GlaxoSmithKline

January 19, 2001

GlaxoSmithKline
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA
19101-7929

Tel. 215 751 4000
Fax. 215 751 3400
www.gsk.com

Ms. Nancy Cherry, Executive Secretary
Vaccines and Related Biological Products Advisory Committee
Food and Drug Administration
Center for Biologics Evaluation and Research
Room 11-13, Rockwall-2 Building
5515 Security Lane
Rockville, Maryland 20852

SUBJECT: ADVISORY COMMITTEE BRIEFING DOCUMENT FOR JANUARY 31, 2001

Dear Ms. Cherry,

Reference is made to our vaccine, LYMErix®, Lyme Disease Vaccine (Recombinant OspA), which is scheduled to be discussed at the next Vaccines and Related Biological Products Advisory Committee (VRBPAC) meeting, on January 31, 2001.

Please find enclosed fifty copies of the Advisory Committee Briefing Document, and a floppy disc with one copy of the submission. We also commit to providing you with copies of the slides which are to be presented, at the meeting.

This document may be released in full to the public and may be displayed on the Food and Drug Administration's website before the Vaccines and Related Biologicals Products Advisory Committee Meeting of January 31, 2001.

Should there be any questions regarding this document or the arrangements for the meeting, please contact me at (215) 751-6593.

Yours sincerely,

Paula Bursztyn Goldberg, Ph.D.
Director
North American Regulatory Affairs

LYMErix®
Lime Disease Vaccine (Recombinant OspA)

Briefing Document

for

Vaccines and Related Biological Products Advisory Committee (VRBPAC)

of

January 31, 2001

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List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
AE(s)	Adverse Event(s)
B. b.	*Borrelia burgdorferi*
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CMI	Cell mediated immunity
DSMB	Data Safety Monitoring Board
ELA	Establishment License Application
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
HLA	Human Leukocyte Antigen
HMO	Health Maintenance Organization
HPHC	Harvard Pilgrim Health Care
ICD-9	International Statistical Classification of Diseases, Injuries and Causes of Death, Ninth Revision
IgG	Immunoglobulin G
i.m.	Intramuscular
IND	Investigational New Drug
LD	Lyme disease
LFA-1	Leukocyte associated antigen 1
Lipo-OspA	Lipoprotein-outer surface protein A
MHC II	Major histocompatibility complex II
NS1-OspA	Unlipidated OspA fused to 81 amino acid of the Non-structural protein 1 of influenza virus
OD	Optical Density
OR	Odds ratio
OspA	Outer surface protein A
PI	Prescribing Information
PMS	Post marketing surveillance
PLA	Product License Application
SBBio	SmithKline Beecham Biologicals (note: SmithKline Beecham is now GlaxoSmithKline)
SAE	Serious Adverse Event
Th1	T-helper type 1
TRLA	Treatment resistant Lyme arthritis
VAERS	Vaccine Adverse Events Reporting System
VRBPAC	Vaccines and Related Biological Products Advisory Committee
WB	Western Blot

1.0 Introduction and Overview

1.1 Executive Summary

The purpose of this document is to provide an overview of the developmental and regulatory history of LYMErix® [Lyme Disease Vaccine (Recombinant OspA)] from initial IND (Investigational New Drug Application) to the present, with particular emphasis on its safety profile.

Section 1 provides background information on Lyme disease (LD) itself, the vaccine, and the regulatory history, which includes interactions between SmithKline Beecham Biologicals (SBBio), its advisors, and The Agency.

Section 2 provides a summary of the data, specific issues of interest, and overall safety assessment for *LYMErix* that supported licensure and launch.

Section 3 addresses the post licensure commitments and the activities that were instituted in order to continue and expand safety assessment, together with the current view of the safety profile of *LYMErix*. Several of the commitments, especially those which were to be accomplished in the short-term post approval, are completed; others, which were to take longer to accomplish have been implemented and are ongoing. Efforts are underway to enhance the enrollment of subjects in a post marketing safety assessment cohort study being conducted out of Harvard Pilgrim Health Care (HPHC).

As a result of post marketing experience, two changes to the safety section of labeling are being proposed: one describes the reporting of concomitantly occurring symptoms, already individually described in the product label; the other describes the occurrence of hypersensitivity reactions not previously observed in clinical trials. The proposed labeling changes have been shared with The Agency and are being prepared for submission; they are also described in this document (see Sections 3.6.1 and 3.6.2).

As compared to the safety profile of *LYMErix* at approval, data from post marketing surveillance (PMS) and Phase IV studies do not indicate any additional safety concerns.

To date, available results do not indicate any increased incidence of an inflammatory arthropathy or chronic neurologic events, nor do they provide support for theoretical concerns.

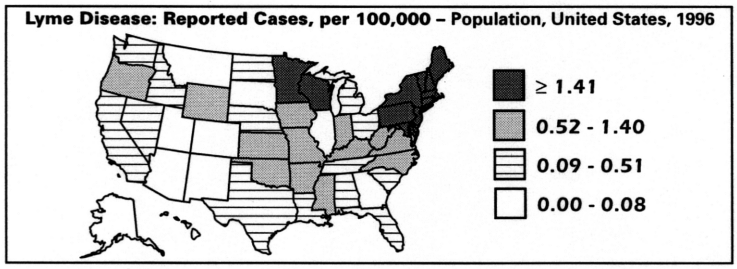
Finally, additional clinical trials being conducted since licensure have not brought to light any novel safety concerns to date.

Note: A full copy of the current Product Information (PI) for *LYMErix*, together with associated references, is provided in Attachment 1.

1.2 Background

1.2.1 Lyme Disease

Lyme disease is a multisystem disease caused by infection with the bacterial spirochete, *Borrelia burgdorferi (B. b.)*, which is transmitted by *Ixode*s ticks. Since its recognition in 1975, LD has become the most commonly diagnosed vector-borne disease in the United States, with over 99,000 cases reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1996. Although most cases have been reported in the Northeast, upper Midwest and Pacific coastal areas of the United States, infections have been reported in almost all states (see map below).



The early stage of LD is usually characterized by a rash (erythema migrans) and may be accompanied by fever, fatigue, myalgias and/or arthralgias. Early disseminated manifestations include secondary skin lesions, neurologic involvement, cardiac involvement, and musculoskeletal symptoms usually consisting of migratory pain in joints and the surrounding soft tissue structures. Late stage disease occurs months to years after initial infection and may be manifested as chronic arthritis, chronic neurologic abnormalities or acrodermatitis chronica atrophicans. Not all patients with LD have this characteristic progression of symptoms. Late stage disease usually requires more intensive therapy and may result in permanent sequelae.

Currently, there are no clinically proven methods to control enzootic *B. b.* infection or to prevent its spread. Personal protection measures to avoid tick bites are cumbersome, inconvenient and infrequently implemented, and are thus largely ineffective in eliminating the risk of LD in endemic regions. Moreover, late stage disease may result from early disease that is either unrecognized or fails to respond to treatment, or from asymptomatic infection. As a result of these factors, the introduction of a preventive vaccine was considered to be a critical approach to the primary prevention of LD in the United States.

For additional information on LD and its epidemiology, please refer to the PI provided as Attachment 1.

1.2.2 Description of the Vaccine

LYMErix is a noninfectious recombinant vaccine developed and manufactured by SBBio. The vaccine contains lipoprotein OspA (Lipo-OspA), an outer surface protein of *B. b. sensu stricto* ZS7, as expressed by *Escherichia coli*.

Each 0.5 mL dose of vaccine consists of 30 mcg of Lipo-OspA adsorbed onto 0.5 mg aluminum (as aluminum hydroxide). Each dose of the vaccine preparation contains 10 mM phosphate buffered saline and 2.5 mg of 2-phenoxyethanol, a bacteriostatic agent.

Primary immunization against LD consists of a 30 mcg/0.5 mL dose of *LYMErix* given by the intramuscular (i.m.) route at 0, 1 and 12 months. The vaccine is currently indicated for active immunization against LD in individuals 15 to 70 years of age.

1.2.2.1 Mechanism of Action

LYMErix stimulates specific antibodies directed against *B. b.* The organism contains several outer surface proteins. Administration of Lipo-OspA to mice results in the formation of specific IgG anti-OspA antibodies. These antibodies have demonstrated bactericidal activity.

Borrelia burgdorferi express OspA while residing in the midgut of the infected tick, but OspA is downregulated after tick attachment and is usually undetectable or absent when *B. b.* is inoculated into the human host. Thus, a novel hypothesis has been proposed to explain the effectiveness of Lipo-OspA vaccination: when infected ticks bite humans who have been vaccinated with *LYMErix*, the vaccine-induced antibodies are taken up by the tick and interact with the *B. b.* in the midgut of the tick, thereby preventing transmission of the organism to the host. This mechanism has been suggested by a pre-clinical study in which *B. b.* were detected by immunofluorescence assay in none of the ticks that fed on OspA-immunized mice, compared with 72% of ticks that fed on control-immunized mice.

1.3 Regulatory History of *LYMErix*

An outline of the regulatory history of *LYMErix* (pre-IND to present) is provided in Figure 1.

1.3.1 Pre-Licensure Activities

Following extensive preclinical testing and preliminary clinical testing in Europe, which lead to the selection of the Lipo-OspA candidate for further development, SBBio and the Food and Drug Administration (FDA) convened a pre-IND meeting in October, 1993, in order to agree upon initial US development plans. The IND application was submitted in February, 1994, in order to initiate Phase II clinical studies.

Subsequent to IND filing, five seminal regulatory meetings were held during the pre-licensure period:

- In June, 1994, FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) convened to discuss and advise on the conduct of a pivotal efficacy and safety study for Lyme vaccine. At this time, the Advisory Committee made recommendations on the following:
  - case definition.
  - 1º and 2º study endpoints.
  - two-year follow-up for safety and efficacy.
  - inclusion of patients with previous history of LD.
- In December, 1994, SBBio held an End of Phase II meeting with the Center for Biologics Evaluation and Research (CBER) in order to discuss and agree upon the final design of the extensive Phase III efficacy and safety trial, Lyme-008, based on available data and the recommendations of the June, 1994 VRBPAC meeting. The pivotal efficacy trial was initiated in January, 1995.
- While the efficacy trial was in progress, another VRBPAC hearing was convened in April, 1996, to discuss the basis for proceeding to a pediatric indication for Lyme vaccine. In addition, the following theoretical questions were raised for the vaccine:
  - the potential for exacerbation of *B. b.* pathology in individuals with a previous history of LD (see Section 2.2.4.1).
  - the possibility that the vaccine might alter or mask the presentation of LD in vaccine failures, with the result that no treatment is administered (see Section 1.3.2).
  - possible induction of autoimmune arthritis due to production of anti-OspA antibodies (see Section 2.2.4.2).

- Based on demonstration of efficacy in Lyme-008, a pre-PLA (Product License Application) meeting was held with CBER in January, 1997. This resulted in the submission of the PLA and ELA (Product and Establishment License Application) in September of that year.

- The last VRBPAC was convened to discuss the basis for approval of *LYMErix*, in May, 1998. In the clinical trials which were completed at the time of licensure, a total of 6,478 individuals received more than 18,000 doses of the ultimately licensed formulation and strength of *LYMErix* (30 mcg OspA adsorbed onto aluminum), on a 0, 1, 12-month schedule. The vaccine was deemed safe and efficacious.

1.3.2 Licensure of *LYMErix*

A summary of the data, specific issues of interest and overall safety assessment for *LYMErix* that supported licensure is outlined in Section 2 of this briefing document.

In brief, a large body of data was accrued prior to licensure which, *in toto*, provided evidence that *LYMErix* had an acceptable safety profile. The retention rate of volunteers in the clinical studies was very high (approximately 95% in the phase III pivotal trial), underscoring the tolerability of the vaccine in such individuals. The body of safety information is summarized in Section 2; however, the following is worth highlighting:

- Serious adverse events (AEs) did not occur more frequently in the vaccine group than in the placebo.

- An evaluation of the safety of the vaccine in subjects with a previous history of LD did not point to any evidence of exacerbation of *B. b.* pathology.

- No evidence of induction of an autoimmune arthritis as a result of vaccination was seen (see Sections 2.2.4.2 and 3.6.3). Additionally, no cases of hypersensitivity reactions to vaccination were reported in clinical trials.

- With regard to the potential impact of vaccination on the course of LD and its diagnosis (as raised at the 1996 VRBPAC), it was concluded that there was no impact of vaccination on the clinical presentation of disease. However since vaccination may result in positive IgG ELISA in the absence of infection, Western Blot (WB), should be used to confirm LD (see labeling in Attachment 1, *Laboratory Test Interactions*).

Based on the above safety data and in conjunction with the demonstrated efficacy of *LYMErix* in preventing LD (78% efficacy against definite LD and 100% efficacy against asymptomatic infection after 3 doses), the product was approved in December, 1998. At

approval, commitments were made to continue evaluation of the safety profile of *LYMErix* post approval; these activities are outlined below (Section 1.3.3), and progress on the commitments is detailed in Section 3 of this document.

1.3.3 Post Licensure Commitments

LYMErix was launched in January, 1999, and has thus been in the US marketplace for exactly two years. The vaccine continues in development for evaluation of potential new indications and for completing the ongoing commitments. A status report on these activities is provided in Section 3; below is provided a brief summary of the status of the commitments.

1.3.3.1 Post Marketing Safety Assessment Cohort Study

- To address the theoretical concern that immunization with a vaccine containing OspA might induce an autoimmune arthritis, a post marketing safety assessment cohort study based on automated record linkage methodology, was initiated at HPHC. The primary objective of the study is to evaluate whether exposure to *LYMErix* is a risk factor for new onset inflammatory arthropathy, and also to evaluate whether exposure to *LYMErix* is a risk factor for LD, treatment resistant LD, rheumatoid arthritis, certain neurologic diseases, allergic events, hospitalizations and death.

- Twenty five thousand HPHC members who are expected to receive *LYMErix* vaccine are being matched to a group of non-vaccinated (unexposed) HPHC members with a 1:3 ratio for age, gender and primary care practice. The follow-up period is four years. The planned exposure will provide more than 80% power to rule out an incident rate ratio of 2 for an adverse event which occurs at a rate of 3 per 10,000 in the control group.

- Following submission of the draft protocol to CBER in May, 1999, discussions were held which resulted in submission of an agreed protocol in May, 2000. Database queries were initiated at that time, going back to entries beginning with January, 1999. To date, three quarterly interim reports have been submitted for review by The Agency (June 15, 2000, September 15, 2000 and December 15, 2000).

- For the most recent report, HPHC automated record systems identified 3,677 individuals with codes indicating *LYMErix* immunization through November 15, 2000. Matched data were available for 2,568 vaccines and 7,497 controls encompassing the initial six months since *LYMErix* licensure.

- At this preliminary stage, the available results of this study do not suggest that rheumatologic, neurologic, or allergic outcomes of interest, identified *a priori* in the

study protocol as being of particular interest, were more frequent among *LYMErix* recipients than among unexposed subjects.

- Having recognized that enrollment of vaccinees into the database is at a slower rate than anticipated in the protocol, in order to augment accrual, HPHC has identified two additional Health Maintenance Organizations (HMOs) in LD endemic areas (Health Partners of Minnesota and Tufts Health Plan) as collaborators on the study. These institutions will begin contributing data in the very near future.

1.3.3.2 Study on Cell Mediated Immunity

Additional analyses of data on cell mediated immunity (CMI) and HLA typing from the pivotal Lyme-008 study were completed after licensure and a study report submitted to CBER in December, 1999.

The results of HLA typing, CMI responses, and evaluation of specific adverse reactions in these subsets did not provide support for an association between vaccination with *LYMErix* and an increased incidence of an inflammatory arthropathy.

1.3.3.3 Reproductive Toxicity Study and Pregnancy Registry

At the time of licensure, the following Guidance documents pertaining to products for use in adolescents and adults were in draft at The Agency:

(a) "Considerations for Reproductive Toxicity Studies for Preventive Vaccines for Infectious Disease Indications" (subsequently published in draft form for comment in August, 2000 and,

(b) " Establishing Pregnancy Registries" published for comment in June, 1999.

Based on alerts to these Guidances at the time of approval in 1998, SBBio made commitments to conduct a preclinical reproductive toxicity study with *LYMErix* and to establish a pregnancy registry (with design agreed by The Agency) as a condition of licensure.

The reproductive toxicity study has been completed; a report was submitted to The Agency in January, 2000. There were no pertinent adverse findings. The registry of pregnancies has been established and is in operation; its review to date has indicated no pattern of abnormal pregnancy events.

1.3.4 Post Marketing Surveillance

Post marketing surveillance (PMS) has been in effect since launch of the vaccine in January, 1999, and seven quarterly reports have been submitted to The Agency. Approximately 1.4 million doses have been distributed. An overview of the current safety profile for *LYMErix* is provided in Section 3.

Correspondence describing all activities related to capture of AE reports between SBBio and CBER during the past six months has resulted in the following additional steps to quality assure the complete and timely capture of AEs from clinical trials and the PMS process, as follows:

- expedition of reporting on musculoskeletal and neurological events.

  Submission of musculoskeletal and neurological events, regardless of seriousness, was expedited (submitted to The Agency within 15 days of receipt by SBBio). This practice was instituted at CBER's request in a letter to SBBio of June 28, 2000, to ensure the earliest accrual of this information.

- Letter to Investigators.

  In order to further assure that investigators remain receptive to reports of AEs from subjects who completed participation in clinical trials, so that such reports could be recorded in our database and provided to The Agency, SBBio wrote a letter to investigators on November 29, 2000, requesting their attention to this matter. The letter noted that it had been brought to our attention that some subjects who participated in past clinical trials have met with some resistance when reporting AEs to their original trial sites. The letter reiterated the procedures and responsibilities in reviewing and reporting AEs; additionally, it encouraged investigators to report any AE to SBBio regardless of attribution, if a subject/parent/guardian seemed particularly concerned about an event.

The current safety profile of LYMErix is as follows:

- The profile is generally similar to that observed at licensure. For other reported AEs, causal relationships with Lyme vaccine have not been established.

- The following findings, however, have led to a proposed labeling change, a preliminary draft of which was shared with CBER by facsimile on January 3, 2001:

  - Reporting of concomitantly occurring symptoms already described individually in the product label (arthralgia, fatigue, fever, influenza-like symptoms, chills/rigors, headache, achiness, myalgia and nausea), with an early onset (see Section 3.6.1)

- Hypersensitivity reactions not previously observed in clinical trials (see Section 3.6.2).

- Based on the data available, no pattern, except that described above as a proposed labeling change, was identified and there is no evidence that arthritic conditions reported were related to an autoimmune process. Furthermore, there were no unexpected patterns in age, gender, time to onset, vaccine dose, or number of reports of rheumatoid arthritis and facial paralysis. The musculoskeletal data will be evaluated by an independent panel of rheumatology experts.

1.3.5 Clinical Trials Completed and Ongoing Since Licensure

In addition to the data accrued in the PMS system, additional clinical data have been forthcoming from controlled clinical trials reported or initiated after the licensure of *LYMErix*. These trials involve alternate vaccination schedules and pediatric subjects, (n = 3 900), and booster studies (n = 1 800). The data available from these investigations do not bring to light any new safety concerns.

1.4 Conclusions

Based on the review of the efficacy and safety of *LYMErix* by FDA and the VRBPAC, licensure was granted on December 21, 1998. It has thus been marketed in the US for two years.

All regulatory activities and commitments to expand our knowledge of the safety of *LYMErix* are completed or in place and ongoing, including efforts to ensure timeliness and completeness in the accrual of post marketing trial and surveillance data.

To date, there are two changes proposed to the approved labeling: one describes the reporting of concomitantly occurring symptoms, already individually described in the product label; the other describes the occurrence of hypersensitivity reactions not previously observed in clinical trials.

Figure 1: Regulatory History of *LYMErix*

Oct 28, 1993	**Pre-IND Meeting**
Feb 2, 1994	**IND Submitted**
Jun 7, 1994	**VRBPAC Meeting on Lyme Disease & Vaccine Pivotal Development**
Dec 6, 1994	**End of Phase II Meeting and Lyme–008 discussion**
Apr 10, 1996	**VRBPAC Meeting on Theoretical Safety Concerns and Plan for Pediatric Development**
Jan 28, 1997	**Pre- PLA Meeting**
Sep 15, 1997	**PLA/ELA Submitted**
May 26, 1998	**VRBPAC Meeting to Evaluate Safety and Efficacy for Approval**
Dec 21, 1998	***LYMErix* Approved**
Jan 08, 1999	***LYMErix* Launched**
May 10, 1999	**Post Marketing Cohort Study Protocol Submitted (Commitment)**
Dec 22, 1999	**Final Report of CMI Study Submitted (Commitment)**
Jan 20, 2000	**Reproductive Toxicity Study Report Submitted (Commitment)**
Jun 15, 2000	**Post Marketing Cohort Study First Interim Report Submitted (Commitment)**
June 28, 2000 and July 24, 2000	**Letter from FDA and Response from SB, respectively, on questions of safety reporting**
Sep 15, 2000	**Post Marketing Cohort Study Second Interim Report Submitted (Commitment)**
Dec 15, 2000	**Post Marketing Cohort Study Third Interim Report Submitted (Commitment)**
Dec 19, 2000	**Copy of Letter to Investigators (distributed Nov 29, 2000) Submitted**
Jan 3, 2001	**Proposed label change sent to The Agency**

2.0 Safety Assessment of *LYMErix* for Licensure/Approval

2.1 Overall Summary of Studies

In support of licensure of the vaccine, SBBio conducted a total of 16 clinical trials (including ongoing trials) by the time of submission of the PLA (see Table 1).

In Phase I clinical trials in Europe (Lyme-001 to Lyme-004), several candidate LD vaccines containing NS1-OspA or Lipo-OspA antigen were evaluated. The Lyme vaccine candidate, Lipo-OspA adsorbed on aluminum hydroxide, was found to be highly immunogenic with an acceptable safety profile and therefore was selected for Phase II.

Two Phase II trials (Lyme-005 and Lyme-007) were performed in the U.S. Lyme-005 was a double-blind placebo controlled dose range study, conducted at three highly endemic sites, with approximately 90 subjects per group. Subjects were vaccinated with 3, 10, or 30 mcg of the candidate vaccine or placebo. The data demonstrated that the vaccine was immunogenic in a dose dependent fashion for anti-OspA antibodies. In regard to safety, there was no increase in the incidence of local or general symptoms following each successive dose. This study also provided valuable experience with regard to methodologies for surveillance of LD, documentation of disease, and case definitions, which supported the very thorough and robust design of the pivotal efficacy trial.

Lyme-007 was initiated to address the issue of the safety of the vaccine in subjects with previous, well-documented LD. In the Lyme-007 trial, subjects previously infected with *B. b.* did not demonstrate vaccine-induced serious adverse effects when vaccinated with Lipo-OspA. Specifically, the vaccine did not induce any Lyme-like pathology. Based on the immunogenicity and safety data from Lyme-005 and -007, clinical development of the 30 mcg Lipo-OspA/0.5 mg aluminum vaccine proceeded to the Phase III pivotal efficacy trial.

Vaccine efficacy was demonstrated in a prospective, multi-center, randomized, double-blind, placebo controlled trial (Lyme-008). This study was conducted over two tick transmission seasons, utilizing investigators located at 31 sites highly endemic for LD, most of which were in the northeastern United States. Beginning in January, 1995, a total of 10,936 healthy adolescents and adults (15-70 years) at risk of LD were randomized to received vaccine or placebo. Doses were administered at baseline, 1 and 12 months (0, 1, 12 months). Subjects were followed for a total of 20 months in Lyme-008 and then for an additional 4 months in an open label fashion in a follow-up study, Lyme-013. Therefore, 24 months of safety data were available for the original Lyme-008 cohort at the time of licensure. As such, the majority of the safety data which was the basis for licensure of *LYMErix* was gathered in the context of this pivotal efficacy trial.

The Lyme-008 study accrued more than 17,000 person-years of observation and provided a large body of evidence that the candidate Lyme vaccine was efficacious, immunogenic and safe. The retention rate of volunteers in this study was very high (almost 95%), underscoring the tolerability of this vaccine. Vaccine efficacy against definite LD was 78% (95% CI: 59% to 88%) after three doses of *LYMErix* (13 cases among 4,765 subjects in the vaccine group; 58 cases among 4,784 subjects in the placebo group). Vaccine efficacy against asymptomatic infection was 100% (95% CI: 30% to 100%) after three doses of *LYMErix* (0 cases among 4,765 subjects in the vaccine group; 13 cases among the 4,784 subjects in the placebo group).

In the clinical trials which were completed at the time of licensure, a total of 6,478 individuals received more than 18,000 doses of the ultimately licensed formulation and strength of *LYMErix* (30 mcg OspA adsorbed onto aluminum) on a 0, 1, 12-month schedule.

Table 1 Summary of Studies by Study Design and Objectives

Study	Phase	Age (yrs)	Design/Objectives	Formulation (N)	Schedule (months)	Duration (months)
001	I	18-40	O	NS1 5 mcg (12) NS1 20 mcg/AL (12)	0, 1, 2	6
002	I	18-49	O, R	NS1 10 mcg/AL (60) NS1 10 mcg/AL + MPL (60) L 10 mcg/AL (60)	0, 1, 2	24
003	I	18-49	DB, R	NS1 10 mcg/AL + MPL (80) L 10 mcg/AL (80) NS1 10 mcg/AL (80)	0, 1, 2	3
004	I	18-47	O, R	L 10 mcg/AL (30) L 30mcg/AL (30) L 30 mcg (30)	0, 1, 2	3
005	II	18-83	DR, R, DB, PC, MC HLA typing	L 10 mcg/AL (89) placebo/AL (87) L 3 mcg/AL (88) L 30 mcg/AL (89)	0, 1, 2	12
007	II	21-79	O, NR Safety in previous LD	L 3mcg/AL (5) L 10 mcg/AL (5) L 30 mcg/AL (20)	0, 1, 2	6
008	III	15-70	DB, R, PC, MC Pivotal Efficacy	L 30 mcg/AL (5,469) placebo/AL (5,467)	0, 1, 12	20
009	II	18-49	O, R	L 30 mcg/AL (30) L 30 mcg (60)	0, 1, 2	13

010	II	18-48	O, R different lots	L 30 mcg/AL (20) L 30 mcg/AL (20)	0, 1	2
013	III	15-70	O, MC Extended F/U of 008 Placebo Crossover	L30 mcg/AL	4 months pre-vaccine then 0, 1, 12 in prior placebo recipients	17
014	III	14-50	O, R, MC Alternate Schedule	L 30 mcg/AL	0, 1, 6 or 0, 1, 12	13
015	II	5-15	DB, R Pediatric Population	L 30 mcg/AL L 15 mcg/AL	0, 1, 2	3
016	III	15-70	O, R, MC Alternate Schedule	L 30 mcg/AL	0, 1, 2, 12 or 0, 1 , 12	13
017	III	15-70	O Booster	L 30 mcg/AL	Booster	36
018	II	18-50	O	L 30 mcg/AL	0, 7, 28 days	2
019	II	15-50	DB, R	L 30 mcg/AL	0, 1	3

Design Codes	Formulation Codes
DB = Double Blind	AL = Aluminum (as aluminum hydroxide)
DR = Dose Ranging	L = Lipoprotein OspA
MC = Multicenter	MPL = Monophosphoryl lipid A
N = Number	NS1 = NS1-OspA (fusion protein)
NR = Non-randomized	
O = Open	
PC = Placebo Controlled	
R = Randomized	

2.2 Safety and Reactogenicity of LYMErix in the Pivotal Efficacy Trial (Lyme-008)

Adverse events were recorded at each study visit. In addition, all subjects were asked to describe any AEs which might have occurred between vaccinations or visits *via* postcards.

Unsolicited events were recorded at early (≤ 30 days) and late (> 30 days) post vaccination intervals (through month 24); any cases of suspected but unconfirmed LD were considered and analyzed as "general unsolicited AEs."

Serious unsolicited events were recorded for all subjects throughout the entire study.

Solicited events (reactogenicity data recorded on diary cards on the day of vaccination and through three additional days) were solicited from a subset of the total cohort consisting of all subjects at a single site.

In addition, events/areas of special interest for safety assessment were evaluated (see Section 2.2.4):

- safety profile in the subset of study participants with previous LD.
- autoimmune arthritis (addressed prospectively).
- rates of neurological and cardiac events in vaccinees and placebo recipients.

A summary of the data obtained is provided below.

2.2.1 Unsolicited Adverse Events

The most frequently reported (≥1%) unsolicited AEs within 30 days of vaccination for all subjects receiving at least one dose (n = 10,936) in the double-blind, placebo-controlled efficacy trial (Lyme-008) are shown in Table 2 of the *LYMErix* Prescribing Information (PI, see Attachment 1). More vaccine than placebo recipients reported local and general unsolicited symptoms within 30 days of each dose. This difference was more pronounced for local symptoms than for general symptoms. Statistically significant differences were found between the vaccine and placebo groups for fever, influenza-like symptoms, injection site pain, injection site reactions, myalgia and rigors.

The most frequently reported (≥1%) unsolicited AEs occurring more than 30 days following vaccination for all subjects (n = 10,936) in Lyme-008 are shown in Table 3 of

the *LYMErix* PI (see Attachment 1). As can be seen, no statistically significant differences were found between placebo recipients and vaccinees in the comparison of AEs by body system or the incidence of any frequently reported late AE by preferred term after any dose or overall.

2.2.2 Solicited Adverse Events

The frequency of solicited local and systemic AEs was evaluated in a subset of the study population (n = 938) who comprised the total enrollment at one study center in the Lyme-008 efficacy trial. Of these 938 subjects, 800 completed a 4-day diary card following each of three doses, and were evaluable according to protocol. Table 4 of the *LYMErix* PI (see Attachment 1) shows the percentage of subjects reporting a solicited symptom following any one of the three doses and overall.

The majority of subjects (vaccinees, 96.5%; placebo recipients, 82.4%) reported at least one symptom. Most solicited events were mild to moderate in severity and limited in duration. As with most vaccines, soreness was the most common solicited injection site reaction, which often lasted for several days. Redness and swelling at the injection site were reported to occur less frequently. Severe soreness was reported to occur following only approximately 5% of doses administered. General symptoms were reported at a lower frequency than local symptoms. Headache and fatigue were the most commonly reported systemic AEs following vaccination. Severe systemic reactions, including fever, were reported rarely. For both local and general symptoms, reaction rates did not appear to increase with successive doses.

As can be seen from Table 4 of the PI (see Attachment 1), statistically significant differences were found between the vaccine and placebo groups for the overall rates of each solicited symptom with the exception of headache and fever, and the rates were higher for vaccinees than for placebo recipients.

2.2.3 Serious Adverse Events Including Deaths

Serious AEs did not occur more frequently overall or for any body system in the vaccine group as compared to placebo (refer to Adverse Reactions Section of the *LYMErix* PI in Attachment 1). Among the 10,936 subjects enrolled in the efficacy trial and followed for 20 months, a total of 15 deaths occurred (10 vaccine, 5 placebo). None of these deaths were judged by investigators to be treatment-related. In the vaccine group, causes of death included: cancer (5), myocardial infarction (3), sudden death (1), cardiac arrest (1). In the placebo group, causes of death included: cancer (1), sudden cardiac death (1), cardiac arrest (1), septic shock (1), homicide (1).

2.2.4 Areas of Special Interest

2.2.4.1 Subjects with Previous LD

Due to a theoretical concern regarding exacerbation of *B. b.* pathology, the safety profile of *LYMErix* was evaluated in Lyme-008, in subjects with a previous history of LD. Subjects with previous LD were assessed using two definitions:

- those whose baseline sera were evaluated for Western blot (WB) (n = 250); and
- those who at study entry self-reported a previous history of LD (n = 1,206).

The nature and incidence of AEs (either early or late) did not differ between vaccinees determined to have been WB-positive at baseline (n = 124) compared to vaccinees determined to have been WB-negative at baseline (n = 151).

Interestingly, using the more subjective definition, vaccinees with a self-reported history of LD had a greater incidence of the following symptoms than vaccinees with no prior history of LD: musculoskeletal symptoms (early and late), psychiatric disorders (early and late), central, peripheral and autonomic nervous system disorders (late), and gastrointestinal disorders (late). The same pattern was observed in the placebo subjects for all the above mentioned symptoms, except for musculoskeletal symptoms of early onset, where no statistical difference was observed in the placebo group self-reporting LD. The data indicate that subjects self-reporting a history of LD tend to report more adverse events in general.

In summary, although an excess of certain adverse events have been reported in subjects self-reporting LD, as compared to subjects who did not report LD, this has not been confirmed if prior LD status is determined using the more objective WB positivity criterion. The data, therefore, do not provide a basis for the theoretical concern that the vaccine might exacerbate symptoms in subjects with previous LD.

2.2.4.2 Induction of Autoimmune Arthritis

Introduction

In June 1998, Allen Steere published a paper entitled "Identification of hLFA-1 as a candidate autoantigen in treatment resistant Lyme arthritis" (Gross DM et al. 1998, *Science* 31;281:703-6), presenting the hypothesis that OspA is responsible for treatment resistant Lyme arthritis (TRLA) because its sequence contains a peptide homologous to LFA-1, an integrin receptor present on the surface of lymphocytes. This publication had been preceded by a series of others suggesting that TRLA could be an autoimmune disease triggered by a *B. b.* infection.

The observations presented in this paper suggest an association between a *B. b.* infection and the subsequent development of TRLA in a fraction of infected individuals. They also indicate that OspA contains a peptide that is partly homologous to an LFA-1 peptide, one that has a high probability to be a DRB1*04 epitope.

Preclinical evaluation

By themselves, these results do not support formally the demonstration of a link between the presence of an anti-OspA response in the joint and the development of TRLA *via* an autoimmune phenomenon, since:

- it has been shown recently that epitope mimicry by itself, or even *in vitro* T-cell cross-reactivity are not sufficient to explain a potential auto immune disease (Maier et al., 2000, *Eur J Immunol* 30:448-57)

- the presence of arthritic symptoms in one (or only a few) joint(s) in TRLA upon infection, does not support an autoimmune phenomenon due to a cross-reactivity between OspA and LFA-1. Indeed, this molecule is present throughout the body, so that multiorgan autoimmune phenomena should be expected. The monojoint nature of the arthritis rather suggests the undetected presence of *Borrelia* or *Borrelia* antigens in the synovial fluid or in the synovium.

In any case, even if demonstrated true, the development of TRLA resulting from the molecular mimicry between OspA and hLFA-1 has requirements that are not achieved by vaccination. It indeed implies that a *B. b.* infection has taken place in the affected joint. This infection is essential to generate a strong Th1 response in the joint, which is not expected to result from vaccination with OspA.

Since the May, 1998, meeting of the VRBPAC and the publication of the Gross et al. paper, no data have been published to further demonstrate the hypothesis put forth in that paper.

The above data have been reviewed by an independent panel of experts in autoimmunity, who reached the conclusion that the data available do not support the hypothesis of autoimmune origin of TRLA.

In addition, SBBio has recently initiated a series of experiments in mice to analyze the impact vaccination with OspA on joints. C3H mice have been shown to be susceptible to the development of arthritis upon *B.b.* infection, and have therefore been selected for these experiments.

Mice were either immunized twice in one leg with 1/10 of a *LYMErix* dose or were infected with *B.b*. The animals were observed for the development of clinically visible arthritis, and subsequently sacrificed to evaluate histologically the presence of OspA and/or of an inflammatory process in the proximal, as well as distal joints.

Preliminary results show the following:

- the infected animals developed a clinical arthritis, as judged by joint swelling.
- none of the vaccinated animals showed signs of joint swelling.
- no inflammation was visible in the joints of the vaccinated mice.
- no OspA was seen in the joint of the vaccinated mice.

The results of this experiment indicate that upon vaccination with OspA, none of the requirements identified for the development of TRLA (presence of OspA in the joint, inflammatory process) are met.

Clinical Observation

From a clinical point of view, the theoretical concern that high anti-OspA titers from vaccination may induce an inflammatory arthropathy was addressed prospectively in Lyme-008 by the sponsor and the Data Safety Monitoring Board (DSMB). Additionally, HLA typing was performed in Lyme-005 (Phase II trial), as well as in a subset of Lyme-008 subjects. Cell mediated immunity was also evaluated in the same Lyme-008 subset. A preliminary report of the CMI findings from this subset was reviewed prelicensure; a final report was submitted following approval in fulfillment of a post approval commitment (see Section 3.5).

HLA typing in Lyme-005 and Lyme-008

In Lyme-005, a double-blind, placebo controlled dose-ranging study in which subjects were vaccinated with 3, 10, or 30 mcg of Lipo-OspA/aluminum or with placebo (approximately 90 subjects per group), most subjects were tested for HLA. A total of 32% of vaccinees were positive for the DR4 allele and 0.8% for the DR2 allele, a prevalence which is representative of the population at large. Within the limited power of the study, there was no evidence of an increased risk of AEs, specifically arthritis, in this population.

In Lyme-008, HLA typing was performed at one study site; results are available for 85 samples from 100 consecutive subjects: 41 vaccine or 44 placebo recipients, regardless of the presence or absence of symptoms. The HLA profile in vaccinees who reported pain

or inflammation at the injection site was similar to that in vaccinees and placebo recipients who did not report such symptoms. Two vaccine recipients were reported to have had arthralgias, headache, or malaise for one or two days after vaccination, and one placebo recipient had a viral syndrome for 16 days that was thought to be possibly related to vaccination. All 3 of these subjects did not have the DR4 or DR2 allele. Additionally, HLA typing was available in 9 of 15 subjects (4 vaccinees, 5 placebo recipients) from any site, who had evidence of arthritis or tendinitis on examination, and in whom an alternative diagnosis for joint symptoms was not found. In this subset, the DR4 allele was present in one vaccinee and one placebo recipient, respectively; the DR2 was not present.

Musculoskeletal Events in Lyme-008

At study initiation, the investigators were asked to report any subject who developed new onset of arthritis or arthralgia following vaccination. One hundred and seven (107) subjects developed joint symptoms within one month of vaccination, which lasted at least one month. There was no evidence of unequal distribution of symptoms by study group, nor of the vaccine causing these symptoms.

The safety data from Lyme-008 on the occurrence of arthralgia were collected as part of the 4-day diary card which solicited reactogenicity data from a subset of subjects. The incidence of arthralgia was statistically different ($p = 0.001$) in the vaccine group (25.6%) compared to placebo (16.3%). However, most of the cases were mild to moderate in severity and self-limited, with only four severe cases in the vaccine group and two in the placebo group.

Separate *post hoc* analyses were conducted to assess two subsets of musculoskeletal events, those which occurred early (≤30 days) or late (>30 days) post vaccination in Lyme-008. There were no significant differences, either early or late, between the vaccine and placebo recipients with regard to experiencing arthritis, aggravated arthritis, arthropathy or arthrosis. However, vaccine recipients were significantly more likely than placebo recipients to experience early events of arthralgia or myalgia after each dose [for dose 1: odds ratio (OR), (95% CI) = 1.35 (1.13, 1.61); dose 2: OR = 1.28 (1.05, 1.56); dose 3: OR = 1.59 (1.18, 2.16)]. With regard to late events of arthralgia or myalgia, there were no significant differences between vaccine and placebo recipients.

Overall, approximately 18% of subjects enrolled in the study had a prior history of some musculoskeletal condition (19% vaccinees, 18% placebo recipients). In a *post hoc* subgroup analysis, there was no significant difference between vaccine and placebo recipients with regard to development of musculoskeletal events (defined as arthritis, arthropathy, arthrosis, synovitis, tendinitis, polymyalgia rheumatica, bursitis or rheumatoid arthritis, and lasting more than 30 days) in those with a prior history of

musculoskeletal conditions. However, both vaccine and placebo recipients with a prior history of musculoskeletal conditions were more likely to experience musculoskeletal events than subjects without such prior history.

Four members of the DSMB reviewed blinded data on 304 subjects who experienced late onset AEs or SAEs that were categorized as "Arthritis". A questionnaire was also sent to each investigator to obtain additional information regarding the type of arthritis (traumatic, osteoarthritis, inflammatory arthropathy, etc). The dataset from each DSMB member was analyzed separately by the DSMB statistician, who found no difference between the vaccine and placebo groups.

Therefore, Lyme-008 did not detect any difference in the incidence of rheumatologic disorders between vaccine and placebo recipients during the 20 months after the initial dose.

2.2.4.3 Neurological and Cardiac Events

There was no significant difference in the rates of cardiac AEs between vaccine and placebo recipients. Neurologic AEs, which occurred at a rate <1% in the vaccine group and were noted to occur with a similar frequency in placebo recipients included: carpal tunnel syndrome, migraine, paralysis, tremor, coma, dysphonia, ataxia, multiple sclerosis, myasthenia gravis, meningitis, trigeminal neuralgia, nystagmus, neuritis, neuralgia, nerve root lesion, neuropathy, hyperesthesia, hyperkinesia, and intracranial hypertension.

2.3 Conclusion

A large body of data was accrued prior to licensure which, *in toto*, provided evidence that Lipo-OspA vaccine had an acceptable safety profile in the clinical trials which had been conducted up until the time of licensure of the product. The retention rate of volunteers in these studies was very high, underscoring the tolerability of the vaccine.

- With respect to early onset unsolicited AEs, significant differences were found for fever, influenza-like symptoms, injection site reactions, myalgia, and rigors with rates which were higher in vaccinees than in placebo recipients. For late onset unsolicited AEs, no such significant differences were found.

- Soreness was the most commonly reported solicited local symptom, and headache and fatigue were the most frequently reported general solicited symptoms. The majority of solicited events were mild to moderate in severity and limited in duration. Interestingly, the reporting rate of adverse events was very high in the placebo group: 82% of subjects reported at least 1 symptom.

- Serious AEs did not occur more frequently in the vaccine group than in the placebo group.
- An evaluation of the safety of the vaccine in subjects with a previous history of LD did not provide a basis for the theoretical concern that the vaccine might exacerbate symptoms in subjects with previous LD. Further, no evidence of induction of an autoimmune arthritis as a result of vaccination was seen in the pre-licensure setting.
- Additionally, no cases of hypersensitivity reactions to vaccination were reported in clinical trials up until the time of licensure.

Based on the above safety data, in conjunction with the demonstrated efficacy of *LYMErix* in preventing LD (78% effective against definite LD and 100% effective against asymptomatic infection after 3 doses), the product was approved in December, 1998, and was made commercially available in January, 1999.

Commitments to continue the study of the *LYMErix* safety profile of were agreed at the time of product approval. The status of these commitments is discussed in Section 3 of this document.

3.0 Post Licensure Safety Assessment

3.1 Introduction

This section will review the status of the clinical commitments which were agreed at the time of approval of *LYMErix*:

- A Post marketing cohort study to assess whether exposure to *LYMErix* is a risk factor for new onset of inflammatory arthropaty, LD, treatment resistant LD, rheumatoid arthritis, certain neurologic diseases, allergic events, hospitalizations and death. Three quarterly interim reports have been submitted to The Agency by end of 2000. The status of this study, together with currently available data, is discussed below (Section 3.2).

- A reproductive toxicity study was conducted in rats according to the agreed protocol. Results have been submitted to The Agency in January, 2000, and are summarized below (Section 3.3).

- A pregnancy registry has been established and maintained to track the outcome of pregnancies reported for women vaccinated with *LYMErix*. The procedure that has been established to capture and evaluate reports, as well as the current observations, is described below (Section 3.4).

- The final report of a study on CMI induced by the vaccine, ongoing at time of registration, has been submitted to The Agency in December, 1999; it is described below (Section 3.5).

In addition to the activities on the above-described commitments, the findings of the post marketing surveillance (PMS) ongoing since launch of the vaccine in January, 1999, and submitted through the Periodic Quarterly Reports to The Agency, are reviewed (Section 3.6).

Finally, the safety experience from clinical trials completed or still ongoing since licensure, including longer term safety follow up, alternate dosing regimens and booster studies, and safety in a pediatric population is discussed (Section 3.7).

3.2 Post Marketing Cohort Study to Assess the Safety of *LYMErix*

To address the theoretical concern that immunization with a vaccine containing OspA can induce an autoimmune arthritis and as a commitment at the time of licensure, the sponsor has undertaken a large prospective Phase IV study to evaluate the safety of *LYMErix*.

This study is ongoing at HPHC, the largest non-profit mixed model HMO in the New England region; it has a long history of research employing automated record linkage methods. All ambulatory encounters, emergency room visits, and hospitalizations of HPHC members generate claims with up to two primary and secondary diagnosis codes and one procedure code. The claims records are computerized, updated at least quarterly and become available for automated searches approximately six weeks after the end of a quarter. Diagnoses are coded according to the International Statistical Classification of Diseases, Injuries and Causes of Death, ninth revision (ICD-9). Each HPHC member has a unique membership number, which can be used to link records from different claims files. The HMO maintains a membership file that contains membership number, gender, date of birth, date of initiation of membership and the date of termination of membership. In addition to automated records, full text medical records from service providers can be requested for manual review.

3.2.1 Objective

The primary objective of the study is to evaluate whether exposure to *LYMErix* is a risk factor for new onset inflammatory arthropathy. The secondary objective is to evaluate whether exposure to *LYMErix* is a risk factor for LD, treatment resistant LD, rheumatoid arthritis, certain neurologic diseases, allergic events, hospitalizations and death.

3.2.2 Methods

Twenty five thousand HPHC members who are expected to receive *LYMErix* vaccine will be matched with respect to age, gender and primary care practice to a group of non-vaccinated (unexposed) HPHC members with a 1:3 ratio. Clinical events of interest after vaccination will be identified from ambulatory and inpatient claims data and selected outcomes will be confirmed by blinded review of the full medical record. Each subject will be followed for at least four years after being identified as exposed (the first dose of *LYMErix*) or unexposed. The incidence of predefined AEs in the exposed cohort will be compared to the incidence of AEs in the unexposed cohort.

Assuming 25,000 exposed subjects (immunized with *LYMErix*), and 75,000 unexposed subjects, all of whom will be followed for a minimum of 4 years, and taking into account attrition for disenrollment from HPHC as well as eventual vaccination of some unexposed subjects, a total of 108,627 person-years of exposure should accrue and provide more than 80% power to rule out an incident rate ratio of 2 for an adverse events which occur at a rate of 3 per 10,000 in the control group.

3.2.3 Results

To date, three quarterly interim reports have been submitted for review by The Agency (June 15, 2000, September 15, 2000 and December 15, 2000). The results below summarize data from the interim report.

A search of the HPHC automated record systems on November 24, 2000, identified 3,677 individuals with codes indicating *LYMErix* immunization through November 15, 2000. Automated records showed that 889 had claims for one dose, 1,670 had claims for two doses, 1,077 had claims for three doses, 40 had claims for four doses, and one had claims for five doses.

A total of 2,568 of these HPHC members received *LYMErix* during the first six months of 1999, and 7,497 unexposed HPHC members were matched to them with respect to age, gender, and affiliation of primary practice. Data for this matched cohort was confined to the first six months post-licensure due to the fact that there is a lag time between medical care, claims submitted by the provider, and claims processed at HPHC. Therefore, diagnosis codes that appear beyond this timeframe may not be complete.

3.2.4 Conclusions

The preliminary results of this study do not suggest that rheumatologic, neurologic, or allergic outcomes of interest identified *a priori* in the study protocol as being of particular interest were more frequent among *LYMErix* recipients than among unexposed individuals. However, it is acknowledged that the diagnosis codes are proxies for diseases, and more reliable estimates of disease incidence will come following the planned review of medical records to confirm the diagnoses.

As accrual of approximately 10,000 vaccinees was expected at HPHC in the first year of the study through commercial use of the vaccine, and as this target was not reached, two other HMOs (Health Partners of Minnesota and Tufts Health Plan) will now be included in the *LYMErix* post marketing safety assessment cohort study. The addition of these two HMOs, whose databases will be searched retrospectively to January, 1999, is expected to double the number of exposed individuals to date. Since the uptake of the vaccine is less than had been expected at the time of study design, it appears that more time will be required to attain the goal of 25,000 vaccinees. Accordingly, the accrual period of the protocol will be extended.

3.3 Reproductive Toxicity Study in Rats

3.3.1 Methods

The influence of *LYMErix* on embryo-fetal, prenatal and post natal development was assessed in sexually mature female CD rats. One group of 60 animals received 50 microL of the vaccine (containing 3 mcg of Lipo-OspA on 50 mcg aluminum) by i.m. administration 30 days before pairing. A second group was treated with 50 microL of saline by the same route and on the same occasion. After mating, 44 females of each group were selected to continue treatment during gestation, both groups receiving the vaccine on days 6, 8, 11 and 15 of gestation. A control group received saline on the same occasions that *LYMErix* was administered during gestation. From each group, a total of 22 females were killed on day 20 of gestation for examination of their uterine contents, and the remaining 22 females in each group were allowed to give birth and rear their offspring to weaning at day 21 of age.

3.3.2 Results

Intramuscular administration of vaccine to the female rats on days 6, 8, 11 and 15 of gestation was well-tolerated, even following a pre-mating immunization. Treatment was without maternal toxicity or any effect upon survival and development *in utero* of fetuses, or the birth, survival and development of offspring to day 21 of age.

3.4 Pregnancy

LYMErix is labeled as Pregnancy Category C (animal reproductive studies had not been conducted prior to licensure and it is not known whether *LYMErix* could cause fetal harm when administered to a pregnant woman or if it could affect reproductive capacity).

3.4.1 Experience from Completed and Ongoing Clinical Trials

A total of 63 reports, describing 66 pregnancies, were received from clinical trials (data lock point December, 2000). Maternal age was provided for 60 (95%) of the reports: the median age was 32 years (range 17-48 years).

Forty one outcomes (41/66 = 62%) were described as "normal." Six reports (6/66 = 9%) of spontaneous abortions and 4 reports (4/66 = 6%) of elective (therapeutic) abortions were received (2 ectopic pregnancies described in the same report). No outcome was available for 15 reports (15/66 = 23%).

3.4.2 Post Marketing Experience

The *Pregnancy Section* of the *LYMErix* labeling includes language which encourages healthcare providers to contact SBBio, using a toll-free number, in the event that they become aware of receipt of the vaccine by a pregnant individual (see PI in Attachment 1). Although the data are limited, to date there has been no pattern of abnormal pregnancy events (e.g., pregnancy-induced hypertension) or outcomes (e.g., fetal malformation, growth retardation, miscarriage/intrauterine fetal demise/stillbirth) in the post marketing setting. A total of 30 reports of pregnancy were received (data lock point December, 2000). Maternal age was available for 21 of the 30 reports, with a median age of 32 years (range 23-42 years). Information regarding outcome was available for 13 of the 30 reports, as follows: 9 deliveries of normal infants, 2 therapeutic abortions (1 ectopic pregnancy and 1 blighted ovum), and 2 spontaneous abortions.

3.4.3 Conclusion

No trends or patterns are apparent from reviewing the pregnancy reports from all sources, i.e., clinical trials and spontaneous reports, in the post marketing setting.

3.5 Study on Cell Mediated Immunity following LD Vaccine Administration during the Lyme-008 Trial

CMI analyses were performed by Dr. Allan Steere on two subsets of subjects participating in the Lyme-008 trial.

- The first subset consisted of 100 consecutive subjects at a single site (site 27) of whom 47 are from the vaccine group and 53 from the placebo group. Evaluation of the CMI response was possible on samples from 85 of these volunteers (41 vaccinees, 44 placebo recipients). The CMI response was measured by T-cell proliferation, IL-4 and IFN-γ production, upon stimulation with unlipidated OspA or overlapping peptides covering the whole sequence of the protein.

  The results of HLA typing, CMI responses and possible adverse reactions were compared and an attempt was made to identify statistically significant correlations between these parameters.

- The second subset was comprised of 15 volunteers from any site (presumably any subject of the study) who developed joint or tendon pain with abnormalities on examination during the study and in whom an alternative diagnosis of joint symptoms was not found. Samples from 12 individuals were available for CMI evaluation, and from 9 individuals for HLA typing.

In the data obtained in these two subsets of volunteers, there is no suggestion that the vaccine induced an unexpected adverse event pattern. Similarly, there is no definitive evidence of an association between adverse events and HLA haplotype or CMI.

In summary, we agree with this statement from the conclusion of the report: "The prolonged pro-inflammatory conditions needed for the development of autoimmunity in the joints of patients with Lyme arthritis may never be duplicated in vaccinated individuals." There are no clinical data to support an association between vaccination and an increased incidence of an inflammatory arthropathy.

3.6 Passive Post Marketing Surveillance Data

Since launch of *LYMErix* in January, 1999 and through October 30, 2000, more than 1.386 million doses of vaccines have been distributed. Seven Quarterly Periodic Adverse Event Reports have been prepared and forwarded to The Agency.

SmithKline Beecham has received a total of 984 adverse event reports to November 30, 2000. Review of these 984 reports showed that the general profile, as reported following the clinical development and as described in the *LYMErix* PI has not changed, except as described below (see Sections 3.7.1 and 3.7.2)

3.6.1 Concomitantly Occurring Adverse Events

During the medical review of AEs received by the pharmacovigilance department of SBBio, it appeared that some of the symptoms described in the product information circular for *LYMErix* as occurring individually might occur concomitantly, with a rapid onset after vaccination.

At the time of the database query, 833 AEs had been reported since launch, with 161 cases presenting at least three of the following AEs: arthralgia, fatigue, fever, influenza-like symptoms, chills/rigors, headache, achiness, myalgia and nausea.

Having reviewed the data, SBBio considered that, although all these symptoms are individually listed in the PI as part of the clinical experience, the PMS experience should be added to the labeling.

This proposed labeling change has been shared with CBER on January 3, 2001 and is being prepared for submission.

3.6.2 Hypersensitivity

Hypersensitivity reactions had not been reported during clinical trials with *LYMErix* in the prelicensure period; accordingly, none were included in the PI (see Attachment 1). The spontaneous reports database was queried to determine whether sufficient evidence of acute hypersensitivity had been reported to propose a labeling change.

Based on a review of the spontaneous reports consistent with hypersensitivity (n = 43), the following labeling change is proposed for *LYMErix:*

3.6.3 Risk of Inducing Autoimmune Arthritis

In view of the discussions regarding LD-induced arthritis, special attention has been given to the spontaneously reported arthritis cases. From launch in January 1999, 70 cases of arthritis have been reported.

No evidence has been found that the incidence of these arthritic conditions was higher than that reported for the general population or that they were associated with an autoimmune process. Nonetheless, SBBio is undertaking to have the cases of arthritis evaluated by an independent panel of rheumatologists.

3.7 Additional Experience from Clinical Trials

Several studies which were ongoing at the time of licensure were completed and a number of studies were initiated following the licensure of *LYMErix*.

3.7.1 Lyme-008 Follow Up

Following unblinding of the pivotal Lyme-008 efficacy trial, subjects were offered participation in a follow-up study, Lyme-013. This study provided an opportunity to collect safety data for an additional period of observation (a little over one year beyond

the 20-month follow up period in Lyme-008) in those who had received active vaccine. Those who had received placebo in Lyme-008 were offered the opportunity to receive active vaccine and were followed during the 3-dose primary course given on a 0, 1, 12-month schedule, up to and including one month after the third dose. In both of the above cohorts, safety data were collected using postcards which queried the subjects as to the occurrence of any new medical conditions evaluated by a specialist, hospitalizations, and new onset of certain symptoms of interest (rash, flu-like illness, arthritis/arthralgia, facial paralysis, numbness/tingling/weakness/tingling of an extremity, faintness or loss of consciousness, and memory difficulty). These data are currently under evaluation.

3.7.2 Alternate Schedules

Two open, randomized, multicenter studies were undertaken in order to evaluate accelerated vaccination schedules. Lyme-014 evaluated approximately 400 subjects who received the vaccine on a 0, 1, 6-month schedule and 400 subjects who received the vaccine according to the licensed 0, 1, 12-month schedule. Lyme-016 evaluated approximately 500 subjects who received the vaccine on a 0, 1, 2-month schedule with a booster at month 12, and 500 subjects who received the vaccine on the licensed 0, 1, 12-month schedule.

3.7.3 Booster

In order to address the need for and safety of booster doses, three studies involving approximately 1800 adults were undertaken in subjects who had participated in previous trials, which evaluated a 3-dose primary series. In these booster studies, subjects have received multiple booster doses (up to a 6th dose including the 3-dose primary series); safety has been followed for 48-60 months after receiving the first dose of the vaccine. In these studies, there was no unexpected pattern of AEs identified.

3.7.4 Pediatric Population

To address the need for data in a pediatric population, a large double-blind, randomized, placebo controlled safety and immunogenicity trial (Lyme-022; N = 4000) was initiated in children and adolescents 4-18 years of age, at multiple centers in LD endemic areas within the US. In this study, approximately 3000 subjects received *LYMErix* and 1000 subjects received placebo, according to the licensed 0, 1, 12-month regimen. Note that a booster study in the pediatric population involving approximately 3000 subjects is currently ongoing.

3.7.5 Conclusion

In the alternate dose and adult booster studies (data in approximately 3,600 vaccinees) as well as the large pediatric study (N = 3000 vaccinees), the nature and frequency of AEs were similar to those seen at the time of *LYMErix* licensure (i.e. for the primary series on a 0, 1, 12-month schedule in adults 15-70 years of age). Additionally, no unexpected pattern of AEs was observed in these trials.

3.8 Conclusion of Post Marketing Safety Assessment

Since licensure of *LYMErix*, SBBio has initiated the agreed commitments and completed those that were to be accomplished within the short-term post licensure period. The status of the PMS activities can be summarized as follows:

- a reproductive toxicity study in rats has been conducted, completed, and the report submitted.
- a CMI study report has been completed and submitted.
- the pregnancy registry has been established and is ongoing.
- The post marketing cohort study to assess the safety of *LYMErix* has been initiated. Data on 3,677 vaccinees have been reported so far. No unexpected observations have been made. Although the accrual is below expectations due to the low vaccination rate of the searched population, activities have been undertaken to increase the number of vaccinated subjects. Interim reports will continue to be submitted to The Agency on the agreed schedule.
- The post marketing surveillance, as well as the analysis of safety data from clinical trials ongoing or reported since licensure of *LYMErix* show that the general profile, as reported following the clinical development, is maintained although some of the symptoms reported in the labeling as appearing individually seem to occur concomitantly in the immediate post vaccination period; and hypersensitivity, not observed during clinical development, has been reported since the launch of the vaccine. These observations prompted a labeling change proposal, which has been shared with CBER and is being prepared for submission.
- Review of the literature and in-house animal experimentation have not confirmed the theoretical concerns discussed at the time of licensure of *LYMErix*.

SmithKline Beecham will continue its post marketing surveillance and work on the continuing commitments, and keep The Agency informed of any further data, including

the report of the independent expert panel of rheumatologists who will review all arthritis cases.

4.0 References

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Identification of LFA-1 as a Candidate Autoantigen in Treatment-Resistant Lyme Arthritis

Dawn M. Gross, Thomas Forsthuber, Magdalena Tary-Lehmann, Carey Etling, Kouichi Ito, Zoltan A. Nagy, Jodie A. Field, Allen C. Steere, Brigitte T. Huber

Treatment-resistant Lyme arthritis is associated with immune reactivity to outer surface protein A (OspA) of *Borrelia burgdorferi*, the agent of Lyme disease, and the major histocompatibility complex class II allele *DRB1*0401*. The immunodominant epitope of OspA for T helper cells was identified. A homology search revealed a peptide from human leukocyte function–associated antigen-1 (hLFA-1) as a candidate autoantigen. Individuals with treatment-resistant Lyme arthritis, but not other forms of arthritis, generated responses to OspA, hLFA-1, and their highly related peptide epitopes. Identification of the initiating bacterial antigen and a cross-reactive autoantigen may provide a model for development of autoimmune disease.

Lyme disease is a multisystem illness caused by infection with the spirochete *Borrelia burgdorferi* (*1*). A prominent late manifestation of the disease is Lyme arthritis (*1, 2*). About 10% of patients with Lyme arthritis develop what we have termed antibiotic treatment–resistant Lyme arthritis, which typically affects one knee for months to years after multiple courses of antibiotics (*1*). Such patients have no detectable spirochetal DNA in joint fluid after antibiotic therapy, which suggests that the spirochete has been eliminated by this treatment (*3*). Because there is increased frequency of the *HLA-DRB1*0401* allele in these patients (*4*), an autoimmune etiology should be considered. The hypervariable 3 region (HVR3) at residues 67 to 74 of *DRB1*0401* is associated with susceptibility to rheumatoid arthritis (RA) and is contained in at least 15 different *DRB1* alleles (*5*). Most patients with prolonged treatment-resistant Lyme arthritis have one of these homologous alleles (*4*). What antigen are these class II molecules presenting?

Borrelia burgdorferi induces an immune response of expanding reactivity to an array of spirochetal proteins over months to years (*6*). Antibody reactivity to outer surface protein A (OspA) typically develops near the beginning of prolonged episodes of arthritis (*7*). T cell lines from patients with treatment-resistant Lyme arthritis preferentially recognize OspA, compared with patients with treatment-responsive disease. OspA-reactive type 1 T helper (T_H1) cells are detectable in the synovial fluid of individuals with treatment-resistant arthritis years after antibiotic treatment (*7*). Thus, these patients may have progressed into an autoimmune state by developing a cross-reactive response between OspA and a self-antigen.

We used the DRB1*0401 peptide-binding algorithm (*8*) to determine the scores for all nine-residue peptides in the OspA protein sequence that contained an appropriate pocket 1 anchor residue—F, I, M, L, T, V, or Y —necessary for binding in the DRB1*0401 peptide-binding cleft. According to this algorithm, only peptides with scores greater than 2 are likely to bind and be able to be presented by the DRB1*0401 molecule (*8*). The highest scoring peptide that was identified, $OspA_{165-173}$, had a predicted binding score of 6.5, and the next best scoring peptide, $OspA_{237-245}$, achieved a score of 3.7. To verify that these peptides can bind to DRB1*0401 in vitro, the binding of ^{125}I-labeled m1 7 (YRAMATL; predicted DRB1*0401 binding score = 5.9), which has the consensus binding motif for DRB1*0401 (*9*), was measured when in competition with unlabeled 20-residue peptides from OspA. Only $OspA_{154-173}$, which contains the DRB1*0401-predicted dominant epitope $OspA_{165-173}$, inhibited binding of the radiolabeled peptide m1–7 to purified DRB1*0401 (Table 1), confirming the algorithm's prediction.

To test for T cell reactivity in vivo, we made use of class II–deficient mice transgenic for a chimeric DRB1*0401 molecule (DRB1*0401-tg) (*9*). Any CD4$^+$ T cell response generated in these mice can be directly attributed to the presence of the DRB1*0401 molecule. The ElisaSpot assay was used for measuring anti-

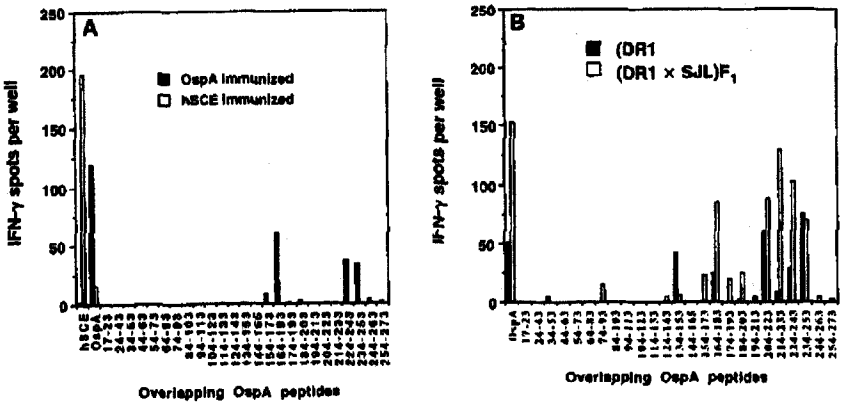

Fig. 1. IFN-γ ElisaSpot analysis demonstrates $OspA_{165-173}$ as the functional, immunodominant epitope of OspA in DR4-tg mice. (**A**) Class II–deficient, DRB1*0401-tg mice, immunized with whole OspA, but not a control protein, recall whole OspA and $OspA_{164-183}$ specifically. DRB1*0401-tg mice were immunized in both hind footpads with either 50 μl of OspA (44 μg/ml) or human spinal chord extract (hSCE; 100 μg/ml) in complete Freund's adjuvant. Eight days later, draining popliteal lymph nodes were isolated and 5×10^5 cells were cultured with either a positive control stimulant, CD3 antibody, mAb 145.2C11, or one of the following test antigens: hSCE (50 μg/ml), OspA (10 μg/ml), overlapping OspA 20-mer peptides (10 μg/ml each), or medium alone. IFN-γ production was analyzed 24 hours later by ElisaSpot (*10*). Values from wells with medium alone were subtracted from values from wells that contained antigen. Antigens are listed as overlapping 20-mer peptides spanning OspA, beginning with amino acid 17. Residues 1 to 17 contain the leader sequence and are therefore cleaved during export through the bacterial membrane. Representative experiments of six OspA-immunized and two hSCE-immunized mice are shown. (**B**) DRB1*0101-tg and (DRB1*0101-tg × SJL)F_1 mice immunized with whole OspA recall $OspA_{165-173}$ as well as other epitopes. In contrast to the DRB1*0401-tg mice, the DRB1*0101-tg mice express murine class II; therefore, a broader array of OspA epitopes is recognized. Experiments were performed as described above. One of three and one of two representative experiments are shown for DRB1*0101-tg and (DRB1*0101-tg × SJL)F_1 mice, respectively.

D. M. Gross and B. T. Huber, Department of Pathology, Tufts University, Boston, MA 02111 USA. T. Forsthuber, M. Tary-Lehmann, C. Etling, Department of Pathology, Case Western Reserve University, Cleveland, OH 44106–4943, USA. K. Ito and Z. A. Nagy, Department of Immunology, Hoffmann–La Roche, Nutley, NJ 07110, USA. J. A. Field and A. C. Steere, Department of Rheumatology, New England Medical Center, Boston, MA 02111, USA.

gen-specific T cell reactivity, a sensitive and efficient technique that allows detection of cytokine production at the single cell level, which may occur in the absence of proliferation (*10*). We initially assayed for production of T_H1 and T_H2 cytokines, interferon-γ (IFN-γ), and interleukin-5 (IL-5), respectively. Both IFN-γ–producing and IL-5–producing cells were detected when cells were activated with a polyclonal stimulus [anti-CD3; monoclonal antibody (mAb) 145.2C11]. In contrast, when cells were stimulated with OspA antigen, IFN-γ production was dominant, with essentially no detectable IL-5 secretion (*11*). Therefore, detection of IFN-γ was used as the readout for antigen-specific T cell reactivity in all subsequent assays. DRB1*0401-tg mice were immunized with OspA and lymph node cells were stimulated with overlapping 20-residue peptides of OspA: the immunostimulatory epitopes correlated precisely with the epitopes predicted by the DRB1*0401 algorithm (Fig. 1A). Immunization of the DRB1*0401-tg mice with $OspA_{165-173}$ resulted in a recall response to whole OspA in vitro (*11*). Hence, we have identified the immunodominant epitope of OspA in the context of DRB1*0401. To test the ability of $OspA_{165-173}$ to be presented by *DRB1* alleles related to DRB1*0401 (*5*), we performed the same experiment in mice transgenic for DRB1*0101 (*12*). These transgenic mice possess a full complement of murine class II genes, thereby providing distinct major histocompatibility complex (MHC) alleles for OspA peptide presentation. ElisaSpot analyses of OspA-immunized DRB1*0101-tg or (DRB1*0101-tg × SJL)F_1 mice showed reactivity to $OspA_{165-173}$ as well as to an array of other epitopes (Fig. 1B). In contrast to DRB1*0401-tg mice, reactivity toward $OspA_{165-173}$ developed as a subdominant epitope, suggesting that alternative determinants are available for binding that could influence disease development. Interestingly, the F_1 mice had a response to $OspA_{165-173}$ that was three times the response of DRB1*0101-tg mice. This is likely because of expression of the murine I-Eβ^k chain, which is homologous in the HVR3 to DRB1*0401 (*5*), thereby providing twice the number of class II molecules for presentation of this particular peptide. Thus, we have identified the immunodominant OspA peptide recognized in the context of DRB1*0401 and found that DRB1 and murine class II alleles homologous to DRB1*0401 in their HVR3 can also present this epitope.

We searched the Genetics Computer Group gene bank for human proteins containing sequences homologous to $OspA_{165-173}$. Of the 20 peptides retrieved with the highest identity and homology scores, two were of human origin: hLFA-1 (CD11a/CD18, integrin $\alpha_L\beta_2$) and 40*S* ribosomal protein. Only the peptide contained in hLFA-1, hLFA-$1\alpha_{L332-340}$, attained a significant DR4-binding score (7.3), with *six*–amino acid identity (YVIEGT*SKQ*; nonconserved residues in italics), suggesting hLFA-1 as a potential autoantigen. The peptide contained within the 40*S* ribosomal protein sequence (YVLEG*KELE*) attained a DR4-binding score of 0, mostly because of Lys at position p6, which is not tolerated in the DR4-HVR3 (*13*). The hLFA-$1\alpha_{L332-340}$ peptide is located extracellularly in the interactive or I-domain that mediates the binding interaction between LFA-1 and its ligand, intercellular adhesion molecule–1 (ICAM-1) (*14*). When the DR4-binding algorithm was applied to the entire I-domain (amino acids 170 to 349), hLFA-$1\alpha_{L332-340}$ achieved the highest predicted bind-

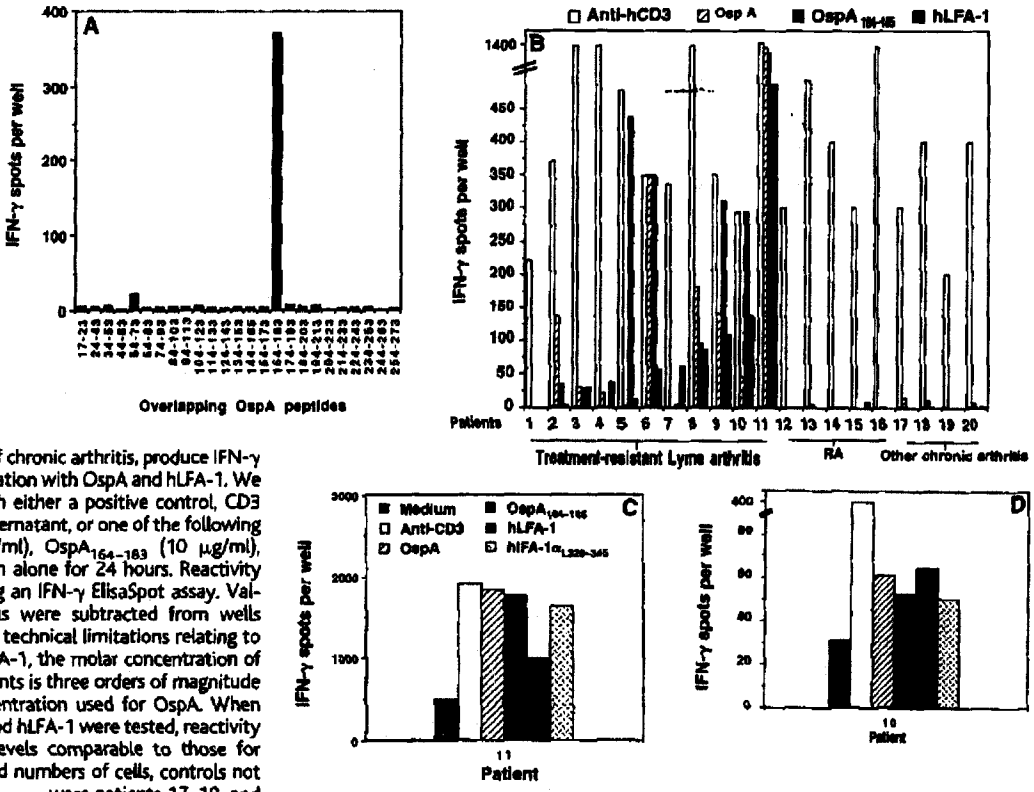

Fig. 2. SF T cells from patients with treatment-resistant Lyme arthritis generate a response to hLFA-1. (**A**) IFN-γ ElisaSpot analysis of 3 × 10^5 SF T cells per well, from patient 4, cultured with each of the overlapping OspA peptides at 10 μg/ml, revealed $OspA_{164-183}$ as the immunodominant epitope (*10*, *15*). Reactivity to whole OspA was positive as determined by proliferation assay (medium, 2552 177 248 cpm; OspA, 24,497 177 2079 cpm) (*16*). (**B**) SF T cells from patients with treatment-resistant Lyme arthritis, but not other forms of chronic arthritis, produce IFN-γ in response to in vitro restimulation with OspA and hLFA-1. We cultured 3 × 10^5 SF cells with either a positive control, CD3 antibody hybridoma OKT3 supernatant, or one of the following test antigens: OspA (10 μg/ml), $OspA_{164-183}$ (10 μg/ml), hLFA-1 (70 ng/ml), or medium alone for 24 hours. Reactivity was determined by performing an IFN-γ ElisaSpot assay. Values from medium-alone wells were subtracted from wells containing antigen. Because of technical limitations relating to the purification process of hLFA-1, the molar concentration of hLFA-1 used in these experiments is three orders of magnitude lower than the optimal concentration used for OspA. When equimolar amounts of OspA and hLFA-1 were tested, reactivity to OspA was depressed to levels comparable to those for hLFA-1 (*11*). Because of limited numbers of cells, controls not tested for reactivity to $OspA_{164-183}$ were patients 17, 18, and 20; and, for hLFA-1, patients 17 and 18. (**C**) Treatment-resistant Lyme arthritis patient 11, who is homozygous for *DRB1*0401*, demonstrates SF T cell reactivity to the 20-mer containing the OspA homologous, *DRB1*0401*-defined dominant epitope within the I-domain, hLFA-$1\alpha_{L326-345}$. We cultured 3 × 10^5 SF cells with hLFA-$1\alpha_{L326-345}$ (25 μg/ml). IFN-γ ElisaSpot assay was performed as described above. (**D**) Treatment-resistant Lyme arthritis patient 10, who is heterozygous for an RA-associated allele (*DRB1*0102*), demonstrates SF T cell reactivity to the 20-mer containing the OspA homologous, *DRB1*0401*-defined dominant epitope within the I-domain, hLFA-$1\alpha_{L326-345}$. We cultured 3 × 10^5 SF cells with equimolar amounts of $OspA_{164-183}$, hLFA-1, and hLFA-$1\alpha_{L326-345}$. IFN-γ ElisaSpot assay was performed as described above.

ing score (7.3), nearly twice that of the next highest scoring peptide, hLFA-1$\alpha_{L196-204}$ (binding score = 4.3), and higher than that of OspA$_{165-173}$. We determined, by performing the peptide binding competition assay [median inhibitory concentration (IC_{50}) = 0.7825 mM], that hLFA-1$\alpha_{L331-345}$, a 15-mer containing the core residues 332 to 340, was capable of binding DRB1*0401 in vitro.

To test the hypothesis that hLFA-1 is an autoantigen in patients with treatment-resistant Lyme arthritis, but not in other forms of chronic inflammatory arthritis, we mapped the immunodominant epitope of OspA in synovial fluid (SF) cells from a patient (4) with treatment-resistant Lyme arthritis (Fig. 2A) (*10*). As in the DRB1*0401-tg mouse, OspA$_{164-183}$ was immunodominant. We then analyzed the antigen reactivity profile of SF T cells from patients with treatment-resistant Lyme arthritis as well as patients with other forms of chronic arthritis (*15*). ElisaSpot for IFN-γ production (*10*) and proliferation assays (*16*) showed that people in a panel consisting of only those with treatment-resistant Lyme arthritis have varying degrees of SF T cell reactivity to whole OspA, OspA$_{164-183}$ as well as hLFA-1 (Fig. 2B). Reactivity to hLFA-1 is due to recognition of hLFA-1$\alpha_{L326-343}$, the region homologous with OspA$_{164-183}$ (Fig. 2, C and D). This reactivity appears to develop over time, as patients who initially showed no response to hLFA-1 had marked reactivity when tested 3 to 3 months later (*11*).

Borrelia burgdorferi sensu stricto is the only spirochetal strain associated with treatment-resistant Lyme arthritis (*17*) and the sole strain that contains the OspA$_{165-173}$ sequence that is highly related to hLFA-1$\alpha_{L332-340}$. Murine LFA-1a differs significantly from hLFA-1 at this particular epitope, providing an explanation for why chronic Lyme arthritis does not develop in DRB1*0401-tg mice exposed to *B. burgdorferi* (*12*).

Our demonstration of autoreactivity against hLFA-1 (in particular, the predicted cross-reactive epitope) in patients with treatment-resistant Lyme arthritis suggests that this disease involves an autoimmune process. However, although the genetic predisposition for development of treatment-resistant Lyme arthritis has been correlated with DR4, we cannot rule out other genetic, environmental, and infectious factors that might be involved. As mentioned above, the HVR3 of the DRB1 chains associated with RA possesses a shared epitope at residues 67 to 74 (*5*). Most patients with severe RA carry at least one allele that contains the shared epitope sequence of DRB1*0401, henceforth referred to as an RA-associated allele (*5*). Individuals who develop the most severe form of RA typically have two RA-associated alleles (*18*). HLA typing of our panel of 11 treatment-resistant Lyme arthritis patients revealed that 7 possessed at least one RA-associated allele (*15*), and 9 made a response to hLFA-1. Patient 11, who was homozygous for DRB1*0401, responded four times more vigorously to both OspA and hLFA-1 than the next highest responder. In patients with other forms of arthritis, the presence of an RA-associated allele by itself was not sufficient for induction of an OspA or hLFA-1 response, as at least five of the nine control patients possessed an RA-associated allele (*15*) yet made no response to OspA or hLFA-1. Thus, priming by *B. burgdorferi* infection or at least with OspA may be required for development of an autoimmune response to hLFA-1. Other factors may also be involved in development of treatment-resistant Lyme arthritis, as some treatment-resistant patients who do not possess an RA-associated allele make a response to hLFA-1 and some patients with treatment-resistant Lyme arthritis do not respond to either OspA or hLFA-1 (Fig. 2B).

On the basis of our DRB1*0401-restricted OspA T cell epitope mapping data, as well as previous work on immune reactivity and cytokine production in response to infection with *B. burgdorferi* (*7*), we propose a model on how an immune reaction to *B. burgdorferi* might result in development of an autoimmune response against hLFA-1: *B. burgdorferi* enters the host via a tick bite and disseminates to multiple tissues. Months later, a highly inflammatory immune response develops in the joint, and this response is dominated by T_H1 IFN-γ-producing cells that contain OspA reactive cells. We propose that the high local concentration of IFN-γ up-regulates expression of ICAM-1 (*19*) on synoviocytes and synovial fibroblasts as well as of MHC class II molecules on the local professional and nonprofessional antigen-presenting cells (APCs) (*19*). This enhanced ICAM-1 expression leads to recruitment of LFA-1 expressing cells, in particular activated T_H1 cells. The combination of elevated LFA-1 expression on T cells and macrophages plus MHC class II up-regulation on APCs may result in increased LFA-1 peptide presentation by macrophages and synoviocytes that have processed either endogenous or phagocytosed LFA-1 (*20*). Hence, a vicious cycle is initiated so that, even after elimination of the spirochetes by antibiotic therapy, the OspA-primed T cells remain activated by stimulation with LFA-1. The release of inflammatory cytokines by these activated T cells and macrophages may then result in tissue damage and joint destruction (*21*).

Table 1. Inhibition of m1–7 peptide binding to DRB1*0401 (*15*) by 20-residue peptides of OspA.

OspA peptide*	IC_{50} (μM)	Nine-residue peptides with appropriate p1 anchor residue†	DR4-algorithm scores for peptides with an appropriate p1 anchor residue‡
154–173	4.381	161, 162, 165, 166	(−) 0.4, (−) 0.8, (+) 6.5, (−) 5.4
54–73	>100	54, 55, 58, 61, 63	(−) 1.1, (−) 4.1, (−) 6.3, (−) 2.8, (−) 0.1
74–93	>100	75, 76, 79, 86	(−) 6.6, (+) 1.1, (+) 2.4, (−) 1.9
124–143	>100	126, 132, 136, 137	(−) 4.7, (−) 4.3, (−) 3.3, (−) 2.4

*Testing was limited to peptides with both sufficient quantity of material and a broad range of DR4-predicted binding scores. OspA$_{164-183}$ was not available for testing. †The number of potential DRB1*0401-binding 9-residue peptides contained within a 20-residue sequence was determined by the presence of an appropriate p1 anchor residue (F, I, L, M, V, T, or Y). p1 anchor residue amino acid numbers are listed for each candidate peptide. ‡Scores were calculated for OspA nine-residue peptides beginning with F, I, L, M, V, T, or Y (9). Scores are listed, respectively, for each 9-mer peptide contained within the 20-mer peptide tested.

References and Notes

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9. In vitro binding studies were performed as described, and generation of DR4-tg mice are documented in [K. Ito *et al.*, *J. Exp. Med.* **183**, 2635 (1996)]. Overlapping 20-mer OspA peptides were synthesized by R. Woods and were a generous gift from M. Hanson (Medimmune, Gaithersburg, MD). OspA 15-mer (SYVLEGTLTAEKTTL) and 9-mer (YVLEGTLTA) peptides, as well as hLFAα$_L$ 15-mer (IYVIEGTSKQDLTSF) and mLFA-1 15-mer (IYAIEGTNRQDLTSF) peptides were purchased from Bio-Synthesis. The hLFAα$_L$ 20-mer (ELQKKIYVIEGTSKQDLTSF) was purchased from Research Genetics.
10. Single-cell suspensions of popliteal lymph node cells from immunized mice, or Ficoll-Hypaque (Sigma) centrifugation isolation of human lymphocytes from peripheral blood mononuclear cells or SF, were prepared and coincubated with appropriate antigen [5 × 10^5 cells per well (mouse) or 3 × 10^5 cells per well (human) and OspA or OspA peptides (10 μg/ml), hLFA-1 (70 ng/ml), or anti-CD3 supernatant] to T-

Spot plates (Autoimmune Diagnostika) precoated with capture monoclonal antibody to IFN-γ (4 μg/ml) and blocked with Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Plates were washed at 24 hours and probed with a sandwich biotinylated antibody to IFN-γ. Spots were detected with an anti-biotin alkaline phosphatase (AP) (murine ElisaSpot) or streptavidin–horseradish peroxidase (human ElisaSpot) with detection enzyme reactions of either NBT/BCIP (Pierce) or 3-amino-9-ethylcarbzole and *N,N*-dimethylformamide (Pierce/Fisher), generating purple or red spots, respectively. Scores were determined by the Series I T-Spot Image analyzer (Autoimmune Diagnostika) as the difference between the number of spots produced with and without antigen. OspA protein was a kind gift from B. Lade and J. Dunn (Brookhaven National Lab) and purified hLFA-1 was a kind gift from D. Staunton (ICOS Corporation). Human spinal chord extract was prepared according to standard procedures. The following antibodies were used for murine in vitro assays: 145.2C11 (murine antibody) or OKT3 (human antibody), CD3 antibody (hybridoma supernatant); R4-6A2, coat, IFN-γ antibody and XMG1.2, capture, biotinylated IFN-γ antibody (PharMingen); biotin-AP antibody (Vector). The following antibodies were used for human in vitro assays: OKT3, CD3 antibody (hybridoma supernatant); coat, IFN-γ antibody, and capture, biotinylated IFN-γ antibody (Endogen); streptavidin–horseradish peroxidase (Zymed).

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12. B10.m/Sn mice transgenic for DRB1*0101 were a kind gift from D. Zaller (Merck Research Laboratories); S. Feng, S. W. Barthold, L. K. Bockenstedt, D. M. Zaller, E. Fikrig, *J. Infect. Dis.* **172**, 286 (1995).
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15. We studied 11 patients (7 male, 4 female; between 12 and 40 years old) with treatment-resistant Lyme arthritis and 9 control patients (4 male, 5 female; between 17 and 78 years old) with RA or other forms of chronic inflammatory arthritis. All Lyme patients met the case definition of the U.S. Centers for Disease Control and Prevention for diagnosis of Lyme disease. They had arthritis affecting the knee and serologic reactivity with *B. burgdorferi* by ELISA and protein blotting. The 11 Lyme arthritis patients and 5 of the control patients were evaluated in the Lyme Disease Clinic at New England Medical Center (NEMC). The remaining 3 RA (patients 12, 13, and 15) and 1 psoriatic (patient 19) control patients' samples were a generous gift from R. Schumacher (Department of Medicine, University of Pennsylvania Medical School). The protocol was approved by the Human Investigations Committee, and informed consent was obtained from each subject. Patients with Lyme arthritis were treated with both oral and intravenous antibiotic regimens. The duration of arthritis after antibiotic therapy ranged from 2 to 33 months. High-resolution HLA-DR typing with sequence-specific amplification was performed by the Clinical Laboratory of Immunology (NEMC) and by Lee Ann Baxter-Lowe (University of South Carolina, Columbia, SC). Patient *DRB1* alleles are as follows: 10, *0102* and *1501*; 6, *0102* and *1501*; 5, *0401* and *1501*; 7, *0701* and *1601*; 2, *0301* and *1201*; 1, *1* and *11*; 4, *14* and *15*; 11, *0401* and *0401*; 8, *0402* and *7*; 9, *0301* and *1302*; 3, *0404* and *13*, 12, *0401* and *1*; 13, *15* and *7*; 16, *4*; 15, *0401* and *7*; 17, *4* and *17*; 18, *11*, *3*, or *13*; 20, *1* and *13*. Insufficient DNA was available from patients 14 and 19, so *DR* typing was not performed on them.
16. Patient SF cells were plated in 96-well U-bottomed plates (Costar) at a density of 2×10^5 cells per 200 μl in complete RPMI medium (Sigma). Cells were stimulated for 5 days with antigen (2 days with phytohemagglutinin), pulsed with 0.5 μCi of [^{3}H]thymidine during the final 16 to18 hours, and harvested for scintillation counting. Insufficient cells were available from patients 5 and 11; therefore proliferation assays were not performed. All Lyme arthritis patients' cells responded to OspA (except for patient 1) and $OspA_{164-183}$ (except for patients 1 and 2). Responses ranged from 254 to 2552 cpm (background) and from 2275 to 56,725 cpm (antigen).
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Pioneer Axon Guidance by UNC-129, a *C. elegans* TGF-β

Antonio Colavita, Srikant Krishna, Hong Zheng, Richard W. Padgett, Joseph G. Culotti*

The *unc-129* gene, like the *unc-6* netrin gene, is required to guide pioneer motoraxons along the dorsoventral axis of *Caenorhabditis elegans*. *unc-129* encodes a member of the transforming growth factor–β (TGF-β) superfamily of secreted signaling molecules and is expressed in dorsal, but not ventral, rows of body wall muscles. Ectopic expression of UNC-129 from ventral body wall muscle disrupts growth cone and cell migrations that normally occur along the dorsoventral axis. Thus, UNC-129 mediates expression of dorsoventral polarity information required for axon guidance and guided cell migrations in *C. elegans*.

Axon guidance along the dorsoventral (D/V) axis of animals of diverse phyla involves secreted, laminin-related, UNC-6/netrin guidance cues (*1*). The signaling pathways activated by these molecules require the UNC-5 and UNC-40/DCC transmembrane receptor families (*2–4*). In *C. elegans*, mutations in *unc-129* (*5*) cause defects in the dorsally oriented trajectories of motoraxons that resemble those present in *unc-5*, *unc-6*, and *unc-40* mutants (*5*, *6*).

A 6.5-kb genomic subclone of cosmid C53D6 was able to rescue the uncoordinated phenotype of *unc-129* mutants after germline transformation (*7*, *8*) (Fig. 1A). Sequence analysis by the *C. elegans* genome-sequencing consortium (*9*) revealed a single open reading frame on this fragment that encodes a protein related to the TGF-β superfamily. The corresponding 1.5-kb cDNA (*10*) includes 5 exons, 34 base pairs (bp) of 5′ untranslated region (UTR), and 281 bp of 3′ UTR and is predicted to encode a protein of 407 amino acids (Fig. 1B). Northern (RNA) analysis of wild-type mRNA revealed a single transcript (*11*) consistent with the size of the cDNA. The 6.5-kb rescuing genomic fragment includes 3 kb of 5′ promoter sequence. A minigene containing 4.5 kb of 5′ promoter sequence fused to the *unc-129* cDNA was able to rescue the phenotype of *unc-129* mutants, indicating that there are no essential regulatory elements in introns or the 3′ sequence (*12*).

UNC-129 shares features with the TGF-β superfamily, including a signal sequence, a prodomain, and a COOH-terminal region that contains seven conserved cysteines (*13*). The UNC-129 COOH-terminal sequence identity ranges from 33% with human BMP-7 to 24% with TGF-β2. Thus, *unc-129* likely represents a subfamily of the TGF-β superfamily.

Sequence analysis revealed the absence of residues in UNC-129 that would be expected between the α-helical region and β sheet of TGF-β molecules (Fig. 1C) (*14*). This interdomain region forms a β turn with a protruding loop accessible to solvent. The three-dimensional structures of TGF-β1 and TGF-β2 differ at this site, which may promote their differing receptor-binding affinities (*15*). Deletion of the loop in TGF-β1 abolishes certain TGF-β1–mediated responses (*16*). Without knowledge of the crystal structure of UNC-129, it remains unclear whether the missing residues form the COOH-terminal end of the long α-helix or affect receptor specificity.

In *C. elegans*, TGF-β signaling pathways

A. Colavita, H. Zheng, J. G. Culotti, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada, and Department of Molecular and Medical Genetics, University of Toronto, Ontario M5S 1A8, Canada. S. Krishna and R. W. Padgett, Waksman Institute, Department of Molecular Biology and Biochemistry, and the Cancer Institute of New Jersey, Rutgers University, Piscataway, NJ 08854–8020, USA.

*To whom correspondence should be addressed. E-mail: culotti@mshri.on.ca

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Multiple cross-reactive self-ligands for *Borrelia burgdorferi*-specific HLA-DR4-restricted T cells

Bert Maier[1], Marc Molinger[1], Andrew P. Cope[2], Lars Fugger[2], Jens Schneider-Mergener[3], Grete Sønderstrup[2], Thomas Kamradt[1, 4], Achim Kramer[3]

[1] Deutsches Rheumaforschungszentrum, Berlin, Germany
[2] Department of Medicine, Stanford University, School of Medicine, Stanford, USA
[3] Institut für Medizinische Immunologie, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Berlin, Germany
[4] Medizinische Klinik m. S. Rheumatologie und Klinische Immunologie, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Berlin, Germany

T cell recognition of self antigens is a key event in the pathogenesis of autoimmune diseases. To date, the initial events that trigger autoreactive T cells are unknown. The "molecular mimicry" hypothesis predicts that during an infection T cells that recognize both a microbial antigen and a related self peptide become activated and cause autoimmune disease. We have systematically examined the recognition of self antigens by HLA-DR4-restricted T cells specific for peptides of the outer surface protein A (OspA) of *Borrelia burgdorferi*, the etiological agent of Lyme disease. We used the peptide spot synthesis technique for complete peptide substitution analyses of two immunodominant OspA epitopes. Each amino acid residue of the epitopes was substituted with all 20 naturally occurring amino acids and the altered peptides were tested for recognition by a panel of OspA-specific T cells. The binding motifs (supertopes) revealed by these analyses were used to screen public databases for matching human or murine peptides. Several hundred peptides were identified by this search and synthesized. Of these, 28 were recognized by OspA-specific T cells. Thus, T cell cross-reactivity is a common phenomenon and the existence of cross-reactive epitopes alone does not imply molecular mimicry-mediated pathology and autoimmunity.

Key words: Infectious disease / Autoimmunity / Molecular mimicry / Peptide library / *Borrelia burgdorferi*

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1 Introduction

Autoimmune diseases are sometimes triggered or exacerbated by infections. One hypothesis to explain the link between infection and autoimmunity predicts that sequence similarity between microbial and self antigens (molecular mimicry) can activate autoreactive lymphocytes, thus enabling such cross-reactive lymphocytes to cause autoimmune damage in the host (reviewed in [1, 2]). Numerous reports demonstrate cross-reactive T cells which recognize both a defined microbial peptide and a highly homologous self peptide [3–7]. In some cases autoimmunity could be elicited by immunization with microbial peptides [8, 9]. Frequently, however, autoimmunity occurred only at a much reduced incidence and severity [7, 10] or significantly higher antigen doses [11] as compared with the self antigen.

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Abbreviation: OspA: Outer surface protein A

Evidence for autoimmune disease triggered by cross-reactive T cells which continue to cause damage in the absence of microbial antigen has been difficult to obtain. Furthermore, there is increasing evidence that individual T cells can recognize a variety of peptides, which do not possess strong sequence homology (reviewed in [12]). Structural analyses have further illustrated the degenerate recognition of peptide-MHC complexes by individual TCR (reviewed in [13]). Therefore, we wished to re-examine the molecular mimicry hypothesis systematically using peptide spot synthesis, a high-throughput approach to testing modified peptide ligands [9, 14]. As a model, we chose the T cell response against *Borrelia burgdorferi* outer surface protein A (OspA). Cross-reactivity between *B. burgdorferi* and self antigens has been suspected to cause chronic neurological disease [15] or treatment-resistant Lyme arthritis [6, 16]. We have shown that T cell lines (TCL) from patients with treatment-resistant Lyme arthritis preferentially recognized *B. burgdorferi* OspA. In contrast, TCL from patients

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with treatment-susceptible Lyme arthritis rarely recognized this protein [16, 17]. Furthermore, both HLA-DR4 and IgG reactivity against OspA were found to be associated with treatment-resistant Lyme arthritis [18, 19]. Therefore, HLA-DR4-restricted T cell recognition of an arthritogenic OspA repitope is one possible way in which the immune response might be involved in the pathogenesis of treatment-resistant Lyme arthritis. Interestingly, immunization with recombinant OspA was effective in preventing Lyme disease in two recent clinical trials [20, 21] and is now available as a vaccine against Lyme borreliosis.

We tested if HLA-DR4-restricted T cells specific for immunodominant OspA epitopes could cross-recognize self peptides. OspA-specific T cell hybridomas were isolated from mice transgenic for HLA-DRA*0101/HLA-DRB*0401 and human CD4 on a background deficient for murine class II expression (hereafter called DR4+/I-Aβ- mice). Global amino acid replacements to determine the structural features necessary for HLA-DR4-restricted T cell recognition of the immunodominant OspA epitopes, followed by database searches yielded approximately 30 human and murine peptides capable of activating the OspA-specific T cell hybridomas.

2 Results

2.1 Identification of four immunodominant OspA epitopes

We immunized DR4+/I-Aβ- mice with recombinant lipidated OspA. T cell hybridomas (n = 982) were obtained from two independent fusions. T cell hybridomas were tested for OspA recognition using spleen cells from DR4+/I-Aβ- mice as APC; 560 hybridomas were OspA specific. Of these, 118 were randomly chosen and tested using both recombinant OspA and overlapping 20-mer peptides spanning the entire OspA sequence. Epitopes were then defined using N- and C-terminal truncations of the 20-mer peptides. Of the 118 hybridomas 104 recognized one of four immunodominant epitopes (Fig. 1). For these experiments a human EBV-transformed B cell line (EBV-BCL), homozygous for HLA-DRA*0101/HLA-DRB*0401 (Priess) was used as APC. Thus, the epitopes that we have identified can be processed by both murine and human APC.

2.2 Substitution analysis and supertope definition

Peptides prepared by spot synthesis were used for a substitution analysis of the two dominant epitopes, $OspA_{164-175}$ and $OspA_{235-246}$. Each position of the peptides was substituted with all 20 naturally occurring amino acids. The resulting 240 peptides for each epitope and synthesized spots of the original OspA peptides were tested for recognition by seven randomly picked hybridomas of the appropriate specificity ($OspA_{164-175}$ or $OspA_{235-246}$). Results for four characteristic hybridomas are shown in Fig. 2. This substitutional analysis revealed the binding motifs (supertopes) and, thus, the structural requirements for T cell recognition for each of the 14 hybridomas (Table 1). The individual supertopes for seven cloned hybridomas specific for $OspA_{164-175}$ differed substantially. Hybridoma 26/1 tolerated no substitutions at two positions: glutamic acid at position 168 of the OspA molecule (E168) and G169; this hybridoma also tolerated only one substitution for T170. Other hybridomas tolerated multiple substitutions at these positions, and hybridoma 170/1 tolerated 10 or more different substitutions at all positions except E168, G169, L171, and T172 (Table 1 and Fig. 2). Two of the seven hybridomas 26/1 and 224/2, Table 1) did not tolerate any substitution for G169. Hybridomas 26/1 and 170/6 did not tolerate any substitution for E168. Hybridoma 170/1 and hybridoma 169/5, on the other hand, tolerated many different substitutions for both E168 and G169 (Table 1). Similarly, the individual $OspA_{235-246}$-specific hybridomas had different supertopes (Table 1). Whereas hybridoma 21 tolerated no substitution of K239 (Fig. 2 and Table 1), the other six hybridomas tolerated between 2 (hybridoma 195) and 13 (hybridoma 844) different amino acid substitutions at that position.

Peptide	Sequence	No. of Hybridomas
$OspA_{146-155}$	EYTEIKSDGS	6
$OspA_{149-160}$	EIKSDGSGKAKE	28
$OspA_{164-175}$	GYVLEGTLTAEK	43
$OspA_{235-246}$	LVFTKENTITVQ	27

Fig. 1. Immunodominant epitopes of OspA. 118 OspA-specific hybridomas from HLA-DR4 transgenic mice were tested for recognition of OspA-peptides. The four immunodominant OspA-epitopes and the number of hybridomas recognizing these epitopes are shown.

2.3 Identification of self peptides mimicking $OspA_{164-175}$

The supertopes defined for the $OspA_{164-175}$-specific hybridomas (Fig. 2 and Table 1) were used to screen the SwissProt and TREMBL databases; 387 human or murine peptides matched one of the supertopes. A conventional sequence alignment search in the SwissProt

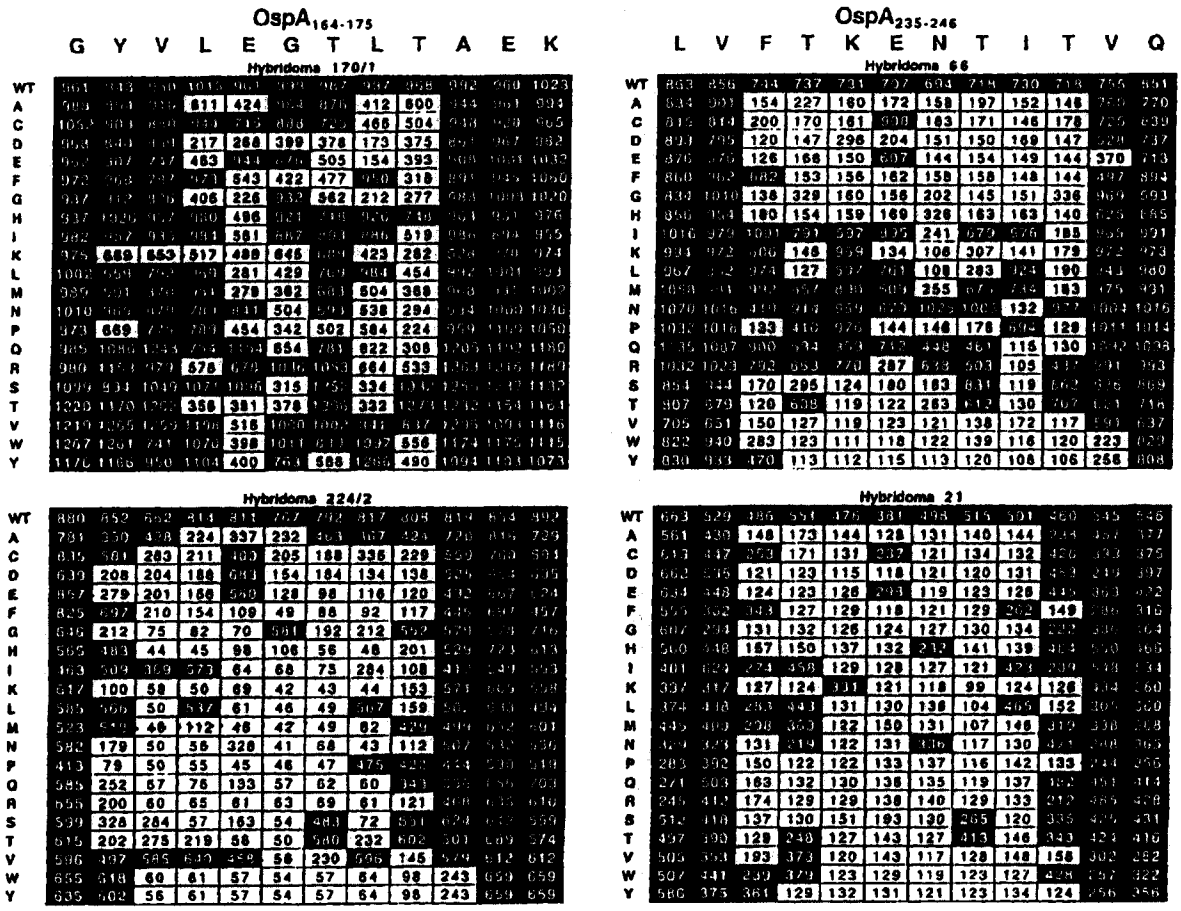

Fig. 2. Substitutional analysis of $OspA_{164-175}$ and $OspA_{235-246}$. Each position of the epitopes was substituted by all 20 naturally occurring amino acids. Peptides were prepared by spot synthesis and T cell hybridomas were tested for IL-2 production at a peptide concentration of ~ 1 μM. Absorbance (A) readings from the IL-2 ELISAs are shown. Dark boxes indicate A values that were considered positive as defined in methods. Values in the top line represent the wild-type (WT) peptide, all other values correspond to single substitution analogues. Data for two characteristic $OspA_{164-175}$-specific hybridomas and two characteristic $OspA_{235-246}$-specific hybridomas are shown. A values for background + 2 SD were < 150 for all four hybridomas.

and TREMBL databases yielded additional 57 human and 31 murine peptides with sequences homologous to $OspA_{164-175}$ (Table 2). The 387 supertope-matching peptides and the 88 peptides identified by sequence alignment were prepared by spot synthesis and tested for recognition by the seven cloned $OspA_{164-175}$-specific hybridomas. Of the 387 peptides identified by the supertope search 13 were recognized by at least one of the clones (Table 2). These peptides represented 11 human and 5 murine proteins. The mimic peptides shared three to five residues with $OspA_{164-175}$. Of the mimic peptides 11 conserved the glutamic acid at position 168 of the OspA molecule (E168) and G169 (Table 3). Of the remaining 2 peptides, 1 had G169 conserved and a conservative E168D substitution. L171 was conserved in 10 of the 13 mimic peptides. Other amino acids, *e.g.* A173 and K175, were not conserved in any of the 13 mimic peptides. Of the 88 peptides which were identified by sequence alignment, 3 activated at least one of the seven hybridomas (Table 2). These represented two human and two murine proteins (Table 3). None of the three peptides would have been predicted by the "supertope-analysis" (compare Fig. 1 and Table 3). The 10 "best matches" among the 88 sequences and the

Table 1. The "supertopes" resulting from the substitution analyses, for the 14 cloned hybridomas *i.e.* the amino acids allowed at each individual position of the 12-mer epitopes $OspA_{164-175}$ or $OspA_{235-246}$. [] indicate the allowed substitutions; X: all amino acids; ( ): all aa except those in the brackets.

	$OspA_{164-175}$											
#H*	G	Y	V	L	E	G	T	L	T	A	E	K
26/1	X	(ADEKNPST)	[ACGV]	[CFILMVY]	E	G	[ST]	[AILPTV]	[GILSTV]	[W]	X	X
169/5	X	[D]	[P]	[CFILMNPQSV]	[AEGNQS]	[GHIRVW]	[AGILFRSTVW]	[HILNPV]	[DNPQR]	[DFY]	X	[HKR]
170/1	X	[KF]	[EFKLPW]	[CFHILMSVWY]	[ENQS]	[ACGHIRVW]	[AINSTVW]	[FHILVWY]	[STV]	X	X	X
170/6	X	[CFHILMVWY]	[ACGINQSTV]	[ILMY]	E	[GH]	[EFKRWY]	[HIL]	[ELP]	X	X	X
224/2	X	[ACFHILMVWY]	[AIV]	[ILV]	[ACDEV]	G	[AST]	[ALPV]	[AGHPQST]	X	X	X
257/4	X	[DP]	[EIMPQ]	[CFILMQ]	[CEIQ]	[GHI]	[AS]	L	[CFITV]	[DEFSTVWY]	[TVWY]	[HK]
376/5	[Q]	[DEP]	[SV]	[EKRWY]	[CEQ]	[GH]	[EFIKLMQWY]	L	[DPVY]	[T]	[WY]	[VWY]

	$OspA_{235-246}$											
#H*	L	V	F	T	K	E	N	T	I	T	V	Q
21	X	X	[CFILMWY]	[ILMNTVW]	K	[CE]	[HN]	[ST]	[FIL]	[FKLPVY]	X	X
66	X	X	[FIKLMNQRY]	[HMNPQRT]	[IKLMNPQR]	[CEILMNQ]	[NQR]	[CHNQRST]	[ILMP]	[NRST]	X	X
168	X	X	[CFILNQSVWY]	[ACGHMNSTW]	[CFILVWY]	[CDEHI]	[AHNSW]	[ACDGHNST]	[ILV]	[DHNST]	X	X
195	X	X	[DEGKNPT]	[AGST]	[DKR]	[ACDEGHS]	[HNRST]	[ASTV]	I	[GHST]	X	X
281	X	X	[FILMVWY]	[ACST]	[DGHKNS]	E	[NSW]	[DNST]	[	T	X	X
296	X	X	[CFILMVWY]	[ACGNSTVW]	[ADGHKNPQS]	[DIE]	[AHNSW]	[ACDNST]	[ILV]	[DHNT]	X	X
844	X	X	[ADEGKNPT]	[DEFKLPQY]	[FILVWY]	[CDEHILKQSV]	[AHNSW]	[EFKLPQRWY]	[ILMPV]	[DHNST]	X	X

* indicates the number of the hybridoma.

peptides that activated at least one of the $OspA_{164-175}$-specific hybridomas are shown in Table 3.

2.4 Identification of self peptides mimicking $OspA_{235-246}$

Of the murine or human peptides 469 matched at least one of the seven supertopes defined for the $OspA_{235-246}$-specific hybridomas. Conventional sequence alignment yielded additional 63 human or murine peptides with sequence similarity to $OspA_{236-245}$ (Table 2). The 469 supertope-matching peptides and the 63 peptides identified by sequence alignment were prepared by spot synthesis and tested with four of the seven for $OspA_{235-246}$-specific hybridomas. Of the 469 peptides, identified by supertope analysis, 15 were recognized by at least one hybridoma. These 15 peptides represented 11 human and 9 murine proteins (Table 2). Several peptides had little sequence homology with the original OspA sequence (Table 4). Of the 15 peptides, 6 did not share a single residue with $OspA_{235-246}$. H21, H195, H296, and H844 each recognized more than one mimic peptide (Table 4). H296 recognized 8 different mimic peptides. The 40 human and 23 murine peptides identified by sequence align-

Table 2. Numbers of peptides identified by supertope analysis or sequence alignment, respectively.

	Supertope analysis		Sequence alignment	
Epitopes	Candidates[a]	Mimics[b]	Candidates[c]	Mimics[b]
$OspA_{164-175}$	387	13 11 human/5 murine[d]	88	3 2 human/2 murine[d]
$OspA_{235-246}$	469	15 13 human/9 murine[d]	63	0

a) Number of peptides matching the supertope for at least one of the hybridomas.
b) Number of peptides recognized by at least one of the hybridomas.
c) Number of peptides identified by sequence similarity search.
d) For some peptides the murine and human sequences are identical.

Table 3. Murine and human peptides recognized by $OspA_{164-175}$-specific hybridomas. (A) The Swissprot and TREMBL databases were searched for microbial peptides containing the supertopes depicted in Table 1. The 13 peptides listed here were recognized by one or more of the seven $OspA_{164-175}$-specific hybridomas examined. (B) The Swissprot and TREMBL databases were searched for homologs of $OspA_{164-175}$ by sequence alignment. The ten best matches, two of which were recognized by one $OspA_{164-175}$-specific hybridoma are shown here. In addition, a peptide derived from glycerolkinase which ranked 17th on the "homology-list" and was recognized by hybridoma 257/4 is also shown.

Sequence $OspA_{164-175}$	Source	Recognized by hybridoma: 376/5	169/5	170/6	170/1	26/1	257/4	224/2
A	Mimics identified by substitution analysis							
GYVLEGTLTAEK	Outer surface protein A *B. burgdorferi*	•	•	•	•	•	•	•
ASVPEGNLHGQD	IL-2R β chain precursor (CD122 mu) mouse	•						
CWVLEGIIIPNR	Macrophage-stimulating protein precursor, human	•	•	•				
EFIMEGTLTRVG	Son of sevenless homolog (SOS1) human, mouse	•		•	•			
GLVMEGHLFKRA	Hypothetical protein KIAA0050 human	•						
KLQLDGSLTMSS	Autoantigen (Q13025/Q13826) human				•			
LLGVEGTTLREA	Myosin I β mouse			•				
LVGIEGSLKGST	Opa-interacting protein OIP5 (fragment) human			•		•		
PLALEGSLQKRG	Insulin precursor human	•						
QVGVEGTASLKA	Lak-1 human			•				
RFVMEGGLLDKP	Insulin-like growth factor I receptor precursor human, mouse	•						
SFTIEGPLTSFG	Apolipoprotein B-100 precursor (Apo B-100) human			•				
SGRLQVRLVLGQ	Melanoma associated chondroitin sulfate proteoglycan (MCSP) human	•						
YWVYEGSLTIPP	Carbonic anhydrase-related protein human, mouse	•		•		•	•	
B	Mimics identified by homology search							
GYVLEGTLTAEK	Outer surface protein A *B. burgdorferi*	•	•	•	•	•	•	•
GYVLEGKELE	40s ribosomal protein S8 RS8 human							
GYILEGNECV	Fibrillin 1 precursor FBN 1 mouse							
GYMLEGSPQS	Complement receptor type II precursor CR2 human							
IYVIEGTSKQ	Leukocyte adhesion glycoprotein LFA-1 α human							
GYVPEDGLTA	Inosine-5′-monophosphate dehydrogenase 1 human, mouse							
GYTLEGSPWS	Complement receptor type I precursor (CD35) human			•				
TPNLEGTLTG	Neuroblast differentiation associated protein AHNAK (Desmoyokin) human, mouse							
GYTLEGSPQS	Complement receptor type II precursor (CD35) mouse						•	
LYMLVGTLAA	Multidrug resistance protein 3 MDR3 mouse							
GYTLRGTSIF	Alkaline phosphatase intestinal precursor mouse							
YYALEGSVAI	Glycerolkinase GLPK human, mouse						•	

Table 4. Murine and human peptides recognized by $OspA_{235-246}$-specific hybridomas. (A) The Swissprot and TREMBL databases were searched for microbial peptides containing the supertopes depicted in Table 1. The 15 peptides listed here were recognized by one or more of the seven $OspA_{235-246}$-specific hybridomas examined. (B) The Swissprot and TREMBL databases were searched for homologs of $OspA_{235-246}$ by sequence alignment. The ten best matches, none of which was recognized by one of the $OspA_{235-246}$-specific hybridomas are shown here.

Sequence $OspA_{235-246}$	Source	Recognized by hybridoma: 21	125	296	844
A	**Mimics identified by substitution analysis**				
VFTKENTITV	Outer surface protein A *B. burgdorferi*	•	•	•	•
AIAGDAGLSP	Erythroid krueppel-like transcription factor mouse	•			
EYASDASLDP	4-1BB ligand human	•			
IQTGISAIDG	Vacuolar ATP synthase subunit B human, mouse	•		•	
WMRRMRILRR	60S Ribosomal protein L19 human mouse	•			•
ICGEDSDLDG	Thrombospondin 2 precursor human, mouse		•		
NQNHCSAINN	Gonadotropin-releasing hormone receptor human		•		
RFHKIANVNK	α-Actinin 2, skeletal muscle isoform, α-actinin 3, skeletal muscle isoform human, mouse			•	
IKQIIRILSK	Neurofibromin human mouse			•	
ALNQEADVSG	Homebox protein HOX-A1 human			•	
DVVDESNINK	Neurofibromin mouse			•	
IVCADADLDL	Aldehyde dehydrogenase 6 human			•	
LSSAENSLSG	MAPK/ERK kinase kinase 3 human, mouse			•	
KFNRLNRLTT	SS-B Sjögren syndrom type B antigen human, mouse			•	
HWTSESSVSG	Fibrinogen α and α-E chain precursors human				•
RVWDISTVSS	Periodic tryptophan protein 1 homolog human				•
B	**Mimics identified by homology search**				
VFTKENTITV	Outer surface protein A *B. Burgdorferi*	•	•		•
NFHKENTVTN	Heparin cofactor II precursor mouse				
DFHKENTVTN	Heparin cofactor II precursor human				
KFTKNNFITI	Interferon-activatable protein 205 mouse				
SFTRENTLMF	NADH-ubiquinone-oxidoreductase chain 4 human				
ISTKENTLSK	β-Neoendorphin-dynorphin precursor human				
IFTKENLTAP	Ceruloplasmin precursor human				
LGESENTITV	Protein-tyrosine phosphatase PCP-2 precursor human				
VHTKEQMLTV	Zinc finger protein ZFP-29 mouse				
VFTETNTLEN	Versican core protein precursor human				
LFSKENSVWN	1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase γ1 human				
RRLKENQITI	Thyrotroph embryonic factor human				
TFTKDNRVHI	GTP-binding protein ARD-1 human				
AFTRSSTLTL	Zinc finger protein 139 human				
STGKENKITI	Heat shock-related 70 kDa protein 2 human, mouse				

ment were tested for recognition by three of the seven $OspA_{236-245}$-specific hybridomas. None of the 63 peptides induced IL-2 production in any of the three hybridomas. The 10 "best matches" among the 63 sequences identified by sequence-alignment are shown in Table 4.

2.5 Dose-dependent activation of OspA-specific T cells by self peptides

The $OspA_{164-175}$ and $OspA_{235-246}$ peptides, the 13 peptides cross-reactive with $OspA_{164-175}$-specific hybridomas (Table 3), and the 15 peptides cross-reactive with

$OspA_{235-246}$-specific hybridomas (Table 4) were synthesized conventionally and used in titration experiments. The hybridomas recognized the OspA and mimic peptides dose dependently. Two characteristic examples are shown in Fig. 3. Some of the human and murine peptides induced IL-2 amounts that were comparable to those induced by the OspA peptides albeit at higher doses (Fig. 3, hybridoma 26/1). Other mimic peptides induced less IL-2 than the OspA peptides throughout the tested dose range (Fig. 3, hybridoma 257/4). Altogether, all peptide mimics identified using the spot-synthesis technique also induced significant IL-2 production in the T cell hybridomas when synthesized conventionally.

3 Discussion

We and others have found that an immune response to *B. burgdorferi* OspA, especially in HLA-DR4$^+$ patients, is associated with an increased likelihood of developing treatment-resistant Lyme arthritis [16–19]. Additional interest in the immune response to OspA has emanated from the fact that recombinant OspA was successfully tested as a vaccine against Lyme disease [20, 21]. Here, we further analyzed the T cell response against OspA and show that HLA-DR4-restricted OspA-specific T cells frequently recognize self antigens. Global amino acid substitution of two immunodominant OspA epitopes allowed us to define the structural motif (supertope) recognized by individual OspA-specific T cell hybridomas.

Database searches yielded 387 human or murine peptide sequences that matched one of the supertopes defined for the $OspA_{164-175}$-specific hybridomas and 469 sequences that matched one of the supertopes defined for the $OspA_{235-246}$-specific hybridomas. However, only 13 of the 387 and 15 of the 469 peptides induced IL-2 production in the hybridomas. Similarly, when we used supertope analysis to predict microbial peptides that could activate murine T cells specific for the CNS autoantigen myelin basic protein (MBP), only 61 of 832 peptides induced T cell activation [9]. Why does only a small percentage of peptides matching the supertope criteria activate the T cells? It is likely that particular amino acid replacements are tolerated when they occur individually but abolish T cell recognition when they occur simultaneously. Importantly, a substitution that eliminates T cell recognition when it occurs individually can be tolerated in the context of other substitutions. This is illustrated by the fact that some of the peptides that were recognized by an individual hybridoma were not predicted by that hybridoma's supertope. An example is the peptide of erythroid krueppel-like transcription factor (EKLF, mouse), which matches the supertope for the $OspA_{235-246}$-specific hybridomas 168 and 844 but was recognized by hybridoma 21 (compare Tables 1 and 4). Similar results have been obtained by other investigators using different approaches [22–25]. Thus, neither a precise knowledge of an epitope's MHC and TCR contact

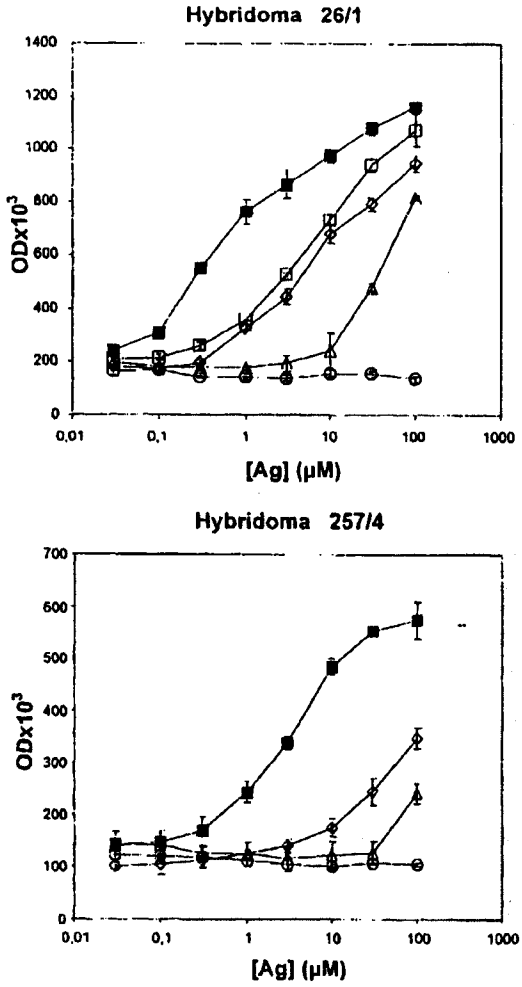

Fig. 3. Comparison of IL-2 production induced by $OspA_{164-175}$ and murine or human peptides. Peptides were used at the indicated concentrations using the cloned T cell hybridomas 26/1 and 257/4 in IL-2 assays as described in Sect. 4.3. Filled squares indicate $OspA_{164-175}$ peptide, open symbols represent mimic peptides. The "son of sevenless" peptide (open circles) did not induce IL-2 production in the spot-assays and was therefore used as negative control. Results shown are the mean of triplicate wells, and representative of three independent experiments. Upper panel: (△) LAK-$1_{216-227}$, (◇) carbonic anhydrase-related protein$_{215-226}$, (□) OPA-interacting protein $OIP5_{122-133}$. Lower panel: (◇) pro-insulin$_{79-90}$, (△) complement receptor type $II_{934-943}$.

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sites, nor a complete set of single substitutions can predict all the peptides recognized by a given TCR. Moreover, our analysis of a panel of 14 cloned T cell hybridomas could only cover a fraction of the cross-reactivities occurring during a polyclonal immune response *in vivo*. An illustrative example is a peptide derived from the human LFA-1 molecule that is highly homologous to $OspA_{164-175}$ (see Table 3) and was recently reported to be recognized by HLA-DR4-restricted OspA-specific T cells [6]. However, this LFA-1 peptide was not recognized by any of the seven $OspA_{164-175}$-specific T cell hybridomas examined here, illustrating again that individual TCR possess different patterns of cross-reactivity. Taken together, the data illustrates that even a careful analysis of autoantigens recognized by a panel of T cells specific for a bacterial antigen will underestimate the full extent of cross-reactivity occurring during a polyclonal immune response *in vivo*.

How do these findings relate to the molecular mimicry hypothesis? Our data together with other available evidence [9, 23, 24, 26] suggest that cross-reactivity leading to T cell activation is a very frequent event. Autoimmunity, however, does not occur as frequently, suggesting that T cell reactivity between a microbial peptide and a self peptide alone is not sufficient to induce autoimmune disease. Several mechanisms normally prevent coss-reactive T cells from causing injury. These include antigen sequestration, proteolytic destruction of epitopes during antigen processing, low concentration of the self antigen, a low frequency of cross-reactive T cells, peripheral deletion or anergy of self-reactive T cells, regulatory cells, and "non-pathogenic" patterns of cytokine production or migratory behavior of cross-reactive T cells [27–29]. Therefore, it is important to note that some of the mimic peptides identified by our analysis are ubiquitously exposed to the immune system (Table 3 and 4, *e.g.* 4-1BB ligand), thus ruling out antigen sequestration. Some of the mimic peptides are derived from self proteins, which are known to be targets of autoimmune responses (Table 3 and 4, *e.g.* insulin precursor, SS-B antigen). Importantly, the peptide PLALEGSLQKRG recognized by one of the $OspA_{155-176}$-specific hybridomas has been identified as the immunodominant proinsulin peptide recognized by HLA-DR4$^+$ diabetic patients [30], demonstrating that this peptide is available for T cell recognition *in vivo*. Thus, it is likely that in most cases the cross-reactivity between a particular microbial and a particular self peptide remains harmless. In fact, some degree of autoreactivity is a necessary survival feature for naive T cells [31] and possibly memory cells [32]; and it is even possible that in some circumstances autoreactive T cells help in controlling autoimmunity [33]. The HLA-DR4 transgenic mice used here should provide a useful system to analyze further the *in vivo* correlates of cross-reactivity between microbial and self peptides.

In summary, we have used the peptide-spot synthesis technique to perform a systematic analysis of T cell cross-reactivity by global amino acid substitutions of two bacterial epitopes. This high throughput approach to epitope mutagenesis will likely have broader applications in the analysis of infectious diseases, autoimmunity, and vaccine development. Multiple endogenous peptides were recognized by OspA-specific T cells. Based on our *in vitro* studies it is not possible to judge finally the role of cross-reactivity between OspA and a self antigen in treatment-resistant Lyme arthritis. Molecular mimicry remains an attractive hypothesis for the pathogenesis of autoimmune disease, for example by maintaining the memory T cell pool specific for a particular autoantigen. It is clear, however, that the mere demonstration of T cell cross-reactivity is inconsequential for the understanding of any autoimmune disease.

4 Materials and methods

4.1 Mice

HLA-DR*0101/HLA-DRB*0401, human CD4 triple-transgenic mice have been described previously [34] and were maintained on the DBA/1J background. These mice were mated with $A\beta^{-/-}$ mice [35]. Offspring were screened for HLA-DR and huCD4 expression by standard FACS analysis as described [34].

4.2 Antigens

4.2.1 OspA

Recombinant lipidated OspA (lOspA) from *B. burgdorferi* strain ZS7 (GenBank accession number X1647) was provided by Dr. Y. Lobet (SKB, Rixensart, Belgium). Unlipidated recombinant OspA was purchased from TibMolBiol (Berlin, Germany).

4.2.2 Peptides

A set of 52 20-mer peptides overlapping by 15 amino acids each and spanning the entire 273 residues of the complete *B. burgdorferi* N40-OspA-sequence was synthesized by Fmoc chemistry and purchased from BioTeZ (Berlin-Buch, Germany). Cellulose-bound peptides were prepared by automated spot synthesis as described before [9]. For titration experiments peptides were conventionally synthesized as described [9].

4.3 Generation of hybridomas and assay conditions

HLA-DRA*0101/HLA-DRB*0401, human CD4 triple-transgenic I-Aβ$^{-/-}$ mice were immunized s.c. with 50 μg of purified lOspA in incomplete Freund's adjuvant (IFA; Difco, Detroit, MI). T cell hybridomas were obtained from two independent fusions as described [30]. Where indicated, we used cloned hybridomas. An FACS Vantage (Becton Dickinson) sorter was used to plate the cells at a density of 1 cell/well onto 96-well plates under sterile conditions; 5×10^4 – 10×10^4 hybridoma cells were plated with 2.5×10^5 irradiated APC. Spleen cells from DR4$^+$/I-Aβ$^-$ mice, or a human EBV-transformed B cell line (EBV-BCL), homozygous for HLA-DRA*0101/HLA-DRB*0401 (Priess) were used as APC as indicated. After 48 h, supernatants were transferred to replicate 96-well plates and IL-2 concentrations were determined using a streptavidin-europium based immunoassay [30] or a conventional IL-2 sandwich ELISA [9] as described. Absorbance (A) values ≤ mean background values + 2 SD were considered negative; A values > mean values – 2 SD for the OspA peptides were considered strongly positive. The range of A values in between these two cut-off values was divided into three equal intervals. For supertope definition only peptide analogs corresponding to A values within the top two intervals were considered positive.

4.4 Database searches

The SwissProt and TREMBL databases were searched for human and murine peptides matching the supertopes (software ExPasy) [36]. A conventional sequence homology search of the SwissProt and TREMBL databases was performed using the blastp software [37].

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Correspondence: Thomas Kamradt, DRFZ, Monbijoustr. 2, D-10117 Berlin, Germany
Fax: +49-30-28460-764
e-mail: Kamradt@drfz.de

Attachment 1: LYMErix® Prescribing Information

PRESCRIBING INFORMATION

LYMErix®
Lyme Disease Vaccine (Recombinant OspA)

DESCRIPTION

LYMErix [Lyme Disease Vaccine (Recombinant OspA)] is a noninfectious recombinant vaccine developed and manufactured by SmithKline Beecham Biologicals. The causative agent of Lyme disease is *Borrelia burgdorferi*; in North America, all Lyme disease is due to *Borrelia burgdorferi sensu stricto*. The vaccine contains lipoprotein OspA, an outer surface protein of *Borrelia burgdorferi sensu stricto* ZS7, as expressed by *Escherichia coli*. Lipoprotein OspA is a single polypeptide chain of 257 amino acids with lipids covalently bonded to the N terminus. No substance of animal origin is used in the commercial manufacturing process. Fermentation media consist primarily of inorganic salts, and vitamins, with small quantities of antifoam (contains silicon), kanamycin sulfate (an aminoglycoside antibiotic), and yeast extract. Silicon and kanamycin are removed to levels below detection (<7 ppm and <10 ppb, respectively). The vaccine is adsorbed onto aluminum hydroxide.

LYMErix is supplied as a sterile suspension in single-dose vials and prefilled syringes for intramuscular administration. The vaccine is ready for use without reconstitution; it must be shaken before administration to ensure a uniform turbid white suspension.

Each 0.5 mL dose of vaccine consists of 30 mcg of lipoprotein OspA adsorbed onto 0.5 mg aluminum as aluminum hydroxide adjuvant. Each dose of the vaccine preparation contains 10 mM phosphate buffered saline and 2.5 mg of 2-phenoxyethanol, a bacteriostatic agent.

The potency of the vaccine is evaluated by immunizing mice with LYMErix® [Lyme Disease Vaccine (Recombinant OspA)] and measuring their serum antibody response to OspA by ELISA.

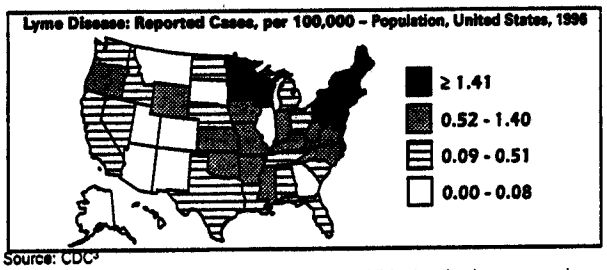
CLINICAL PHARMACOLOGY

Microbiology

Lyme disease is a multisystem disease caused by infection with the bacterial spirochete, *B. burgdorferi*, which is transmitted by *Ixodes* ticks. The enzootic life cycle of *B. burgdorferi* is dependent upon its transmission between a vector, the *Ixodes* tick, and a reservoir host, most commonly the white-footed mouse. Tick larvae usually feed in the late summer and acquire *B. burgdorferi* from an infected animal host. Nymphal ticks feed in the late spring and summer, and serve as the most common source of human infection. Adult ticks feed in the fall, winter and early spring, with the white-tailed deer being the preferred host. Adult ticks can also transmit *B. burgdorferi* to humans.[1] Both deer and rodent hosts are necessary to maintain the enzootic cycle of *B. burgdorferi*.

Epidemiology

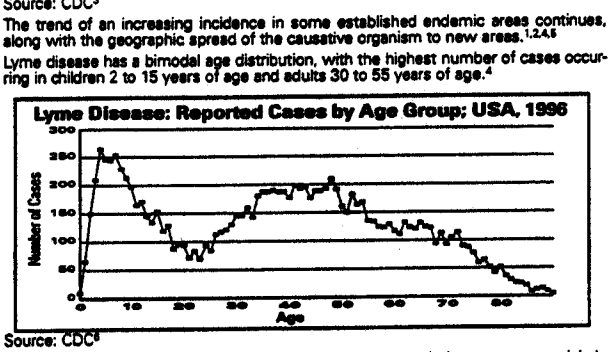
Lyme disease is the most commonly diagnosed vector-borne disease in the United States, with over 99,000 cases reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1996. During that time, the incidence of reported cases increased by at least 32-fold. Although most cases have been reported in the Northeast, upper Midwest and Pacific coastal areas of the United States, infections have been reported in almost all states.[2] The incidence rates vary considerably from state to state and even within states at the county level.[2]



Source: CDC[2]

The trend of an increasing incidence in some established endemic areas continues, along with the geographic spread of the causative organism to new areas.[1,2,4,5]

Lyme disease has a bimodal age distribution, with the highest number of cases occurring in children 2 to 15 years of age and adults 30 to 55 years of age.[4]



Source: CDC[6]

The primary risk factor for Lyme disease is exposure to wooded or grassy areas inhabited by *B. burgdorferi*-infected ticks. Such areas may include woodlands, meadows, or residential yards in endemic areas.[5] Cases have been reported in people whose only exposure to *B. burgdorferi* has been while on vacation in an endemic area.[1]

Lyme disease has been reported to occur throughout the year.[7,8] Peak incidence of Lyme disease varies by region and may vary annually based on fluctuations in local climatic conditions.[1,5,7,8] For example, the peak occurs in the late spring and summer in the Northeast United States, coincident with the feeding of nymphal ticks, the most common source of human infection. Transmission can occur also in the fall, winter, and early spring when adult ticks are feeding.[1]

Clinical Manifestations: Lyme disease has a variable incubation period.[5] Lyme disease is a multisystem disease, which has been described as having early and late stages. The early stage is usually characterized by a rash (erythema migrans) and may be accompanied by fever, fatigue, myalgias and/or arthralgias. Erythema migrans represents a localized cutaneous infection and is the presenting symptom in 60% to 80% of cases. Early disseminated manifestations include secondary skin lesions, neurologic involvement (meningitis, facial palsy, other cranial neuritides, radiculoneuritis), cardiac involvement (atrioventricular block, myocarditis), and musculoskeletal symptoms usually consisting of migratory pain in joints and the surrounding soft tissue structures.[9]

Late stage disease (persistent infection) occurs months to years after initial infection and may be manifested as chronic arthritis, chronic neurologic abnormalities or acrodermatitis chronica atrophicans. Not all patients with Lyme disease have this characteristic progression of symptoms. Late stage disease usually requires more intensive therapy and may result in permanent sequelae. In particular, late neurologic involvement is associated with chronic, slowly progressive disease.[10]

The rate of asymptomatic infection has not been well studied in adults. In the LYMErix [Lyme Disease Vaccine (Recombinant OspA)] study, the rate of asymptomatic infection (for definition, see *Clinical Efficacy*, Asymptomatic *B. burgdorferi* infection) was approximately 0.25% per year with one case of asymptomatic infection occurring for every four cases of erythema migrans.

Late stage disease may result from early disease that is either unrecognized or fails to respond to treatment, or from asymptomatic infection. The relative importance of these conditions in predisposing to the development of late stage disease is unknown.

Diagnosis: Diagnosis is based on clinical manifestations, epidemiologic information and laboratory evaluation. Confirming the diagnosis may be difficult in some cases.

At a consensus meeting of the CDC and ASTPHLD (Association of State, Territorial and Public Health Laboratory Directors), a two-step approach was recommended if serologic evaluation of Lyme disease is required.[11] A sensitive screening test such as an enzyme-linked immunosorbent assay (ELISA) or immunofluorescent assay (IFA) is recommended as the initial laboratory test and, if positive or equivocal, immunoblot (Western blot) testing should be performed to confirm the results (see Laboratory Test Interactions).

LYMErix Mechanism of Action: LYMErix stimulates specific antibodies directed against *B. burgdorferi*. The organism contains several outer surface proteins, with lipoprotein OspA being immunodominant.[12] Administration of lipoprotein OspA to mice resulted in the formation of specific IgG anti-OspA antibodies, including those directed against a specific epitope, LA-2 (designated LA-2 equivalent antibodies). These antibodies have demonstrated bactericidal activity. Studies have shown that mice immunized with recombinant lipoprotein OspA are protected against disease after tick challenge with *B. burgdorferi*.[13] LA-2 equivalent antibody titers have been shown to correlate with protection against infection in laboratory animals.[14]

B. burgdorferi express OspA while residing in the midgut of the infected tick, but OspA is downregulated after tick attachment and is usually undetectable or absent when *B. burgdorferi* is inoculated into the human host.[15] Thus, a novel hypothesis has been proposed to explain the effectiveness of lipoprotein OspA vaccination: when infected ticks bite humans who have been vaccinated with *LYMErix*, the vaccine-induced antibodies are taken up by the tick and interact with the *B. burgdorferi* in the midgut of the tick, thereby preventing transmission of the organism to the host. This mechanism has been suggested by a pre-clinical study in which *B. burgdorferi* were detected by immunofluorescence assay in none of the ticks that fed on OspA-immunized mice, compared with 72% of ticks that fed on control-immunized mice.[13]

Clinical Efficacy

A randomized, double-blind, multicentered, placebo-controlled trial has shown that *LYMErix* confers protection against Lyme disease.[16] This trial was conducted in highly endemic areas of the United States, primarily in the Northeast, and enrolled 10,936 subjects (5,469 vaccinees; 5,467 placebo recipients) ages 15 through 70 years. Subjects with a history of previous Lyme disease were not excluded from this trial.

Subjects vaccinated with three doses of *LYMErix* or placebo at months 0, 1 and 12 were observed for 20 months after the first injection (January 1995 through November 1996). The primary endpoint of the trial was the incidence of definite Lyme disease after two doses of vaccine. Each subject was actively followed for symptomatic disease during the entire observation period and was assessed for possible asymptomatic infection (as evidenced by IgG Western blot seroconversion) at months 12 and 20.

Definite Lyme disease

In the pivotal efficacy trial, definite Lyme disease was defined as clinical manifestations (erythema migrans, neurologic, musculoskeletal or cardiovascular involvement) with laboratory confirmation (positive culture for *B. burgdorferi* from skin biopsy; positive polymerase chain reaction [PCR] result for *B. burgdorferi* from skin biopsy, synovial fluid, or CSF; or IgM or IgG Western blot seroconversion) as defined by CDC/ASTPHLD criteria.[11]

Post-second dose efficacy was measured beginning at 4 weeks following the second dose through to month 12. Post-third dose efficacy was measured from the third dose through to month 20.

Prevention of Definite Lyme Disease: Vaccine efficacy against definite Lyme disease was 78% (95% CI: 59% to 88%) after three doses of vaccine administered according to protocol (13 cases among 4,765 subjects in the vaccine group; 58 cases among 4,784 subjects in the placebo group). Vaccine efficacy against definite Lyme disease was 50% (95% CI: 14% to 71%) after two doses of vaccine administered according to protocol (20 cases among 5,148 subjects in the vaccine group; 40 cases among 5,166 subjects in the placebo group).

Asymptomatic *B. burgdorferi* infection

In the pivotal efficacy trial, subjects were defined as having asymptomatic infection when, in the absence of recognizable clinical symptoms, IgG Western blot seroconversion occurred either between months 2 and 12 of the first year, or between months 12 and 20 of the second year.

Prevention of Asymptomatic Infection: Vaccine efficacy against asymptomatic *B. burgdorferi* was 100% (95% CI: 30% to 100%) after three doses of vaccine administered according to protocol (0 cases among 4,765 subjects in the vaccine group; 13 cases among 4,784 subjects in the placebo group). Vaccine efficacy against asymptomatic *B. burgdorferi* was 83% (95% CI: 25% to 98%) after two doses of vaccine administered according to protocol (two cases among 5,148 subjects in the vaccine group; 12 cases among 5,166 subjects in the placebo group).

Possible Lyme disease

In the pivotal efficacy trial, possible Lyme disease was defined as a flu-like illness (fever, chills, fatigue, headache, joint or muscle aches) with IgM or IgG Western blot seroconversion, or physician-diagnosed erythema migrans with negative laboratory results.

Prevention of Possible Lyme Disease: Following the three-dose course of vaccine administered according to protocol, efficacy was 48% (95% CI: 1% to 73%) against possible Lyme disease. Fourteen of the subjects in the vaccine group developed a possible case of Lyme disease, compared to 27 placebo recipients. Following two doses of vaccine administered according to protocol, the vaccine efficacy against possible Lyme disease was 21% (95% CI: -45% to 56%). Nineteen subjects who received two doses of vaccine developed possible Lyme disease, compared to 24 placebo recipients.

The data regarding flu-like illnesses due to possible Lyme disease may be confounded by possible cross-reactivity and/or co-infection with *Ehrlichia*, which may cause a flu-like illness and false-positive IgM Western blot for *B. burgdorferi*.[17]

Lyme Disease Manifestations and Laboratory Diagnosis in the Efficacy Trial: The clinical presentation of the 131 cases of definite Lyme disease was as follows: erythema migrans, 128 (32 vaccine, 96 placebo); arthritis, 1 (vaccine); trigeminal neuralgia, 1 (placebo); and facial palsy, 1 (placebo). Of the 128 cases with erythema migrans, additional presenting clinical manifestations included: facial palsy, 3 (1 vaccine, 2 placebo) and trigeminal neuralgia, 1 (placebo). The duration of erythema migrans was similar for both vaccine and placebo recipients.

Subjects were treated at either acute presentation of Lyme disease symptoms, following laboratory confirmation of symptoms, or following laboratory confirmation of asymptomatic infection. Active surveillance and prompt treatment of identified cases may have accounted for the low incidence of late Lyme disease manifestations.

A similar proportion of definite Lyme disease cases in both vaccine and placebo groups were confirmed by positive culture, PCR analysis, or Western blot seroconversion.

Immunogenicity in Persons 15 to 70 Years of Age: In the pivotal efficacy trial, immunogenicity of LYMErix [Lyme Disease Vaccine (Recombinant OspA)] was assessed by measuring IgG anti-OspA antibodies and LA-2 equivalent antibodies in a subset of subjects 15 to 70 years of age enrolled at one study center.

Table 1 shows the seropositivity rates and geometric mean titers (GMTs) following the second and third doses of *LYMErix*.

Table 1. Immunogenicity in Vaccinees

Antibody	Sampling Time	Seropositivity* % (n/N)	GMT-EL.U./mL (95% CI)
Total IgG Anti-OspA	1 mo. after dose 2	99% (260/264)	1227 (1029, 1463)
	Pre-dose 3†	83% (201/241)	116 (96, 139)
	1 mo. after dose 3	100% (267/267)	6006 (5180, 6963)
	8 mos. after dose 3	98% (262/267)	1991 (1686, 2351)
			GMT-ng/mL (95% CI)
LA-2 Equivalent	1 mo. after dose 2	96% (236/245)	909 (773,1067)
	Pre-dose 3†	58% (150/258)	132 (118, 149)
	1 mo. after dose 3	99% (220/222)	4402 (3686, 5257)
	8 mos. after dose 3	97% (217/223)	1935 (1628, 2300)

(continued)

LYMErix® [Lyme Disease Vaccine (Recombinant OspA)] continued

* Seropositivity defined as an IgG OspA antibody titer ≥20 EL.U./mL or a LA-2 equivalent antibody titer ≥100 ng/mL.
† At month 12.
n/N = number of seropositive subjects/total subjects tested.
% = percentage of seropositive subjects.

Subjects in the placebo group did not develop detectable anti-OspA seropositivity at the sampling time points indicated in the above table.

INDICATION AND USAGE

LYMErix is indicated for active immunization against Lyme disease in individuals 15 to 70 years of age.

Individuals most at risk may be those who live or work in *B. burgdorferi*-infected tick-infested grassy or wooded areas (e.g., landscaping, brush clearing, forestry, and wildlife and parks management),[4,19-21] as well as those who plan travel to or pursue recreational activities (e.g., hiking, camping, fishing and hunting) in such areas. Most cases of Lyme disease in the United States are thought to be acquired in the peri-residential environment, through routine activities of property maintenance, recreation, and/or exercise of pets.[19,22]

Previous infection with *B. burgdorferi* may not confer protective immunity.[23] Therefore people with a prior history of Lyme disease may benefit from vaccination with *LYMErix*.

Safety and efficacy for this vaccine are based on administration of the second and third doses several weeks prior to the onset of the *Borrelia* transmission season in the local geographic area (see DOSAGE AND ADMINISTRATION).

LYMErix is not a treatment for Lyme disease.

As with any vaccine, *LYMErix* may not protect 100% of individuals. The vaccine should not be administered to persons outside of the indicated age range.

CONTRAINDICATIONS

LYMErix is contraindicated in people with known hypersensitivity to any component of the vaccine.

PRECAUTIONS

General

LYMErix will not prevent disease in those who have unrecognized infection at the time of vaccination. *LYMErix* will not provide protection against other tick-borne diseases such as babesiosis or ehrlichiosis.

Treatment-resistant Lyme arthritis (antibiotic refractory), a rare complication of *B. burgdorferi* infection, has been associated with immune reactivity to OspA of *B. burgdorferi*.[24] Since the underlying etiology is not clearly understood, it is recommended that *LYMErix* not be administered to such patients.

As with other vaccines, although a moderate or severe febrile illness is sufficient reason to postpone vaccination, minor illnesses such as mild upper respiratory infections with or without low-grade fever are not contraindications.[25]

Before the injection of any biological, the physician should take all reasonable precautions to prevent allergic or other adverse reactions, including understanding the use of the product concerned, and the nature of the side effects and adverse reactions that may follow its use.

Prior to immunization with any vaccine, the patient's history should be reviewed. The physician should review the patient's immunization history for possible vaccine sensitivity, previous vaccination-related adverse reactions and occurrence of any adverse-event-related symptoms and/or signs, in order to determine the existence of any contraindication to immunization and to allow an assessment of benefits and risks. Epinephrine injection (1:1000) and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

Packaging for the *LYMErix* Tip-Lok® syringe contains dry natural rubber, which may cause allergic reactions; packaging for the vial does not contain natural rubber.

A separate sterile syringe and needle or a sterile disposable unit must be used for each patient to prevent the transmission of infectious agents from person to person. Needles should be disposed of properly and should not be recapped.

As with any vaccine administered to immunosuppressed persons or persons receiving immunosuppressive therapy, the expected immune response may not be obtained. For individuals receiving immunosuppressive therapy, deferral of vaccination for three months after therapy may be considered.[26]

Information for Patients

In addition to vaccination with LYMErix [Lyme Disease Vaccine (Recombinant OspA)], people can further decrease their risk of acquiring tick-borne infections by taking standard preventive measures (e.g., wearing long-sleeved shirts, long pants rather than shorts, tucking pants into socks, treating clothing with tick repellent, and checking for and removing attached ticks).[2]

Patients, parents or guardians should be informed of the benefits and risks of immunization with *LYMErix*, and of the importance of completing the immunization series. As with any vaccine, it is important when a subject returns for the next dose in a series that he/she be questioned concerning the occurrence of any symptoms and/or signs after a previous dose of the same vaccine and adverse events be reported. The U.S. Department of Health and Human Services has established a Vaccine Adverse Events Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine. The VAERS toll-free number is 1-800-822-7967.

The duration of immunity following a complete schedule of immunization with *LYMErix* has not been established.

It is important to note that subjects with a prior history of *B. burgdorferi* infection may not have protection against subsequent disease[23] or asymptomatic infection.

Individuals should be informed that vaccination with *LYMErix* may induce a false-positive ELISA result for *B. burgdorferi* infection (see Laboratory Test Interactions). Patients should be advised to inform health care professionals that they have been immunized with *LYMErix*, since it may affect laboratory testing for diagnosing Lyme disease.

Laboratory Test Interactions

LYMErix immunization results in the generation of anti-OspA antibodies, which can be detected by an enzyme-linked immunosorbent assay (ELISA) for *B. burgdorferi*. The incidence of positive IgG ELISA tests is dependent on the sensitivity and specificity of the ELISA assay and the titer of anti-OspA antibody. In general, there is an association between anti-OspA titer and IgG ELISA index or Optical Density (OD) ratio; the higher the titer of anti-OspA achieved, the higher the IgG ELISA index or OD ratio.

Therefore, because vaccination may result in a positive IgG ELISA in the absence of infection, it is important to perform Western blot testing if the ELISA test is positive or equivocal in vaccinated individuals who are being evaluated for suspected Lyme disease.

Following vaccination, the appearance of a 31kD OspA band, possibly accompanied by other lower molecular weight bands on an immunoblot (Western blot), should not interfere with the determination of positivity when assessed by CDC/ASTPHLD criteria.[11]

Drug Interactions

No data are available on the immune response to *LYMErix* when administered concurrently with other vaccines. As with other intramuscular injections, *LYMErix* should not be given to individuals on anticoagulant therapy, unless the potential benefit clearly outweighs the risk of administration.

Carcinogenesis, Mutagenesis, Impairment of Fertility

LYMErix has not been evaluated for carcinogenic or mutagenic potential, or for impairment of fertility.

Pregnancy

Teratogenic Effects: Pregnancy Category C. Animal reproductive studies have not been conducted with *LYMErix*. It is also not known whether *LYMErix* can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. *LYMErix* should be given to a pregnant woman only if clearly needed.

Health care providers are encouraged to register pregnant women who receive LYMErix [Lyme Disease Vaccine (Recombinant OspA)] in the SmithKline Beecham Pharmaceuticals vaccination pregnancy registry by calling 1-800-366-8900, ext. 5231.

Nursing Mothers

It is not known whether *LYMErix* is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when *LYMErix* is administered to a nursing woman.

Pediatric Use

Safety and efficacy in pediatric subjects younger than 15 years of age have not been evaluated. Therefore, the vaccine is not indicated for this age group at this time.

ADVERSE REACTIONS

During clinical trials involving 6,478 individuals receiving a total of 18,047 doses, *LYMErix* has been generally well tolerated.

Subjects with the following conditions: chronic joint or neurologic illness related to Lyme disease; diseases associated with joint swelling (including rheumatoid arthritis) or diffuse musculoskeletal pain; second- or third-degree atrioventricular block or a pacemaker were excluded from the efficacy trial because such conditions could interfere with the assessment of Lyme disease in the trial. Therefore, data are limited regarding the safety of the vaccine in subjects with these conditions (see below).

Unsolicited Adverse Events

The most frequently reported (≥1%) unsolicited adverse events within 30 days of vaccination for all subjects receiving at least one dose (n=10,936) in the double-blind, placebo-controlled efficacy trial are shown in Table 2.

Table 2. Incidence (≥1%) of Unsolicited Adverse Events Occurring Within 30 Days Following Each Dose* and Overall (after Doses 1, 2 or 3)

	Dose 1		Dose 2		Dose 3		Overall	
	Vaccine (N = 5489)	Placebo (N = 5467)	Vaccine (N = 5367)	Placebo (N = 5417)	Vaccine (N = 5001)	Placebo (N = 5018)	Vaccine (N = 5489)	Placebo (N = 5467)
Events	%	%	%	%	%	%	%	%
Local								
Injection site pain	17.96[c]	4.90	8.76[c]	2.95			21.87[c]	6.91
Injection site reaction							1.54[b]	0.91
General								
Body as a Whole								
Achiness	1.57	1.19	1.22	0.90			2.78	2.25
Chills/Rigors							2.05[a]	0.73
Fatigue	2.03	1.86	1.72	1.42			3.86	3.42
Fever	1.35[a]	0.91					2.59[a]	1.61
Infection viral	1.88	1.66					2.83	2.45
Influenza-like symptoms	1.44[a]	0.93					2.54[a]	1.86
Nausea							1.12	1.04
Musculoskeletal System								
Arthralgia	3.22	2.67	3.11	2.60	1.24	1.16	6.78	6.05
Back pain							1.90	1.55
Myalgia	2.69[a]	1.72	1.52[a]	0.98			4.83[c]	2.94
Stiffness							0.95	1.21
Nervous System								
Dizziness							1.01	1.08
Headache	3.51	2.96	2.39	2.33			5.61	5.09
Respiratory System								
Bronchitis							1.10	1.28
Coughing							1.50	1.46
Pharyngitis	1.39	1.12	1.15	1.20			2.52	2.45
Rhinitis	1.50	1.46					2.41	2.47
Sinusitis	1.74	1.57	1.26	1.27			3.16	2.93
Upper respiratory tract infection	2.63	3.22	1.65	1.75			4.35	4.98
Skin/Appendages								
Rash							1.37	1.08

*Includes events obtained through spontaneous reports following each dose and events reported 1 month after doses 1 and 2 (when all subjects were queried regarding the occurrence of any adverse event since the previous vaccination).

a. *p*-value <0.05. b. *p*-value <0.01. c. *p*-value <0.001.

The most frequently reported (≥1%) unsolicited adverse events occurring more than 30 days following vaccination for all subjects (n=10,936) in the double-blind, placebo-controlled efficacy trial are shown in Table 3.

Table 3. Incidence (≥1%) of Unsolicited Adverse Events Occurring More Than 30 Days Following Doses 2 and 3* and Overall (after Doses 1, 2 or 3)

	Dose 2		Dose 3		Overall	
	Vaccine (N = 5367)	Placebo (N = 5417)	Vaccine (N = 5001)	Placebo (N = 5018)	Vaccine (N = 5489)	Placebo (N = 5467)
Events	%	%	%	%	%	%
Body as a Whole						
Achiness	1.50	1.38			2.30	2.18
Chills/Rigors	1.30	1.05			1.74	1.76
Fatigue	3.24	3.43	1.86	1.81	5.01	4.98
Fever	2.28	2.60	1.34	1.30	3.58	3.82
Infection viral	1.43	1.74			2.19	2.34
Influenza-like symptoms	2.33	2.10			2.87	2.76
Cardiovascular System						
Hypertension					0.93	1.24
Gastrointestinal System						
Diarrhea					1.01	1.19
Musculoskeletal System						
Arthralgia	9.93	10.04	4.72	4.46	13.64	13.55
Arthritis	1.98	1.74	1.04	1.12	2.91	2.84
Arthrosis	1.22	1.09			1.66	1.50
Back pain	2.69	2.73			3.58	3.46
Myalgia	2.78	2.22	1.14	1.28	4.02	3.40
Stiffness	1.82	1.59			2.47	2.40
Tendinitis	1.45	1.05			1.92	1.63
Nervous System						
Depression					1.02	1.10
Dizziness					1.02	1.26
Headache	3.56	3.05	1.36	1.49	5.06	4.72
Hypesthesia	2.20	2.66			2.96	3.60
Paresthesia	2.69	2.20	1.06	0.98	3.60	2.98
Respiratory System						
Bronchitis					1.32	1.39
Pharyngitis	1.70	1.68			2.19	2.12
Rhinitis	0.94	1.07			1.41	1.37
Sinusitis	2.33	2.53			3.07	3.11
Upper respiratory tract infection	2.02	2.29			2.80	3.00
Skin/Appendages						
Contact dermatitis	1.50	1.75			1.68	1.94
Rash	2.39	1.99			3.07	2.71

* Data for adverse events occurring more than 30 days after dose 1 are not provided because most subjects received dose 2 approximately 30 days after dose 1.

Note: No significant differences in adverse events were noted between treatment groups after any dose and overall.

Separate post hoc analyses were conducted to assess two subsets of musculoskeletal events which occurred either early (≤30 days) or late (>30 days) post-vaccination. There were no significant differences, either early or late, between the vaccine and placebo recipients with regard to experiencing arthritis, aggravated arthritis, arthropathy or arthrosis. However, vaccine recipients were significantly more likely than placebo recipients to experience early events of arthralgia or myalgia after each dose [for dose 1: odds ratio (OR), (95% CI) = 1.35 (1.13, 1.61); dose 2: OR = 1.28 (1.05, 1.56); dose 3: OR = 1.59 (1.18, 2.16)]. With regard to late events of arthralgia or myalgia, there were no significant differences between vaccine and placebo recipients.

There was no significant difference in the rates of cardiac adverse events between vaccine and placebo recipients. Neurologic adverse events which occurred at a rate <1% in the vaccine group and were noted to occur with a similar frequency in placebo recipients included: carpal tunnel syndrome, migraine, paralysis, tremor, coma, dysphonia, ataxia, multiple sclerosis, myasthenia gravis, meningitis, trigeminal neuralgia, nystagmus, neuritis, neuralgia, nerve root lesion, neuropathy, hyperesthesia, hyperkinesia, and intracranial hypertension.

Overall, approximately 18% of subjects enrolled in the study had a prior history of some musculoskeletal condition (19% vaccinees, 18% placebo recipients). In a post hoc subgroup analysis, there was no significant difference between vaccine and placebo recipients with regard to development of musculoskeletal events (defined as arthritis, arthropathy, arthrosis, synovitis, tendinitis, polymyalgia rheumatica, bursitis or rheumatoid arthritis and lasting more than 30 days) in those with a prior history of musculoskeletal conditions. However, both vaccine and placebo recipients with a prior history of musculoskeletal conditions were more likely to experience musculoskeletal events than subjects without such prior history.

Solicited Adverse Events

The frequency of solicited local and systemic adverse events was evaluated in a subset of subjects (n=938) who comprised the total enrollment at one study center in the efficacy trial. Of these 938 subjects, 800 completed a 4-day diary card following each of three doses, and were evaluable according to protocol. Table 4 shows the percentage of subjects reporting a solicited symptom following any one of the three doses and overall. The majority of the solicited events were mild to moderate in severity and limited in duration.

Table 4. The Incidence of Local and General Solicited Adverse Events (including Severe Events) Reported After Each Dose and Overall

	Dose 1		Dose 2		Dose 3		Overall	
Events	Vaccine (N = 402) %	Placebo (N = 398) %	Vaccine (N = 402) %	Placebo (N = 398) %	Vaccine (N = 402) %	Placebo (N = 398) %	Vaccine (N = 402) %	Placebo (N = 398) %
Local Symptoms								
Redness, any	21.64[c]	8.29	16.67[c]	7.04	25.12[c]	11.81	41.79[c]	20.85
Redness, severe*	2.2[b]	0.0	1.0	0.0	2.5[b]	0.0	4.2[c]	0.0
Soreness, any	81.59[c]	36.68	76.37[c]	30.90	82.59[c]	52.26	93.53[c]	68.09
Soreness, severe†	1.2	0.0	1.0	0.3	3.0[b]	0.3	5.0[c]	0.0
Swelling, any	14.43[c]	4.27	11.44[c]	3.27	19.15[c]	6.78	29.85[c]	11.31
Swelling, severe*	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0
General Symptoms								
Arthralgia, any	11.94[c]	4.52	10.70	8.29	13.43[b]	7.54	25.62[b]	16.33
Arthralgia, severe†	0.7	0.0	0.2	0.3	0.0	0.3	1.0	0.5
Fatigue, any	20.90	16.83	20.15[c]	11.81	21.89[a]	16.33	40.80[a]	32.91
Fatigue, severe†	0.5	0.05	1.5	1.3	1.0	1.0	3.0	2.3
Headache, any	20.65	19.10	14.43	12.31	19.90	18.34	38.56	37.19
Headache, severe†	0.5	0.05	1.2	0.5	1.2	1.8	3.0	2.8
Rash, any	4.23[a]	1.51	4.98[a]	2.01	5.47[b]	1.76	11.69[b]	5.28
Rash, severe*	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0
Fever ≥99.5°F	1.49	0.75	1.00	0.50	1.00	1.01	3.48	2.26
Fever >102.2°F	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Severe = measuring >3.0 cm and persisting longer than 24 hours.
† Severe = preventing everyday normal activity.
a. *p*-value <0.05. b. *p*-value <0.01. c. *p*-value <0.001.

Subjects with Previous Lyme Disease

Subjects with previous Lyme disease were assessed using two definitions: subjects whose baseline sera were evaluated for Western blot (WB) positivity and subjects who at study entry self-reported a previous history of Lyme disease.

Study participants did not routinely have baseline sera tested by WB for Lyme disease. WB at baseline was performed for subjects who were noted to have a positive or equivocal WB during a visit for suspected Lyme disease or when tested at months 12 or 20. Baseline serology was thus found to be positive in 250 subjects out of 628 tested. The nature and incidence of adverse events (either early or late) did not differ between vaccinees determined to have been WB-positive at baseline (n=124) compared to vaccinees determined to have been WB-negative at baseline (n=151).

There were 1,206 subjects enrolled in the study who self-reported a previous history of Lyme disease (610 vaccinees, 596 placebo recipients). For adverse events occurring within the first 30 days, there was an increased incidence of musculoskeletal symptoms in vaccinees with a history of Lyme disease compared to vaccinees with no history of Lyme disease (20% vs. 13%, $p<0.001$). No such difference was observed in the placebo group (13% vs. 11%, $p=0.24$). Subjects with a previous history of Lyme disease had an increased incidence of late (>30 days post-vaccination) musculoskeletal symptoms compared to subjects without a history of Lyme disease in both the vaccine and placebo groups. There was no significant difference in late musculoskeletal adverse events between vaccine and placebo recipients with a history of Lyme disease (33% vs. 35%, $p=0.51$).

Subjects with a self-reported prior history of Lyme disease had a greater incidence of psychiatric disorders (early and late); central, peripheral and autonomic nervous system disorders (late); and gastrointestinal disorders (late) than subjects with no prior history of Lyme disease. However, there was no significant difference in the incidence of any of these disorders between vaccine and placebo recipients with a prior history of Lyme disease.

Among the 10,936 subjects enrolled in the efficacy trial and followed for 20 months, a total of 15 deaths occurred (10 vaccine, 5 placebo). None of these deaths were judged to be treatment-related by investigators. In the vaccine group, causes of death included: cancer (5), myocardial infarction (3), sudden death (1), cardiac arrest (1). In the placebo group, causes of death included: cancer (1), sudden cardiac death (1), cardiac arrest (1), septic shock (1), homicide (1).

As with all pharmaceuticals, it is possible that expanded commercial use of the vaccine could reveal rare adverse events not observed in clinical studies.

DOSAGE AND ADMINISTRATION

Primary immunization against Lyme disease consists of a 30 mcg/0.5 mL dose of *LYMErix* given at 0, 1 and 12 months.

Vaccination with all three doses is required to achieve optimal protection.

Safety and efficacy for this vaccine are based on administration of the second and third doses several weeks prior to the onset of the *Borrelia* transmission season in the local geographic area (see INDICATION AND USAGE). For example, in the pivotal efficacy trial performed primarily in the Northeast United States (see *Clinical Efficacy*), individuals were vaccinated between January and April in both years of the trial.

LYMErix [Lyme Disease Vaccine (Recombinant OspA)] should be administered by intramuscular injection in the deltoid region. *Do not inject intravenously, intradermally or subcutaneously.*

Preparation for Administration: Shake well before withdrawal and use. Parenteral drug products should be inspected visually for particulate matter or discoloration prior to administration. With thorough agitation, *LYMErix* is a turbid white suspension. Discard if it appears otherwise. Any vaccine remaining in a single-dose vial should be discarded.

The vaccine should be used as supplied; no dilution or reconstitution is necessary. The full recommended dose of the vaccine should be used.

As with other intramuscular injections, *LYMErix* should not be given to individuals on anticoagulant therapy or with clotting disorders, unless the potential benefit clearly outweighs the risk of administration.

No data are available on the immune response to *LYMErix* when administered concurrently with other vaccines. When concomitant administration of other vaccines is required, they should be given with different syringes and at different injection sites (see Drug Interactions).

STORAGE

Store between 2° and 8°C (36° and 46°F). Do not freeze; discard if product has been frozen.

HOW SUPPLIED

LYMErix [Lyme Disease Vaccine (Recombinant OspA)] is supplied in Single-Dose (30 mcg/0.5 mL) Vials and Prefilled Syringes

NDC 58160-845-11 Package of 10 Single-Dose Vials
NDC 58160-845-32 Package of 1 Prefilled Disposable Tip-Lok® Syringe with 1-inch 23-gauge needle
NDC 58160-845-35 Package of 5 Prefilled Disposable Tip-Lok® Syringes with 1-inch 23-gauge needles

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