exceeding 30 per 100,000. And even in these counties, the disease is highly focalized.

This summarizes some of the studies that have been done in the past, looking at the most highly endemic communities, to try to get some understanding of the prevalence and incidence rates that do occur in these almost outbreak situations, in some instances.

And you can see that using serologic and clinical -- and these are both on standardized serologic and standardized case definitions, that the prevalence was found to be in the range of eight to fifteen percent, in an incidence in the range of two-and-a-half to three-and-a-half percent, in the most highly endemic foci communities that have been studied.

Now, the Center for Disease Control, working with others, have developed diagnostic tools for surveillance of immunologic studies of Lyme Disease. Most important is the case definition based primarily on clinical findings.

The simplest description of this case definition

is a physician diagnosed erythema migrans rash, or at least one objective manifestation of a major later stage illness in the musculoskeletal, cardiovascular, or neurologic systems, with so-called laboratory confirmation.

I will just show you how we have qualified the clinical aspects, just using erythema migrans as an example. It can't just be a rash that occurs after a tick bite. It is a rash that has particular characteristics -- a solitary lesion for surveillance purposes must reach five centimeters or greater in diameter, it must be diagnosed by a physician -- it cannot be something that arises quickly and disappears quickly. It usually occurs three to thirty days after the tick bite.

In addition to that, the serodiagnostic tests, CDC, working together with clinical researchers, manufacturers, with the state territorial public health laboratory directors, the FDA, NIH, and others, had a primary research priority to standardize and improve serodiagnostic tests.

We have been working with a flagellant ELISA and have standardized this, and have used it in conjunction with the Western blot.

We now, with a broad range of highly characterized serum specimens, many coming from patients from whom Borrelia burgdorferi has been isolated, achieve a

sensitivity of about 85 percent and a specificity of 98 percent or greater when this combination of flagella and ELISA and Western blot are used, and a high degree of precision with cases, non-cases, and serum specimen from patients with the disease thought to be cross reactive.

Also, we have had a chance to look at the performance of this testing schema in patients with early disease which would be most important to following patients immunized in a vaccine trial.

And you can see that, even in early stages of disease, we have a fairly high sensitivity. In patients seen in the period day zero through thirty -- and I will say day zero through seven is underrepresented in this sampling -- we have nearly 80 percent sensitivity.

These are patients that all erythema migrans. Most of them had Borrelia burgdorferi isolated from their lesions. They were seen early and treated.

Similarly, we got about an 80 percent sensitivity in persons in the period of 30 to 100 days, and it fell off after 100 days, and we had follow ups up to more than a year.

So, using this two test approach, we have a fairly sensitive test now, even for early stage disease.

There has been a big movement, not only to standardize the ELISA but also to standardize the Western

blot. We have made progress. We think that in the coming months we will be able to have a standardized western blot.

A working group of people most active in the United States in the clinical and research development of serodiagnostic tests, met at CDC and have come up with the interim recommendations on serodiagnosis, in which they are recommending a two test approach with an ELISA, with taking care for setting the negative cut-off using certain criteria, and then testing all persons who have a positive or equivocal test obtained by the ELISA with the immunoblot, using a low passage strain of Borrelia burgdorferi as antigen, and with the immunoblot using the criteria of Dressler, et al, discussed by Dr. Mitrane.

It appears that these immunoblot criteria will be simplified, and it appears that three bands along in the IgM -- p45, p31 and the OpaC -- will probably be sufficient for use in the IgM criteria, when two of those three, and including the OpaC, are used as the criteria.

So, we expect that this will be standardized and simplified in the fairly near future.

Just some thoughts on trial design issues. Lyme Disease and the testing of vaccines, obviously you can't have experimental challenge of subjects. But there are animal models -- particularly the primate and the canine models -- that are very good models of human disease, not

only for the clinical aspects but also the immunologic aspects in the primate, mimicking the response that we see in humans, very closely.

Population sampling, I think it is very important that we use a sampling that tries to achieve a representative sampling of the population that we think would be targeted for vaccine, because of their risk.

Obviously, in thinking about statistical power and alpha and beta errors, we not only have to think about efficacy, but also safety issues. And some of the safety issues are ones that theoretically may be of very low frequency of occurrence and, because of that, there will be a need for setting into place epidemiologically placed surveillance systems for monitoring not only of the effect over time, but also some of the safety issues over time.

The targeting of the use of the vaccines, I think we have to work very closely with state and local health departments and research groups that are working to really define the risk of population groups.

Obviously, epidemiologic studies can be useful in developing cost benefit analyses of the vaccine, and certainly for helping develop strategies of distribution of the vaccine, if and when it becomes available.

Thank you very much.

DR. LEMON: Thank you, Dr. Dennis. Perhaps I

could open the discussion with a couple of questions that came to mind listening to you.

One of your first slides is very impressive, in that it showed an increase in reported cases over the past decade or more. And one question is, how much does this reflect a true increase in the incidence of the infections, the disease, versus an increase in physician recognition and the availability of diagnostic tests.

DR. DENNIS: I should have mentioned, there are considerable caveats in that bar graph. There is a big problem -- but a decreasing problem -- of misclassification of cases. And I think that there has been a big effort by state health departments over the past three or four years in particular, in order to validate the cases that are reported to them, as cases that truly meet the surveillance case definition.

There still is a big problem with misdiagnosis, not only because the clinical signs and symptoms can be protein and not as specific as we would like, but also because we haven't had the best serodiagnostic tools.

On the other hand, there is a very big problem with underreporting. And i think this is most important in those states that have the most endemic disease. And it would not surprise us if, in states like Connecticut, New York, New Jersey and Pennsylvania that there may be 70, 75

percent underreporting. So, this graph represents a combination of things.

DR. LEMON: And the second question, you may have already answered for me, and that deals with the role of the OspA antigen in the immunoblot. The data you showed utilizing a combination of the flagella and ELISA immunoblot, with a high degree of sensitivity and specificity, if you were to eliminate the OspA band from the immunoblot in that analysis -- because that may become irrelevant in a vaccinated population -- how does that affect the overall sensitivity and specificity of the combination of the serologic procedure.

DR. DENNIS: It won't, because OspA is not an important band for diagnostic criteria. And as I mentioned, it looks, at least for the IgM, that the three bands -- p39, p41, p23 -- will probably be as sensitive and specific as what is in place now.

DR. LEMON: Will it be different for the IgG, do you think.

DR. DENNIS: Perhaps others can best address that. Dr. Steere may be able to later.

DR. LEMON: Are there other questions about this to the community.

DR. EICKOFF: Could you go back to the underreporting issue for just a second. Is this, in your

view -- well, Lyme Disease is almost a little too upper socioeconomic strata disease, I would infer. It is not a disease in the urban ghetto.

Is the underreporting a physician failure to diagnose, a patient failure to seek medical attention, or simply physician failure to report, having made the diagnosis, or all of the above, or can't you tell.

DR. DENNIS: I think it is all of the above, but I think it is probably mostly a failure of physicians to report.

The Connecticut Department of Health did a study a year ago. They searched their reportable diseases records, and they searched their records of primary care physicians in the state, and they found that all cases of Lyme Disease reported to them, had been reported by only seven percent of primary care physicians.

They then went and did a survey of primary care physicians, and they got a very good sampling and a good rate of response. And 65 percent of the physicians -- primary care physicians -- sampled, said that they had seen and diagnosed and treated at least one case in that same year.

So, there is an obvious significant underreporting by physicians of cases that they do see.

There is a problem with asymptomatic infection,

sub-clinical infection and misdiagnosis of cases of true Lyme Disease. But we don't know how important this is, as it relates to our surveillance data.

DR. JOHNSTON: The chronic arthritis is worrisome, of course. It has received a lot of play in the lay press. In trying to understand what is going on there, I have a couple of questions.

Are there enough data in children to detect any difference in the likelihood of getting a persistent arthritis. Number one.

And number two, is immune response -- has it been studied enough -- let's say in toddlers, even -- to know whether there is any difference in the immune response in children.

DR. DENNIS: I am probably not the best person to answer those questions. But I will say from surveillance data -- hematologic studies that we have done -- that the spectrum of illness in children now seems to be about the same as what we are seeing in adults, I think as physicians and the public become more sensitized and aware and understanding of the disease.

As far as the questions of immunologic response, I think Dr. Allen Steere and Dr. Dattwyler and others would be better placed to answer that.

DR. GLOBE: Back to the issue for a second, of

sub-clinical disease. If you look at your seroprevalence studies that I think you showed us from endemic areas, were those done with any questionnaires to know what percent of those, I think it was 8 to 15 percent seropositive had had disease that might have been compatible with Lyme Disease, just to try to get a handle on how much asymptomatic self limited infection there is.

DR. DENNIS: Yes. Actually, I should have been more clear in the title of the slide, but that represents -- there were seroepidemiologic studies and that represented input both from serodiagnostic testing as well as questionnaires on past history of disease.

And each of those investigators asked it in a different way, and with a different degree of sensitivity and specificity, I would think. But that was a combination.

If you look at asymptomatic seropositivity, if you look at sero conversion over a transmission season, the ratio of symptomatic to asymptomatic is about one to one, or a little bit greater than one to one.

If you look at those who are seropositive at a community prevalence bleed at one time, more than half of them in the studies that have been reported do give a history of having had disease compatible with Lyme Disease.

DR. LEMON: Let's have this be the last question before we move on.

DR. O'BRIEN: I am not sure I am asking the right person. In the background material that we got for description of Lyme Disease, there is a comment that not all strains of Borrelia make OspA, OspB, or OspC. How does that -- it is a concern when it comes to making a vaccine made of OspA, but also in serodiagnosis, when you are relying on OspC in your western blot, how is that -- is that taken into consideration and is that really a major problem in diagnosis.

DR. DENNIS: We don't know how large a problem it is. We are just now trying to do the comparisons with different geographic strains from throughout the United States. But we do know that the strains that have been identified in the areas that are highly endemic in the northeast and in the north central part of the country, have a considerable homogeneity and would be expected to have both OspA and OspC.

There are greater differences in organisms isolated in the Pacific coast and from some enzootic cycles that we do not think cause a public health risk of any significant amount in the south and in the Rocky Mountain states.

DR. LEMON: I think we had better move on. Thank yo, Dr. Dennis. I am sure we will come back and revisit some of these issues in later discussions.

We are scheduled next to hear from Dr. Raymond Dattwyler from the State University of New York at Stonybrook, who will speak about the clinical overview of Lyme Disease.

Agenda Item: Clinical Overview of Lyme Disease.

DR. DATTWYLER: I think Dr. Mitrane has outlined a lot of the clinical stuff, so it allows me to sort of expand on what I was going to talk about a little bit more.

I think that, you know, one has to put this disease in the context of an infectious disease. Right from the beginning, I should state, I don't think that we have fully delineated all of the various clinical manifestations associated with this infection.

We tend, when we have an emerging infectious disease, to first look at the most dramatic aspects of the disease -- and I think that is what is in the literature. Some of the more subtle abnormalities, especially the more subtle neurologic abnormalities, I think remain to be somewhat defined.

Be that as it may, I mean conceptually the disease is viewed as an infectious disease that starts as a local infectious process at the site of a tick bite. Characteristically, erythema migrans appears which, as has been pointed out, is not a universal phenomenon.

The early part of the disease may or may not be

associated with some viral-like illness, which is quite non-specific, consisting of arthralgias, myalgias, occasional headache.

Early in the course of infection, there is acute dissemination, with seeding of all major organ systems. Usually, by this phase in the disease, individuals have constitutional signs and symptoms, including fever.

Dr. Ben Loft and myself, and a number of our colleagues, have looked at early CNS invasion using PCR, and found that, in individuals which multiple erythema migrans lesions, or a single lesion and erythema migrans and major constitutional symptoms, that about two-thirds of these individuals will have Borrelia burgdorferi DNA in their cerebral spinal fluid, which we take as evidence of the early central nervous system invasion of this organism.

As has already been pointed out, acute neurologic involvement, of course, in about 20 percent of individuals at this phase of infection, with meningitis and cranial neuropathy -- particular seventh grain cranial nerve involvement being the most dramatic.

There is also peripheral nerve involvement early in the course. Classically, it has been described in Europe as a painful raticulopathy or a reticular neuritis. It can present as a brachial or a polexopathy or a sciatical-like syndrome. Peripheral neuropathies, which are also painful,

can occur.

The hallmark of dissemination, I think, is erythema migrans. And studies have demonstrated an incidence of that from anywhere from 10 to 50 percent. Generally, in our experience, it runs somewhere around 15 percent.

An enteric hepatitis can occur quite commonly, and this is just a transemanitis, by and large. Acute arthritis has been reported in this phase of the infection but, as has already been pointed out, arthritis is usually a later manifestation.

Cardiac involvement, in our experience at Stonybrook, occurs now less than one percent of the time.

There are differences in the clinical presentation of disease, perhaps some region. In Europe there seems to be a link, perhaps, between certain strains of this Borrelia and the clinical manifestations. But this has not been demonstrated in the United States, although it remains a possible explanation.

The chronic phase of the infection occurs months to years after the onset of infection. Arthritis has certainly been well described. It is generally a large joint arthritis.

Individuals begin usually with a more vague type of symptom complex of myalgias and arthralgias and, only

after some period of having this sort of prodromal syndrome begin to develop, a good arthritis.

The knee is overwhelmingly the most commonly infected joint. And quite interestingly, it is usually associated with very large effusions.

The small joints are very uncommonly involved, and symmetrical arthritis is also uncommon. So, by and large, it is a mono or oligoarticular arthritis of the large joints.

Acrodermatitis chronic A. trophicans is seen in Europe. It is only rarely reported in the United States, but it is a manifestation that one has to pay attention to.

Peripheral neuropathies tend to be axonopathies, and they tend to be diffuse in nature, not involving any one specific nerve.

The neurologic involvement in late disease can be encephalitis, chronic meningeal encephalitis, or a vaguer symptom complex and then encephalopathy.

Again, this has not been terribly well studied. And I don't know of any very long term population based study that has delineated the full repertoire of disease.

We have, for example -- these are some things on erythema migrans -- some problems in how we define it. There is even, in something as classic as erythema migrans, there is a considerable amount of variability of the

presentation.

The classic description is this target-like lesion where the tick bite occurred centrally. The tick bite occurred centrally here, and one sees erythema, clearing, erythema and then clearing skin. That is the classic description. We can get other types of things where this a more homogeneous erythematous area, still others where it is almost like a patch, and others where it is raised as opposed to being flat.

One can see it is almost like a bruising central area in this particular lesion, and still others where there is some vesiculation which occurs centrally.

One of the problems that we have observed is that many physicians are not terribly familiar with erythema migrans, and have failed to understand the fully range of this particular skin lesion, which is associated with this disease, and it is easy to make mistakes.

And even people who are somewhat experienced with Lyme Disease frequently have difficulty with erythema migrans.

Anybody that is familiar with dermatologic manifestations realizes that, although the classic target lesion is easy to recognize, some of these others may be confused with such things as fixed drug eruptions, and a detailed history is important.

What about, can we get some hints about disease based on natural history studies. Unfortunately, there are few natural history studies of this disease. Dr. Steere reported one in the annals of internal medicine a number of years ago, in which he took 55 patients who had presented to the clinic at Yale with erythema migrans who remained untreated.

They remained untreated because this was in the era when people didn't realize that this was caused by this spirochete.

These individuals were followed from anywhere from three to eight years, and there is a fair age range and fairly equal distribution of males to females.

The interesting thing in this is that the highest incidence -- greatest incidence of problem in this area was intermittent arthritis with arthralgias occurring in a significant number of individuals. Only six of the fifty-five developed chronic arthritis.

The intermittent arthritis in these individuals tended to be self limited and just resolved with time, even untreated. The arthralgias in this group didn't progress.

So, we seem to see a spectrum of rheumatological manifestations in this disease, even in its untreated state. Of course, treatment would change those numbers.

As far as the non-rheumatological manifestations

in this population, one saw fatigue, fever, headache or stiff neck, myalgias and recurrent erythema migrans.

Certainly, of these, the first four are fairly non-specific and, I think, would be difficult to categorize from the point of view of a study population when one talks about vaccine trials.

Another natural history of Lyme Disease studied also came from Dr. Steere's group, which was of 46 children who had been selected for arthritis. These were individuals who presented to Dr. Steere's group with arthritis. None had been treated for four years after the diagnosis, and there was a 10 to 13 year follow up in 39 of them.

Erythema migrans was the most common initial manifestation of the disease, with a viral-like illness alone in 15 percent. And I think that highlights one of the difficulties with this disease, in that the best marker of early infection is not a universal marker.

Neurologic involvement, as was expected, was seen in facial palsy in seven percent and meningitis in fifteen percent.

The interesting thing, these were individuals all with arthritis, but there were latent neurologic complications -- encephalopathy in 2 of the 39, a seizure disorder in 1 of the 39, and a demyelinating disease in 1 of the 39.

It is unclear whether the latter two manifestations were associated with Borrelia burgdorferi infection, although if one looks at these types of numbers and compares it to what we know about untreated T. pallidal infection, these numbers are not terribly different than that.

The other manifestations of disease that were not associated with frank arthritis were continuing arthralgia, marked fatigue and keratitis, in 2 out of the 39 individuals.

So, what we see from this, I think, is something that had not initially been described in the classic literature -- i.e., the keratitis -- which again, I think, points out that we perhaps don't know the full repertoire of this disease.

Well, what can we learn from other studies. It is very difficult to do a non-treatment trial. I just pulled this out of a recent trial which we were involved in where amoxicillin was compared to azithromycin. And what we saw in this was that the azithromycin protocol had a higher failure rate.

But it is interesting what the manifestations were. Certainly, arthritis was very common. But muscle tenderness was also quite common in this patient population. And these were -- all these individuals had erythema

migrans. So, this is, by definition, Lyme Disease and they were photographed and reviewed, so that we have a fair confidence.

But pain on flexion of the neck was also quite common, paresthesia was seen in one and meningitis was seen in one. So, I think that non-classical manifestations can be a part of someone who developed Borrelia burgdorferi infection.

Again, going on with this group, even to make it more difficult, fatigue, joint pain, headache, muscle pain, stiff neck, numbness and tingling, were also quite common manifestations of failure in this group of patients who had been treated for erythema migrans.

Now, to change tacks and talk to you a bit about what laboratory has to contribute to the clinical evaluation of individuals.

Certainly, one in any infectious disease, would like to have microbiological proof of infection. Unfortunately, this has proved difficult in many instances.

The best results in culture come from individuals with erythema migrans in which, now, with modern culture techniques, 90 percent-plus in individuals can have the organism isolated from their skin.

Unfortunately, this is the area where it is least necessary to provide culture results because, in the right

hands, the diagnosis of erythema migrans is straightforward.

ACA is the next most common place where one can culture the organism. Again, unfortunately, ACA is usually not seen in the United States -- that is acrodermatitis chronical antrophicans.

Where I think it becomes more interesting, especially from the view of a trial like this, would be we would like to isolate it from the central nervous system or the joints.

And in these instances, even in untreated individuals, the ability to get cultures under these circumstances is low.

Now, this is older data and perhaps with more modern techniques and better culture media, that we can get more isolates from CSF -- we certainly hope so. But still, I think it points out a significant problem, that certainly culture doesn't seem to be the answer in defining this. I think that is fair.

Well, what about PCR. I didn't put my PCR slide in there. I think PCR offers a real opportunity, and certainly, one can do PCR on cerebral spinal fluid or synovial fluid, and get fairly high yields, as has been demonstrated in the literature. And this could be something that is, I think, a very important adjunct in the microbiological definition of this disease.

PCR, though, is not an easy technique and it has to have very vigorous controls. As anyone who has done it realizes, if you have amplicons contaminating your laboratory, that you can turn everything positive.

The other thing that you must take great care in handling samples and preparing them for PCR, in that it is very easy to contaminate it. So, you have to have no Borrelia in the area where you are alloquatting your samples, or you can easily contaminate it.

And it is my understanding that some results of PCR, when analyzed further, it turns out that the organism was really high passage, laboratory strain bacteria which, somehow, accidentally contaminated the sample.

So, PCR, in well controlled circumstances, can be quite good.

Obviously, the question of serologies comes up and that is probably one of the most useful tools that we have. This is a study of 217 individuals with erythema migrans, and they are serial serographies -- and this is combining both IgM and IgG responses.

And what we see in these individuals is that, by day 20, everyone who was going to seroconvert, seroconverted. So, we see that we can utilize early serologic testing to define things. We don't have to, as sometimes, wait four to six weeks. So, I think the feeling

that there is a marked delay in the serological responses in this disease are not supported by out studies -- our most recent studies.

And the way this is done, day zero would be presenting time with erythema migrans and the duration after presentation with erythema migrans.

What also should be said under these circumstances is that if we recognize erythema migrans as an infection, that every one of these individuals was put on antibiotic therapy at time zero.

So, whether that had some influence in the subsequent seroconversion and whether, if we didn't put them on antibiotics we would have seen 100 percent seroconversion, we don't know, because I don't think anybody could ever do that study today.

Now, what serologic assays could one use in a study such as a vaccine trial. Would a single ELISA be adequate. Would a single Western Blot be adequate. Or, should one do serial ELISAs and serial Western Blots.

It is my opinion that the best way to assess most infectious diseases is to get an acute and a convalescent serology. If one thought that the person was acutely infected, I think that that is a classic way of assessing.

We will know that, if you immunize someone with a vaccine and get an appropriate immune response, that they

should have some antibody and perhaps be positive in a single ELISA. So, a single ELISA under those circumstances, I don't think, would be terribly useful.

A single Western Blot, since we are immunizing -- at least in this discussion -- with OspA, would that be useful. The answer is, I think, yes, and I will get back to that in a minute.

A serial ELISA certainly could be helpful if one did it in an acute and convalescent. A rising serologic response would suggest an infection. And the same, I think, would be true about serial Western blots, where one would see an increase repertoire of immune response against various antigens to the bacteria.

Now, when one looks at some of the difficulty with serology in this disease, one has to look at the major antigens. And the problem, I think, becomes very apparent. The 41 kilodalton flagellant antigen induces an early immune response. But it has been fully sequenced, and there is a high degree of homology with other flagellant antigens from other spirochetes or other organisms -- things like trepanimadenticolon borrelia bucalus, which can cause gum infection and induce an immune response. There, flagellant antigens are highly cross reactive, with the flagellant of Borrelia burgdorferi.

The other thing is that Borrelia burgdorferi

expresses a number of common bacterial antigens. The best characterized ones are at 60 to 66 range, and another at the 73 kilodalton range. These belong to the heat shock family 70 and 60 family members, respectively.

Antibodies directed against these are non-specific and we have been able to demonstrate that in individuals with subacute endocrinities causes by strep varigans, that one can see the productions of antibodies against these common bacterial antigens, which are highly cross reactive with Borrelia burgdorferi.

Some of the more specific antigens of this bacteria tend to be the outer surface protein antigens. The OspC is particularly interesting in that it produces an early immune response.

The difficulty with OspC, though, is that it is a plasmid encoated antigens. And most strains of this bacteria that express OspC, as you passage them repetitively over time in tissue and in culture, they will lose the plasmid that encodes for this, and that is a problem.

And there are numbers of commercial laboratories right now that have organisms which are simply not expressing this any longer.

The 93 is an antigen which is of import. And we recently went over, at the meeting that Dr. Dennis alluded to, and the committee at that CDC meeting really specified a

number of bands which we felt were important in developing Western Blot criteria. And these included 18, 22, the 23 which we call OspC -- because OspC is a fairly variable molecule, and some papers have put its molecular weight anywhere from 20 to 25; we arbitrarily called it 23 -- a 28 kilodalton antigen, a 30 kilodalton antigen -- which is not in our surface protein antigen -- 39, 41, 60, 66, and 93.

The proposed criteria, which are going to be studied, which is going to be five out of these ten bands on an IgG blot, will be considered to be positive.

The proposed criteria for an IgM blot is that IgM blots are only important in the first month of infection. And one must have two out of three antigens. And the antigens of import for IgM are 41, 39, and the OspC. And one must have two out of the three to be considered positive under those circumstances.

Those are preliminary proposals. I hope I didn't step on the CDC by telling people about that, but I think that that is important.

With regards to a question that I heard from the panel, the OspA band is not included in this, because the immune response to OspA usually only occurs late, and it occurs in only a minority of individuals. So, it would not felt to be a critical band in the development of IgG Western blot criteria. I think that is somewhat important.

Now, there are other problems with serologic samples. Because of the lack of reproducibility, samples run at different times cannot be compared. If one takes a serum sample and divides it, say, into 10 and runs it at 10 different times, the amount of variability in that serum sample is usually quite high, particularly when you use a commercial lab.

So that, if one were to compare two samples, I think that a prerequisite would almost be that it has to be run on the same ELISA plate, one right next to the other.

Another problem which we delineated is, we have been unable to find a correlation between serologic response and clinical response. This is both in early and late disease.

And in fact, in early disease, there seems to be somewhat of a negative correlation, in that individuals who failed to mount a vigorous immune response tend to have a higher risk of subsequent failure.

And as already has been pointed out, rarely patients may fail to mount a measurable immune response. These are generally individuals who have been given antibiotics early in the course of infection and the course has been inadequate. I should emphasize the word, rarely, in here, since seronegative disease are rare for that.

DR. DENNIS: Do those three comments apply to

immunoblot as well as ELISA.

DR. DATTWYLER: Yes.

DR. DENNIS: The inter-assay variability.

DR. DATTWYLER: I think there is less variability in the Western Blot. But again, I think it is important, if one were to do these assays, especially the ELISA, one would do them at the same time.

Western Blots, I don't think that is important, if appropriate controls are used in the assays. I think the key with Western Blotting is to do appropriate controls. And also very critical is what antigen substrate one uses.

One should use a low passage Borrelia burgdorferi for these assays. And if one carefully monitors that, and makes sure that that organism is expressing a full range of its protein antigens -- if has lost, say, the OspC, then it should not be utilized. And I think that that is a key thing.

So, in summary, I think that what we have is that we have, perhaps, an incomplete view of the full range of clinical manifestations. We are now just evolving and standardizing serological tests to the point that they can be utilized. But that is still work in progress that I think the CDC is doing a great job, but we are still not totally there yet.

I would think that we should do epidemiological

studies on large populations of heavily-at-risk people, both following serology and also applying clinical responses, to fully delineate the full range of this disease. I am not sure that I know the answers yet, or anybody does at this point. Why don't I stop there.

DR. DENNIS: Thank you, Dr. Dattwyler. Are there questions from members of the panel.

DR. O'BRIEN: I was concerned about your last slide where you said there was a poor correlation between serologic response and clinical disease. And as I heard you to say, some people who mount better responses get worse disease. Did I hear you say that.

DR. DATTWYLER: No, no, I said the reverse. The better responses tended to have a better response. And I should clarify where this is from. This is from antibiotic trials. These are treatment trials of erythema migrans, in which individuals given an antibiotic regimen which was not optimal -- we didn't know that it was not optimal at the time -- the ones that failed to mount a vigorous immune response tended to do worse, clinically. So, there was an inverse correlation between the degree of serologic response and the outcome.

So, individuals with a poor immune response tend to have worse disease.

DR. O'BRIEN: One other question, back to your

natural history that you presented. There were some patients who were not treated for four years because they were not recognized to have the disease. And this had to do with the development of understanding Lyme disease.

DR. DATTWYLER: Yes.

DR. O'BRIEN: What happened to patients that were treated after four years. How did they, people that had been ill for a long time and then were treated.

DR. DATTWYLER: Actually, the best person to answer that, because I was quoting Dr. Steere's data, is Dr. Steere. I think he can answer that question much better than I can.

DR. STEERE: It is the usual response to antibiotics whenever you treat it, although it may be more difficult later. You may have to treat longer. And neurologic disease requires intravenous antibiotic therapy.

DR. O'BRIEN: Sort of like syphilis.

DR. STEERE: It is like syphilis. But if a person already has deficit -- and particularly neurological deficit -- it usually improves, but it may not become totally normal.

DR. O'BRIEN: Thank you.

DR. DATTWYLER: I must comment, that is our experience as well, in people that present with late disease, that the antibiotic response is usually quite good.

DR. ROOS: There was a question before, about how valid serologic tests are with different types of genotypes. (Portion of question off microphone.) Or making it more broad with respect to serology, how about PCR. There are other kinds of Borrelia which may be not wanted --

DR. DATTWYLER: I don't think we know a full answer to that. We know, from studies in Europe, that there are three genotypes -- genospecies -- that have been defined.

Studies in the United States would suggest there is only one genospecies, but it has really not been studied fully at this particular point in time, and we know that, from tick isolates, that there appear to be some variability in Borrelia from ticks.

Whether these additional isolates can cause human disease, I don't think we know at this particular point in time.

As far as serologic import, there is enough cross reactivity between the various Borrelia at this particular point in time, that serologic assays should pick them up, because the flagellant and the common bacterial antigens are well served across spirochetal species.

With regard to other tick-borne infectious diseases, these ticks certainly carry a number of other things. I guess the best characterized thing would be

lovizia(?), and that can present with a viral like illness after a tick bite.

And it does not produce erythema migrans lesions. There could be some confusion, however, in an individual who presents after a tick bite with a viral-type illness. What is it. And that would create some difficulty in the differential diagnosis. And one would have to back off and look at, perhaps, serologic responses in those circumstances.

DR. LEMON: I wonder if you could speculate a little bit about the pathogenesis in patients with multiple EM lesions. Is this really hematogenous dissemination from a primary site with a single tick bite, or is it possible that these are actually representative of multiple tick exposures.

DR. DATTWYLER: Multiple tick exposures would be, frankly, uncommon. I live in a very highly endemic area, in Suffolk County, Long Island, and multiple tick bites would be, frankly, uncommon.

So, I think, by and large, these represent hematogenous dissemination of the organism.

The other thing in support of that is that individuals with other symptoms and other abnormalities would suggest a systemic disease.

DR. LEMON: There is no evidence that multiple EM

lesions are less common in low prevalence areas, for example.

DR. DATTWYLER: I am not aware of any data that would suggest that.

DR. LEMON: Does that men that the parasitemia is very low level, if you are seeing such a few number of lesions.

DR. DATTWYLER: No. I think that it speaks to the variability of the disease. I should also say that there may be regional differences, because in Connecticut, in Dr. Steere's work, they had a much higher incidence of multiple erythema migrans lesions, than we had observed.

Also, from different regions of the country, we seem to see different incidence of other manifestations, say, carditis.

On Long Island, one sees carditis occurring less than one percent of the time in individuals with erythema migrans. And I have talked to physicians in Connecticut and they report a higher incidence of that.

From treatment trials, which are national based treatment trials of erythema migrans, there appears to perhaps be some regional differences in response to antibiotic therapy.

Now, there is not enough statistical power in those studies to prove that. That is just an off-hand

observation. So, I really don't think we know at this point.

DR. FERRIERI: Could you comment on the universality of the antigenic profiles relative to the European strains and whether one can expect the immune responses to be similar, so that the criteria for WB would be similar.

DR. DATTWYLER: Yes. I think that, fortunately, there is enough shared proteins between various genospecies that the Western Blot criteria should hold up fairly well.

There are some strains in Europe which don't express OspA or OspB, but that is not an important criteria.

There seems to be a universality to the flagella and the common bacterial antigens -- 93, some of the others. So, I think that from that perspective on Western Blots, we are fairly safe.

DR. EICKHOFF: With other tick-borne infections, the tick-borne recipsiosis(?), in particular, a history of tick exposure is highly variable. What proportion of patients, who fit the CDC case definition of Lyme, actually give a history of tick exposure.

DR. DATTWYLER: A little less than half.

DR. EICKHOFF: Just like other tick-borne diseases.

DR. DATTWYLER: And the difficulty in certain

regions, though -- say, Suffolk County -- if you ask the population of a very heavily endemic areas in Suffolk County, have you ever had a tick bite, the answer is yes. So that, it fails to be much of historic import, because tick bites are so common in certain populations.

And I have been bitten by ticks, my children have been bitten by ticks, my wife has been bitten by ticks. We don't have Lyme Disease. So, it is a fairly universal thing.

In our region, one of the scientists at Stonybrook tried to look at dogs to find some negative dogs to study. And he couldn't find any seronegative dogs. All the dogs had been exposed to Borrelia burgdorferi in our area.

DR. ROOS: Just one further point regarding the serological differences. There are some articles in the literature about similarities in the central nervous system, syndromes in European Lyme and American Lyme, but differences regarding at least some aspects of the serological response in the spinal fluid so that oligoclonal bands, I guess, are commonly seen in Europe and rare here, suggesting that there are certain important qualitative differences.

Now, I don't know how much confirmation there is at present in those studies, and whether those qualitative differences also have some quantitative differences and

aspects, and whether you have done Western Blot studies on those European spinal fluids, et cetera.

DR. DATTWYLER: The last question, no, we haven't done studies on the European cerebral spinal fluids. I can tell you, though, that there appears to be a real difference between European serologic responses in the central nervous system and North American responses in the central nervous system.

It is almost universal in Europe to have a serologic response in the CSF and it is uncommon in the United States. And we have individuals who we have PCRed the DNA out of the cerebral spinal fluid, who failed to mount a serologic response, and clinically had Lyme meningitis -- had erythemal migrans and meningitis.

So, I think that the serologic response in the CSF in North American patients is of less value than it is in Europe. It is a less reliable marker of disease. And there, the PCR might be more useful.

DR. BROOME: Two questions. One is just following up on the point of the CSF antibody. Is that using the same serologic tests.

DR. DATTWYLER: Yes.

DR. BROOME: And there is no explanation.

DR. DATTWYLER: There is just no explanation, using the same type of serologic methodologies.

DR. BROOME: I wanted to ask about the variant erythema migrans lesions you showed, and the feasibility of using those as part of a case definition for a vaccine trial. I guess part of it is just the specificity of such variant lesions on clinical grounds.

And the second part is whether people have done biopsies and cultures of such lesions, and is that a reasonable approach for the less typical clinical.

DR. DATTWYLER: The answer is yes, people have done biopsies and cultures of those types of lesions. And yes, they have isolated Borrelia from that type of lesion.

I think, in experienced hands, it is easy to recognize those lesions as erythema migrans. I think the difficulty becomes, in inexperienced hands, if one were to show those slides, blinded, to an experienced dermatologist, I think there would be no trouble in identifying that as erythema migrans. So, I don't see that that is a problem.

I think that most individuals who become used to seeing all the varieties of this would have no difficulty under those circumstances.

DR. LEMON: Perhaps we should move on. Thank you very much. I would like to see if we could have Dr. Steere give his presentation prior to our break. And then, after the break, we can proceed with the manufacturers presentations in open session.

///

Agenda Item: Immune Response to OspA in Natural Infection.

DR. STEERE: Good morning, and thank you for the opportunity to present here. We have been interested in studying the immunopathogenesis of Lyme arthritis. And it is because of that, that we have learned that there is an association between Lyme arthritis and the immune response to outer surface protein A.

That immune response in the natural infection is very unusual. You might even say weird. And I wish that I could tell you today that we understood it, that I could present to you how it works. I cannot do that. All I can do today is show you certain associations. And how this works in the natural infection is something that we don't understand yet.

In a way, it means that this presentation is like work in progress. I apologize for that, but I still wanted to present what is known about the immune response to outer surface protein A in the natural infection.

As you heard from previous speakers, the tick inoculates the spirochete Borrelia burgdorferi, at a site where a skin lesion develops, and then often there is dissemination of the spirochete hematogenously. And that occurs in the first days to the first several weeks of

infection. And one of the sites that, at least in the United States, and at least in New England, there is common hematogenous spread, is to joints.

Usually, that event is rather vague, in terms of joint symptoms, at that time. They seem to be dampened. And it is only months later -- usually within the context of an expanded immune response to the spirochete -- that one gets a picture like this.

This is a child who, six months into the illness, had the sudden onset of marked knee swelling. And one gets huge infusions in this disease.

Typically, they don't last very long, at least in initial attacks. This will often go away within several weeks to at least several months, but it may recur. And it is only at the far end of the spectrum that this lasts for a longer period of time.

This is a study that Ray showed, that we did with patients back when we -- well, it was started in 1976, when we suspected that erythema migrans was a manifestation of this disease, and in which one also got arthritis.

And we simply identified patients with these skin lesions and followed them prospectively to see what would happen. We did not know about antibiotic therapy for the disease or Borrelia burgdorferi at that time.

In about 20 percent of the patients, nothing else

ever happened. They had erythema migrans alone, and it went away.

In about another 20 percent of patients, they did develop mile arthralgia, usually of one joint at a time, only lasting for several days in a given joint, and maybe several episodes of that. And that is all that ever happened in the ensuing months to several years.

More commonly, people did develop this kind of attack of arthritis later, months later. It may be brief -- and there were people with untreated disease who only had one attack of arthritis and never more. But usually it recurred.

It was only at the far end of the spectrum that one saw what we defined as chronic arthritis, more than one year of continuous joint inflammation. The longest that we saw was four years.

This is not a type of arthritis that lasts forever. Even in untreated disease, and even at the far end of the spectrum, the joint involvement eventually resolves, although there may be an erosion of cartilage and bone at this point in the disease, and certainly one would not get a completely normal joint again, after the arthritis resolves.

I think that in roughly 80 percent of these patients, and maybe more, the spirochete did spread to the joint early in the illness.

Well, now maybe the number of spirochetes that got to joints were different, maybe there are differences in virulence of the spirochete that we don't know anything about that.

We have investigated the issues of, are host responses different in these people, and in particular, rheumatologists are interested in immunogenetic markers because so many of the types of arthritis that we deal with have associations with Class I or Class II MHC molecules.

So, we looked at, well, both class I and class II MHC molecules in 80 patients who had Lyme arthritis. And they had a spectrum of disease, including arthritis of short duration which was defined of less than five months, moderate was six to twelve months, and chronic was one to four years, not forever.

There was an increased frequency of HLA DR4 in the patients who were at the severe end of the spectrum, compared to the mild end of the spectrum. And this is the only significant difference, in this group of 80 patients.

There was a suggestion of an increased frequency of HLA DR2, also, in the group with chronic or moderate arthritis. But this was not a significant difference.

If these things are increased in frequency, what was less. And the answer to that was HLA DR5 and maybe DRW6, although the number of patients with that specificity

was small.

At any rate, there seemed to be a suggestion of an increased frequency of DR5 in short duration versus chronic duration. Still, the only significant difference was with HLA DR4.

This next group of observations is about patients with untreated disease, specifically -- well, not that all 80 patients had untreated disease, but it includes patients who had untreated disease, and that is what I would like to show you next.

We were interested in looking at the antibody response in patients with Lyme arthritis. And we were trying to address the issue of, is it different in any way in patients with arthritis of short duration versus arthritis of long duration.

And we had serial sera on some patients that were followed throughout the course of the disease. This would be a patient with severe involvement, disease onset which, incidentally, included both carditis and meningitis which resolved by itself, which it typically does.

Then, one started to see less of a systemic disease and, instead, short attacks of arthritis and then, in the second year of the illness and the third year of the illness, what we called chronic arthritis, which was continuous for a period of four years.

And this is the IgG antibody response to whole spirochetal lysates that went along with that. Typically, the response early on seems to be -- well, I will use the word suppressed. That may not be the correct word. But then, one sees development of the antibody response over a period of months to even several years.

Typically -- and this patient shows it -- there is an early response to OspC, outer surface protein C, the flagellar antigen of the spirochete, and this heat shock protein of the spirochete. And then gradual expansion.

We wondered whether there would be a typical response that one would see with the first attack of arthritis. There is not. Or, we have not been able to identify it. But typically, this is what one would see.

The last point in the expansion of the immune response would be reactivity with outer surface protein A and outer surface protein B of the spirochete.

These are related proteins. They are coded for by the same plasmid, a 49 kilobase plasmid of the spirochete, and those two proteins share 56 percent sequence identity.

This event typically occurred near the beginning of these prolonged episodes of arthritis.

We were next interested in trying to dissect out these responses using recombinant proteins. The bottom first, is the clinical course. And this, again, would be a

patient with severe involvement, disease onset -- which also included neurologic involvement, but it went away. Remember, this is all untreated disease.

Then, during the second year of the illness was the period of arthritis, which there was some fluctuation but he had continuous involvement for about a one-year period, and then it went away.

During the sixth year of the illness and through the eighth year of the illness, he had a chronic encephalopathy, and he was treated with antibiotic therapy at this point in the illness.

First, I would like for you to focus on the IgM and the IgG response to these non-outer-surface proteins -- p39, p41, and p93. These are probably the immunodominant proteins that are non outer surface proteins.

One does see an IgM response to these proteins and then, quickly, as one would expect, an IgG response, which just remains high throughout these years of the illness.

The responses to the outer surface proteins are somewhat different -- this is OspA, OspB, OspC. And incidentally, there are other outer surface proteins. We have not been able to find an antibody response yet in anyone to outer surface protein D, but outer surface proteins E and F have now been described, and there may be more as well.

But at any rate, this is what we were able to measure. We were actually somewhat surprised to find an IgM response to all of these outer surface proteins early in the illness. But it is even somewhat difficult -- well, maybe I should point this out first. One does then see an IgG response to outer surface protein C, which is shown here. But it does increase some during the period of arthritis, and then falls off.

The point that I really want to point out is that there is an IgM response to outer surface protein A early in the infection, but then no IgG response until two years into the illness, when one gets a marked IgG response to outer surface protein A, during this period of arthritis, which decays somewhat over the coming years.

I would like to focus particularly, now, on outer surface protein A.

This is the same patient. And now we are looking at his antibody response to outer surface protein A, using a full length recombinant protein, but also truncated fragments of the protein that divided roughly into thirds.

By the way, this is an HLA DR4 positive patient, and is an example of someone at the severe end of the spectrum.

One sees an IgM response to outer surface protein A, and to all fragments of it, suggesting that there are

epitopes throughout the protein that are being recognized early on.

Then this delay into the second year of illness, when there is a marked response to outer surface protein A, and often the part of it that is highest is to this third fragment, the C terminal end of the protein which is the part that, in mice, seems to provide protection, although one also sees epitopes recognized in other parts of the protein, less so in the middle of the protein.

This is another example, also, an HLA DR4 positive patient. We looked at his course earlier -- disease onset, short attacks of arthritis, prolonged periods of arthritis. Maybe it should be remembered -- or you wouldn't know this -- that these courses were drawn long before it was possible to measure outer surface protein A responses with recombinant proteins.

And we were really amazed to see this early IgM response, but then, several years into the illness, a marked IgG response that parallels the course of the arthritis. And one is also seeing an IgM response at the same time, in a lot of patients that parallels the course of the arthritis.

DR. LEMON: Dr. Steere, I wonder if I could ask you a question about, I guess it is three back, the color slide with the multiple panels.

I am not too clear on one point. In the second panel from the top, where you are showing the p39 and p41, and I guess p93 responses, are those tests being done in a linear range so that we are not seeing any increases in antibodies against these antigens, or are these tests saturated at the maximum points on that graph.

DR. STEERE: We have not done all of these points by dilutions, and consequently, it is difficult for me to answer that with certainty. But I think they are in a saturated range.

DR. LEMON: Thank you.

DR. STEERE: Here is another example of a course with disease onset, some shorter attacks of arthritis and, in the second year of the illness, prolonged episodes of arthritis. And then, in the fourth and fifth year of the illness, prolonged episodes of arthritis.

Again, this clinical course was drawn long before it was possible to measure the response to outer surface protein A.

One does see -- we are catching, perhaps, the end of the IgM response early on. Then one sees a marked IgM response to the spirochete, along with a marked IgG response, during this period of arthritis. Those responses are dampened during these years of the illness. In the fourth and fifth year of the illness there is, again, a

marked response to outer surface protein A, primarily an IgG response.

I have been asked, could this be due to re-infection, that one would see a picture like this. I don't think so for two reasons. One is, this is a classic clinical course to see in prolonged infection. But also, the response to the other antigens of the spirochete, one is seeing an early IgM response, and then an IgG response that just persists.

It is this response to outer surface protein A that has these marked peaks and valleys.

We wondered whether new epitopes of the spirochete were being recognized as a way of explaining this is the reason these studies were done with fragments of the spirochete. But we have really not been able to identify any pattern.

Most patients have had responses to epitopes on all three fragments of the spirochete, both early and late.

This would be -- actually, all three patients that I showed you previously are HLA DR4 positive patients. This is a patient who has the HLA DR6 specificity. And actually, this is an example of a patient who we would have said is negative for reactivity with outer surface protein A.

And incidentally, during the arthritis period of the illness, roughly 60 to 70 percent of the patients, one

does show a response to outer surface protein A, and in roughly 30 percent one does not.

But this would be an example of someone who we would have said would be in that 30 percent.

And yet, when you have serial sera, and you look at them over time, there is still something of a suggestion of an early IgM response to the spirochete and an IgG response during the period that one is having arthritis, albeit briefer attacks than the patients that I showed previously.

In a statistical analysis, when one correlated the height of the IgA antibody response with the total duration of arthritis, according to HLA DR specificity, there was quite a significant association between the height of that response and the duration of arthritis.

So, one could say that, the higher the antibody response to outer surface protein A, the longer the duration of arthritis. And that statistical correlation was dependent primarily on patients with the HLA DR4 specificity.

We now treat this disease with antibiotic therapy. And most patients respond. These are patients with Lyme arthritis -- swollen knees. They were randomized to either receive doxycycline or amoxycillin and probenecid, standard doses, for a one-month period.

Many of the patients had resolution of arthritis during that period of antibiotic therapy, or during the subsequent two or three months.

There were some patients -- a few patients -- however, in whom it took longer. So, this would be an example, in some patients in whom it took much longer.

At the time, we were not able to do PCR testing as a way of showing whether the spirochete were in the joint. We have now been able to go back and do that. And what we have learned is that, prior to antibiotic therapy, in the great majority of patients, one is able to show spirochetal gene segments, and specifically the OspA genome, prior to antibiotic therapy.

Afterwards, in these patients, we were no longer able to show the OspA genome in synovial fluid.

These patients were still treated with antibiotic therapy multiple times, with the idea that perhaps there are spirochetal organisms that are persisting and, if we treat with antibiotic therapy, we will be able to eradicate them. In these patients, it seemed like we were doing nothing.

I do think that the spirochete must be in the joint to get the process started, but there is a small percentage of patients in whom the arthritis seems to go on for a period of time after antibiotic therapy.

We were interested in the hypothesis of, is the

hypothesis of, is the immune response different in these people compared with these people. And we supplemented this with other people that we knew of, who had had persistent arthritis despite antibiotic therapy.

There is also an increased frequency of HLA DR4, in this group of people.

And as the final thing that I would like to present today is looking at the T cell response in patients with treatment-responsive arthritis, which this is an example of, versus what I will call treatment-resistant arthritis, the sort that goes on after apparent eradication of the spirochete from the joint.

These are T cell lines that are generated from synovial fluid by growing them with Borrelia burgdorferi lysates. It is through multiple cycles and it is a process that requires about three months to grow the lines.

So, this is an example of the lines that could be generated that were B. burgdorferi specific from joint fluid in this particular patient.

And we have been able to test them against five recombinant proteins to try to identify what their specificity is.

The important point is here. We have had a very difficult time generating OspA reactive T cell lines from anyone with treatment responsive arthritis.

One can often generate lines against OspB. This particular patient also had a lot of lines that would could generate against the p39 protein of a spirochete.

In contrast, here is a person with treatment resistant disease. The major things that we have been able to find, the major specificities, are T cell lines at OspC, OspA and OspB, and particularly, OspA-reactive lines.

The majority of lines that we have been able to generate see this protein.

This type of analysis is very time consuming and labor intensive. And we have been able to do it so far in four patients with treatment responsive disease and in five patients with what we are also calling treatment resistant disease.

The one difference that we can find in these patients is this. It is difficult to find T cell lines that see OspA in treatment responsive patients where the majority of lines have that specificity in treatment-resistant disease.

To a much lesser degree, one sees some difference in OspB reactivity as well.

What does all of this mean, in terms of vaccine development, against immunizing a large number of people with the OspA protein.

The first thing that we asked ourselves and

wondered about was, would patients immunized in this way be more likely to develop arthritis.

And it seems that the answer to that question is no, that the spirochete needs to be in the joint for this process to go on.

And in essence, one needs both. One needs the spirochete in the joint, and apparently an immune response to it, of this sort, in order to have prolonged episodes in this sort of treatment resistant course.

Since the spirochete is not in the joint in patients who are vaccinated, might this ever be a problem with the vaccine.

The one question that I would ask is, how long with the immunity last with the vaccine. Is it three years, is it ten years, is it longer than that. But how about in the patient in whom immunity has waned. And let's say they would acquire, then, possibly the natural infection again, but they have been immunized previously.

Would the fact that one has generated, with the vaccine, this type of OspA reactive T cell line, would that then potentiate the arthritis in a patient who acquires the disease after vaccination, after the vaccine response has waned.

As I said, this is really work in progress, and I wish it were possible to explain the mechanisms better.

That is what we are working on. Thank you very much.

DR. LEMON: Thank you Dr. Steere. I am sure there will be a number of questions for you.

I wonder if I can just start off by asking you to comment on animal models that have been developed for Lyme Disease. Are any of these models relevant to this complication of long disease that you are referring to.

DR. STEERE: I don't know that we really know. What we know from animal models is that it takes a live spirochete in order to induce arthritis in a mouse. And I believe the same thing is true of a person, in a human.

We also know that the severity of the arthritis varies in mice according to certain inbred strains, and that the number of spirochetes that they are permissive to is a factor in that, and it may well be in people as well. But that type of information we don't have in humans.

What we don't know in mice is whether, if you treat them with antibiotic therapy, you can find a subset in whom the subset progresses over a period of months even to several years, despite antibiotic therapy and despite presumed eradication of the spirochete. That is simply not known yet.

DR. LEMON: I guess my concern would be that, since mice don't have the DR4 marker, that what we might be seeing in mice is simply the response of acute arthritis,

and arthralgias that we see in patients.

DR. STEERE: Absolutely, and it might take a transgenic mouse with the human HLA DR4 gene to actually produce the same situation.

DR. JOHNSTON: I think you had data on this point, but I didn't get it clearly. When you detect T cells sensitized to OspA, is it possible that you are dealing -- that that is a consequence of the fact that you have got a protracted arthritis. You have got an immune response that is amplified and you are getting a kind of polyclonal response to an otherwise -- at the cell level, to an otherwise rather relatively weakly antigenic protein.

In other words, I think you did look -- you showed us a slide that showed the T cell responses to a variety of antigens. Was OspA clearly different than the response to C or B or other antigens.

DR. STEERE: Well, these are generating T cell lines from synovial fluid. The thing that we can say is that, in patients who have had what we are defining as a treatment resistent course, the major thing that we have been able to find is T cells that react with OspA and OspB of the spirochete.

Now, in patients with treatment responsive disease, which is the majority, what is different. We have had a very hard time finding anyone who has T cells that see

OspA.

DR. JOHNSTON: And that sampling for comparison was done at exactly comparable times in those two patients.

DR. STEERE: I would emphasize that this is still quite a small number of patients, and I would also emphasize how labor intensive doing this type of analysis is.

The one thing that we can say is that, in the treatment responsive patients, we were able to show, prior to treatment, that they have spirochetal genome in joint fluid. And in these patients with treatment-resistant disease, after treatment they were no longer able to show evidence of the spirochetal genome in synovial fluid.

And instead, what we see is marked T cell reactivity with OspA and OspB. Now, this is in this small number of patients. Whether it will hold up is another story.

DR. JOHNSTON: But you concluded that, to have the chronic arthritis, you still had to have the spirochete there.

DR. STEERE: I think it has to be there to trigger it. But this suggests that if you kill it with antibiotic therapy, that in these patients, it is a problem to turn off the immune response, and that it takes some time for that to happen.

In other words, it seems to be hard to turn it on

in the natural infection. You get it and then, for some reason, it is dampened -- I will use that word, I don't know if that is the mechanism, but it looks like that.

And then, that is the case for several years. And then, at the beginning of the period of long episodes of arthritis, one sees a huge response to OspA. And at that point, it certainly doesn't look like it is a poor immunogen. It is dominating the response -- it seems to be dominating the response, at that point.

And then, once you get it turned on, in genetically-susceptible people, it is hard to get it turned off. Now, what is the mechanism of that.

I mean, we don't have another infection that you can point to where that has been worked out, where that is the equivalent so you could say, well, in some other disease it was such and such.

I mean, you can make postulates about why that might be. You can postulate that something about OspA within the joint is close to us, and that that response needs to be dampened. And within the cytokine-rich milieu of the joint, perhaps that becomes up-regulated and that is something that is close to us -- I will even use the word, auto-reactivity, but maybe I shouldn't -- is hard to get turned off. But that may not be the mechanism.

DR. JOHNSTON: Just as an aside, and another quick

question to keep going as far as I can on this, one of the exciting things about this disease is that you have the potential of making a relationship that we haven't been able to make with other kinds of arthritis, in which it has been postulated that there may be a microorganism at the base.

We have the end results. We have relationships to certain HLA types, but no organism to deal with.

In the situation in which you have the chronic arthritis and PCR negativity, is it possible that the organism is in a macrophage or it is in a non-phagocytic cell, it is still there, it is still eliciting cytokine response, et cetera.

DR. STEERE: Sure, absolutely. It is possible. I mean, we really do not know how this organism survives in its protected niches. There are some in vitro systems to suggest that the organism can survive within macrophages. But it is really not clear that they survive very long there. The studies are 24 hours.

So, in looking in vivo at tissue, I mean, no one has been able to see that. And consequently, it is really not clear that this organism survives because of an intracellular location.

And I think one can say the same thing in syphilis, that there is an organism that is able to survive within the immune system of some people for 30 years. But

how it does it is really not clear.

But sure, it is possible that the organism is still there.

DR. LEMON: Dr. Boone has a question and then Dr. Karzon.

DR. BROOME: Some clarification of the studies that you presented with OspA linked with treatment resistant disease. Could you break the T cell line data down by patient. As I understand it, you summed all the results.

DR. STEERE: Well, the slide I showed was examples of individual patients.

DR. BROOME: No, but I mean, the four versus the five.

DR. STEERE: Yes. Could I break it down.

DR. BROOME: How many of the five with resistant disease had the about 50 percent response.

DR. STEERE: Four of the five did. In one of the five, it was less than that. Still, what we were able to identify were OspA and OspB reactive clones. But most of the clones in that patient, we were not able to identify what their reactivity was toward.

DR. BROOME: And the treatment responsive had the five out of the eighty-seven break out.

DR. STEERE: Regarding the reactivity with OspA. In those people we were only able to identify one or two

clones that reacted with OspA.

And actually, we have not really been able to propagate them. Where the work is now that we are trying to identify the T cell epitopes that those patients see, and we have had trouble with being able to even grow the C cell lines for prolonged periods of time, in the treatment responsive group, with the few lines that we have had.

DR. BROOME: In the correlation slide that you showed, you just had a correlation coefficient of .54, which was statistically significant, but is not very strong. I wondered if you could tell us, first of all, is there one point per patient, what were the percent with the response, you know, looking at it from different --

DR. STEERE: It varied from patient to patient how many sera we had. And so, that is a factor. What we did was to pick the high point that we knew of for a given patient. And there were patients in whom we only knew of one point. And I think that may be one reason why the statistical association is not as strong as it might be.

DR. BROOME: But there are multiple points for some patients.

DR. STEERE: It includes the slides that I showed you.

DR. BROOME: I guess -- I think you can get a lot of information in different ways, but that also means that a

lot of the points aren't independent. So, if you were breaking it out both by something that used a value of just one per patient, as well as the way you have done it.

DR. STEERE: For instance, the first point, or which one would you take.

DR. BROOME: That is why I was interested in how you had done it, because I think it is sort of tricky.

DR. STEERE: We took the high point.

DR. BROOME: The high point of the OspA response, and then whether or not that was the point at which the patient had chronic arthritis.

DR. STEERE: Well, I mean, yes, that is when patients had chronic arthritis, that had chronic arthritis.

DR. KARZON: If I could draw the general paradigm that replicating organism and non-replicating antigens have different antibody responses; if you give non-replicating antigens you can make very good antibody response, and yet the organism fails to do so generally.

Now, if that is true, one could pose several questions that might be approached experimentally. For example, there are other instances in microbiology, where probably this is true as mediated by the type of T helper cell which is invoked, and the consequent cytokine patterns that have different pathogenetic consequences.

You mentioned one risk situation. Would another

risk situation of getting vaccine be in a low grade or an old or non-recognized disease, where live spirochetes are present, and you invoke and induce a large amount of anti-OspA antibody. Could that be a stimulus for pathology.

But one could design experiments to approach this sort of question through looking at various mechanisms of this sort.

We talk about the mouse model, which has certain features which may or may not resemble man in their delicacy. I would like to learn more about the rhesus macaque model, and might that be a good system to work on.

DR. STEERE: I mean, I would be very interested in that, too, and I don't know the answer. And I don't really think that it is known whether a particular monkey has the equivalent of our HAL DR4 allele.

And actually, maybe it is important to point out as well that the HLA DR4 specificity is a grouping, and there are many subgroups of HLA DR4. And in rheumatoid arthritis, as an example, some of the subtypes are susceptible to the disease and others are not at all.

So, actually the molecular basis of the HLA DR4 susceptibility isn't known here.

DR. LEMON: I have one question. I find quite impressive the association that you are showing between the antibody ELISAs to OspA in the chronic long term arthritis,

even with the statistical questions that Claire is posing.

But I am still concerned that maybe there is a global response to multiple antigens that may be missed, because it looked to me on the immunoblot as if other bands were also much stronger in those sera, that had the strong OspA reactivity.

And if the ELISA test were really at a saturating level, can you assure us that there are not hundred-fold increases to other antigens accompanying those OspA increases in those sera.

DR. STEERE: Can I assure you. Well, it is really what I said before. We have not done limiting dilution studies of all of these points. To the degree that we have done limiting dilution studies, I think we are at saturation levels. But the point is still well taken.

There are antigens that we are not measuring here. And that is why I mentioned about OspE and F. And there are supposed to be more outer surface proteins than that. So, no, that is not everything that is going on.

DR. ROOS: Are there other studies in other countries, even, looking at DR4 overrepresentation and chronic arthritis and Lyme disease.

DR. STEERE: No, there are not. And actually, I would like to point out that what we are observing here may be a phenomenon of this disease with group one strains in

the United States, or within New England.

It is not clear that the equivalent process happens in Europe. And as a matter of fact, finding an immune response to outer surface protein A and the European disease at all is unusual. And apparently, or Sobatina Wilska tells me, that she has not seen a response to OspB in the European disease.

Well, we see these huge responses during the period of arthritis, to OspA and OspB.

DR. LEMON: I guess there are no other questions at this time, and that works out perfectly because we are just five minutes behind our scheduled break. Thank you very much, Dr. Steere.

Let's take a break and reconvene in 15 minutes.

(Brief recess.)

DR. LEMON: We will continue now with the first presentation by the manufacturers, and that will be by SmithKline Beecham.

Agenda Item: Presentation by SmithKline Beecham.

DR. MATRIONE: I would like to introduce Dr. Michel DeWilde, who is vice president of SmithKline Beecham Biological's research and development department.

Dr. DeWilde will overview the approach SmithKline has taken, toward the development of a Lyme vaccine, briefly reviewing some of our supportive pre-clinical information.

I would like to remind the committee that confidential preclinical information, as well as confidential clinical information will be presented in closed session this afternoon.

DR. DE WILDE: Thank you. It is an honor for me to have an opportunity to present to you, briefly, this morning, some of the rationale that led us to choose one of the major surface proteins as a bacteria, namely, the OspA gene product, as a candidate for vaccine development.

A few historical considerations maybe first. Even so, it is already 1985, when Bob, in his science paper, pointed to the use of OspA as a potential vaccine target. It is not until 1980 that the first positive evidence were gathered.

Originally, German groups in Frieburgh and Eidelburgh, using an animal model that is developed, which consisted of using SCID mice as a model for both infection and disease -- I may want to point out here one further piece to the puzzle, that these type of mice who are not able to mount an immune response to the antigen do develop symptoms.

So, in this model, using monoclonal antibody directed against the OspA protein, as well as using animal sera, raised again a recombinant form of OspA. Those workers were able to demonstrate the protective efficacy in

this particular model.

Shortly thereafter, the group at Yale, using this time a C3H model that was already alluded to this morning, demonstrated active protection following active immunization with recombinant OspA protein.

Further evidence were gathered later on. Those experiments that I mentioned made use of syringe as a mode of challenge with the spirochete. And further evidence was gathered, again by the Yale group more recently, using a more natural route of challenge -- that is, using for a challenge things that were fed infected animals.

And also, the Yale group made a very interesting observation which is, upon feeding vaccinated animals, the ticks were actually cleared from detectable viable spirochetes.

As was already mentioned this morning, and it is certainly one of the challenges of using the antigen of the nature of OspA as a target, is the issue of variability.

Based on different typing techniques, both immunological or genetic, one today recognizes three so-called genospecies -- B. burgdorferi, B. garinii, and B. afzelli. The vast majority of strains that have been collected in the United States, indeed, belong to burgdorferi genospecies.

Looking in more detail to the actual sequence of

the OspA gene in some of the isolate, this is represented in the red here in this venn diagram, that this is an actual distance in between different strains, different sequences.

All the U.S. isolates are presented in red on this slide, and you can see that, indeed, variation in sequence in amino acid sequences is very limited, with the notable exception of one strain that has been isolated, which is right here, 25 015.

In contrast to that, in the rest of the world, or in Europe, I should say, there are two other genospecies, and for instance, here in Germany you can also see that the diversity or the heterogeneity of sequence in this group is considerably higher than in the American isolate, up here, as depicted by greater distances between the two sequences.

Even so, these are groups that are found in Europe. You also find isolates in Europe, and namely the ZS7 isolate here, which is a German isolate that we actually used for further development. It gives you a better feel for the differences between ZS7 here and the prototypic b31 U.S. isolate is actually three amino acid differences among the protein sequence.

On the next slide, we indeed choose ETA(?)-7 as the source of our sequence, and produce recombinant protein in E. coli. These proteins were then purified through near homogeneity, as you can see here on this chromatin stained

gel. And in the following couple of slides I will give you examples of some of the studies that were done. I have some additional ones in the committee's briefing document but I thought I would focus this morning on two experiments, both of them, as I said, in an attempt to be as close as possible to the natural situation for the efficacy studies in animal models used as a way of challenge ticks that were fed infected animals.

In this particular experiment, three sets of mice were used, of differing susceptibility to disease, and were vaccinated with recombinant OspA protein and OspA protein and compared in this little control group.

They received three immunizations prior to challenge, the result of which was assessed by culture of the spirochetes from biopsies. And as you can see, all the control animals were positive by that criteria, whereas protection was complete in the vaccinated animal.

Also, as estimated by immunofluorescence assay on the ticks that were recovered after challenge, or after dropping off of the animals. You can see by the number of positives here, that there is quite a significant clearing of the infectious bacteria from the ticks, once they have fed a vaccinated animal, again, pointing to a somewhat unusual, or eventually unexpected mechanism of protection in this setting.

The next slide is, again, challenge experiment using infected ticks. But this time, to go a step further closer to the natural situation, ticks were actually collected in nature and then applied to the animals. And this is why obviously the infectious rate in the control group is not 100 percent, since 10 to 20 percent of the ticks are infected out here where these are collected.

Despite that, I think the experiment is quite conclusive. As you can see, either looking at seroconversion by Western blot focusing on p39 as one of the sensitive markers as well as by culture, we observed full protection in the vaccinated animal and, again, here, as assessed by culture of the ticks that fell off the animals and were recovered, complete clearly by the criteria of the ticks that fed on the vaccinated animals.

So, I think this is the essence of what I have to say. I think that, on the base of this data, that indicate that for recombinant OspA is, indeed, protective in this system. I think the data are consistent with developing it further.

They are also consistent with antibody as the mechanism of protection in this system. Also to point out -- I may not have done this point clear enough in the presentation -- but those in the two challenge experiments, obviously, there were differences even from within the

burgdorferi genogroups, there were differences between the immunogen and the challenge organism. Thank you.

DR. LEMON: Thank you. Are there questions for Dr. DeWilde.

DR. JOHNSTON: Do you know what happens to the infected ticks. Do the ticks fall off, or are the ticks still there and they don't have the Borrelia in them.

DR. DE WILDE: Excuse me.

DR. JOHNSTON: You had a reduction in the number of infected ticks. How do you explain that. Do the ticks fall off, or do the ticks stay there and the Borrelia is gone.

DR. DE WILDE: No, those ticks feed normally, and so actually, experimentally, they are collected in the bottom of the cage in water, and obviously we compare the situation between the ticks that fed on the control animals. They don't fall off if they are not fed to repletion, and there you have 100 percent infection.

I think the most likely mechanism is that, during the course of a blood meal, which takes a substantial amount of time actually, to take up the antibodies and the bactericidal mechanism must take place within the tick itself.

DR. BROOME: The graph that you showed of the OspA, could you tell us where the 13 U.S. strains came from,

what is the distribution geographically over time, et cetera.

DR. DE WILDE: I will have to confess that I cannot. Maybe my molecular biologist colleague can help me. While I know for a fact a portion of p31, also the CA strain, which is this one, and CS7, CA8, and maybe, as in a comment made this morning, there may be further variation on the west coast, 25 or 15 is the strain collected by year, but I am not sure of the location in the east coast. While all the rest are east coast, those that are now CA.

DR. BROOME: Also, this is presumably based on sequence variability.

DR. DE WILDE: Yes, this is protein sequence.

DR. BROOME: How does that match up with antigenic homology.

DR. DE WILDE: There is full cross reactivity at the antigenic level. Using the reagent that we know of, it is quite possible. Actually, I would not call them hot spots at this point, because they are not that hot, but they are in a position that usually you find the differences. But we don't have monoclonal antibodies which would distinguish the strains.

DR. BROOME: It would be nice to know over how many years were the strains collected, and do they represent a good geographic distribution. I mean, it looks very good.

DR. DE WILDE: I can look it up in the computer but I think it is more of a historical isolate, and others are fairly recent isolates. So, it does span the time this work is going on, for sure.

DR. ROOS: On one of the slides it says, the average number of spirochetes per tick.

DR. DE WILDE: Yes.

DR. ROOS: And how did you assess that subject.

DR. DE WILDE: Well, it is a fairly crude assessment that was done by Dr. Fikrig at Yale. It is based on how much bacteria he actually recovers, after culturing for a fixed period of time. And from there, that is assessing the initial inoculants of OspB.

DR. ROOS: So, that is the result of a culture.

DR. DE WILDER: It is by culture.

DR. ROOS: By titer.

DR. DE WILDER: In that case, yes. This assessment is by culture, yes.

DR. FERRIERI: Despite the sequence variability, do we know whether the protective epitopes for OspA are similar for B. garinii and B. afzelli, compared to burgdorferi.

DR. DE WILDE: From the published literature, there is actually no cross protection between genospecies, as assessed by needle challenges. I know tick challenges

are ongoing, but those data have not published.

It could actually be that the vector, itself, has an impact on the mechanics of all this. That is why we like to use ticks as our system of challenge.

DR. LEMON: If there are no further questions, we should move on, then. Thank you very much, Dr. DeWilde.

The next presentation will be by Connaught. Dr. Six will begin this presentation.

Agenda Item: Presentation by Connaught.

DR. SIX: I would like to thank the members of CBER and the advisory committee, for giving us the opportunity to talk about our vaccine project for Lyme disease.

Connaught became interested in developing a vaccine about five years ago. We have chosen OspA as the candidate antigen for that vaccine. And John Mays is going to describe some of our rationale and the animal data that led us to that conclusion.

Don Marks will then follow and will present the data from our phase I trial and from one of our early phase II trials. This afternoon, we will be reviewing the status of trials that are currently ongoing and some animal model studies that we have done with the committee.

With that, I would like to introduce John Mays.

DR. MAYS: As Dr. Six said, we began our work on

development of a Lyme vaccine back in 1989, first, focusing on the whole cell organism, and then quickly moving into the acellular fractionations of the organism. And finally, in 1990, we established collaborations with Dr. Allen Barber at the University of Texas, and Dr. Sven Bergstrom at the University of Umio(?) in Sweden.

As Dr. DeWilde pointed out, there is a wealth of scientific literature that clearly demonstrates that antibodies against the outer surface protein A are protected in the mouse model, in the gerbil model, in the guinea pig, and in the rat.

We have used, for a lot of our preclinical studies, the mouse model using the C3H as described earlier. We have concentrated our work on the unabsorbed vaccine formulations using the lipidated form of the outer surface protein A. And we feel that this protein alone is sufficient for priming the immune system so that, after two doses, you have a very strong immune response.

Also, our animal experiments, working with Dr. Barber, have clearly demonstrated that the lipidated form of the OspA antigen is non pyrogenic, it is very immunogenic and, as you will see in a minute, it is also protective.

Our current vaccine that is in clinical trials is a lipidated form of OspA. It is a highly purified recombinant protein, that is identical in gene sequence and

in amino acid sequence to the lipidated OspA from Borrelia burgdorferi.

This is a protocol describing the routine mouse experiments that we perform, in collaboration with Dr. Allen Barber. The protocols are randomized placebo controlled.

In this study, we used five vaccine formulations with placebo thrown in. Two of the vaccine formulations were lipidated OspA absorbed to alum as an adjuvant. And the other three vaccine formulations were various concentrations of the lipidated OspA.

Our vaccine schedule, in all of our animal studies, is typically two doses at three or four-week intervals.

Following the two doses, all the mice are challenged by subcutaneous syringe challenge. And in our challenge we used 10^4 Borrelia burgdorferi spirochetes that are of a heterologous Borrelia burgdorferi strain, from our vaccine. These occur three weeks post-dose-two.

In all our mouse experiments to date, all our mice show no increase in IgG titers directed against the lipidated form of OspA early in infection, and after challenge.

We have not taken these challenge studies out for very long periods of time, because the animals are euthanised.

All of our vaccinated mice show rises in IgG titers directed against the lipidated OspA at each dose, and also the functional antibody as measured by an in vitro growth inhibition assay that is performed in Dr. Barber's lab, shows that we have very good functional antibody response.

All of our control mice are culture positive from all of the tissues sampled, which includes blood, joint, bladder and heart. And they are culture positive at all time points.

And again, all of our vaccinated mice are culture negative from all the tissues that we examined.

With this information, we moved into phase I studies and phase II studies. And Dr. Marks will present that information now.

DR. MARKS: Good morning. Today I will report on clinical trials Connaught Laboratories has performed on outer surface protein A Lyme vaccine.

First, I will present data on our phase I trial of recombinant outer surface protein A.

We used the University of New Mexico as the study center, to avoid background positive changes in Lyme serology during the study. The design of the study was randomized, double blind, and placebo controlled. We evaluated one dose -- 10 micrograms -- with and without

adjuvant, which was alum, and we evaluated placebo.

The immunization schedule was two doses given 30 days apart. Individuals in the non-adjuvant and OspA group received a third dose 20 weeks after the second dose.

The primary end point of this study was safety and the secondary end point was immunogenicity. The data which I will show demonstrates acceptable safety and immunogenicity of outer surface protein A Lyme vaccine.

There were 12 subjects randomized to each group. The age, sex, and ethnic origins of the three groups were similar. Two doses of both outer surface A Lyme vaccine formulations were well tolerated, as was a third dose of non-absorbed vaccine.

Local adverse events were primarily pain and tenderness at the injection site, which began within 24 hours after vaccination, were of mild severity and resolved spontaneously.

The majority of systemic adverse events, were reported within the first 72 hours. None were severe, and all resolved spontaneously.

The local and systemic adverse event profile was similar to that seen with other adult vaccines. Headache and joint ache, or pain, were the most common reported systemic adverse events.

The adverse event profile of the third dose

appeared to be the same as for the first two doses.

There was no significant difference in immunogenicity between the OspA groups. This is the placebo group, lipidated OspA, and lipidated OspA with adjuvant. And these are the GMTs, post-dose one, post-dose two, and post-dose three. And there is no difference between these numbers.

We found up to a 100 percent response rate as defined by a four-fold increase in titer.

We observed a good amnestic response for the second and third doses. This is the response of the second dose compared to the first, and of the third dose compared to the second.

The antibody response profile is similar to that seen in animal studies, which have demonstrated protection.

The antibody generated is functional. CLI, working with Dr. Allen Barber at the University of Texas as a collaborator, show that the antibody generated has neutralizing activity.

Based on these promising results, we conducted a randomized, double blind, placebo controlled escalating dose study of outer surface protein A Lyme vaccine in four centers.

The doses used were one, five, ten, and thirty micrograms, on adjuvant of lipidated outer surface protein A

lyme vaccine and placebo.

Three hundred and thirty-seven seronegative individuals participated in this study. We again evaluated two doses 30 days apart.

The primary end point of this study was immunogenicity, and a secondary end point was safety.

There were 67 to 69 individuals per group. The age, sex, and ethnic origins of the five groups were uniform.

The OspA Lyme vaccine, 30 days post-dose 2, generated a good dose response relationship with increasing doses of OspA, as represented by the closed circle. This is the GMT anti-OspA ELISA, 32 days post-dose 2, with increasing ELISA and increasing doses of OspA. And this curve with the closed circles corresponds so there is a very good dose response.

A good dose response is also seen when looking at the percentage of subjects demonstrating a four-fold rise in OspA titer, as represented by the open boxes. This might be a little bit difficult to see in the light, but there is a second curve going up to 98 percent response rate in individuals who received the highest dose of OspA, and this Y axis is percentage of individuals exhibiting a four-fold response.

All doses of OspA vaccine were well tolerated.

The most common reported local adverse events, again, were pain and tenderness at the injection site. Local reactions usually occurred with less frequency post-dose 2.

All OspA vaccine and placebo groups had similar systemic adverse event rates. The most common systemic adverse events reported by individuals in all groups -- vaccines and placebo -- were headache, fatigue and joint pain. All adverse events resolved spontaneously, or with symptomatic treatment -- for example, a non-steroidal anti-inflammatory drugs -- and none were severe.

Chronic arthritis can be a systemic complication of Lyme disease. Because of the published observations implying a temporal relationship between OspA and Lyme arthritis, we searched for any possible relationship between OspA and joint pain.

This slide shows the frequency of reported joint pain in individuals who received one of the four doses of OspA -- these are increasing doses -- one, five, ten and thirty -- or a placebo. And the frequency of the reported joint pain is listed for the first and the second dose in each of the groups. And these are days post-vaccination up to day ten.

As shown on this slide, the percent subjects reporting joint pain ranged from zero to seven percent. Joint pain occurred with equal frequency in all OspA groups

and placebo -- one, five, ten, and thirty and placebo, equal frequency of joint pain.

In fact, there is no difference in the frequency of joint pain between the highest dose of OspA and the placebo group -- 30 micrograms and placebo.

This is unlike the immune response that I just showed in which there was clearly seen a dose effect, both in the phase I and phase II studies. Since there is no dose effect with joint pain, and since there is as much reported joint pain in the placebo group, as in any vaccine group, the highest and the lowest, therefore, we think that there is no reason to suspect an immunologic relationship to the occurrence of joint pain. Nonetheless, we continue to evaluate this issue.

It is our conclusion, based on our clinical experience in more than 400 OspA Lyme vaccine recipients, that there is no relationship between vaccination with a recombinant lipidated OspA Lyme vaccine and the occurrence of joint pain.

In summary, Dr. Mays and I have presented data showing that lipidated OspA vaccine is safe, immunogenic and protective in animals.

Lipidated OspA Lyme vaccine is safe and immunogenic in humans.

Based upon the data presented today, we are

currently performing a large scale, placebo-controlled efficacy study, which will be reported in the following closed session. Thank you.

DR. LEMON: Thank you, Dr. Mays. Are there questions for Dr. Mays from members of the panel, or for Dr. Marks, either individual.

DR. ROOS: Were there any differences in the severity of joint pain among the different groups.

DR. MARKS: No, as a matter of fact, there was not. I think in some of the instances where investigators called and had reports of rather marked joint pain, and we broke the code immediately for those individuals, some of the most severe cases were the placebo groups. But there was not a relationship between severity and the dose received.

DR. EICKHOFF: The ELISA titers that you showed, are they measuring IgG or IgM or both.

DR. MARKS: IgG.

DR. EICKHOFF: Second, could you put some numbers around the vaccine side effects profile. You used words like infrequent or most common, or not severe. But what do the numbers actually look like.

DR. MARKS: We do have some back-up slides that I could show that discuss the adverse event rates local and systemic. If you like, I could show an overhead of a local

adverse event -- pain, tenderness, and swelling. The numbers are rather similar for the phase II dosage study.

(Slides)

This is the incidence of erythema at the injection site by vaccine group. And the erythema only appears within the first day or two, and then resolves spontaneously. And there does appear to be more in the 10 microgram and 30 microgram doses than in the one in five, and there is none in the placebo, as you would expect.

And we could show, next, tenderness at the injection site. Again, there is more tenderness in the 10 and 30 microgram group, than in the one in five, with one microgram at day one for the first dose, there is 13 percent reporting, and for the second dose 7, which is an observation we have noted with almost all of our adverse events, that the second dose gives a lower reported frequency of both local and systemic adverse events than the second dose. The second dose gives a lower frequency than the first dose.

For five, there is 57 percent reporting for the first dose, at 5 micrograms, and then it goes to 75 and 80 percent are reporting local tenderness at the day one after the first dose.

But again, you can see the frequency rapidly drops off, and by day three, there is no tenderness, erythema,

pain at the injection site. And I think, for the most part, the resolved spontaneously. Some people may have required ice or tylenol, but did not have any complications from that.

DR. EICKHOFF: How about systemic.

DR. MARKS: Our most frequent systemic adverse events, we have our fatigue, headache and joint pain. I already have reviewed the joint pain.

We did look for other systemic adverse events and really didn't see any. But for the temperature, for example, greater than 99, there was a clustering in the, I think, the placebo group about the most, and there were a few in the 30 and some scattered throughout the other groups, but there really wasn't very much elevated temperature.

Again, for headache, we are also seeing a lot of this in the beginning with one-and-a-half percent after dose one in the one microgram group, 10 percent in the 5 microgram group, 18 percent in the 10 microgram group, and 17 percent. So, it is about the same. And the 30 microgram group, and 11 percent in the placebo group.

And this is fatigue, also tending to cluster in the beginning with three in the one microgram group, three percent in the five microgram group after dose one, eight percent in the ten, nine percent in the thirty, and three

percent in the placebo group.

DR. LEMON: Was there any follow up beyond the 10 days after the second dose, Dr. Marks.

DR. MARKS: Yes, these individuals are followed for 30 days. And actually, the group that received the third dose, which was one-third of the subjects who received the unadjuvanted OspA, were followed for seven or eight months, because they received a third dose -- or, this is the phase I study. They received a third dose at 20 weeks after the second dose. And in the phase II study, these individuals that I am reporting now were followed for some time.

And actually, a subgroup of them did receive a third dose at six months, and were followed -- I think for most of these subjects, they were followed up for nine to twelve months.

DR. LEMON: Do you know anything about the prevalence of the HLA DR4 marker in the phase II subjects. Was that looked at at all.

DR. MARKS: In all of the subjects, in all of the studies, in concert with the FDA, we planned in the protocol for the rheumatologic evaluation of all joint pain. Most of the joint pain that we saw didn't last long enough to be evaluated by a rheumatologist.

There was no difference in joint pain between the

groups, as I mentioned, and there was no relationship with OspA, as I have also discussed.

We did study HLA on those individuals who reported joint pain, and whose joint pain lasted long enough to be evaluated by a rheumatologist. Again, we tried to have everybody seen within the first 24 hours or so after reporting joint pain, but a lot of it was quite ephemeral.

We don't have the complete data on the HLA distribution in those individuals at this time. But there doesn't appear to be a relationship between dose and HLA serotype.

DR. HOSBACH: I just want to add as a qualifier, of the 21 people who did make it to a rheumatologist, only 6 were DR4 positive. And they were disbursed equally amongst all the groups, plus placebo.

DR. KARZON: Would you tell us something about the kinetics of the antibody response. Of particular interest would be the half life after a dose. You have given titers at an optimal period for measurement 30 days after dose.

So, what happened to the antibody. For example, what was the antibody titer before the following dose, and in particular in those individuals who received a booster -- I believe you said some at six months and some at twelve months. What happened to their antibody titers.

The other thing, a second sort of question would

be of interest -- perhaps we can come back to it later -- is what threshold titer do we have information about that would be a protective level.

DR. MARKS: I don't think that we have established a serologic correlate for protection. So, we don't have a threshold titer. The titer that we are looking at for response is a fourfold serologic response.

There was a dropping off of titer before the second dose and after the first dose which, after receiving a second dose, there was a boost. Again, there was a drop-off in titer after the second dose. And again, a booster response was seen after the third dose. But the titers don't persist at a high level between doses.

DR. KARZON: How far down did it drop. For example, if you took the geometric mean 30 days post-dose and just before the following dose a month later.

DR. MARKS: I was going to put the slide back up. It is shown on your handout of the slides. This would be number 8.

After the second dose, 14 days after the second dose, which is the first time we measured the OspA, there was a very good anamnestic response, both for the lipidated unadjuvanted and the lipidated adjuvanted. Thirty days after the second dose, there was some decrease. And 150 days after the second dose and before the third dose, there

was a decrease further. But the decrease was not down to the baseline level. It was at a much substantially greater level.

DR. LEMON: I wonder if we could ask one last question before we move on, and this would be either for you or for Dr. Steere, and it may not be possible to answer it. But could you comment on the magnitude of these OspA antibody levels, and how they would compare with the antibody levels measured by Dr. Steere in patients with chronic arthritis. Is there any way to compare these, different tests, are they apples and oranges. Dr. Steere is shaking his head negatively.

DR. STEERE: I don't know how to compare them.

DR. MARKS: And I don't have any comment on that either.

DR. LEMON: Maybe we should move on, then. Thank you, Dr. Marks. Dr. Karzon, do you have a last comment.

DR. KARZON: Just to home in further on these antibody titers, if you did an extinction curve of the transfer titer, say, in a mouse, of these materials, what do you need for a protective level.

There are some surrogate estimates one could make even at this stage, and I am curious to know about the value of, say, a titer of one to two hundred, if that is a dilution, whatever that scale is.

DR. LEMON: I think we should move on to the presentation by Medimmune. Dr. James Young will give this.

Agenda Item: Presentation by Medimmune.

DR. YOUNG: Good morning. Let me start off by first thanking Dr. Mitrane for the invitation and the opportunity to present to the committee today.

What I would like to do this morning is tell you about a vaccine candidate for Lyme disease that we have been developing at Medimmune for the last three or four years now. And, like the previous two speakers, it is also based with its active ingredients on OspA, and I don't plan now on reiterating some of the reasons or rationale for why we think OspA is a good molecule for a candidate vaccine.

I would make one other point that I haven't heard made yet today, and that is that in the passive transfer studies that have been done in animals, those studies have shown that the OspA antibody has to be on board at the time of challenge, and that antibody given post-challenge does not have a significant effect on the outcome of infection in those animals.

So, it reminds me of my previous days and trying to develop a malaria vaccine based on sporozoite antibody, where you need to have very high levels of antibody present at time of challenge, in order to prevent the infection, and you really don't have time, apparently, for a good

anamnestic response to get natural boosting.

So, we are really trying to prevent infection moreso than disease in this situation.

The vaccine we have been developing is based not on a purified subunit vaccine, but rather, a live recombinant BCG organism, which expresses the Borrelia burgdorferi B31 OspA protein, as a chimeric lipoprotein on the surface of BCG.

Now, just -- I am sure the committee, having talked a lot in recent months about BCGs, is very familiar with it. For the rest of you, I thought I would give you a little brief background on BCG and why we think that it makes sense as a live vaccine delivery vehicle.

Of course, the organism was developed in the early 1900s by two French scientists at the Institute Pasteur, and is based on an attenuated derivative of microbacterial bovus, through serial passaging culture for 13 years.

And it has been used for the prevention of tuberculosis and is licensed in the United States for that indication. It is also licensed for treatment of carcinoma in situ of the bladder.

It has an excellent safety record with over three billion vaccinees receiving the vaccine. Of course, should a problem develop with the vaccine, unlike viral live vectors, you can treat the organism with antibiotics to

suppress its replication.

We know from numerous studies that the organism is higher immunogenic, and has been used to immune infants, children and adults, both by interdermal and percutaneous routes of administration.

Many of you may not know, but it was first used through oral immunization. Therefore, we feel it has some significant advantages and opportunities for both delivery and for inducing mucosal immune responses.

And I might add that it has also been used in booster immunizations.

At Medimmune, we have been developing technology which allows us to genetically manipulate BCG, and have developed a variety of extrachromosomal integrating vectors that allow us to stably introduce foreign genes into the BCG organism.

We have developed the means by which we can express proteins from these imported genes, if you will, as either cytoplasmic proteins in the BCG, expressed on the surface of BCG, or actually secreted from the expressed organism.

We have also been able to develop systems which allow us to consider using this in a multivalent mode, where we have been able to express multiple antigens in the same BCG organism.

That allows us to either do the same antigen from one agent or to think of using multiple antigens for multiple agents, or simply to mix different constructs of BCG which are each expressing different antigens.

We have shown that we can take a recombinant BCG expressing a foreign antigen and prime animals and then come back with a booster dose or either the same recombinant BCG or a purified subunit form of the antigen and get very nice booster responses.

We have also shown that we can take animals that have been immunized with a wild type parental or standard BCG non-recombinant strain, and come back and immunize those animals with a second strain, expressing a foreign antigen, and get very nice primary and secondary responses in those animals.

So, prior immunization with BCG does not preclude coming back with a new organism and getting a good immune response to the new antigen.

And we have also been able to show that, by mucosal delivery of the organism, primarily internasal delivery, we have been able to generate very nice systemic responses, long lived sustained responses, as well as mucosal response, as measured by T cell and antibody responses in the respiratory track or in the gut, or, in fact, we have seen antibody responses in vaginal secretions.

Now, to develop the candidate Lyme disease vaccine, we have utilized the expression vector shown on this slide. It is a shuttle vector that has origin of replications for both the maintenance of this plasmid in E. coli and microbacteria. It contains a gene-encoding kanamycin resistance in order to select recombinant organisms, and then, in the upper left-hand quadrant, contains the expression cassette for the OspA protein.

It is essentially the entire mature coding sequence of the OspA protein, attached to an export and acylation signal sequence from the 19 kilodalton microbacterium tuberculosis lipoprotein, one of the major surface proteins on MTB.

It is drive by the BCGH's p60 promoter, and expression of this cassette results in the expression of a protein which is exported to the surface of BCG.

We have done, on the next slide -- this is just a Western Blot showing the expression of the OspA protein. We have taken whole cell extracts of the organism shown here -- either the prototypical Borrelia burgdorferi string at b31, the recombinant BCG expressed in the OspA -- MEDI-490 -- or the parent non-recombinant BCG, and separated those out on polychromide gel, blotted them to nitrocellulose, and probed them with a monoclonal antibody specific to the OspA.

As you can see here, the BCG parental strain does

not express the OspA protein, whereas the recombinant strain and the Borrelia burgdorferi both have proteins of the expected molecular weight of about 31 kilodalton.

We have done numerous biochemical and biophysical studies to examine the state of this protein, and have shown that it is acylated, that it is associated with the detergent phase, or the membrane component of the organism.

And in this slide, I show you a cytometric profile of an analysis of parental BCG along with the recombinant OspA expressing MEDI-490.

In this experiment, we took the organism, exposed it to antibody, to OspA, and then a secondary fluorescent antibody, and then analyzed the organisms by fluorescence activated cell sorter.

And what you see on the Y axis is the number of cells, and on the X axis, the fluorescence intensity. The profile of the wild type BCG is shown here, and you can see there is a significant shift of fluorescence intensity of the recombinant organism, indicating the presence of the OspA protein on the surface of the BCG organism.

And because of this, we feel it may actually represent a more natural presentation of the OspA protein on the surface of the organism, much like it would be on the surface of the Borrelia burgdorferi.

Now, we have done numerous animal immunogenicity

studies with this candidate vaccine, and have shown it to be highly immunogenic in mice, guinea pigs and sheep. And I will show you much of this data.

We have used it in several routes and shown very nice immune responses.

We get very nice booster response. We can get responses to very low doses of the recombinant organism. And the antibody responses generated in mice are highly potent, have good growth inhibition activity against Borrelia burgdorferi, are long lived and provide sterilizing immunity against challenge with virulent organisms.

Now, this is a slide showing an example of an immunization experiment done in C3H mice, with either 10^3 colony forming units, or 10^6 colony forming units of the MEDI-490 organism. There are a few points I would like to make.

First of all, as you can see, even with doses as low as 1,000 organisms -- and this will represent about 10 picograms of the actual protein present in the vaccine inoculum.

You can see, we get very nice responses that build over time and actually are quite long lived. We can see booster responses -- in this case about a 10-fold booster response -- I should say that what we are measuring here is anti-OspA antibody responses, these are the end point titers

of the serum from animals that received the vaccine at day zero, followed by a booster dose at sixteen weeks. And notice that these are a logarithmic scale on the left.

So, we see a very nice response built. It is boostable and long lived. You can see, in this experiment we went out about a half a year and saw very good maintenance of those titers.

In some animals, we see boosting as high as one to two hundred thousand end point titer, in other experiments as high as one to a million in these animals.

This is another example of where we have immunized animals with a single dose of the recombinant BCG. And here you can see very long lived responses out almost a year now where the titer has not dropped at all after a single parenteral dose of this organism.

We have also looked at these antibodies in terms of their biological activity, and in collaboration with Allen Barber, examined the responses for their ability to inhibit the growth of Borrelia burgdorferi in vitro. There are two different experiments here.

In these experiments, we used three different vector constructs, all of which express the OspA protein in a lipidated form on the surface of BCG.

Various strains of mice were used, either BALBs, C3Hs or an outbred NIH Swiss mice, and either one dose or

two doses of the vaccine were given.

And what you can see is, animals that received a negative control BCG had no growth in inhibitory activity against Borrelia, and in strains receiving the recombinant BCGs expressing the OspA on their surface, developed titers as high as one to thirty-two thousand growth inhibition titers against Borrelia.

And then finally, we have done a number of protection experiments, which are summarized here, again, in collaboration with Dr. Barber.

And these four experiments were done, again, with the different vector constructs that all express the lipidated form of the OspA on the surface of BCG.

Animals were challenged following immunization, in this case, where they received two immunizations they received an immunization at day zero followed by a second immunization at week 17, in most cases.

They were then challenged either by, in the first experiment IP challenge with 10^6 Borrelia low passage virulent SH2 strain of Borrelia or, in the other experiments 10^4 ID of the same virulent strain.

Then, two weeks after challenge, the animals were euthanized, tissues were harvested, minced, and deposited into BSK media. Two weeks later they were examined for the growth of the organism.

And any animal showing a single spirochete in any of the tissue samples in the medium after two weeks of growth, were scored as a positive animal. So, it is a very stringent test. If you find a single organism, that means the animals are infected.

As you can see, in the case of the control animals, in all experiments, they were 100 percent infected.

In all other experiments, you can see those receiving the OspA protein in a lipidated form, on the surface of BCG, you can see that almost all cases, with this one exception, animals were all totally protected against this challenge.

I should point out, the last experiment here was done with the accession bank material for MEDI-490 with a single dose either given IP or intranasally, where we get 100 percent protection.

So, in summary, this recombinant vaccine containing OspA, elicits long-lasting potent immune responses in animals that present sterilizing immunity against challenge. And consequently, we believe that studies in humans to examine the safety and immunogenicity of this vaccine are indicated.

Now, we expect to proceed down that pathway and are planning to initiate a phase I clinical trial shortly. We have, as primary objectives for that study, obviously, to

evaluate the safety of this vaccine and the tolerance in primary immunization, in dose ranging study in health adults. We will also, obviously, evaluate the immunogenicity and evaluate the OspA specific responses, both antibody and T cell mediated responses.

And we will also evaluate the frequency and duration of PPD skin test conversion, of subjects receiving this recombinant vaccine.

As a secondary objective, we plan to boost half of these patients with the same dose they received in their primary immunization and examine the immunogenicity and safety of those responses and also to evaluate two different lots of the vaccine which have been prepared.

And hopefully, in the not-too-distant future, I can come back and tell you about those responses. Thank you for your attention.

DR. LEMON: Thank you, Dr. Young. Are there questions for Dr. Young and the Medimmune group.

DR. GLODE: Do you have a sense, from working with this in other animal models or with other vaccine candidates, of the potential efficacy of the different routes of administration. I mean, there was really no difference in yours with interperitoneal, intradermal, internasal, although there were a limited number of experiments with those other ones.

DR. YOUNG: Pretty much all the studies we have done, we have actually done more experiments IP, simply because it is easier to deliver a reproducible dose. But those experiments where we have done it ID and internasal, we have not done dose responses in terms of the immunizing dose. We can't say which route is better or worse.

But typically, with a ten to six dose, which is in the range of a human dose, we see equivalent protection, regardless of the route of delivery.

DR. GLODE: I would encourage you to pursue the oral route.

DR. LEMON: Do you have information about the relative response one could expect in larger animals.

DR. YOUNG: As I said, we did do sheep. It turns out it wasn't a planned experiment where we were going to look for OspA responses. It turned out, we used that as a control group in a study with another antigen. And when we look at those responses, I believe the priming response gave us a titer of about one to twenty-five thousand, end point titer against OspA, and boosting responses we saw approximately one to a hundred, to one to two hundred thousand in those animals.

DR. ROOS: Do you have any problems with repeated BCG injections, with only one booster, as far as reactogenicity and sequelae.

DR. YOUNG: Obviously, that is something that we are going to be looking for in phase I studies.

DR. LEMON: Could you repeat the question.

DR. YOUNG: The question was, what can we expect in terms of reactogenicity on boosting of subjects.

I can tell you that we have done a lot of experiments in animals with these preparations and really have seen very minimal effects at the site of injection.

I should point out that we prepare our vaccines a little differently than the way the standard TB vaccines which are sold are produced, which are normally done in a static mode in a pellicle, usually, which is harvested and ground up and lyophilized.

We prepared our vaccine by a disbursed culture method in roller bottles, which gives us a very high viability culture. The vaccine preparations typically will have viabilities greater than 50 percent viability. We are using a fresh frozen culture. And at times we have even seen as high as 98 percent viability in the culture. So, we have very high viability in the preparations we are using.

And that may, in fact, result in a lower reactogenicity at the site of infection, because generally the organism disseminates away from the site of injection within 24 hours. Biopsy studies that have been down have shown that the organism does, in fact, leave the site very

quickly.

So, we may not be seeing significant reactions at the site of the injection on boosting, because of that.

The other thing I should point out is, we know from studies we have done in animals and in vitro growth characteristics of this recombinant, it does grow a little more slowly in vitro than the standard parental BCG. And it also does not persist as long in the host as the parental BCG strain. So, that might also have an effect on the reactogenicity.

We have gone as high as $5X10^8$ into animals, both guinea pigs and in monkeys, and see very little reactogenicity at the site, which is about 500 times a human dose.

DR. LEMON: You had suggested that the processing might more closely mimic the processing that occurs with OspA in natural infection, with Lyme. Is that a good thing or a bad thing.

DR. YOUNG: I am not sure I said processing as I did presentation on the surface, because, of course, this replicates in macrophages, unlike Borrelia.

DR. LEMON: Okay, but focusing on processing for a minute, though, it is quite likely that the immune response to such an antigen will be different if it is presented and processed from a living organism, such as Dr. Karzon had

alluded to earlier today.

DR. YOUNG: Right.

DR. LEMON: What does that mean as far as the potential for induction of arthritis if, in fact, Dr. Steere was correct, that in a subset of persons there may be a risk for induction of long-term arthritis in association with an OspA response.

DR. YOUNG: Obviously, that is something that we are going to look at very closely and follow in patients. In fact, in the phase I studies, we are planning to look out as far as four years follow up on these patients, just to address some of the safety issues.

Again, I am not sure that it will be processed and presented in the same way. Certainly, we wouldn't expect the organism to be in the joints. It normally replicates in lymphoid tissue. So, you wouldn't expect it to be present there, where it might catalyze the arthritic response that Allen referred to.

DR. LEMON: Do you plan to do such studies in a Lyme endemic area or a non-Lyme endemic area.

DR. YOUNG: Initially, the phase I studies are planned at the Center for Vaccine Development and the University of Maryland, in individuals who are defined as seronegative and PPD negative.

DR. O'BRIEN: Two questions. Really, they are

related more to BCG as a vector. The first had to do with your comment that BCG has been due orally. That is true, but is it my understanding, from what I remember, that there were problems in small children giving it orally, there were really serious side effects, so that, in that one group is not a good idea. Is that correct.

DR. YOUNG: That is correct. The reason I think it was stopped is two-fold. One, you had to go to much higher doses. You had to use about 100 times more organisms orally than you did perentally, because of the acidity of the stomach killing the organism.

The second reason is that you did see significant lymph adenitis develop in small children, because the entire dose was not swallowed. It may be that by different formulations, particularly enteric coding of the organism, you may be able to around both of those problems.

DR. O'BRIEN: I know those were old studies.

DR. YOUNG: Yes.

DR. O'BRIEN: The second thing is, of course, your plague, and that is, are you going to have everybody PPD positive and then you are going to deal with that issue.

Do you have any data, anything, that says about your formulation and PPB positivity.

DR. YOUNG: Yes, we do, not in people yet, unfortunately. But we have done studies both in guinea pigs

and mice. Studies done in guinea pigs were with David McMurry down at Texas A&M, where we looked at single dose administration of this organism and saw very nice PPB conversion, DTH responses, in those guinea pigs.

We also did studies in mice with Ian Orem at Colorado State University, where a similar study was done, and we did not see very good DTH responses develop.

We saw very nice responses develop to the parental BCG but not to the recombinant BCG. So, we have got conflicting data. In mice, it did induce the DTH response, in mice it did not induce a DTH response. Those were both given either 10^6 or 10^7 recombinant organism.

What we don't know is -- well, first of all, we don't know whether the mouse or the guinea pig is more representative of what we want to see in people. Secondly, we don't know what the immunizing dose is going to be in people which will give us a good OspA response versus PPD conversion. So, we will have to look at that in the phase I studies.

DR. O'BRIEN: Thank you.

DR. KARZON: When you introduced foreign nucleic acid material in a vector, you are likely to change the virulence of the vector, depending on how it is put in, what is interrupted. So, it would be of interest to know whether this is more of less virulent than the original BCG. And

that is a complicated, as you point out, by the fact that preparation is quite different than the ratio of PFU and antigenic mass differs.

I think one of the questions that anyone would have is just exactly what are the properties of the final vector product when it is put into man, and what significance would it have on the population in various ways.

DR. YOUNG: Well, obviously, that is a big unknown. I am sure there are people who are debating BCG in general, in terms of its efficacy for TB.

We know from studies we have done thus far in animals, that the recombinant organism is very safe in guinea pigs. We see virtually no systemic responses and only very minor responses at the site of injection, giving $5X10^8$ organism, five times the typical human dose.

So, we see very little adverse effects and those have been in about 80 guinea pigs so far.

We have looked in mice in terms of the replication or the persistence of the organism, the recombinant organism versus the non-recombinant BCG. And it does not persist as well as the non-recombinant strain. So, the expectation is that it will probably be much less reactogenic.

DR. LEMON: Dr. Ferrieri address BCG generally, or is it more the --

DR. FERRIERI: It is Lyme, regarding the autopsy data and persistence in macrophages and lymphoid tissues. And how far out from the immunization did you do such studies of persistence.

DR. YOUNG: We have done studies going out, I would say, in well executed experiments, in three to four months. Typically, after about two months, it is very difficult to recover the recombinant organism from the host.

We have, with certain recombinants, been able to recover organism as long as eight or nine months post-immunization, very very few organisms. The ones we have recovered are still expressing the antigen.

DR. FERRIERI: Harvested from abdominal lymph nodes. Do you have joint data, synovial tissue data.

DR. YOUNG: No. All we have looked at are usually lung, liver, and spleen, from BCG immunized animals.

DR. LEMON: I think it is important that we keep in mind the questions that FDA is posing to us today vis-a-vis Lyme. And maybe we should conclude the Medimmune presentation. I think it is a very exciting and interesting approach, but I would like to ask Dr. Mitrane to recapitulate some of the comments that she made in her introductory speech earlier this morning, and to pose for us again the questions that she wishes us to address specifically.

And then, I believe it would be best that we broke for lunch, then, and try to reconvene around 1:00 o'clock, and to entertain those questions at that time. Dr. Mitrane.

Agenda Item: Concluding Remarks and Questions.

DR. MITRANE: I want to thank our guest speakers and the companies for their excellent presentations this morning. Now, I would like to take the opportunity to present the CDC case definition of Lyme Disease in some further detail, which is as follows:

Physician-diagnosed erythema migrans at least five centimeters in diameter, or at least one late manifestation and laboratory confirmation of infection.

Laboratory confirmation for diagnosis consists of isolation of Borrelia burgdorferi from clinical specimen, or demonstration of diagnostic levels of IgM and IgG antibodies to the spirochete in serum or CSF, or significant changes in IGM or IgG antibody response to Borrelia burgdorferi, impaired, acute, and convalescent base serum samples.

A confirmed case of Lyme Disease meets one of the clinical case definitions.

Late manifestations include any of the following, when an alternate explanation is not found:

Musculoskeletal system, recurrent, brief attacks, lasting weeks or months in one or a few joints, sometimes followed by chronic arthritis in one or a few joints.

Musculoskeletal manifestations, not considered as criteria for diagnosis include chronic, progressive arthritis, not preceded by brief attacks, and chronic symmetrical chronic arthritis.

Arthralgia, myalgia or fiber myalgia syndromes are not criteria for musculoskeletal involvement.

For the nervous system, lymphocytic meningitis, cranial neuritis, particularly facial palsy, reticuloneuropathy or, rarely, encephalomyelitis. Encephalomyelitis must be confirmed by showing antibody production against Borrelia in the CSF, demonstrated by a higher titer of antibody in the CSF than in the serum.

Cardiovascular system, acute onset, high grade, atrial ventricular conduction defects, that resolve in days to weeks, and are sometimes associated with myelocarditis.

Palpitations, bradycardia, bundle branch block or mild carditis alone are not criteria for cardiovascular involvement.

I would like to conclude with the questions for the committee discussion. Is the CDC case definition for Lyme Disease appropriate for a pivotal efficacy trial.

Please comment on laboratory assays to support the diagnosis of the disease; that is, culture, western blot and polymerase chain reaction.

The CDC case definition was developed for national

reporting of Lyme Disease. It may not be appropriate for clinical diagnosis in a phase III efficacy trial. Laboratory criteria for confirmation of infection need to be specific.

Lyme Disease has a wide range of clinical manifestations which occur in the acute and chronic phases of infection by Borrelia.

Please comment on appropriate primary and secondary end points that provide specificity and diagnosis of the disease for a pivotal efficacy trial with an OspA vaccine.

Prevention of early versus late manifestations of Lyme disease may need to be addressed. Immunization may prevent certain manifestations -- for example, erythema migrans -- but not other manifestations.

And it is possible that immunization may modify the symptom complex of the disease.

How should the safety of OspA vaccines be evaluated, especially as it relates to individuals with HLA DR2 or DR4 haplotype.

How long should immunized individuals be followed to obtain adequate safety and efficacy data.

How should the safety and efficacy in children be assessed. If safety and immunogenicity data are available in children, adult efficacy studies in which an immunologic

correlate of the protection has been identified, may be adequate to extend to a pediatric population.

What other studies could be performed to answer additional safety and efficacy question with the OspA vaccine. For example, how should the use of vaccine be evaluated in seropositive individuals, and in those with a history of Lyme disease. Thank you.

DR. LEMON: Thank you, Dr. Mitrane. I think now would be appropriate for us to recess for lunch. I think it is not going to be possible for us to get into these questions in a substantive fashion in the time remaining otherwise.

Could we please try to reassemble here so that we could start promptly at 1:00 o'clock.

(Whereupon, at 12:15 p.m., the meeting was recessed, to reconvene at 1:00 p.m., that same day.)

///

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

(1:09 p.m.)

DR. LEMON: Just before we broke for lunch, we heard from Dr. Mitrane, a series of questions that the FDA has posed to us. And I think during the next hour we need to do our best to try to answer these questions, to see if we can develop a consensus amongst us with the help of our experts.

Before proceeding, though, I would like to ask Dr. Dattwyler to give us a few additional comments concerning the serologic response.

DR. DATTWYLER: Just after the break someone raised a point that I think just needs clarification. I presented a slide and I think that it is important to -- which is cumulative seropositivity in patients with erythema migrans with time. And what I had said was that everybody who seroconverted did so by day 30.

I think the operative word here is cumulative, because that is important, because you don't maintain your serologic responses, once you have seroconverted, if you have been treated. And I think this slide will clarify that quite nicely.

These are individuals who were in a prospective randomized double blind, double dummy study comparing azithramycin to amoxicillin. And the maintenance of

serologic response was not as maybe had been implied in that earlier slide, in that most people, after time with treatment, become seronegative.

The other point that I think is very important is that the azithramycin arm of this study was not adequate, in that we had a significantly greater number of failures in the azithramycin arm.

At the time of failure, approximately 50 percent of the failures -- and these are people with objective clinical abnormalities compatible with Lyme disease, were seronegative.

So, serologic response was not a good marker in this study, and it raised the question, at least to us, are diminished numbers of spirochetes or partially treated infection associated with a blunted immune response. And I think the answer is perhaps, yes. So, I just wanted to clarify that point.

DR. LEMON: Is this by western blot or by lysate ELISA.

DR. DATTWYLER: Both.

DR. LEMON: Either/or.

DR. DATTWYLER: Either/or, correct. Now, the western blot criteria we used I outlined prior to that. So, it is not just any one band. IgM had to had 41/39 or OspC. And for IgG positive, you had to have five out of the ten

specific bands. Thank you.

DR. LEMON: Thank you, Dr. Dattwyler.

Agenda Item: Committee Discussion.

DR. LEMON: In considering the questions before us, I think it is important to remind the committee that all of us have seen information in briefing documents related to the closed session later this afternoon. It is important not to confuse that information from what we have seen earlier in the day and bring that up during the discussion. This is an open session.

The first question deals with the appropriate case definitions to be used in a pivotal efficacy trial, and specifically asks us to comment on whether the CDC case definition for Lyme disease is an appropriate end point definition for a pivotal efficacy trial.

And we are asked to comment on laboratory assays to support the diagnosis of the disease -- that is, culture, western blot, or polymerase chain reaction.

I had hoped that Claire would be here to lead off in this discussion, but she must have been prescient, because she is not. But is there any member of the committee who would like to take a stab at this question.

DR. EICKHOFF: Well, I will take a stab at it, but only from the -- this is interpreted as opening up a discussion.

The CDC case definition is, I think, a very appropriate one for epidemiologic purposes. Probably some would even argue with that. But at least for an initial assessment of what Lyme disease is and what the scope of the problem is, I think the CDC case definition will probably work fine.

When it comes to vaccine efficacy, I don't think it will work fine. And there are parts of it that I simply would not accept as a case definition for Lyme disease because, if I read the definition correctly, part of it, physician diagnosed erythema migrans at least five centimeters in diameter or. So, that means a physician diagnosed erythema migrans of that size would be sufficient for a case definition, which I would frankly, flat out, disagree with.

There were a number of comments made by people who are far more knowledgeable, in fact the experts on Lyme disease today that, in capable hands, a capable physician or a trained physician can recognize erythema migrans, but that leaves out many of the rest of us, I think myself included. So, enough said about that part of the definition.

So, I think there has to be pretty stringent serologic guidelines built into this, to include either ELISA plus western blot, according to that new more rigid definition that was presented, or PCR or culture

confirmation. So, that is what I would say at the outset.

DR. ROOS: Would you accept a dermatologist looking at a photo. I kind of agree with you that I might be skeptical of local physicians. But if you could document what the skin lesion was and have an expert look at it, would that be adequate as far as a case of Lyme disease.

DR. EICKHOFF: Well, as far as a pivotal vaccine trial, I would far prefer serologic support.

DR. LEMON: What about culture, Ted.

DR. EICKHOFF: Laboratory confirmation. Let me put it a little more broadly than that.

DR. LEMON: Would one of the experts that talked this morning wish to comment on the practicality of culture, in terms of transport of specimens and the technical difficulties.

DR. STEERE: I think it is pretty easy. I think the culture of erythema migrans is pretty easy. But one thing it does -- the best results are from biopsy. It is an invasive procedure. And one can take a two millimeter punch biopsy and simply put the tissue in BSK medium, and do it right there where one is doing the biopsy.

It can be taken to the laboratory and incubated. In other words, I think that culture should be a part of the evaluation of erythema migrans. And we can even hope that it would be positive in -- I will say the majority -- of

people who do have erythema migrans.

It may be a bit optimistic to hope that it is going to 90-plus percent. But I will still say that I would like to think that it is going to be 50 percent or more.

While I am at it, could I also comment that there may be two levels of what one requires. One is confirmation, a definite case. And I think having culture or serologic proof of that is needed. However, one can also record information on whether a physician thinks that it was erythema migrans, even in the face of having no culture proof or no serologic proof.

If, when the code is broken, all those people are in the placebo group, it would worry me.

DR. LEMON: You told us this morning that serology may be approaching 90 percent or greater.

DR. DENNIS: We have two groups of patients that have taken part in a study of cultural isolation. One is in conjunction with clinical research groups, such as Dr. Wormser, or other persons. And there, under those very good conditions of physicians who are quite experienced with the diagnosis and have the tools for biopsy in the laboratory right on hand, then the isolation rate has exceeded 60 percent.

We also have an ad hoc program whereby we promote physicians throughout the country, who think that they are

seeing erythema migrans, send us biopsy specimens after having received a media from us. And under those circumstances, where the level of expertise may be varied and where there may be more problems with handling and sending, we get from endemic areas about a 35 to 40 percent recovery.

DR. LEMON: How does that compare with the combined flagellant, ELISA and immunoblot.

DR. DENNIS: I don't have a direct comparison of those two, but the data that I showed this morning are quite similar. Our experience is quite similar to what Dr. Dattwyler has presented.

DR. DATTWYLER: Two comments. The high degree of culture positive biopsies came from people that are extraordinarily experienced, and that would be Bernie Berger and Russ Johnson.

I totally agree with what Dave said. I think in routine hands it is much lower.

As far as the ability of physicians to diagnose erythema migrans, I agree that it is problematic. But one thing that can occur is that, a, the lesion can be photographed.

And erythema migrans lasts. So, if someone presents with it, there is not an immediacy where one has to immediately make a decision. That person will have that

lesion in a day or two and if a study could send it to a regional place where it could be appropriately photographed and evaluated by a knowledgeable individual who is quite used to looking at erythema migrans -- and it doesn't necessarily have to be a dermatologists. As a matter of fact, in our region, the dermatologists are not as familiar with it and they send their cases to us.

But I think that there are individuals in endemic areas, at university medical centers or other places which could readily identify erythema migrans lesions, photograph it and provide culture material.

DR. STEERE: I do think that anyone presenting in a vaccine trial, or participating in a vaccine trial, is likely to come quite early. And it is not really going to be an option -- well, it is an option, but it may not be the one taken -- to say, well, we will wait and see what happens to it, because this is a disease that is treatable with antibiotic therapy. And we have said, the earlier one treats, the better.

And consequently, a given patient, even though there seems to be a small lesion, may prefer to be treated immediately, at which point it is going to be somewhat harder to tell by the clinical impression whether or not that was erythema migrans.

I still think that the lesions should be biopsied

and a culture should be taken. And we can hope that in the majority of people, if it was erythema migrans, one can prove it that way.

The earlier they are seen and treated, the less likely that they are going to be seropositive. So, when one gives figures like maybe 30 percent are seropositive acutely during that first one to two weeks, if one is getting there within the first one, two, and three days, it is going to be less than that.

One will, however, pick up some people in a convalescent sera, who will seroconvert, even after antibiotic therapy and after the skin lesion goes away.

DR. BROOME: I wanted to just get some clarification of the expected natural history in promptly treated EM. I guess even though you could spend a lot of time on the other parts of the case definition, I would think that in this kind of a vaccine trial setting, where there will be prompt therapy, that the expected frequency of complications is very low. But I wonder if Allen or Ray could comment on that.

DR. DATTWYLER: Our group sees about 150 to 200 erythema migrans cases a year, and under those circumstances, I can say that prompt treatment is usually associated with a rapid clinical response and, with resolution of the lesion within a day or two -- very rarely

more than that -- and a lack of clinical symptomatology -- especially in individuals with single lesion erythema migrans without constitutional systems.

Individuals with constitutional symptoms can sometimes take longer to resolve and have a higher risk of failure on routine antibiotics. It also depends on what you treat people with.

The other thing I would like to say is, as far as my suggestion of a central location, I wasn't talking about a long delay, I was talking about a delay of, at most, a day.

DR. LEMON: With regard to the second half of Claire's comment, though, as I recall, the data presented this morning, there are a substantial proportion of patients who presented with late complications who never had ECM.

DR. DATTWYLER: That is correct. I think the statistics are that anywhere from 40 -- up to 40 percent, say 25 to 40 percent of individuals -- never develop erythema migrans.

And another question, you know, in a study like this, you know, would you have attenuated disease. I don't think we have an answer to that. Would you skip through an erythema migrans stage, I don't know.

DR. STEERE: There is another issue here. It is true that, in the natural disease, Lyme disease can present

with a late manifestation of the disease, and the early manifestations are asymptomatic.

And those people who present with late manifestations of the disease, particularly if it is arthritis, are strongly seropositive.

In those people, we don't know when they seroconverted. In this vaccine trial, you have an opportunity to know that, because these people are going to be followed with blood samples drawn.

And consequently, you stand the chance of knowing that people seroconverted, and they may seroconvert without any symptoms.

Well, we know that can happen. You can have asymptomatic infection. On the other hand, it could be someone who would later come down with arthritis or neurologic disease.

Therefore, are you going to treat people who seroconvert. Are you going to tell them they seroconverted. And if so, when. And how would you treat them, if it is asymptomatic seroconversion. We really don't know how that should be treated.

DR. LEMON: I think that gets into a different issue. If we could focus first on the clinical manifestations of Lyme and the clinical endpoints to be used in a trial.

What I am hearing around the table -- and I think it is a consensus that I tend to agree with is that, the CDC case definition which relies upon a physician's diagnosis, by visual inspection of a lesion, is probably not adequate, that we ought to try to go one step beyond that for a laboratory confirmation of Lyme infection, recognizing that that may be a difficult thing to accomplish, particularly in early infection if it is treated promptly.

DR. JOHNSTON: Can I ask a slight modification of that. What if there is no clinical presentation -- I mean, which could be the circumstance in the vaccine trial. How do you deal with that.

I mean, you would like to have both. You would like to have EM and some definitive assay to tell you that the spirochete is there.

DR. LEMON: I would tend to agree. I think that goes beyond where we use the CDC case definition. Do we accept the CDC case definition by itself or do we require something more than that, recognizing that, in a study that is done in a double blinded, randomized fashion, the physician's assessment does carry some importance, as Dr. Steere pointed out. If all those cases show up in the placebo group, that is potentially very significant.

In terms of the supporting laboratory assays, we talked about serology. And I am left with the feeling that

there is virtually no standardization of tests that are being done in different laboratories right now, for this infection.

So, it becomes difficult to even suggest what serologies might be done. It sounds like western blot may be the most close to being standardized in a reasonable fashion. It might be nice to know a little more about the flagellant ELISA we heard about earlier this morning.

Culture was talked about. Polymerase chain reaction, we haven't really talked about in this setting here. Does anybody want to comment on the use of PCR for diagnosis in this setting, and confirmation of the ECM rash, for example.

If you can culture the organism from skin, you would think that you would be more likely to recover it by PCR, given the success of PCR and chronic arthritis.

DR. WORMSER: Well, in our experience with PCR, it was identical to the culture results, in terms of the sensitivity. But the cases were not identical necessarily, in our initial studies.

In other words, there were some PCR positives that were culture negative and some culture positives that were PCR negative.

DR. GLODE: Can I ask a follow up question. How about PCR of blood. How long are spirochete anemic, or

whatever is the correct word.

DR. WORMSER: We just reported in this month's JID about 25 percent of our erythema migrans patients were PCR positive on blood. So, it is not very sensitive.

DR. ROOS: I think one especially important place for PCR in diagnosis is central nervous system disease, because we have heard a lot about unreliable serological testing in spinal fluid.

And I know there have been some studies in the literature. And I think that this, as far as I am concerned, would have an important role in helping with the diagnosis generally, and specifically in the central nervous system disease.

DR. JOHNSTON: And also the chronic arthritis and 10 percent or so non-responsive.

DR. LEMON: What about other aspects of the CDC case definition beyond EM. I think we all have, in our briefing documents, the actual case definition that CDC accepts. And Dr. Mitrane went over these points with us earlier.

Are there reasons why the epidemiologic criteria for chronic complications of Lyme cannot be used in a study such as this.

DR. ROOS: Well, from the point of view of the nervous system diseases, I think there has to be further

clarification of what these terms mean, and definitions.

For example, with retriculneuropathy, it is not clear to me whether this is a subjective complaint of the patient, or whether there is laboratory data to support or physical examination finding to support it. What does that actually mean.

Or people talk about a late complication being encephalopathy. Here they talk about encephalomyelitis. And it is not clear to me, is that the same and what does that really mean. What is the definition of that.

So, I think that terms are basically used to describe these late effects, but there are no definitions. And I think this is a special concern for a vaccine study in which you are going to talk about nervous system problems. And it is a special problem out in the neurological community at present, when patients come in with one or another complaints, and serological studies are done and it is said, this is neuroborreliosis. And is it.

So, I think that for the vaccine study and maybe even for the CDC also, we really need more specific clarifications.

I think things are a bit easier with arthritis. You have a swollen red hot joint. But these terms are much more diffuse and vague ones in the way that they are used. So, I have some discomfort. You know, a facial palsy is

pretty clear but after that it is complicated.

DR. STEERE: For surveillance purposes, it seemed that a clinical definition had to be used. In other words, we really weren't able to require, for instance, EMG evidence, in order for someone to report that that is what they thought a patient had.

But I think in a vaccine trial there are, in fact, objective tests that one can use for most of these manifestations of the disease. And to take the reticuloneuritis as an example, the great majority of those people do have evidence of a diffuse axonal polyneuropathy, or diffuse axonal polyreticuloneuropathy.

And consequently, one can use test results, or neurologic tests, to help clarify that the patient actually does have that particular manifestation.

Then, how does one link it to Lyme disease. I would like to say that I think serologic testing in that instance, in the patient with neurologic disease, is generally better -- in my opinion, it is generally better than we have heard this morning, including that particularly patients with meningitis, in my experience, have abnormalities in CSF that include intrathecal antibody production against the spirochete.

The hardest one to diagnose and be certain about is the late encephalopathy of the disease.

We are currently working on a combination of using MRI and spec scanning to actually image abnormalities in the brain. And consequently, I believe that they exist and that these late manifestations of the disease that are analogous to tertiary neurosyphilis exist.

My hope would be, though -- or, in my experience, the great majority of these people have earlier manifestations of the disease. And consequently, in a vaccine trial, their earlier manifestations, I think, I hope, would be apparent.

DR. LEMON: Dr. Dennis, did you have a comment about that.

DR. DENNIS: Encephalopathy was left off because we did not have good laboratory markers of that. But encephalomyelitis, we felt, that combined with intrathecal antibody production, indicative of the inflammation, that it could be used in that patient.

DR. LEMON: I wonder if either of you would care to comment on the likelihood that late CNS complications are likely to occur with a sufficiently high frequency to actually impact on the clinical efficacy trial.

EM is going to be a much more likely outcome and I expect arthritis is also.

DR. STEER: If I were to try to anticipate a worst case scenario about that, the worst case scenario that I

could anticipate -- and Ray and I have talked about this -- would be if somehow one would have incomplete protection and a few spirochetes survived. And let's say it made it to the nervous system.

And we know that, with inadequate antibody therapy, that that can happen, and then develop later in an attenuated CNS picture, that serologically is incomplete as well, but still responsive to antibiotic therapy and still, I believe, the spirochete.

So, that would be the worst case scenario, I think, that one could envision. Now, how often would that happen or would it happen at all, I don't know. Do you want to hazard a guess, Ray.

DR. DATTWYLER: I totally agree with what Allen just said. But certainly, less than 20 percent. I mean, if that.

DR. STEERE: Well, in the natural history of untreated infection --

DR. DATTWYLER: It is only five or ten percent. I totally agree with what Allen said.

One of the problems, as I see it, is not so much the more obvious neurologic problems, but if you look at patients who are recovering from erythema migrans, fatigue is not an uncommon occurrence.

The trouble with fatigue, as we all know, is quite

a common occurrence in life. So, that is the type -- you talk about neurologic abnormalities, that is the type of thing that may very well drive people a little crazy when designing a study. But I agree with the other comments.

DR. BROOME: I guess this is really the question I was asking earlier. And probably the only place you can answer it are the seroepidemiological studies. If you took the Hanrahan and the other one, what proportion of those people who presumably were treated pretty expeditiously had anything other than EM.

DR. STEERE: Very few. I mean, we would say, if they did, they were inadequately treated. That is the most likely explanation. So, erythema migrans response to antibiotic therapy.

DR. BROOME: I assume that, since they were doing serology, they also picked up those -- that portion of the cases -- if there were persons with arthritis who either did not have or did notice their preceding EM, those studies would have picked them up in a systematic way. And there were very few.

DR. STEERE: I mean, that happens in the natural history of the disease. You can have late manifestations as the presenting feature of the disease, and they are usually strongly seropositive.

DR. BROOME: What portion are presenting that way,

but in the treatment area.

DR. STEERE: Less than 20 percent.

DR. DATTWYLER: We have some recent data on that. We have looked at some high risk worker groups recently, on Long Island. And even in the highest risk worker group that we found that had a seroprevalence rate of 28 percent, and the incidence of disease was on the order of magnitude of about 6 percent or 7 percent in that population. And that included individuals with neurologic and arthritic involvement, so that it does occur in certain high risk groups in the population, but they are uncommon.

DR. LEMON: You know, we are sort of beating around both question one and question two here. I get the sense from the committee, again, that we don't believe that the CDC case definition can be applied, as it is, to an efficacy trial. I don't think there is any disagreement about that.

We believe that there probably should be a greater attention paid to supporting laboratory evidence of infection and stricter clinical definitions for each of the syndromes to be considered.

I am not sure that I really understand how question two differs from question one, but I wonder whether a vaccine that prevented EM successfully, and did not prevent the late manifestations of disease, whether such a

vaccine would be useful. Obviously, probably not.

So, I am worried about a vaccine trial that might tend to focus on the EM manifestations of the disease, and give us a reasonable efficacy determination with respect to EM, but not leave answered the question of the prevention of the late manifestations of EM. And I wonder if anyone could comment or elaborate further on that issue.

It is one assumption that if you prevent EM, you prevent the later manifestations of the disease. And I don't know if anything that we have heard today tells us that that is going to be the case.

DR. KARZON: I would think that that is pathogenetically possible. And I would also like to bring up, in that regard, extend your notion to look for enhanced disease.

It is a theoretically possibility that some of the known late manifestations might appear in altered forms, or aberrant forms.

I don't know what forms they take, but what it would suggest to me is that the screening process look at joints, heart, neurological status in a broader way and, even before we know the answer, record events that fall into those logical categories.

DR. BROOME: I guess what I am wrestling with it, if you are looking at enhanced disease, you probably have a

reasonable chance to at least identify a substantial increase. But I think if you are dealing with treated individuals, then the only group that you will know if you prevent complicated disease, is that group who do not present with EM.

And that is why I am trying to find out what is the expected frequency of that because it seems to me that, if you care about that group, that should drive the sample size.

DR. KARZON: I agree, and what I meant is to add this thought onto the one that you have already stated.

DR. LEMON: Then that would substantially increase the sample size, of course. Dr. Steere wants to respond to that.

DR. STEERE: I think that the expected frequency of that group, at least in a percentage way, is small. I would like to come back to the idea that seroconversion has occurred, because I am going to postulate that that group has already seroconverted. And if you know that and you treat those people, presumably they are not going to develop late manifestations of the disease.

So, it becomes what is ethical which is, if you go ahead and you draw blood and you do antibody responses, patients are going to know whether they have seroconverted. And then what is one going to do about that. Is one going to

go ahead and treat those people.

Well, if you do, I don't think that you are going to see much late disease.

DR. LEMON: Are you postulating a clinical trial, Dr. Steere, that would be based on prevention of seroconversion. Am I understanding correctly.

DR. STEERE: It isn't postulating it. It is saying that blood is going to be drawn and is one going to go ahead and measure the antibody response at the time you do it.

And if you do, and if you let people know what the results are, you will know who seroconverted.

And I would postulate that someone who might later develop arthritis because they are strongly seropositive when they do, and has had no earlier manifestations of the disease, that you may pick up that person because they have seroconverted.

And if you go ahead and treat them, they are not going to develop arthritis.

DR. LEMON: If you periodically follow people for seroconversion following immunization, and you demonstrate seroconversion, is it not ethical to withhold that information and treatment from them.

DR. STEERE: I mean, to me, we are postulating that it may make a difference in whether they develop late

manifestations of the disease. So consequently, it seems to me, it would be important to let people know.

DR. LEMON: And yet, we have a great deal of seropositive individuals without evidence of disease in the community; right.

DR. STEERE: It depends where. Let's take Lyme, Connecticut, as an example, where maybe the cumulative frequency of seropositivity may now be in the range of 15 percent.

Maybe it is important to say that if a person was treated for erythema migrans they lose positivity in time. But if they had later manifestations of the disease, like arthritis, in our experience, they are all seropositive. And I don't know about re-infectivity in that group either. So, that should give us hope that, well, within the natural infection, indeed, there is such a thing as protective immunity.

DR. LEMON: If you picked them up in a trial based on seroconversion and you treat, you treat the possible risk of the late occurrence but you don't know then, in an unmonitored situation that would occur after licensing the vaccine, what would then happen, in given openly to the population.

DR. GLODE: I just wanted to support Dr. Eickhoff's statement made earlier about not only the need

for a laboratory confirmation of some sort, but the need for standardized assays.

So, if efficacy trials went ahead and each individual company had their own assays and there was not a standardized assay available for comparison purposes, then at least samples should be kept so that, you know, comparisons can ultimately be done.

We went through this with the H flu vaccine issue and it was pretty confusing.

DR. LEMON: We have six questions and we have an hour allotted to this and we have already used 45 minutes. Let me ask Dr. Mitrane whether she has heard enough discussion about one and to, to allow us to move on to the other questions.

DR. MITRANE: Yes, that is fine.

DR. LEMON: Ray, did you have a comment you wanted to make before we moved on.

DR. ROOS: Just something quick and it relates perhaps to primary and secondary end points and diagnosis. And that is that maybe we do need definite diagnoses and there may be diagnoses that are less secure. So, a diagnosis of a skin lesion that looks like erythema migrans, without a serological conversion perhaps isn't forgotten and is dealt with, in some way, in the trial.

We don't really know, perhaps -- or maybe our

experts do, how many of those EMs that are picked up right away and then get blood drawn at that point, and when is the company going to draw the blood again -- let's say in a month -- how many of those might be missed, and it is EM, for example.

So, perhaps we do need two levels and maybe that would be in some of the secondary end points.

DR. LEMON: But if we are to do that, the idea that we are putting an emphasis on laboratory confirmation also will, of necessity, increase the sample size, because of the low sensitivity of those procedures.

Let's move on to the third question, which is how the safety of OspA vaccines, how should they be evaluated, as it relates to individuals with HLA DR2 or DR4 haplotypes.

As I remember the data from this morning, the distribution of DR2 haplotypes among those with long term chronic arthritis was not significant, was borderline significant, a trend perhaps. But we can focus on DR4 as an example here.

DR. ROOS: I just had a question and that is, in the DR4 cases that are resistant to antibiotics, you are talking, really, about patients who already have chronic arthritis.

So that, in this particular situation, with this vaccine trial, we are dealing with patients who will be

recognized as having Lyme pretty soon -- that is, within a relatively short time. And they are going to be treated at the first sign of Lyme.

So, it could be that the particular subset under the circumstances that have been described will never really appear during the vaccine trial itself.

Are those patients going to be more at risk for the later complications. It is possible, but remember, we are giving them early aggressive antibiotic treatment, and that might not have been the case in the cases that you followed that perhaps weren't treated properly in the beginning.

DR. STEERE: I have never seen a patient develop chronic arthritis of the sort I was describing, who was treated with antibiotic therapy beforehand.

I think it is quite important, to develop that complication, that the organism be there and it be in untreated disease.

So, I mean, I agree with what you are saying that it is a complication that may not be seen at all in the vaccine trial.

DR. LEMON: It is probably also important to recognize that these are the patients that perhaps are most in need of such a vaccine, in order to prevent the infection in the first place.

But given the scenario you outlined this morning in a patient with waning vaccine immunity, who becomes exposed to natural infection at a later date, what are the implications to having been primed to OspA, and how does that impact how long you would like to follow such patients following immunization. And how does that also impact the need to know HLA types of the patients that are involved in such studies.

DR. STEERE: That is the worst case scenario, again, which, if you prime a person and then are actually able to give them the infection later, are you worse off because of it.

If that does happen, I would think that people could be typed at that point. That is my own reaction.

I do think that the duration of follow up needs to be long, to answer questions like this.

DR. LEMON: I would tend to agree with that statement, certainly.

DR. FERRIERI: I have a scientific question, perhaps this has been addressed, but are there any cross reactive epitopes with human tissues of OspA, or B or C, whatever.

DR. LEMON: Is there molecular mimicry that has been established.

DR. FERRIERI: Exactly.

DR. LEMON: I would imagine that he would have told us that, if that was known.

DR. FERRIERI: Has it been studied.

DR. STEERE: The sequence of OspA is known, and there are different sequences of OspA, though, of Borrelia burgdorferi group one, all the sequences are very close to being the same.

One can run those sequences through the gene banks, and the various banks of human gene sequences that are known. And there are no long sections of homology.

One can still ask the question, if you knew important T cell epitopes and could probe gene banks with a smaller sequence, that sometimes even a relatively few amino acids can still be important, perhaps, in molecular mimicry between human and bacterial proteins.

DR. FERRIERI: I gather your answer conveys that we don't really understand this area.

I know how to do that kind of searching in the gene bank also, but do we have any information that suggests that there is any mimicry one way or the other.

DR. STEERE: No, not at this time.

DR. LEMON: Claire, I would like to ask you, with respect to a design of a particular study, are you satisfied with the idea of looking at HLA types late, should complications be encountered, doing basically a case control

study, perhaps, on such late cases of arthritis, if that occurred.

Or, do you think it would be reasonable to have at least, in a subset of patients, the HLA distribution known at the outset.

I am asking questions just to try to get some discussion going here.

DR. BROOME: I was trying to remember, what is the expected frequency of DR4.

DR. LEMON: As I recall, DR4 was about 30 percent; was it.

DR. STEERE: Twenty-five percent, in that range.

DR. BROOME: The significance is substantial. Delete the word, significant.

DR. STEERE: So that, in effect, one out of every four patients that is immunized will be a DR4 positive patient. So that, when you are doing your safety and immunogenicity study, you are really looking at the safety in the DR4 population.

DR. BROOME: I was just trying to think of what you could do in the phase II studies that would really help, but it is really a matter of -- to me, if there were any kind of an animal model, that would be the most attractive way to look at this.

I don't know whether there is anything in the

primate area that would let you.

DR. LEMON: In terms of chimpanzee or rhesus, rhesus came up earlier this morning. I would imagine that rhesus haplotypes are significantly different from human haplotypes. I am just extrapolating from my own knowledge.

Is there any evidence or anything known about the antibody response in DR4 positive patients versus DR4 negative vaccine recipients. Is there a more active T cell response or antibody response in the DR4 positives, anything to distinguish those populations.

I am not sure that we are going to get much further with this question, Dr. Mitrane, at this point.

DR. JOHNSTON: Stan, I think you made a salient comment earlier, that this is the population -- since it takes the spirochete to get the chronic arthritis, you can argue on that side of it, that you are reducing, with the vaccine, that opportunity.

It doesn't remove the possibility that the risk could then occur or accentuated disease could occur, and only a timed follow up, I think, will tell you that.

DR. LEMON: Do you want to suggest a time, a duration of follow up which is, in fact, the next question.

DR. JOHNSTON: At least two years.

DR. BROOME: I guess one other comment, in thinking about this. It raises something that is clearly

profoundly concerning in terms of, you don't want to cause problems with this vaccine. And yet, it is based on some very intriguing, very suggestive, but very limited numbers.

And I realize that no one is going to have the kind of freedom that Dr. Steere has, in terms of being able to do the very elegant serial correlations.

But I would think it would be possible to identify other patients with chronic arthritis relative to Lyme, and just confirm whether or not the DR4 OspA correlation is as impressive as suggested.

DR. LEMON: In a larger number of patients with chronic arthritis. Obviously, I think that is something that would be useful to do. But even if you achieved a very strong statistical correlation between the OspA response and chronic arthritis, there is a huge leap from that to an etiological role for OspA, which I think you can only speculate on at this point.

DR. BROOME: But if the correlation didn't persist, it would certainly make life easier.

DR. LEMON: Make it simpler, perhaps, yes.

DR. FERRIERI: Could we address the question of knowledge of HLA DR status of the vaccinees.

DR. LEMON: I was trying to prompt the committee to give me a consensus on that, but I wasn't getting any good feedback.

DR. FERRIERI: I think we should address it. It is part of our major concern here.

DR. LEMON: Do you believe it should be known up front, then.

DR. FERRIERI: I would like to know it up front.

DR. O'BRIEN: I agree with that. I have heard enough to make me worry about not knowing that ahead of time.

DR. LEMON: Let me take the argument on the other side, just for the sake of arguing this, if, in fact, a quarter of the vaccine recipients are going to have this haplotype anyway, why do we need to know which one of four has it at the outset. Why can't we watch and wait for complications and, if we find complications at a later date -- still following this population -- assess haplotypes at that point.

DR. O'BRIEN: You also put forth another suggestion, to know it in a certain subset and look for complications there, and then that would alert for the alternative.

DR. LEMON: That would be an alternative.

DR. O'BRIEN: I still would feel more comfortable with that.

DR. JOHNSTON: The question that I would ask is what would you do differently. And your answer is that you

would just be more sensitive to possible arthritic response.

DR. O'BRIEN: I might treat them sooner. I don't know. I am just concerned.

DR. JOHNSON: But presumably you would want to do that for everybody.

DR. O'BRIEN: I know.

DR. STEERE: I would like to argue for not doing it initially. And my thinking involves several things. One is that, if you do it initially and in a large group of people, you will identify that roughly a quarter of them have the DR4 specificity.

It is true, you could look at that group more carefully in some way, if you knew that. On the other hand, I still hope that all patients will be looked at carefully, in terms of any subsequent difficulty following this vaccination.

I think some of the problems of knowing it up front in a large number of people include that there is a great deal of work involved in determining it. And also, there are subtypes of HLA DR4.

And we really don't know what part of that group is affected. And consequently, if it does prove to be a problem, I certainly think that that group should be focused on.

But I have some hesitancy about doing it up front

in a large group of people.

DR. O'BRIEN: I accept that.

DR. FERRIERI: I understand the difficulties technically, and the amount of time. I guess I would like you to re-think it, because if it were done up front, and everything works out perfectly, you will have addressed the critics as well and you would have your data up front, and that is the end of the story, more or less.

DR. LEMON: You might be able to get to that same point by having just a subset analyzed.

DR. FERRIERI: I agree.

DR. ROOS: If no one gets arthritis in the vaccine group, then it is a lot of data that is collected. I think I would go along with perhaps seeing who has chronic or even acute arthritis, and do HLA typing and taking a look at what that distribution is, versus a comparable number of non-arthritic controls.

DR. LEMON: I think I get consensus building around the table that that is perhaps a reasonable way to go, although it might be good to document up front that a certain proportion of the vaccinees have got HLA DR4 haplotype, in a subset.

DR. KARZON: If our concern about DR4 is confined to one series, I agree that perhaps it would be duplicative and perhaps these trials ought to be a mechanism of doing

so.

But it is also true that insofar as there is variance in response, effectiveness of a vaccine itself, we may want to look at it in terms of DR4.

So, whatever information we, at some point, want to learn, we are going to have to do the large group, not select for the DR4s.

But I suppose that would come down to compromise, to verify the importance of DR4. And we can always answer the second question.

DR. LEMON: The study size would also get enormous, too, if you began breaking down the population in subsets like that.

What about the length of follow up. Dick has said two years.

DR. JOHNSTON: At least two years.

DR. LEMON: Which, to me, sounds like a reasonable minimum estimate, given the point in the infection at which arthritis begins to occur.

DR. JOHNSON: I think two years is the same number I would have selected. And I would like more time for several reasons. One is that I am concerned about the decreasing antibody response and what the population looks like, immunologically, in that second year.

And second of all, in the second year, one would

go into this, at least in the intermediate if not the late stage portion of the disease, and you begin to be able to discern or screen for these second phenomena.

And I don't say three years because that is unwieldy and probably unnecessary to answer the major questions. It always could be extended.

The other reasoning is, do we really need a second dose at the twelve months. In modestly immunogenic antigens, we often have to have a true booster, if you give a short succession of doses. You are giving essentially a primary series.

A booster may be used in a subset to find out, in the long run, how to use the vaccine in the population.

DR. LEMON: Other comments from the committee on this issue, duration of follow up.

DR. FERRIERI: At least two years.

DR. LEMON: That is the third time I have heard at least. Is there a need for a subset of patients to be followed longer.

DR. EICKHOFF: I guess I will submit a minority report. I would have said three years, simply because one of the major overriding concerns that I think all of us have is, we are looking for late effects which may, in fact, be modulated, and may not be expressed the way modern natural wild Lyme disease would be expressed. So, for that reason,

I would probably err on the longer side, rather than at a minimum.

DR. JOHNSTON: The only adverse events we are really worried about are, at least as we project now, are those that are going to occur late, as the antibody dwindles.

DR. LEMON: What about children. Is there anything unique to children that would cause us to change the kind of evaluation we are talking about.

DR. ROOS: I wonder how well the natural history of the disease is known with respect to children and how different it is, in order to really assess the vaccine studies.

DR. LEMON: Perhaps Dr. Steere or Dr. Dattwyler would comment on that, perhaps, the natural history of the disease in children versus older individuals.

DR. STEERE: I think the disease is similar in children to the way it is in adults. A possible exception, though, is that arthritis appears to be milder in very young children.

For instance, we have had children aged two, three, and four, and in those children it has been mild rather than a more florid picture that we have seen in older children and in teenagers that looks the same or similar to what we have seen in adults.

We have seen the late neurologic involvement occur in children as we have in adults. In adults, the encephalopathy appears to be particularly manifested by memory impairment. And that is what the person will tell you about.

Children do not tell you about memory impairment. And actually, the neuropsych testing that we have done in children has suggested that they may have a preferential problem in auditory processing.

But in terms of what they tell you about, it is more like headache or the parent will notice some behavioral change.

And of course, in anything that non-specific, it shows why it takes very specific tests to know that, indeed, that would be due to Lyme disease.

DR. LEMON: So, it sounds like the kind of specific case definitions we were talking about earlier, might need to be modified somewhat for a pediatric population, compared to an adult population, written with children in mind rather than adults.

DR. FERRIERI: Also the issue of other arthritides that we see very commonly in children in JRIA and other entities, for example. So, I think the clinical criteria and laboratory criteria will need to be extremely rigid in order to document failure, for example.

DR. LEMON: But the basic principles that we are talking about would be pretty much the same for children and adults.

DR. STEERE: I think the principles in adults and children are pretty much the same.

DR. O'BRIEN: What about the immune response in young children.

DR. STEERE: Very young children, age two, three, and four, they develop an antibody response. In terms of comparing it according to specific polypeptides, I am not able to do that, or we just haven't done it. We haven't focused on that group.

DR. FERRIERI: You might want to follow the children for a longer time than -- the at least two years would, I think, be insufficient and I would lean in the more conservative direction of three years in childhood vaccinees.

DR. EICKHOFF: I recognize this isn't part of the question being addressed, but while Allen Steere has the mike, I would like to ask him, what is known about the natural history or the immune response in patients who are highly immunocompromised.

DR. STEERE: We know of very few such people and it just hasn't come up. I mean, we are often asked, for instance, do you know of both Lyme disease and AIDS in the

same person. And of course, Fire Island is a place where you would think there would be the potential of that happening.

And we know of a few people -- I know of one -- who had AIDS and developed Lyme disease. And he was treated with antibiotic therapy for his Lyme disease and responded well.

So, I don't really know of an immunocompromised group as being more at risk. I mean, you can think they might be, but you really don't have the patients out there that have shown that it is a greater problem.

DR. JOHNSTON: And could I ask Dr. Steere about, the youngest patients that you have seen, how young has it been diagnosed.

DR. STEERE: Age two is my youngest.

DR. JOHNSTON: Because that relates to when you would start immunizing, obviously. And that, of course, is sociologic, too, when they are out in the woods and things.

DR. STEERE: Yes.

DR. LEMON: If two is the youngest, when do you begin to see cases commonly among children, at what age.

DR. STEERE: Well, David, you must have some data on that. But the group that was on your graph from age one to nine, let's say, was a group that had a high level of disease.

DR. DENNIS: I don't have the figures for the five-year intervals, but that zero through nine group is mostly driven by the five through nines, but there are considerable numbers of cases reported zero through four.

DR. LEMON: Below the age of five.

DR. KARZON: Will there be enough children in the vaccine trials that are anticipated to develop a background from which to make recommendations. And do we want such a distribution, or should it be a secondary trial.

DR. LEMON: David, could you repeat that a little bit louder. I didn't hear to well.

DR. KARZON: Should small children be specifically included in the phase I, II, III trials, in anticipation of need for use.

Could it be, or should it be, that children are separately studied after we have some primary data in adults, and that is often done, in cases with unexpected reactions to be seen.

DR. LEMON: This would be to protect children from something we might expect, if it happened, would happen in both adults and children, not because we think children would be more likely to have an adverse effect from the vaccine. Did I interpret your comments right.

DR. KARZON: Well, sometimes they react differently and sometimes it is not expected. The usual

dividing line is that you get seronegative babies and then they turn seropositive. So, they constitute a naive group. That is not the differential here.

DR. LEMON: Other comments from the pediatricians on the committee.

DR. FERRIERI: What I would be concerned about is the age of those included. And it isn't essential that children be included from the beginning. And it could be a secondary study based on the data that accumulates from adults, and my concerns being the issues of immunologic memory, tolerance, et cetera.

I think that I would not be including children, for example, under the age of four years, perhaps, or some cut off in that area, but I would feel more secure if there was a considerable amount of data on follow up in adults.

DR. JOHNSTON: I would be more comfortable with putting it off also. I wouldn't worry about immunologic memory in the age groups I think we are talking about. The dose phenomena, I don't think, would be a risk, but other things might be, and I would worry particularly about what happens long term.

DR. LEMON: So, what I am hearing is that concern over potential long term adverse consequences as a result of immunization, should be evaluated first in adults, perhaps, before -- there should at least be data accumulated before

studies involve young children.

So, are you saying less than age four or less than age twelve.

DR. FERRIERI: For exclusion purposes.

DR. LEMON: For exclusion purposes.

DR. FERRIERI: Well, I would be willing to have a higher break point, you know.

DR. LEMON: It sounds like if you take four, it is pretty much -- you are excluding those that are at relatively low risk of acquiring the disease anyway, based upon the CDC data, focusing on those who are most likely to develop Lyme.

What about individuals who have previously had Lyme disease or who are seropositive.

Dr. Steere suggested that maybe these individuals develop their own Lyme neutralizing response, because he hasn't seen re-infections, if I understood him correctly.

Does that mean that there are any special precautions that we should take or don't need to take, in considering immunization of that group.

It would seem to me that from the point of view of keeping a study population as clean as possible, it would be nice to exclude those individuals from immunization, to immunize seronegative individuals.

DR. JOHNSTON: But if you do that, then you, in

endemic areas, the areas that you are most worried about, then you are going to -- once the trial is over, again, you are going to have a large number of people. So, the other thing you would like to know is, do they have an anamnestic response, what happens with arthritis, all the things that you are concerned about, and you are concerned about in a general population.

DR. LEMON: So, you would include them without -- include individuals without regard to their serostatus.

DR. JOHNSTON: I would include them.

DR. GLODE: I think that if you screen ahead of time and found cleaner and purer, and only entered seronegative individuals --

DR. LEMON: It is not real life.

DR. GLODE: It is not real life, plus some of those people may have had Lyme disease, been treated, and be naturally immune, even though they do not have detectable serum antibody, I gather. But I guess they would maybe have a history, at least, of antibiotic use.

DR. LEMON: But in designing a sample size, you design this based on the community incidence rates for Lyme disease that takes all of that into consideration, I suppose.

DR. DATTWYLER: A potential problem I see in immunizing individuals who have had Lyme disease is that we

know, from our studies of PCR cerebral spinal fluid that the nervous system is invaded early.

Many of the common oral regimens don't provide great levels into the CNS and there is always a question of, are there a few spirochetes still in there. I would think that would muddy the waters.

What happens if someone is immunized and then develops a neurologic complaint in that population. Is that person a vaccine failure or is that just the natural history of inadequately treated central nervous system disease, and I think that that is a problem.

And that may happen at a fairly low incidence because, even in the untreated state, late neurologic involvement is fairly uncommon.

DR. LEMON: But if you had collected serum at the time of immunization, you would be able to see that that individual was seropositive, probably.

DR. DATTWYLER: That is what I am addressing, the question of, should people who have a history of Lyme disease or seropositivity be included in this type of trial.

When one assesses efficacy, I think one has to factor into the equation that you might have recrudescence of neurologic disease as part of the natural history, which may have nothing to do with the vaccine at all.

DR. LEMON: So, are you saying you would exclude

them from the trial or you would just exclude them from the analysis, perhaps.

DR. DATTWYLER: No, I think we might have to analyze them separately or be aware of it. I certainly think, in someone who had a history of Lyme disease, other than simple erythema migrans, you might want to exclude them, because that clouds the picture a bit.

But the correct thing is that, in certain areas of the country, there is a significant background seropositivity rate, in the magnitude of five to ten percent or even more in very highly endemic areas.

DR. JOHNSON: Yes, it would make sense to exclude those with a prior history of disease because you can do that when you are immunizing.

I guess you might argue that you would much rather find out if you are going to have this kind of response with CNS disease as part of the trial. Then later on, either as the manufacturer or the approving agency, that it could occur and that it could be a problem, you have got a chance to study it.

DR. DATTWYLER: But my feeling is that it is a low incidence process and it just clouds the efficacy analysis of the vaccine. So, from that perspective you may want to exclude that population.

DR. JOHNSTON: From the analysis at least.

DR. DATTWYLER: The other thing is that it is not clear that if you take individuals who already have the disease, and they develop and OspA response, it doesn't seem to have much clinical effect as far as curing.

So, as Allen points out, you have people with arthritis who you can PCR DNA out of and you find that you can do it and they have nice OspA responses.

DR. STEERE: I think you do not want to know what has happened when you vaccinate people who have had erythema migrans in the past, or who have had Lyme arthritis in the past.

I think in the community, many of the people who are seropositive will have had some symptoms of the disease.

I would also like to clarify or say again, if the patient has erythema migrans and is treated for it -- which is the most common thing that is out there now -- the antibody response will go away in time with that patient, and that patient can become reinfected.

It is only in the people who have had an expanded immune response to the spirochete, like one gets with arthritis, where I have not seen them become seronegative, and in that group I have also not seen reinfection.

But I think one does wonder what would happen if you boost, in essence, someone who has had Lyme arthritis, by giving them an OspA vaccine.

DR. LEMON: But that may be a separate study.

DR. STEERE: Absolutely, or it could be analyzed separately.

DR. LEMON: Or separate studies so that you get adequate numbers of patients and so forth to reach the end point you are interested in.

Are there other comments from members of the committee, or consultants.

DR. KARZON: I think we ought to reckon with the fact that the vaccine may have some down sides.

If it does, if it enhances the disease anywhere along the line, because it is marginal. Obviously, if it prevents disease in the very early acute phase, it won't have the opportunity to do possible immunologic enhancement, if that is the phenomenon that drives the late disease.

But if it is less than 100 percent effective, if the immunity wanes, I think there is a possibility to see enhanced disease.

Now, we don't know that. I think a conservative point of view -- and I am not sure how I want to vote in the final decision making process, a conservative point of view would be not to immunize people who have the disease already, because they may be particularly susceptible to some sort of immune enhancement, increasing the antibody against which it may participate in the immune response,

which doesn't make much sense a priori.

So, one way to be conservative is to omit these people, see if the vaccine works, what its efficacy is under various circumstances, determine the correlates of immunity if there are such -- and perhaps in this disease it might be a threshold of a given quality antibody -- and then go ahead and look at that group, because you must, in a public program some day.

You are going to run into many people, on a statistical basis. There will be thousands of them who have had the disease and who would be at risk from taking the vaccine.

Also, we never know, until you do it, whether this is the final vaccine product. We have no idea. And we can assume that it is going to be very effective, but it may not be. Or it may be 50 percent effective, in which case we will be looking for other candidates and we will be doing successive trials.

I don't know how I would vote on this, but I think we ought to face this before we do.

DR. LEMON: I don't think that we need to take a formal vote. I don't know how we might express our proclivity.

Is it a reasonable consensus to say that we feel that seropositive individuals should not be excluded from a

pivotal efficacy study. In other words, serostatus alone should not be a criterion for inclusion or exclusion, but that individuals who have a prior history of Lyme arthritis or other late complications of Lyme, would be best excluded from a pivotal efficacy study, and perhaps studied separately in a separate trial, to address a whole different set of questions than an efficacy study would be designed to address.

DR. MITRANE: A small phase II safety trial could be done in patients, or individuals, who are seropositive. And then, as long as there are no safety concerns that have come up from the trial, then we could permit enrollment of seropositive patients in phase III efficacy trials. And that could be a way to deal with the situation.

DR. LEMON: That is one approach. Does anyone want to comment on that. I guess the question is, how long do you follow these individuals now.

DR. JOHNSTON: It would just delay the incorporation for two or three years.

DR. LEMON: Do you wait two or three years, then, to get an end point answer then. Is that a reasonable thing to do.

Do I hear voices in support of what Dr. Mitrane has outlined, among members of the committee.

DR. JOHNSTON: I think that could be, in my own

opinion --

DR. LEMON: Separately or concurrently, perhaps.

DR. JOHNSTON: Yes, concurrently.

DR. LEMON: I think the reason behind that is that the evidence supporting the possibility of an adverse reaction in that group seems very very small.

I guess concurrently is the same thing as putting it into the general trial, so that would be very different.

DR. KARZON: The only thing that would be modified is if I did them simultaneously or in a phase II trial, which is an interesting idea because you can control the numbers. You may want to look at these with more care. You may want to have more entry points into looking for antibodies and agent along the way.

DR. LEMON: I think perhaps we should move on. I think we have given the open session questions fairly good discussion. I think it has been a productive discussion here.

So, let us take a break, the, for 10 minutes and let me point out that the next session will be closed to everybody except the committee and consultants, the FDA staff, the audio visual staff, and SmithKline staff.

And everyone who is leaving the room should take everything with them. They should not leave any belongings behind in the room, because the FDA is going to sweep the

room, looking for hidden microphones and so forth, I guess, during the break. We will get together again in about ten minutes.

(Whereupon, at 2:27 p.m., the open session was adjourned.)

///