Safety and Immunogenicity of Recombinant Outer Surface Protein A (OspA) Vaccine Formulations in the Rhesus Monkey

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ABSTRACT

Background: The safety and the immunogenicity of OspA vaccine formulations were investigated in the rhesus monkey, to help to ascertain possible deleterious effects that could appear both in naïve and infected individuals during the vaccination process and especially after a challenge infection had been administered.

Methods: Three different vaccine formulations, NS1-OspA/Al(OH)$_3$, a fusion protein composed of recombinant OspA (strain ZS7) lacking the C-terminal cysteine and fused to a fragment of 81 N-terminal amino acids from the nonstructural influenza virus protein NS1, NS1-OspA/Al(OH)$_3$/MPL (mono-phosphoryl lipid A), lipidated OspA/Al(OH)$_3$, and one placebo (Al(OH)$_3$) were used in a vaccination trial involving 19 male 2-to-3-year-old Chinese Macaca mulatta. Three 10-µg-doses of each vaccine were given to each animal intramuscularly at 4-week intervals. Group 1 (n=3) was vaccinated after being infected with Borrelia burgdorferi, as a model to assess vaccine safety in patients with an active infection. Group 2 (n=16) was divided into 4 groups of 4, according to the vaccine formulation received. Safety was assessed in both groups of animals by physical examination, clinical laboratory analyses of blood and urine samples, and quantification of inflammation in the anterior eye chamber (uveitis).

Immunogenicity was assessed by OspA-ELISA, Western blot, antibody-dependent, complement-mediated killing in vitro (ADCK), by the LA$_2$ competitive inhibition assay and by peripheral blood mononuclear cell (PBMC) blastogenesis in vitro in response to OspA.

Results: No local vaccine site reaction or obvious signs of arthritis were seen in any animal at any time. All urinalyses, serum chemistries, and complete blood count results were either normal or, when not, not attributable to vaccination. No significant intraocular inflammatory responses were found at any time in any animal, thus indicating that the powerful adjuvant properties of lipidated OspA do not cause uveitis. All formulations elicited a strong IgG anti-OspA response, with geometric mean reciprocal titers (GMRT) measured by ELISA as high as 2560 x 10^3, 4 weeks after the last vaccine dose. ADCK$_{50}$ GMRT were as high as 3475 in the lipidated OspA group at 2 weeks before challenge, but declined as much as 15-fold in animals of this group 9 weeks postchallenge. A booster effect on the antibody response was not evident after the challenge infection.

PBMC blastogenesis was measured longitudinally in vitro, before and after the challenge infection, in response to the mature form of OspA, both lipidated and nonlipidated. PBMC (from animals of all groups except controls) responded to lipidated OspA and the highest responders were among the animals vaccinated with this form of OspA. Responses to nonlipidated OspA were marginal. The PBMC responses to lipidated OspAdeclined to baseline values by 8 weeks after the challenge infection but resurfaced later and remained high until week 32 postchallenge, the last time point determined. No booster effect was observed.

Conclusion: Within the framework of our study, before postmortem analyses, all vaccine formulations appeared safe, including the lipidated OspA/Al(OH)$_3$ that is currently being used in humans. Immunogenicity at the humoral level was strong but short lived. This, together with the undetectable booster effect, may entail a need for repeated administration of the vaccine.

Key words: OspA vaccine, rhesus monkey, Lyme borreliosis

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INTRODUCTION

Lyme borreliosis is usually curable with appropriate antibiotics.[1] However, long courses of therapy may be required if the infection is allowed to become chronic, and some patients do not respond to therapy at all.[1] At risk of acquiring a persistent *Borrelia burgdorferi* infection are the 20% to 40% of infected individuals who do not show the telltale erythema migrans.[2] This erythematous papular skin rash is the most important marker of infection, largely because diagnosis based on serology, polymerase chain reaction (PCR), or spirochetal culture, although much improved in the last few years, remains imprecise.[3] Thus, the uncertainty of nonclinical diagnosis of Lyme borreliosis explains in part the occurrence of chronic Lyme disease. The fact that the latter is sometimes refractory to treatment underpins the need for immunoprophylactic strategies.

Recombinant outer surface protein A (OspA) is currently the most promising molecularly-defined vaccine candidate to prevent Lyme borreliosis. Proof of the "principle" that OspA is a protective antigen has been achieved in numerous animal experiments using diverse antigen/adjuvant combinations.[4] More recently, vaccine formulations that are compatible with human use also have been shown to be efficacious in mice.[5-7] Safety and immunogenicity of recombinant OspA vaccines have been evaluated in human subjects with and without a previous history of Lyme disease[8-10] but with no evidence of active infection. In addition, Phase III trials are underway.[11]

The present study of safety and immunogenicity of OspA vaccines in the rhesus monkey was undertaken to ascertain possible deleterious effects that could appear in naive individuals not only during the vaccination process but also after a challenge infection had been administered. Vaccine safety was examined also in a small group of animals that were infected with *B burgdorferi* at the time of vaccination. Immunogenicity was investigated both at the humoral and cellular levels. Because of the relatively rapid decline in anti-OspA antibody titers that had been observed in humans,[8] it was important to assess whether a booster effect was detectable in vaccinated rhesus monkeys subsequent to the challenge infection.

Vaccine formulations with and without added adjuvant were compared with regard to differences in safety and immunogenicity. The animal model was chosen because it had been shown previously that rhesus monkeys infected with *B burgdorferi* develop disease signs that mimic both the acute and chronic phases of Lyme disease in humans. These signs include erythema migrans, arthritis, and neuroborreliosis.[12-14] At the serological level, too, the time course and specificity spectrum of the antibody response to *B burgdorferi* in rhesus monkeys had been shown to be similar to their counterparts in humans.[12] The experimental design used in the present study is described below.

EXPERIMENTAL DESIGN

Distribution of Information

All personnel responsible for the clinical examinations, sample collection, vaccine administration, and assays described below were fully informed of the experimental design but were blind to the vaccine formulation or placebo administered to the animals.

Study population

The study population consisted of 19 male 2- to 3-year-old Chinese *Macaca mulatta* (rhesus). Three of these animals, L379, L452, and L453 (Group 1), were infected with *B burgdorferi* 4 months prior to vaccination, as a model to assess safety of vaccination in previously infected patients. The 16 other animals, L457, L549, M021, M581, L458, L594, L971, M585, M243, L537, M219, M107, L476, L712, L642, and M106 (Group 2), were divided into four groups of four animals each, according to the vaccine formulation received.

Animal examination schedule

During and after vaccination, animals of Group 1 were examined on a weekly to biweekly basis. Animals of Group 2 were examined biweekly during the 12-week period before the challenge infection, and weekly thereafter.

Vaccine formulations

Three different vaccine formulations and one placebo were used:

1) NS1-OspA/Al(OH)$_3$, a fusion protein adsorbed onto aluminum hydroxide. The NS1-OspA fusion protein is composed of recombinant OspA (cloned from the *B burgdorferi sensu stricto* isolate ZS7) that lacks the carboxyl-terminal cysteine and is fused to a fragment of 81 N-terminal amino acids from the nonstructural influenza virus protein NS1. This formulation was given to animals L458, L594, L971, and M585;

2) NS1-OspA/MPL/AlOH)$_3$, as above, but combined with 50 µg of the adjuvant (immune modulator) 4′ -monophosphoryl lipid A (MPL). This formulation was given to animals L476, L712, L642, and M106;

3) lipidated OspA/Al(OH)$_3$, recombinant lipidated OspA (ZS7) adsorbed onto aluminum hydroxide. This formulation was given to animals M243, L537, M219, and M107.

4) Aluminum hydroxide: was used as a placebo and was given to animals L457, L549, M021, and M581. All

vaccine formulations contained 10 μg/ml OspA, 0.5 mg/ml of $Al(OH)_3$, and 5 mg/ml of 2-phenoxyethanol (a preservative) in a buffer containing 150 mM sodium chloride, 5 mM sodium phosphate, and 5 mM potassium phosphate, pH 6.8.

Vaccine administration protocol

All vaccines/placebos were administered by intramuscular injection into the cranial thigh, alternating left and right thighs with each injection. Three 10-μg doses of the chosen vaccine formulation were given to each animal at 4-week intervals. The animals in Group 2 were given a challenge infection 4 weeks after the last vaccine dose. The three animals in Group 1 were vaccinated 4 months after being infected with *B burgdorferi*, using the same vaccination regimen as the animals of Group 2. The vaccine formulation chosen for Group 1 was NS1-OspA/MPL/$Al(OH)_3$.

Inoculation with *B burgdorferi*

All animals were inoculated with the B31 strain of *B burgdorferi* by the natural route of tick bite using infected *Ixodes scapularis* nymphal ticks. The procedure used and the source of ticks were the same as those described previously.[12]

Evidence that animals of Group 1 were infected

After 5 days of exposure to 20 ticks each, four ticks had fed upon animal L379, 11 upon L452, and two upon L453. All ticks were shown to be infected with *B burgdorferi* by direct immunofluorescence with a *B burgdorferi*-specific antibody, a procedure described previously.[13] Clinical examinations performed weekly on all animals revealed a mild-to-moderate skin erythema peripheral to the inoculation site in animal L452 by week 1 postinoculation (PI), which lasted for a total of 2 weeks. Animal L379 showed a mild erythema in the same site 2 weeks PI. Histological assessment of skin biopsies obtained weekly revealed deep perivascular lymphocytic infiltrates considered characteristic of the dermatitis associated with human erythema migrans in all animals until week 4 PI. By week 6 PI, the dermatitis was no longer present. Immunohistochemistry performed on these same skin sections using a monoclonal antibody to a 7.5 kDa lipoprotein of *B burgdorferi*[12] showed that *B burgdorferi* antigens were present both within and outside dermal macrophages in sections from all animals obtained after weeks 1 and 2 PI. Biopsies taken prior to infection were normal. Corneal biopsies obtained from all animals showed a marked conjunctivitis microscopically by week 2 PI, and immunostained positively for *B burgdorferi*. Fractions of skin sections from all animals—processed for PCR analysis as described previously[12]—contained detectable *B burgdorferi* DNA by week 3 PI. No spirochetes were cultured in vitro from skin or blood samples collected during the first 4 weeks PI. Western blots of sodium dodecyl sulfate (SDS)-solubilized B31 spirochetes were developed with anti-IgM and IgG antibodies. The IgM response peaked by weeks 2 to 3 PI and gradually waned. In contrast, the number of antigens recognized by anti-*B burgdorferi* IgG antibodies increased gradually over time, an indication of an active infection. By week 4 PI, four to six antigens were recognized, depending on the animal; by week 17 PI, (1 week after the last vaccine dose had been administered), 9 to 12 antigens; and by week 44 PI, 12 to 18. Prominent amongst the antigens recognized were P41 (flagellin) and P39.

Assessment of vaccine safety

Safety was assessed in both groups of animals (1 and 2) by physical examination of the animals, clinical laboratory analyses of blood and urine samples, and quantification of inflammation in the anterior eye chamber. Physical examination involved general observation, thoracic auscultation, determination of body temperature and weight, and gauging of lymph node and spleen sizes by palpation. Joints were examined for presence of swelling or redness. Laboratory analyses included complete blood cell count, serum chemistries, and urinalysis. Ocular inflammation was assessed both using the slit lamp and by laser flare photometry.

Assessment of vaccine immunogenicity

Vaccine immunogenicity was longitudinally assessed in animals of Group 2, both at the humoral and cellular levels. Antibody responses were assessed qualitatively by Western blot of serum samples using SDS extracts of whole *B burgdorferi* B31 as antigen; appearance of both IgM and IgG antibodies was examined. The overall IgG antibody response was quantified by ELISA, using purified recombinant OspA without the NS1 residue (MDP-OspA) as antigen. MDP-OspA also lacks the lipid moiety but contains the tripeptide Met-Asp-Pro covalently attached to the lysine in position 18 of the native OspA molecule. Bactericidal antibody was quantified by antibody-dependent, complement-mediated killing of *B burgdorferi* in vitro (ADCK) and functional, protective antibody by the LA_2 assay, a competitive inhibition assay in which the anti-LA_2 epitope antibody present in a serum sample is quantified by competition with the binding of the LA_2 monoclonal antibody, an antibody that binds to the LA_2 functional epitope of OspA. Cellular immune responses were examined by blastogenesis of peripheral blood mononuclear cells (PBMC), measured in vitro in response to 1) the T-cell mitogen concanavalin A (Con A), as a positive control; 2) recombinant MDP-OspA from *B burgdorferi* strain ZS7; and 3) recombinant lipidated OspA from the same strain.

MATERIALS AND METHODS

Animal care and housing

Animals were cared for and housed as described previously.[12]

Western blotting procedure

The procedure used was described previously[16] but *B burgdorferi* strain B31 (4th passage) was used rather than strain JD1.

Enzyme-linked immunosorbent assay (ELISA)

Plates were coated with 1.2 μg/ml MDP OspA (100 μl per well) in 0.1 M carbonate buffer, pH 9.6. Plates were incubated with antigen O/N at 4°C and washed three times with 200 μl/well of phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBS/T1). Plates were blocked for 2 h at 37°C with 200 μl of 1.5% BSA, 1.5% nonfat dry milk in PBS/T1 per well. A volume of 100 μl of test serum appropriately diluted with PBS/T1 containing 0.75% BSA, 0.75% nonfat dry milk was added to each well and incubated for 1 h at 37°C. Horse-radish peroxidase-labeled goat anti-human IgG (γ-chain specific) (Kirkegaard and Perry Lab Inc, Gaithersburg, Md) was diluted to 1:2000 and 100 μl were added to each well and incubated for 1 h at 37°C. The color development reagent (3,3′,5,5′-tetramethylbenzidine) was used according to the manufacturer's instructions (Kirkegaard and Perry Lab Inc, Gaithersburg, Md). All samples, including the preimmune serum, were titered by serial dilution. Titer is defined as the maximum dilution whose optical density (OD) is ≥ to 3 x OD obtained with the same dilution of the preimmune serum of the same animal.

Antibody-dependent, complement-mediated killing of *B burgdorferi* in vitro

The ADCK assay was performed as described. All determinations for each animal and time point were performed in duplicate. The mean percentage killing of these duplicate determinations was plotted as a function of the serum dilution, and the serum dilution corresponding to 50% killing ($ADCK_{50}$) was obtained from each titration curve by interpolation.

LA_2 inhibition assay

The LA_2 competitive inhibition assay was performed as follows: 96 well plates (Immunoplate Maxisorp, Gibco, Grand Island, NY) were coated with purified recombinant lipidated OspA by incubating the plates overnight at 4°C with 100 μl/well of a 0.5 μg/ml solution of OspA in 0.05 M $NaHCO_3$ buffer, pH 9.6. The optimal coating concentration of OspA was determined for each batch of purified protein. Unbound OspA was removed by washing four times with a 0.15 M NaCl, 0.05% Tween 20 solution and the plates were then "blocked" with 200 μl/well of PBS 1% bovine serum albumin for 30 min at room temperature, and washed again as above. Monkey serum samples and the LA_2 antibody standard solution were diluted two-fold serially, starting at a dilution of 1:2 or 1:10, and a concentration of 4 μg/ml, respectively. Dilutions were made with PBS 0.2% BSA containing 0.05% Tween 20. A volume of 100 μl of each dilution was added to each of duplicate wells and left for 2 h at 37°C. Plates were then washed as above and incubated for 2 h at room temperature, with 100 μl/well of a limiting concentration of LA_2 antibody labeled with horseradish peroxidase (1/10,000 for the antibody batch used) in PBS 0.2% BSA, 0.05% Tween 20. After washing as before, a volume of 100 μl/well of 0.4 mg/ml o-phenylenediamine (Sigma Chemical Company, St. Louis, Mo), 0.15% H_2O_2 in a 0.1 M citrate buffer, pH 4.5 was added. The reaction was stopped after 15 min by adding 50 μl/well of 1.0 N HCl, and the OD were read at 490 nm in a microplate reader. The raw data were analyzed by the four-parameters method. The results are expressed in μg/ml of LA_2 equivalent, using data from the standard LA_2 monoclonal antibody curve as a reference for interpolation.

Analysis of significance

Significance of the differences between mean antibody titers (ELISA, ADCK, or LA_2) elicited by, and laser flare photometry values in groups receiving, different vaccine formulations was established by analysis of variance.

Peripheral blood mononuclear cell blastogenesis

Antigens and mitogens. For each blastogenesis assay, MDP-OspA and lipidated OspA were used at 1, 5, and 10 μg/ml. The concentration of antigen eliciting the maximal response at each time point was used in the data analysis. Con A (Sigma) at 8 μg/ml was the optimal concentration chosen based on a preliminary dose-response analysis.

Culture conditions for in vitro blastogenesis. Blood was obtained from each animal and mixed with preservative-free heparin during extraction. PBMC were obtained by the Ficoll-Hypaque (Sigma) density gradient centrifugation method. The final viable cell counts were adjusted to 2 x 10^6/ml in RPMI-1640 medium (Cellgro, Fisher Scientific, Pittsburgh, Pa) containing 10% heat-inactivated human AB serum (Sigma). The blastogenesis assays were performed in triplicate in round (antigen cultures) or flat bottom (mitogen) microtiter plates (Costar Corporation, Cambridge, Mass). To each well, 100 μl of antigen or mitogen and 100 μl of cell suspension were

added. Cultures were incubated at 37°C in a humidified atmosphere (5% CO_2 and 95% air) for 4 days (mitogen) and 6 days (antigen). Approximately 24 h before harvesting, 1.0 μCi of [³H] thymidine (ICN, Irvine, Calif) was added to each well. The cells were harvested onto glass-fiber mats, washed and dried overnight at room temperature. The dried filters were placed into 5 ml of scintillation fluid, and radioactive incorporation was measured in a liquid scintillation counter. Results are expressed as stimulation index (SI), ie, counts per minute (CPM) of stimulated cells divided by CPM of unstimulated cells. The cut off SIs were 4.15 and 5.13 for MDP OspA and lipidated OspA, respectively, which represent the mean SI for all animals before vaccination began, plus 2 SD for each antigen.

Anesthesia protocol

All animals were anesthetized with ketamine-HCl (10 mg/kg) given by intramuscular injection. Animals known to have excessive myoclonic activity under ketamine anesthesia were anesthetized with tiletamine and zolazepam in combination (Telazol 10 mg/kg) by intramuscular injection. If necessary, injectable anesthesia was supplemented with 1% to 3% isoflurane and O_2. Analgesia was provided after skin biopsies with butorphanol tartrate (0.05 mg/kg).

Hematology, serum chemistries, and urinalyses

Complete blood count and serum chemistry determinations as well as urinalyses were all standard.[17] Serum chemistry analyses included tests for serum electrolytes, glucose, alanine aminotransferase, serum alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, blood urea nitrogen, creatinine, globulin, and albumin.

Slit-lamp examinations and laser flare photometry

Following external inspection of conjunctiva and adnexa, slit lamp and laser flare photometry (LFP) examinations were performed in animals of both Group 1 and 2 at baseline and at 1 and 2 weeks following each of the three immunizations. Animals of Group 2 were also examined at 1, 2, and 3 weeks following the challenge infection. LFP was performed as described elsewhere,[18] using a KOWA laser flare meter (KOWA, Inc., Torrence, Calif).

RESULTS

Vaccine safety

Physical examination: No local vaccine site reaction was noted in any animal (Groups 1 and 2) at any time. In animals of Group 2, a transient splenomegaly was observed in 3 of the 4 animals that were given $Al(OH)_3$ alone at 2 weeks after the challenge infection (PC) and in one of the animals that received lipidated OspA/$Al(OH)_3$ at 3 weeks PC. Clinical signs of arthritis were not seen in any animal. Body temperatures were taken initially at 2-week intervals coinciding with physical examinations; no fever was noted. To determine whether vaccination would cause fever shortly after the time of injection, body temperature was measured 1 day after the application of the third injection in the animals of Group 2, and 1 day after the second and third injections in the animals of Group 1. Some of the animals from Group 2 (uninfected) demonstrated a temperature marginally above the baseline for rhesus monkeys (>102.0 °F) one day after vaccination. These animals were uniformly distributed among all vaccination groups, including that receiving $Al(OH)_3$ alone. None of the animals of Group 1 (preinfected) demonstrated a temperature one day after vaccination. Therefore, these febrile episodes cannot be associated with the effects of OspA in any of the forms employed, including the lipidated form.

Urinalysis: All urinalysis samples were within normal limits in all animals.

Complete blood count: An initial leukocytosis was seen in all animals prior to experimental manipulation and in some cases also thereafter. This is a common phenomenon when animals kept in outdoor breeding groups are moved to indoor single cage housing for project work, and is probably a physiologic response to stress. In most cases seen at the Tulane Primate Center this leukocytosis resolves within 2 weeks. The leukocytosis was mild to moderate in intensity and could not be associated with any particular vaccination protocol in so far as it occurred with similar frequency in all vaccination groups, including that receiving $Al(OH)_3$ alone.

Serum chemistry: With two exceptions, all animals were within normal values for all the serum enzymes tested. The exceptions were animals M585 (Group 2), vaccinated with NS1-OspA/$Al(OH)_3$ and L712 (Group 2), vaccinated with NS1-OspA/MPL/$Al(OH)_3$. Animal M585 showed marginally elevated alanine aminotransferase (ALT>100 units) after the third vaccine dose and at the time of tick removal. Animal 712 also showed elevated ALT at the time of tick removal (198 units) and both elevated ALT and aspartate aminotransferase (AST) (AST>100 units) at 1, 2, and 4 weeks PC. AST and ALT are hepatocellular enzymes which, when elevated, indicate hepatocellular death or increased hepatocyte cell membrane permeability.

Quantitative assessment of inflammation in the anterior eye chamber

Animals were examined at the slit lamp and by LFP. LFP is a procedure which objectively measures intraocular inflammation.[18-20] A pilot study was performed to establish the reliability of the laser cell flare meter in the rhesus monkey. In this study, the results of baseline and follow-up examinations by slit lamp and LFP were compared in 10 animals, to establish the correlation between a subjective and objective examination method. Inflammatory responses were induced surgically in the right eyes by paracentesis of the anterior chamber. A comparison of LFP baseline readings of the inflamed right eyes and simultaneous readings of the untreated contralateral left eyes documented a significant fibrinous inflammatory response (P=0.008) within the right eyes. This correlated well with the slit lamp findings (subjective). Based on these results, photometry data collected during and after the vaccination procedure could be assumed to be reliable.

Rhesus monkeys that received the different vaccine formulations and were subsequently challenged (Group 2) and the animals that were vaccinated after infection (Group 1) were examined at baseline and at 1 and 2 weeks following each of the three immunizations. Animals of Group 2 were also examined at 1, 2, and 3 weeks following the challenge infection. These results are shown in Table 1. Additionally, 40 eyes of normal rhesus monkeys were examined to establish baseline values of normal anterior chamber LFP.

In previous vaccine studies using the simian immunodeficiency virus model, the LFP value of 5.0 was established as the upper limit for normal, uninflamed eyes (M.D. Conway, MD, unpublished data). In the present study, this value was never reached. LFP values from the vaccinated animals or from animals within the "immunopotentiation" Group 1 did not differ significantly from baseline data within each group or from photometry values obtained with the independent group of normal animals. All LFP evaluations were simultaneously confirmed by slit lamp examinations. Therefore, no significant intraocular inflammatory response was found at any time within the framework of this study.

TABLE 1

QUANTITATIVE ASSESSMENT OF INFLAMMATION IN THE ANTERIOR EYE CHAMBER USING LASER FLARE PHOTOMETRY (LFP).

Vaccination Group	Time of observations	LFP (Mean ± SD)
Baseline Normals n = 40 eyes.	NA	Mean 3.9 ± 0.5
Group 1	Baseline 6 eyes flare	3.4 ± 1.2
	Baseline post-infection	4.7 ± 2.4
n = 6 eyes	Baseline prior to V1,V2	3.9 ± 2.1
	Flare @ 1 wk post-V1,V2	3.8 ± 1.9
	Flare @ 2 wks post-V1,V2	3.6 ± 2.1
Placebo [$Al(OH)_3$]	Baseline -Flare	3.4 ± 1.8
n = 8 eyes	1 wk post-V1,V2,V3	3.7 ± 1.7
	2 wks post-V1,V2,V3	3.8 ± 1.8
	Challenge baseline	3.9 ± 1.3
	1 wk PC	3.6 ± 1.5
	2 wks PC	2.9 ± 1.0
	3 wks PC	3.5 ± 1.4
NS1-OspA/$Al(OH)_3$	Baseline -Flare	2.9 ± 1.2
n = 8 eyes	1 wk post-V1,V2,V3	3.3 ± 1.2
	2 wks post-V1,V2,V3	3.5 ± 1.5
	Challenge baseline	3.1 ± 1.6
	1 wk PC	2.5 ± 0.9
	2 wks PC	3.1 ± 1.2
	3 wks PC	2.8 ± 1.0
NS1-OspA/MPL/$Al(OH)_3$	Baseline -Flare	3.3 ± 1.5
n = 8 eyes	1 wk post-V1,V2,V3	3.8 ± 1.9
	2 wks post-V1,V2,V3	3.5 ± 1.5
	Challenge baseline	3.7 ± 1.5
	1 wk PC	2.8 ± 0.9
	2 wks PC	3.3 ± 1.2
	3 wks PC	3.3 ± 0.5
Lipidated OspA/$Al(OH)_3$	Baseline -Flare	3.7 ± 1.0
n = 8 eyes	1 wk post-V1,V2,V3	3.5 ± 1.7
	2 wks post-V1,V2,V3	3.7 ± 1.8
	Challenge baseline	3.3 ± 1.3
	1 wk PC	3.0 ± 1.0
	2 wks PC	3.3 ± 0.7.
	3 wks PC	3.2 ± 0.7

V = vaccination 1,2,3.
PC = post-challenge.

Vaccine immunogenicity at the humoral level

Western blot analysis: Western blots were performed using serum samples from the 16 animals in Group 2 at 2 weeks after the first vaccine/placebo injection and 4 weeks after the second injection. The results are shown in Figs 1A and 1B, respectively. By the second week after the first injection, an antibody response to OspA was already detectable in most animals that had received OspA. The intensity of the OspA bands depended on the vaccine formulation used, and increased in the sequence NS1-OspA/$Al(OH)_3$, NS1-OspA/MPL/$Al(OH)_3$, lipidated OspA/$Al(OH)_3$. This was true of both the IgM and the IgG responses, although it was more marked for the IgG response. Control animals showed no response to OspA (Fig 1A). By week 4 after the second injection, the banding pattern of the anti-OspA IgG antibody response became very complex (Fig 1B). Antibodies reacted with antigens

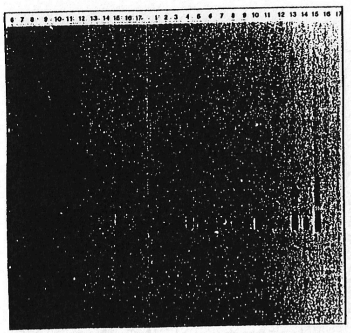

Fig 1A. Serum samples collected 2 weeks after the first immunization.

Pooled serum taken before the first injection from all animals was used as negative control (track 1), and monoclonal anti-OspA antibody H5332 as positive control (track 18). Serum samples from the 4 animals that received placebo are on tracks 2, 6, 11, and 16, respectively; NS1-OspA/Al(OH)$_3$ on tracks 3, 7, 10, and 17; NS1-OspA/MPL/Al(OH)$_3$ on tracks 4, 8, 9, and 12; lipidated OspA/Al(OH)$_3$ on tracks 5, 13, 14, and 15. IgM antibodies are shown on the left-hand panel and IgG antibodies on the right-hand panel. Western blot was done as described in Materials and Methods.

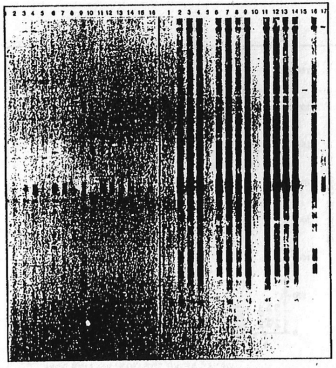

Fig 1B. Serum samples collected 4 weeks after the second immunization.

Monoclonal anti-OspA antibody H5332 was used as positive control (track 17). Serum samples from the 4 animals that received placebo are on tracks 1, 5, 10, and 15, respectively; NS1-OspA/Al(OH)$_3$ on tracks 2, 6, 9, and 16; NS1-OspA/MPL/Al(OH)$_3$ on tracks 3, 7, 8, and 11; lipidated OspA/Al(OH)$_3$ on tracks 4, 12, 13, and 14. IgM antibodies are shown on the left-hand panel and IgG antibodies on the right-hand panel. Western blot was done as described in Materials and Methods.

of molecular weights that were both higher and lower than the 31 kDa B31 OspA. This band is indicated by monoclonal anti-OspA antibody H-5332 (Fig 1B, track 17). The IgM antibody response to OspA contrasted with the IgG response in that it did not evolve the complex banding pattern of the IgG response and appeared to subside 4 weeks after the second injection, as compared with the level observed 2 weeks after this time (not shown). On occasions, serum samples from animals that had received placebo reacted with antigens in the higher molecular weight region (eg, track 15, Fig 1B). Most of this reactivity disappeared when serum samples were used at a dilution of 1:200 (not shown) rather than the 1:50 dilution used in the experiments described above. In contrast, reactivity patterns of serum samples from animals vaccinated with OspA did not change noticeably when used at a dilution of 1:200.

Enzyme-linked immunosorbent assay: Anti-OspA IgG antibody titers were determined by ELISA throughout the vaccination process. Serum samples were titrated on weeks 2, 4, 6, 8, 10, 12, and 21 after the first injection, ie, 2 and 4 weeks after the first injection, 2 and 4 weeks after the second injection, 2 and 4 weeks after the third injection, and 9 weeks PC, respectively. The results, expressed as geometric mean reciprocal titers (GMRT) per "vaccination group" are shown in Fig 2, together with the titer range per group. IgG anti-OspA antibody titers increased as a function of time, and reached remarkably high values, in the range of 1:10^6, regardless of the immunization protocol. By the end of the vaccination procedure, the group that had received the lipidated form of OspA had achieved the highest GMRT of IgG anti-OspA antibody. However, differences between groups were not significant at the 95% level. After the last injection, titers appeared to decline rapidly, for by week 13 after the last injection (9 weeks PC), the GMRT had decreased 14-fold in the NS1-OspA/Al(OH)$_3$ group, 38-fold in the NS1-OspA/MPL/Al(OH)$_3$ group, and 10-fold in the lipidated OspA/Al(OH)$_3$ group.

Antibody-dependent, complement-mediated killing and LA_2 assays: $ADCK_{50}$ titers were determined with serum samples collected on weeks 4, 8, and 10 after the first injection and LA_2 titers only with the latter samples. The results are shown in Table 2. As expected, $ADCK_{50}$ values increased as a function of time during the vaccination process. Both the $ADCK_{50}$ and LA_2 mean values by

TABLE 2

$ADCK_{50}$ AT WEEKS 4, 8, AND 10 AFTER THE FIRST VACCINE DOSE AND LA_2 VALUES AT WEEK 10.

Vaccine Formulation /Animal #	$ADCK_{50}$			LA_2 (µg eq./ml)	$ADCK_{50}$ GMRT	LA_2 GMT
Placebo	Wk 4	Wk 8	Wk 10	Wk 10	Wk10	Wk10
L457	NA	NA	NA	0.3		
L549	NA	NA	NA	0.3		
M021	NA	NA	NA	0.4	NA	0.3
M581	NA	NA	NA	0.2		
NS1 OspA Al(OH)$_3$						
L458	NA	320	1000	49.9		
L594	NA	300	675	122.1		
L971	15	225	750	136.4	854	104
M586	NA	ND	1050	141.1		
NS1 OspA MPL Al(OH)$_3$						
L476	15	1800	2250	115.6		
L712	NA	60	525	97		
L642	NA	750	4200	399.1	2173	172
M106	NA	ND	4500	193.4		
Lipidated OspA Al(OH)$_3$						
M243	18	ND	9000	556		
L537	NA	1800	9000	793		
M219	105	ND	480	115	3475	376
M107	105	ND	3750	396		

NA: not applicable (serum sample does not contain sufficient antibody to promote 50% killing under the conditions in which the ADCK experiment is performed.

ND: Not determined.

week 10 after the first inoculation were significantly higher (95% level) in animals that had received the lipidated form of OspA and Al(OH)$_3$, compared to the group that had received NS1-OspA/Al(OH)$_3$. Other differences between groups were not significant at this time. In selected animals, the $ADCK_{50}$ titer was determined at the time of challenge (week 12 after the first injection) and 9 weeks PC (Table 3). As with the ELISA, a sharp decline in the titer was noted in all animals. Animal L537 (lipidated OspA/Al[OH]$_3$), which had an $ADCK_{50}$ titer of 15 000 at the time of challenge, had a titer of only 960, a 15-fold decrease, 9 weeks later. Animal L712 (NS1-OspA/MPL/Al[OH]$_3$) experienced a 10-fold decrease in titer (from 700 to 70) in the same time interval, and animals L476 (NS1-OspA/ MPL/Al[OH]$_3$) and M585 NS1-OspA/Al[OH]$_3$ a four-fold decrease.

Vaccine immunogenicity at the cellular level

Immunogenicity at the cellular level was investigated by quantifying in vitro the blastogenic responses of PBMC when these cells were stimulated either with recombinant nonlipidated OspA (MDP-OspA) or recombinant lipidated OspA, both from B burgdorferi strain ZS7. The results of a longitudinal study including samples taken at 4, 8, 13, 23, 27, 32, 36, 40, and 44 weeks after the first vaccine dose are summarized in Fig 3. Each time point represents the mean stimulation index (SI) of the four animals in each vaccination group. The error bar represents the standard error of the mean. The SI for each animal is itself the mean value of a triplicate determination. The time of each vaccine injection is indicated on the horizontal axis by each of the first three arrows. The challenge infection occurred 12 weeks after the first injection (fourth arrow in Fig 3). The antigens used for blastogenic stimulation are listed at the bottom of the figure. The horizontal dotted line represents the mean value of the SIs of all animals measured before vaccination, in response to each of the two antigens used, plus 2 times the value of the standard deviation of each mean.

Based on this criterion, the cut-off values were 4.15 and 5.13 for MDP-OspA and lipidated OspA, respectively. PBMCs from the placebo group did not respond to any antigen at any time. Regardless of the vaccination protocol, the response to the nonlipidated (MDP) form of OspA was minimal. In contrast, the response to lipidated OspA was significant in all "vaccine" groups at some time during the vaccination protocol or after the challenge infection. At 15 weeks after the first vaccine dose (3 weeks PC), the mean SI values were 7.7, 17.3, and 37.2 in the NS1-OspA/Al(OH)$_3$, the NS1-OspA/MPL/Al(OH)$_3$, and

lipidated OspA/$Al(OH)_3$ groups, respectively. However, the responses declined to background levels within the first 11 weeks PC (Fig 3).

Interestingly, after this time the mean PBMC response of the animals that had received lipidated OspA increased again, and oscillated around an SI of 16 through the last time point measured. PBMC responses to the T-cell mitogen Con A were also measured for each animal at the same time points, as a positive control for T cell responsiveness. The mean SI values for each vaccine/placebo group and the corresponding SI range are shown in Table 4, for cells obtained 4, 8, 15, and 27 weeks after the first vaccine dose. Throughout this time period (and also thereafter) the PBMCs of all animals continued to show a vigorous response to Con A.

TABLE 3

CHANGE IN $ADCK_{50}$ WITH TIME: $ADCK_{50}$ IN SELECTED ANIMALS AT WEEKS 10 AND 12 AFTER THE FIRST VACCINE DOSE, AND 9 WEEKS POST-CHALLENGE.

Animal #	Vaccine Formulation	$ADCK_{50}$		
		Wk 10	Wk 12	Wk 9 PC
L457	$Al(OH)_3$	NA	NA	20
M021	$Al(OH)_3$	NA	NA	15
L712	NS1-OspA/ MPL/$Al(OH)_3$	525	700	70
L476	NS1-OspA/ MPL/$Al(OH)_3$	2250	2800	720
M585	NS1-OspA/ $Al(OH)_3$	1050	600	135
L537	Lip OspA/ $Al(OH)_3$	9000	15000	960

TABLE 4

MEAN PBMC RESPONSES TO CON A OF ANIMALS THAT RECEIVED THE SAME VACCINE TYPE, AT DIFFERENT TIMES AFTER THE FIRST VACCINE DOSE.

Vaccine Type	Mean SI Range			
	wk 4	wk8	wk15	Wk 27
$Al(OH)_3$ Placebo	520 98 - 1107	568 93 - 1237	409 71 - 982	372 72 - 693
NS1-OspA $Al(OH)_3$	432 281 - 595	617 364 - 1163	609 257 - 1374	366 193 - 506
NS1-OspA MPL/$Al(OH)_3$	451 69 - 382	537 333 - 728	329 74 - 490	199 61 - 365
Lipidated OspA/ $Al(OH)_3$	375 306 - 451	1089 580 - 1439	676 365 - 863	432 327 - 522

DISCUSSION

We investigated the safety and the immunogenicity of three recombinant OspA vaccine formulations in the rhesus monkey. The advantage of performing such a study in an animal model is that it is possible to examine the effects which a challenge or a pre-existing active infection might have on both the safety and the immunogenicity of a vaccination protocol. Postmortem analyses are also possible in animals. The disadvantage, of course, is that in animal models the disease syndrome, the immune response to the infection and to the vaccine, and the interplay between vaccine and infection may differ from that found in humans. With our choice of model we sought to minimize this disadvantage, for both the acute and chronic phases of the Lyme disease syndrome are well mimicked in the rhesus monkey infected with *B burgdorferi*.[12-14] As it turned out, the results of two safety and immunogenicity studies performed in humans[8,9] are comparable to those obtained in the rhesus monkey. In the human studies, vaccine safety was evaluated clinically by recording adverse systemic (fever, headache, malaise, arthralgia) or local (pain, redness, swelling, induration) signs and symptoms observed during the vaccination procedure[8,9] and up to 6 months after the last vaccine dose.[8] In one of the studies, standard urinalyses and hematologic and serum chemistry analyses were performed as well.[9]

Both studies showed no significant adverse effects other than transient local pain and tenderness at the site of inoculation. In our study, no local vaccine site reaction was noted in any animal, before or after the challenge infection (Group 2), or when the vaccine was given to preinfected animals (Group 1). Therefore, all vaccine formulations were locally nonreactogenic in the rhesus monkey, as assessed clinically. Systemically, both control and vaccinated Group 2 animals occasionally exhibited fever,

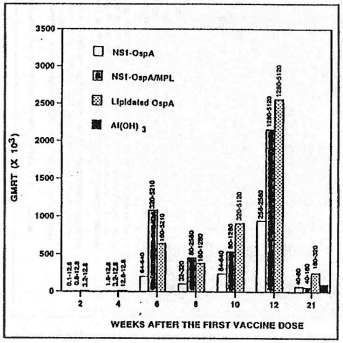

Fig 2. Geometric mean reciprocal antibody titers of serum obtained during and after the vaccination period.

Geometric mean reciprocal titers (GMRT) were determined by ELISA using MDP-OspA as antigen, as described in Materials and Methods. The GMRT range for each vaccination group at each time point is depicted on top of the corresponding histogram bar. Week 21 after the first vaccine dose corresponds to week 9 PC.

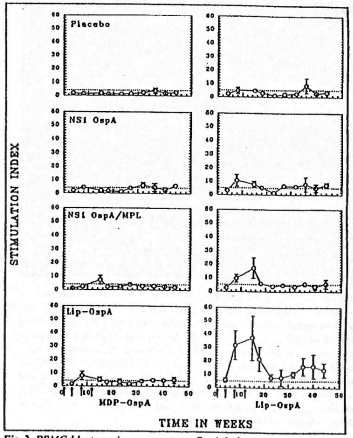

Fig 3. PBMC blastogenic responses to OspA before and after the challenge infection.

Each time point represents the mean stimulation index (SI) of PBMC from the four animals in each vaccination group. The error bar represents the standard error of the mean. The SI for each animal is itself the mean value of a triplicate determination. The time of each vaccine injection is indicated on the horizontal axis by each of the first three arrows. The challenge infection occurred 12 weeks after the first injection (fourth arrow). The antigens used for blastogenic stimulation, non-lipidated OspA (MDP-OspA) and lipidated OspA (L-OspA), are listed at the bottom of the figure. The horizontal dotted line used as cut-off value represents the mean value of the SIs of all animals measured before vaccination, in response to each of the two antigens used, plus two times the value of the standard deviation of each mean.

so this sign cannot be ascribed unequivocally to the vaccine. Clinical signs of arthritis were not seen in any animal at any time. As with humans, no evidence of alterations in medullar or renal functions could be gleaned from the hematologic and urine analyses. Two animals that had received NS1-OspA/$Al(OH)_3$ and NS1-OspA/MPL/$Al(OH)_3$, respectively, showed elevated hepatic enzyme levels, on one occasion before, and on several occasions after the challenge infection. Because changes were transient and mild, their etiology is unlikely to be a toxic effect attributable to the vaccine.

One of the key issues pertaining to vaccine safety is whether an adjuvant such as MPL or a highly immunogenic molecule such as the lipidated form of OspA could promote uveitis.[21,22] The mitogenic and cytokine-producing properties of the lipidated form of OspA have been analyzed recently in vitro using mouse splenocytes and bone marrow-derived macrophages.[23] As little as 5 to 10 ng/ml of lipidated OspA elicited proliferation of splenocytes of naive mice and induced the secretion of interleukin-6, tumor necrosis factor-α, γ-interferon, and nitric oxide in macrophages. In contrast, the nonlipidated form of the molecule was inactive in these assays.[23] Moreover, the lipidated form of OspA, but not the unlipidated homolog, was shown to deliver augmenting costimulatory signals for the activation of T lymphocytes.[24] These results, and the fact that the antibody response produced in mice by the lipidated form of OspA is significantly higher than that induced by the nonlipidated molecule and is directed chiefly to peptide epitopes,[25-27] indicate that the lipid moiety on the OspA molecule acts as a potent adjuvant.[23]

Historically, vaccines containing derivatives of muramyl dipeptide have been implicated in the induction of uveitis and the breakdown of the blood ocular barrier when administered to rabbits.[21] The use of these compounds in vaccine preparations has created concern, especially after the appearance of uveitis in animals that had received muramyl dipeptide derivatives or other adjuvants.[21,22,28] Uveitis production would be a drawback for any candidate vaccine since, once the blood ocular barrier is broken, it may be compromised more easily by subse-

quent nonselective inflammatory events.

Nutrients and metabolites necessary to sustain intraocular structures are supplied by the aqueous humor in the eye. Protection of the delicate nervous tissue is provided by unique tight junctions between the endothelial cells of the ciliary body. This endothelium provides a blood-ocular barrier that normally controls the selective permeability of plasma components and liposoluble substances into the aqueous. If the barrier is damaged by inflammation, protein infiltration increases, raising the concentration of protein and leukocytes in the aqueous humor.[29] Damage to the blood-ocular barrier is usually evaluated subjectively by slit-lamp examination, during which a light beam is narrowed and placed into the center of the pupil to estimate the protein content of the aqueous (flare) and the number of red and white blood cells that appear in the aqueous, or objectively by LFP.

Our evaluations of the animals by slit lamp and LFP indicated that no inflammatory response or permeability change of the blood-ocular barrier was experienced by any animal, either during the vaccination process of preinfected or naive animals, or after the challenge infection. Thus, within the framework of this study, neither the immunomodulator MPL nor the lipidated form of OspA, with its strong adjuvant properties, caused uveitis. Because a similar study was not performed in humans, a comparison is not possible. We expect, however, that in view of the uniformity of our results, uveitis may be either clinically insignificant or may not arise at all.

Our analysis of the antibody response by Western blot indicates that the vaccination protocols employed do not elicit a strong or long-lasting IgM response to OspA. The anti-OspA IgM antibody also did not evolve the complex banding pattern of the IgG response. This banding pattern appearing at molecular masses that are both higher and lower than that of OspA is intriguing, for it is antigen-specific: addition of increasing amounts of soluble recombinant OspA to serum samples prior to their incubation with the nitrocellulose strips, gradually eliminated all the bands by competitive inhibition; the 31 kDa OspA band was the last to disappear (unpublished data). The simultaneous occurrence of SDS-resistant OspA aggregates and OspA degradation products seems unlikely. Alternatively, antibody specificities elicited during the affinity maturation process of the anti-OspA response could bind with low(er) affinity to OspA epitopes shared by other *B burgdorferi* proteins. Although it is reasonable to assume that the lipid moiety is a target for this cross-reactivity, the complex banding pattern was elicited with both lipidated and unlipidated OspA and, moreover, it is very similar to that exhibited by the anti-OspA monoclonal antibody H-5332 used as a positive control. This monoclonal antibody is known to react with a peptide portion of the OspA molecule.[30] A better explanation for its causes notwithstanding, the "OspA" banding pattern is a fact that may complicate the detection of seroconversion to anti-*B burgdorferi*-antibody-positive by Western blot in patients participating in vaccine efficacy trials. In our own study of efficacy of the OspA vaccine in the rhesus monkey (unpublished data) we solved this problem using the competitive inhibition described previously.

In the study in which the same vaccine formulations that were used by us were employed,[9] the highest "functional" (LA_2) anti-OspA antibody titer was achieved with the lipidated form of OspA. Four weeks after the administration of the last vaccine dose, this GMT was about 40-fold the baseline value that is regarded as protective, about two-fold that elicited by NS1-OspA/MPL/$Al(OH)_3$, and six-fold that obtained without MPL. This trend was mimicked in our study regardless of whether functional antibody titers were measured by ADCK or by the LA_2 assay. However, the geometric mean LA_2 titer of functional anti-OspA antibody and also the ELISA titers, were much higher in monkeys than in humans, eg, the LA_2 GMT elicited by the lipidated form of OspA and $Al(OH)_3$ was 86 times higher in monkeys at a comparable time during the vaccination procedure.[9] In this sense then, the antibody response to the OspA vaccine in the rhesus model differs from the human immune response. This divergence is not only of obvious importance when evaluating efficacy of the OspA vaccine in the rhesus monkey but may also be relevant in the present study, for if there were immunopathological signs associated with high titers of anti-OspA antibodies, as has been suggested in view of the correlation found between IgG antibody reactivity to OspA and treatment-resistant arthritis,[31] these signs might be readily apparent in the rhesus model. Our present study shows no such evidence, but a more complete picture will be obtained after joint tissues are examined postmortem.

The bactericidal antibody response to OspA was transient both in humans and in monkeys. In humans, the bactericidal anti-OspA antibody titer was down to baseline values 20 weeks after the last vaccine dose.[8] In monkeys, it had not reached baseline values 13 weeks after the last vaccine dose but it had decreased between four-fold and 15-fold. Because the challenge infection occurred 9 weeks before this time (4 weeks after the last vaccine dose), it follows that the infection could have elicited only a transient booster effect, and probably none at all. The absence of a detectable booster effect is probably due to the mode of action of the OspA vaccine, to the peculiar expression pattern of the OspA molecule, or to both. The absence of a booster effect could have arisen from the annihilation of the spirochetal population within the tick midgut,[32] or the depletion of this population to very low (nonimmunogenic) levels. Furthermore, recent work by de Silva and colleagues[33] (reviewed by Barthold[34]) has demonstrated that OspA is no longer detectable on the spirochetal surface (and probably ceases to be expressed altogether) after the organisms reach the tick salivary glands, immediately before infection. Hence, spirochetes that survived the onslaught of antibody to OspA in the midgut would be unable, all the same, to boost the immune response to this antigen. From a practical standpoint, this entails that it may be necessary to administer OspA vaccines repeatedly on a scheduled basis, so as to maintain bactericidal antibody titers at an effective level.

Like the anti-OspA antibody response, the cellular response to OspA also appeared to be transient. Although blastogenic responses of PBMC were evident by week 8 after the first vaccine dose in all vaccination groups when the cells were stimulated in vitro with lipidated OspA, 4 weeks PC (week 16 after the first vaccine dose) the response appeared to decline, and reached background levels 10 weeks PC. Interestingly, however, the response to lipidated OspA started to climb again by week 32 after the first vaccine dose, and oscillated around an SI of 16 through the last time point measured. Taking into consideration the resurfacing of this response and that T cells within the PBMC compartment continued to respond to Con A throughout the study period, it appears as though the response to lipidated OspA was specifically and temporarily suppressed rather then intrinsically short-lived.

Although speculative, it is possible that a transient *B burgdorferi* infection may have induced the down-regulation of the PBMC response. The subsequent elimination of this putative infection allowed the response to reappear. A tick-induced modulation of the immune response also may have contributed to this phenomenon.[35] Throughout this study, lipidated OspA elicited a stronger blastogenic response than MDP-OspA in all vaccination groups. This was not due to the reported mitogenicity of this molecule,[23] as PBMC from the animals that received placebo did not respond to lipidated OspA and, moreover, stimulation indices were measured after 6 days of incubation with antigen, when mitogenic responses have long been elicited. Rather, it is possible that the reported ability of lipidated OspA to elicit the B7.1 costimulatory pathway[24] may be the cause of this phenomenon.

It was recently reported that T cell lines isolated from PBMC or synovial fluid of human Lyme disease patients with treatment-resistant arthritis recognized OspA significantly more frequently than T cell lines from patients with treatment-responsive Lyme arthritis. This prompted the concern that exposure to *B burgdorferi* after vaccination with OspA, as in our Group 2, or vaccination of a person with an undiagnosed (active) infection, as with Group 1, might induce immunopathologic T cell responses affecting the joints.[36] Postmortem examination of the joints of the animals will allow us to address this concern. Our results thus far do not justify it.

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REFERENCES

1. Steere AC. Lyme disease. *N Engl J Med*. 1989;321:586-596.
2. Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J Clin Microbiol*. 1995; 33:419-427.
3. Rahn DW, Malawista SE. Lyme disease: recommendations for diagnosis and treatment. *Ann Intern Med*. 1991;114:472-481.
4. Philipp MT, Johnson BJB. Animal models of Lyme disease: pathogenesis and immunoprophylaxis. *Trends Microbiol*. 1994;12:431-443.
5. Golde WT, Burkot TR, Piesman J, et al. The Lyme disease vaccine candidate outer surface protein A (OspA) in a formulation compatible with human use protects mice against natural tick transmission with *Borrelia burgdorferi*. *Vaccine*. 1995;13:435-441.
6. Telford SR III, Kantor FS, Lobet Y, et al. Efficacy of human Lyme disease vaccine formulations in a mouse model. *J Infect Dis*. 1995;171:1368-1370.
7. Gern L, Rais O, Capiau C, et al. Immunization of mice by recombinant OspA preparations and protection against *Borrelia burgdorferi* infection induced by *Ixodes ricinus* tick bites. *Immun Lett*. 1994; 39:249-258.
8. Keller D, Koster FT, Marks DH, Hosbach P, Erdile LF, Mays JP. Safety and immunogenicity of a recombinant outer surface protein A Lyme vaccine. *JAMA*. 1994; 271:1764-1768.
9. Van Hoecke C, DeGrave D, Hauser P, Lebacq E. Evaluation of three formulations of a candidate vaccine against Lyme disease in healthy adult volunteers. In: Cevenini R, Sambri V, and La Placa M, eds. *Proceedings of the IV International Congress on Lyme Borreliosis*. Esculapio, Bologna, Italy;1994:123-126.
10. Schoen RT, Meurice F, Brunet CM, et al. Safety and immunogenicity of an outer surface protein A vaccine in subjects with previous Lyme disease. *J Infect Dis*. 1995; 172:1324-1329.
11. Wormser GP. Prospects for a vaccine to prevent Lyme disease in humans. *Clin Infect Dis*. 1995; 21:1267-1274.
12. Philipp MT, Aydintug MK, Bohm RP Jr, et al. Early and early-disseminated phases of Lyme disease in the rhesus monkey: a model for infection in humans. *Infect Immun*. 1993; 61:3047-3059.
13. Roberts, ED, Bohm R, Jr, Cogswell FB, et al. Chronic Lyme disease in the rhesus monkey. *Lab Invest*. 1995;72:146-160.
14. Pachner AR, Delaney E, O'Neill T, Major E. Inoculation of non-human primates with the N40 strain of *Borrelia burgdorferi* leads to a model of Lyme neuroborreliosis faithful to the human disease. *Neurology*. 1995;45:165-172.
15. Piesman J, Mather TN, Telford SR III, Spielman A. Concurrent *Borrelia burgdorferi* and *Babesia microti* infection in nymphal *Ixodes dammini*. *J Clin Microbiol*. 1986;24:446-447.
16. Aydintug M, Gu Y, Philipp M. *Borrelia burgdorferi* antigens

that are targeted by antibody-dependent, complement-mediated killing in the rhesus monkey. *Infect Immun.* 1994;62:4929-4937.

17. Loeb WF. The nonhuman primate. In: Loeb WF and Quimby FW, eds. *The clinical chemistry of laboratory animals.* New York:Pergamon Press; 1989:59-69.

18. Shah SM, Spalton DJ, Smith SE. Measurement of aqueous cells and flare in normal eyes. *Br J Ophthalmol.* 1991;75:349-52.

19. Mermoud A, Pittet N, Herbort CP. Inflammation patterns after laser trabeculoplasty measured with the laser flare meter. *Arch Ophthalmol.* 1992;110:368-370.

20. Moriarty AAP, McHugh DA, Spalton DJ, Fytche JT, Shah MS, Marshall J. Comparison of the anterior chamber inflammatory response to diode and argon laser trabeculoplasty using the laser flare meter. *Ophthalmol.* 1993;100:1263-1267.

21. Waters RV, Terrel TG, Jones GH. Uveitis induction in the rabbit by muramyl dipeptides. *Infect Immun.* 1986;51:816-825.

22. Petty RE, Johnston W, McCormick AQ, Hunt DWC, Rootman J, Rollins DF. Uveitis and arthritis induced by adjuvants: clinical, immunological and histological characteristics. *J Rheumatol.* 1989;16:499-505.

23. Weis JJ, Ma Y, Erdile L. Biological activities of native and recombinant Borrelia-burgdorferi outer surface protein A: dependence on lipid modification. *Infect Immun.* 1994; 62:4632-4636.

24. Simon MM, Nerz G, Kramer MD, Hurtenbach U, Schaible UE, Wallch R. The outer surface lipoprotein A of *Borrelia burgdorferi* provides direct and indirect augmenting/co-stimulatory signals for the activation of CD4+ and CD8+ T cells. *Immun Lett.* 1995;46:137-142.

25. Erdile LF, Brandt MA, Warakomski DJ, et al. Role of the attached lipid in immunogenicity of *Borrelia burgdorferi* OspA. *Infect Immun.* 1993; 61:81-90.

26. Simon MM, Schaible UE, Kramer MD, et al. Recombinant outer surface protein A from *Borrelia burgdorferi* induces antibodies protective against spirochetal infection in mice. *J Infect Dis.* 1991;164:123-132.

27. Fikrig E, Barthold SW, Kantor FS, Flavell RA. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. *Science.* 1990; 250:553-556.

28. Allison AC, Briars ME. Immunological adjuvants: desirable properties and side-effects. *Mol Immunol.* 1991;28:279-284.

29. Cousins SW, Guss RB, Howes EL, Rosenbaum JT. Endotoxin-induced uveitis in the rat: observations on altered vascular permeability, clinical findings and histology. *Exp Eye Res.* 1984;39:665-676.

30. Shanafelt MC, Anzola J, Soderberg C, Yssel H, Turck CW, Peltz G. Epitopes on the outer surface protein A of *Borrelia burgdorferi* recognized by antibodies and T cells of patients with Lyme disease. *J Immunol.* 1992;148:218-224.

31. Kalish RA, Leong JL, Steere AC. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity to OspA and OspB of *Borrelia burgdorferi*. *Infect Immun.* 1993;61:2774-2779.

32. Fikrig E, Telford SR III, Barthold SW, Kantor FS, Spielman A, Flavell RA. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. *Proc Natl Acad Sci USA.* 1992;89:5418-5421.

33. de Silva A, Telford SR III, Brunet LR, Barthold SW, Fikrig E. *Borrelia burgdorferi* OspA arthropod-specific Lyme disease vaccine. *J Exp Med.* 1996;183:271-275.

34. Barthold SW. Lyme Borreliosis in the Laboratory Mouse. *Journal of Spirochetal and Tick-borne Diseases.* 1996;3:22-44.

35. Wikel SK, Ramachandra RN, Bergman DK. Tick-induced modulation of the host immune response. *Int J Parasitol.* 1994;24:59-66.

36. Lengl-Janssen B, Strauss AF, Steere AC, Kamradt T. The T helper cell response in Lyme arthritis: differential recognition of Borrelia burgdorferi outer surface protein A in patients with treatment-resistant or treatment-responsive Lyme arthritis. *J Exp Med.* 1994;180:2069-2078.