A. Sadziene, A. G. Barbour

Experimental Immunization against Lyme Borreliosis with Recombinant Osp Proteins: An Overview

Summary: Interest in human and veterinary vaccines against Lyme borreliosis is growing. Both whole cell immunization and subunit vaccines can protect against infection with *Borrelia burgdorferi*. For development of a human vaccine the focus has been on a subunit vaccine. The most promising candidate is OspA, a major outer membrane lipoprotein of *B. burgdorferi sensu lato*. Of Osp proteins A through D, OspA shows the least variability between strains in its sequence and in the level of its expression. Borreliae in ticks express OspA. Antibodies to OspA kill borreliae *in vitro* and provide passive protection in mice. Active immunization of mice with OspA provides protection against challenge by syringe inoculation or tick bite. The lipid moiety of the OspA is necessary for immunogenicity in the absence of a potent adjuvant. A recombinant OspA-based vaccine is already in clinical trials. Although there is compelling evidence that immunization with OspA will provide protection, questions remain regarding the duration of protection from such immunization, the necessity to have a minimum level of neutralizing antibodies at all times for protection, and the relationship of an immune response to OspA and autoimmune features of Lyme borreliosis. The experimental aspects of immunization with Osp-A based constructs and other Lyme vaccine candidates are reviewed and discussed.

Lyme Borreliosis Vaccine: Is It Justifiable?

Lyme borreliosis, the multisystem illness caused by the tick-borne spirochete *Borrelia burgdorferi sensu lato*, has emerged as a threat to public health worldwide. It is now the most common arthropod-borne disease in the United States as well as in Europe. Lyme borreliosis probably ranks only behind acquired immunodeficiency syndrome in media coverage of infectious diseases in the United States in the last decade. Unfortunately, an accurate estimation of its importance to human and animal health has not been made because of difficulties in diagnosis and inadequate surveillance activities [1]. The full extent to which people are being inappropriately treated with antibiotics cannot be assessed, but it is likely that a large portion of health-care dollars used for the therapy of Lyme borreliosis is paying for misdiagnosis of *Borrelia burgdorferi* infection [2, 3]. Some of these resources would probably better benefit the community if directed towards methods of disease prevention. Strategies for prevention of Lyme disease include vector control and vaccines.

Until recently, vaccination against Lyme borreliosis was considered neither a realistic nor profitable prevention strategy. Lyme borreliosis does not meet traditional public health criteria to warrant protecting the community at large. The disease is usually easily treatable with antibiotics and is not spread from person to person. Moreover, human and animal studies indicate that pathologic changes in Lyme borreliosis are determined in part by the host's immune response [4–9]. More has to be learned about disease pathogenesis and immunity to more accurately predict the risk of provoking disease through vaccination. Lyme borreliosis is rarely fatal, and serious untoward reactions from the vaccine would hardly be tolerated.

Despite these discouraging reasons, public interest in human and veterinary vaccines has increased considerably. Demand for preventive measures other than behavior modification and tick avoidance has prompted efforts to develop human and veterinary vaccines for Lyme borreliosis. There is some justification for this effort. The morbidity from *B. burgdorferi* infection in highly endemic areas is considerable and can reach 10% of the population [10]. Many of these patients have a mild form of infection. However, some of them might develop a disabling form of disease involving joints and nervous system, and would not substantially improve even after parenteral antibiotic therapy [11–18]. Although doubts remain about the many diagnoses of chronic Lyme borreliosis, the large number of patients with unrelieved disabilities prompts further consideration of a *B. burgdorferi* vaccine for high-risk populations, such as outdoor workers, residents of endemic areas, pregnant women in particular, campers and hikers. Family dogs in some endemic areas have been hit by a near epidemic of lameness caused by Lyme arthritis [19]. In some hyperendemic regions of New York and New England, Lyme borreliosis is now such a threat that it interferes with all sorts of outdoor activities and has led to a depreciation of real estate values [19]. Hence, the interest in human and veterinary vaccines is growing.

Ariadna Sadziene, M. D., Sc. D., *A. G. Barbour*, M. D., Dept. of Microbiology and Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7758, USA.

Correspondence to: Dr. *A. G. Barbour*.

New address Dr. *Ariadna Sadziene*: Dept. of Medicine, House Staff, UMDNJ-New Jersey Medical School, 1855 Orange Ave., Newark, NJ07103, USA.

Table 1: Osp protein candidates for Lyme borreliosis vaccines.

Immunogen	MW (kDa)	Location	Comments
OspA	31–33	49 kb linear plasmid	Protective in rabbits, mice, hamsters against the same species. Immunogenic in humans. The least variability between strains. In clinical field trials.
OspB	34–36	49 kb linear plasmid	Protective in mice against the same strain. Possibility of truncation. High variability between strains.
OspC	20–23	27 kb circular plasmid	Protective in gerbils. Highest variability between strains (similar to Vmps of relapsing fever Borrelia)
OspD	28	38 kb linear plasmid	Not comprehensively evaluated
OspE	19.2	? 45 kb plasmid	Not proven for protection
OspF	26.1	? 45 kb plasmid	Partial destruction in ticks
Whole cell			Vaccine available for dogs. Efficacy not proven in field trials. Safety issues for humans.

Lyme Borreliosis Vaccine: Is It Feasible?

Whole Cell Vaccines

There is evidence from experimental studies in animals that a protective vaccine can be developed. Research has demonstrated unequivocally that actively or passively acquired antibodies against whole cell immunization, as well as subunit vaccines, can protect against infection with B. burgdorferi.

Passive and active protection against homologous challenge was obtained in hamsters immunized with killed B. burgdorferi cells [20, 21]. The theoretical advantage of a whole cell vaccine is the more assured presentation of immunogens in their native conformations. It is also possible that single recombinant protein based vaccines will not be efficacious in practice and may need to be supplemented by one or more other components. This could be achieved by adding more recombinant products to the formulation, but this increases the cost of the vaccine. The same end may be achieved by using the whole organism expressing these components. Commercially developed chemically inactivated whole cell "bacterin" is already available for dogs [22]. The vaccine is administered intramuscularly twice, at an interval of 2–3 weeks, with an annual booster dose. This vaccine provided evidence of protection when examined under experimental conditions [23] as well as in field trials [22], however, the latter study was not well controlled. The duration of protection obtained with this vaccine still needs to be clarified. Experimental studies indicated that the vaccine may have to be administered yearly to sustain antibody levels [Jacobson, unpublished studies].

Vaccination with "bacterin" was found to be safe regardless of previous exposure to B. burgdorferi or history of borreliosis. The possibility of a live attenuated vaccine for animals is under investigation. In a recently performed study in mice, the dose protecting 50% of mice was 15 times lower for live cells than for heat-killed cells [Sadziene et al., submitted].

Subunit Vaccines: OspA

For a human vaccine the focus, however, has been on subunit recombinant vaccines, primarily because of safety concerns about whole cell preparations. The most promising spirochete component for this purpose is OspA, a surface-exposed, abundant lipoprotein of B. burgdorferi (Table 1). It has been shown that mouse and human antibodies to OspA kill borreliae in vitro in the absence of complement [24–26, Sadziene, unpublished]. Clear damage to the outer membrane of borreliae was observed when the morphologic effects of sera from volunteers vaccinated with Osp A were examined by electron microscopy [Sadziene, unpublished]. The majority of borreliae exposed to vaccinee sera had large membrane blebs similar to those which were observed with bactericidal Fab fragments [27]. Mice passively immunized with monoclonal or polyclonal antibodies to OspA also have been shown to be protected against experimental B. burgdorferi infection [28–31, Sadziene, unpublished].

The most work, however, was done on an active OspA-based immunization [25, 26, 31–35]. The ability of recombinant OspA to induce an immune response against B. burgdorferi was first demonstrated with rabbits and subse-

quently with mice and hamsters [31, 35–37]. Several OspA constructs and different routes of immunization have been proposed. Both native enriched OspA and recombinant OspA proved to be effective for experimental protection [31, 35]. Protective immunity was elicited by recombinant bacille Calmette-Guérin (BCG) expressing OspA as a membrane associated lipoprotein. Intraperitoneal delivery of this construct resulted in high protective antibody responses observed in heterogeneous mouse strains that vary in their immune responsiveness to OspA [25]. A single intranasal vaccination with recombinant BCG expressing OspA resulted in a prolonged (more than 1 year) protective systemic IgG response and a highly sustained secretory IgA response [38]. Mice orally vaccinated with *Escherichia coli* expressing OspA or attenuated *Salmonella typhimurium* expressing OspA developed high titers of anti-OspA antibodies and were protected against intradermal challenge with spirochetes [39, 40].

Still, the main construct explored by investigators is recombinant OspA protein produced by *Escherichia coli* [26, 32–35, 41]. The difficulties inherent in extracting and purifying lipoproteins have led to the use of genetic engineering to produce a non-lipoprotein version of OspA by expression of a truncated gene lacking the lipopeptide signal sequence [42]. However, active immunization studies have shown that non-lipidated recombinant OspA needs a potent adjuvant, such as Freund's adjuvant or ABM3, to elicit a good immune response, and so would not be practical for human vaccines [26, 28, 31, 32]. Priming and boosting with OspA lipoprotein either without adjuvant or adsorbed to alum, elicited a strong, dose-dependent IgG response. The ability of the purified recombinant lipoprotein to induce a strong protective response in the absence of toxic adjuvants makes it an excellent candidate antigen for a human vaccine against Lyme disease. This particular construct of an OspA vaccine for humans is now in clinical trials in the United States [42].

Efficacy tests of vaccines based on OspA as an immunogen have generally involved needle inoculation of cultured spirochetes as the challenge regimen. Several studies, however, pointed out that the immune responses to needle inoculation and tick transmission of spirochetes differ [43, 44]. It was shown that the antibody response to OspA and another outer surface lipoprotein, OspB, after natural infection in experimental animals is similar to that seen in *B. burgdorferi* infection in humans. In humans, antibody to OspA and OspB is not detectable until late in infection [45, 46]. In contrast, the strong early antibody response to these surface proteins is elicited in experimental animals given a large inoculum by syringe [43–47]. The reason for the delayed or absent immune response to OspA and OspB in patients and in experimental animals infected or immunized with small inocula of *B. burgdorferi* is unknown.

It is possible that borreliae can regulate the presentation of these proteins so that they appear to be different from culture-grown antigens. The latter presumption is sup-

ported by recent data reported by *Schwan* et al. [48]. Adaptation of borreliae to tick and mammalian environments probably involves very different surface components so as to insure their transmission and survival in two very different hosts. The presented theory suggests that two environmental cues, an increase in temperature and tick feeding, trigger a major alteration of the spirochetes' outer membrane when adapting to the new mammalian environment. It has previously been shown that *B. burgdorferi* produces OspA and probably OspB in the midgut of *Ixodes* ticks that have not yet engorged on blood [49]. Yet another surface protein, OspC, stimulates an early antibody response in humans [50, 51]. In unfed *Ixodes scapularis* ticks, spirochetes with OspA, but not OspC, on their surface are primarily restricted to the tick's midgut [52]. Although the spirochetes may be very abundant in the midgut of unfed ticks, they fail to cause infections when experimentally inoculated in rodents [53]. While the tick is feeding, the spirochetes multiply, disseminate through the midgut wall to the hemolymph, invade the salivary glands, and are transmitted to mammals via tick saliva [54, 55]. During this time borreliae under the influence of the occurring environmental changes up-regulate OspC expression and, very likely, down-regulate OspA expression, consequently presenting to the new host OspC, but little or no OspA antigen. This theory would correspond well to yet another observation indicating much higher OspC heterogeneity in comparison to OspA lipoprotein. It is quite reasonable to assume that the more accessible to the host immune system antigen is the more heterogeneous it might be.

In the light of this theory, the experimental tick transmission model becomes significant to assess whether an OspA-based vaccine will be effective against natural *B. burgdorferi* infection. Several groups of investigators have already demonstrated that immunization with recombination OspA protein is efficient in protecting mice from tick-delivered spirochetes [31, 33, 34]. It is possible that, at least in part, the protection by anti-OspA antibodies occurs prior to the entry of the pathogen into the feeding site within the skin of the host. Borreliae appear to be particularly vulnerable to destruction by antibody-mediated mechanisms within the gut prior to dissemination, because ticks concentrate the products of their blood meal. This hypothesis is supported by observations showing that antibodies to OspA eliminate spirochetes in ticks feeding on OspA-immunized mice [32, 33].

Although heterogeneity in OspA has been reported by a number of investigators, the homology of the OspA antigen between borreliae of the same genospecies is higher than that of two other surface lipoproteins, OspB and OspC, which have been investigated as vaccine candidates [29, 50, 56–61]. OspA also shows less variability between strains in its level of expression *in vitro* [59]. There was found to be a strong association of certain OspA serotypes with distinct *B. burgdorferi sensu lato* genospecies. *B. burgdorferi sensu stricto* strains belong to one OspA serotype: *Borrelia afzelii* strains are clustered into another se-

rotype and only *Borrelia garinii* strains expose marked OspA heterogeneity and can be grouped into five serotypes [59]. A similar classification system was proposed by *Schaible* et al. [29]. According to this classification, *B. burgdorferi* isolates can be divided into at least six subgroups based on their distinct OspA/OspB genotypes. These observations play an important role in OspA-based vaccine development, suggesting that European and American vaccine formulations should probably be different. This has been supported by the experimental data showing the OspA-derived protection against strains of the corresponding OspA genotype but not against isolates of a different OspA genotype [29, 33, *Sadziene*, unpublished]. Another study provided evidence that antigenically variable *B. burgdorferi sensu stricto* serotypes delivered by naturally infected ticks fail to infect mice actively immunized with single recombinant OspA protein of one *B. burgdorferi sensu strictu* serotype [34].

Despite all the controversies regarding OspA-based protection, an OspA vaccine for humans is already in clinical trials in the United States [42]. In the study performed in 36 adult volunteers, two or three doses of OspA Lyme vaccine proved to be safe and immunogenic. Although antibodies to OspA develop late in the course of Lyme infection and only in a subset of patients, results of the performed study indicated that immunized humans were able to mount a strong IgG response to OspA protein. A large multicenter study to determine the optimal dosage, extend the safety profile and evaluate the efficacy of this recombinant OspA vaccine is now in progress.

Subunit Vaccines: Other Candidates

Although the OspA antigen is considered the most promising Lyme vaccine candidate, it is still not known whether this construct will provide sufficient and reasonably long protection. Investigators anticipate the duration of Lyme vaccine-conferred protection to be at least for one season. It is possible that for an effective vaccine OspA may need to be supplemented by one or more other components [37]. Recent findings suggest that antigens other than OspA may also provide protective immunity in experimental animals [30, 62]. Other antigens known to do this are the outer membrane lipoproteins OspB and OspC (Table 1), [63, 64].

Similarly to OspA, active immunization with an OspB fusion protein (Table 1) protected mice from experimental infection with *B. burgdorferi*. The difference, however, was the dose of spirochete challenge used to obtain full protection. The active protection with OspA could be overcome with a challenge of 10^7 borreliae, while OspB was insufficient to fully protect against 10^4 spirochetes. It has been shown that antigenic variation in OspB occurs spontaneously in subclones derived from cloned strains [65]. *In vitro* data suggested that truncation or modification of OspB protein might be the means by which borreliae escape OspB-based immunity [66, 67]. Experiments in

mice clearly indicate that the influence of OspB immunity is related to the ability of spirochetes with truncated OspB to survive preferentially within the host [68, 69]. Another doubt about OspB-based vaccination is the higher degree of heterogeneity of this protein among different *B. burgdorferi* isolates in comparison with OspA protein [63]. There are experimental data suggesting that OspB protection might be somewhat strain-specific [43].

About 40% of European and some American *B. burgdorferi* strains express an abundant amount of a 20–23 kDa protein OspC (Table 1). In contrast to OspA, OspC is an immunodominant protein in the early immune response in Lyme borreliosis patients. There is a negative correlation between the amount of OspC, on the one hand, and OspA and OspB, on the other, expressed by the individual strain [57]. It was demonstrated that vaccination with recombinant OspC, but not OspA, protected gerbils from experimental challenge with a *B. burgdorferi* clone abundantly expressing OspC but only a little OspA [64]. The drawback of OspC-based vaccination is the very high variability of this surface protein [58]. The similarity of OspC to the variable major proteins of relapsing fever *Borrelia* has been proposed [70]. It is important, however, to consider OspC, most likely in combination with OspA, as a future vaccine candidate in at least some European regions.

Two other proteins, OspE and OspF have been described and examined for their ability to elicit protective immunity against Lyme disease (Table 1), [71, 72]. Although it was reported that OspF-immunized mice were partially protected from both intradermal syringe challenge and tick-mediated transmission of *B. burgdorferi*, experimental differences were not statistically significant [72]. Vaccination with OspE did not confer protective immunity. *B. burgdorferi* organisms were, however, partially destroyed within ticks that engorged on either OspE- or OspF-immunized mice [72].

Several other outer surface structures, such as OspD, p10, p13 and 66 kDa protein have been reported [73–77]. It was shown that a monoclonal antibody derived against one of these proteins, p13, is bactericidal for Osp-lacking borreliae *in vitro* [76]. Although no data are yet available to show whether these proteins have a role in protective immunity against *B. burgdorferi in vivo*, the alternative of including these constructs into a combined Lyme vaccine cannot be denied.

Flagellar protein was also evaluated for its ability to evoke protective immunity [63]. In *B. burgdorferi* the flagellae appear to consist primarily of this single protein. The flagellar apparatus is a motility mechanism in spirochetes as in other bacteria. It was reasonable to question whether antibodies towards flagellin would destroy borreliae or, at least, affect their virulence. Antibodies towards this antigen appear early in both human and animal infections. However, in spirochetes the flagellum is located not at the cell surface, as it is in other bacteria, but instead is entirely periplasmic in location and oriented along the long axis of the cell [78]. This may explain why active immunization

with recombinant flagellin protein appeared to have no protective effect against experimental *B. burgdorferi* infection in mice [63].

Lyme Disease Vaccine: Further Questions

Despite all the work that has been performed on the development of a Lyme disease vaccine, certain important issues have not yet been clarified.

One of the main questions is how to evaluate vaccine efficacy in humans. Data obtained by different investigators indicate that matrix-based assays, such as ELISA and Western blot might not be sufficient to assess the level of protective antibodies [24, 79, 80, *Sadziene* et al., submitted]. These assays by their nature cannot be used to directly predict the function of antibodies. For this purpose, an assay that measures the ability of antibodies to agglutinate cells, to prevent growth inhibition *in vitro*, to lyse or opsonize cells, or to prevent or ameliorate infection is needed. Several groups of investigators have proposed their own alternative functional tests [24, 79–81]. Recent data suggest that measuring growth inhibition *in vitro* is a much more accurate predictor of protection than matrix-based assays [*Sadziene* et al., submitted]. The duration of active immunity is yet unknown, as is the extent of crossprotection between strains, the possibility of generations of escape mutants and the minimum levels of protecting antibodies needed. If a reliable functional *in vitro* test can be developed, it may be used alone or in combination with animal models to address all these questions.

In humans, the mechanism of immunity remains a bit of a puzzle. The argument for a prominent protective role for circulating antibody is confounded by the fact that the antibody response to *B. burgdorferi* in patients actually widens as infection progresses [3]. Patients with chronic arthritis usually have anti-OspA and anti-OspB responses and may first appear with onset or relapse of arthritis [4]. Antibody-mediated immunity seems to be failing to control chronic Lyme infection; this corresponds with experimental data in the hamster model, when only antibody administered or induced before infection could confer protection [83]. The protective role of cell-mediated immunity is unclear. Although a heightened T-cell blastogenic response to *B. burgdorferi* antigens is found in patients with Lyme disease, such a response also frequently occurs in healthy controls [82]. Whether a T-helper response exists towards OspA antigen is not clear. An involvement of specific T cells in determining the outcome of the disease is suggested by the finding that in *B. burgdorferi* infected mice and humans the outcome is genetically linked to the major histocompatibility locus (MHC), a region which controls immune responsiveness of T cells [9, 12]. It was recently reported that depletion of CD4+ T cells increased the severity of arthritis and the number of spirochetes in different strains of mice [83]. In contrast, the CD8+ T-cell compartment, particularly in mice of the susceptible H2 haplotype, appeared to promote the disease process, as abrogation of this T subset *in vivo* led to a reduction in both arthritis and in spirochete levels [83]. Further studies of T-cell populations are needed to clarify how each of them contributes to the immunity towards *B. burgdorferi* and to identify B and T-cell epitopes necessary for an effective subunit vaccine. The degree of natural protection induced by one infection against the subsequent one is also a subject for debate.

Finally, and most importantly, several safety issues must be resolved with regard to human vaccination. Genetically determined variations in the immune response to the spirochete may cause chronic arthritis and resistance to antibiotic therapy. It was suggested that HLA-DR4 and HLA-DR2 might bind to Osp proteins and initiate the autoimmune response in the joint [84]. Therefore, the immune response to recombinant OspA may complicate efforts directed towards development of a human vaccine using this lipoprotein. Vaccine safety is also highlighted further by the reports of molecular mimicry where antibodies to the spirochete crossreact with nerve axons [85, 86], joint synovia [86, 87], heart [87] and skeletal muscles [86]. There are also some theoretical concerns about Lyme vaccination in general. Waning immunity after vaccination could theoretically leave behind a residually sensitized individual prone to develop a more severe reaction in the event of reinfection or revaccination. Another concern would be whether vaccine-based immunity will protect against the majority of invading borreliae but not all. In such cases, erythema migrans might not occur, vaccinated individuals would easily avoid treatable early Lyme disease, but still develop more severe and less easily treatable late Lyme manifestations.

Acknowledgement

Zusammenfassung: Experimentelle Immunisierung gegen Lyme Borreliose mit rekombinantem OspA und anderen Komponenten: Übersicht. Die Entwicklung humaner und veterinärmedizinischer Impfstoffe gegen die Lyme Borreliose gewinnt zunehmend an Interesse. Ein Infektionsschutz gegen *Borrelia burgdorferi* kann sowohl durch eine Ganzzell- wie durch Subunit-Vakzinen erreicht werden. Bei der Entwicklung einer humanen Vakzine wurde das Hauptgewicht auf Subunit-Vakzine gelegt. Am vielversprechendsten ist OspA, ein wichtiges Lipoprotein der äußeren Membran von *B. burgdorferi sensu lato*. OspA weist in seiner Aminosäuresequenz und dem Expressionsgrad unter der Reihe von Osp-Proteinen von A–D die geringste Stamm-Variabilität auf. In Zecken lebende Borrelien exprimieren OspA. Antikörper gegen OspA töten Borrelien *in vitro* ab und vermitteln bei Mäusen einen passiven Schutz. Die aktive Immunisierung von Mäusen mit OspA bietet gegen die Inokulation durch Injektion oder durch Zeckenstich einen Schutz. Die Lipid-Verbindung von OspA ist für deren Immunogenität erforderlich, wenn nicht ein potentes Adjuvans eingesetzt wird. Eine rekombinante OspA-Vakzine befindet sich bereits in klinischer Prüfung. Obwohl schlüssige Beweise dafür vorliegen, daß die Immunisierung mit OspA protektiv ist, bleiben Fragen zur Dauer des Schutzes durch eine solche Immunisierung, die Notwendigkeit eines anhaltenden Minimalspiegels neutralisierender Antikörper für den Schutz und die Beziehung zwischen Immunantwort gegen OspA und die Autoimmun-Komponente der Lyme Borreliose zu klären. Die experimentellen Aspekte der Immunisierung mit Vakzine-Konstrukten auf der Basis von OspA und anderen Kandidaten von Lyme Impfstoffen werden in einer Übersicht dargestellt und diskutiert.

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