Limitations of the OspA Vaccine for Humans: A Review*

Denise M. Foley*, PhD; David R. Blanco†, PhD; Michael A. Lovett†‡, MD, PhD; James N. Miller†, PhD

INTRODUCTION

Presently, the OspA vaccine is the most developed and defined candidate for generating protection against Lyme disease. As has been reported in the literature, significant protection has been achieved for several animal models including mice,[1-9] dogs,[10] rabbits,[11] and monkeys.[12] An important issue for any vaccine is that it not only be effective, but also safe. In this regard, vaccination studies in monkeys[13] and humans[14-16] have indeed shown that the OspA vaccine is safe with only minor reactions being reported in a small percentage of persons. More importantly, both the Pasteur Merieux Connaught and SmithKline Beecham Laboratories have now reported on phase III human vaccine trials where it was demonstrated that OspA provides significant protection against Lyme disease.[17,18]

While these studies indicate that OspA is a very promising vaccine against Lyme disease, there are limitations based upon experimental studies in animals that may have important implications for humans. The purpose of this paper is to discuss the recombinant OspA vaccine and, in particular, raise issues regarding its limitations for humans by reviewing data obtained from published studies. Many investigators have described their rationale for these limitations that tend to support the necessity for utilizing other protective immunogens in concert with OspA in a "cocktail" vaccine. These concerns will be included in the context of this review.

OspA HETEROGENEITY

While it has been observed that OspA varies greatly or is completely absent in European isolates, it was hoped that OspA vaccination would provide protection for vaccinated persons in North America where there is less OspA heterogeneity.[19] Recently, however, a growing concern about diversity among North American isolates has been noted.[20-31] The ability of some OspA serotypes to avoid killing with antibodies raised against other serotypes has been shown.[24,31] In the study by Lovrich et al,[27] the authors concluded that although cross protection occurred against some strains expressing different antigenic types of OspA, vaccination with a single OspA type did not provide complete protection against challenge with all strains. Even more surprising was the finding that the presence of anti-OspA antibodies elicited from some isolates did not result in protection against challenge with the homologous strain.

The current OspA vaccine utilizes a *Borrelia burgdorferi* sensu stricto OspA molecule which, to date, has been found in the majority of the isolates from North America.[19,26] However, one type of North American OspA variant, typified by strain 25015. has been shown to infect mice vaccinated with N40 OspA,[21] a molecule similar to the current OspA vaccinogen. This variant type, isolated from upstate New York, has also been isolated from Illinois,[32] (presented by Maria Picken, 11th Annual Scientific Conference on Lyme Borreliosis, New York, April 25-27, 1998). It is therefore possible that, individuals infected with this variant strain may not be protected with the current vaccine. Furthermore, the probability of discovering other variants against which the vaccine will fail is high given the propensity for the organism to undergo mutational and recombinational events at the OspA locus,[33-38] and the discovery of variant *Borrelia* strains in locations such as California,[25,39] New York,[30,40] Texas,[40] Missouri,[40,41] Illinois,[32] Georgia, and Florida,[42-44] which have yet to be tested in vaccination protocols.

*From the *Department of Biomolecular Science, Chapman University Division of Natural Sciences, Orange, CA; and the †Department of Microbiology and Immunology; and the ‡Division of Infectious Diseases, UCLA School of Medicine, Los Angeles.*

Address correspondence to: Denise M. Foley, PhD, Divison of Natural Science, Chapman University, 333 North Glassell, Orange, CA 92866.

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While the greatest variation of the OspA molecule occurs in European isolates, the increasing evidence of OspA variability in North America, together with the observation that cross protection is not always achieved with OspA vaccination, implies that even a vaccine that includes several serotypes of OspA molecules will not result in complete protection of the vaccinated North America population.

OspA downregulation in the vertebrate and host adaptation

Another key issue to be considered when using OspA as the sole vaccinogen is the widely accepted fact that OspA is not expressed during vertebrate infection. The studies by Schwan et al[45] and de Silva et al[46] demonstrate that OspA is present on *B burgdorferi* before tick feeding but is lost after initiation of the bloodmeal. Furthermore, in the study by Schwan et al, it was demonstrated that OspC, a more heterogeneous molecule than OspA, is unregulated after tick feeding. They suggest this downregulation of OspA and corresponding upregulation of OspC is crucial for the ability of the organism to infect the vertebrate host.

Further evidence that OspA is not expressed in the vertebrate host can be gathered from studies in which animals inoculated with low numbers of *B burgdorferi*, whether it be from needle injection or tick transmission, do not develop antibodies to OspA in spite of developing an antibody response to other *B burgdorferi* antigens.[45,47-51] It has also been observed that many Lyme borreliosis patients either do not produce antibodies that react with OspA or produce relatively low OspA antibody titers.[52-57] In a study by Schutzer et al,[58] 12 of 16 early Lyme disease patients with neurological involvement were found to have cerebrospinal fluid (CSF) and serum IgM directed against OspC and 5 of these 12 also had IgM to OspA that was restricted to the CSF. These data suggest that in certain neurological Lyme disease patients, OspA may be selectively expressed in the central nervous system (CNS) and not in the peripheral blood or skin. When considering the abundance of the OspA protein in ex vivo cultured organisms and the evidence for its downregulation during tick feeding, the absence of a universal serum antibody response to OspA in humans would seem to support the theory that the majority of spirochetes will not initially express this protein in the infected human. If some organisms do revert to expression of OspA once they reach the CNS or other specific site, this may explain why some patients develop a response to this protein. It is assumed that vaccinated persons will destroy these organisms if the appropriate OspA serotype is being expressed. However, the population of organisms within the OspA-vaccinated host, which continue to keep OspA downregulated, would remain unaffected.

The studies of Barthold et al[59] provide additional evidence that OspA is not expressed by *B burgdorferi* while in the host. These data are particularly convincing because they demonstrate that OspA vaccinated mice exposed to organisms taken directly from a vertebrate host via a skin transplant from an infected syngeneic mouse are susceptible to infection. These studies were extended by de Silva et al[60] who demonstrated that mice passively administered immune mouse serum were still susceptible to infection by *B burgdorferi* following homologous challenge with infected mouse skin or by tick bite. All mice became infected despite being administered immune sera over the course of 14 days. These investigators concluded that the organisms may be resistant to immune serum antibodies through a mechanism of "host adaptation" that results in immune evasion. The ability of these organisms to evade host immune defenses is clear from the course of natural infection where the establishment of chronic infection and late debilitating manifestations is a common feature. This is also illustrated by the fact that in spite of high levels of borreliacidal antibodies present in humans during stages of Lyme disease,[61-63] these patients remain infected. The fact that immune sera administered after challenge does not abort the infection, which is in contrast to the ability of this same sera to prevent infection if administered before challenge,[59] is compelling evidence that the organism quickly "adapts" once inside the host. This adaptation allows the organism to persist in the presence of what would otherwise be an effective immune response. Although these authors concluded in the same study that OspA vaccination was completely protective, it is also pertinent to consider the possibility of host adaptation and immune evasion when examining OspA vaccination.

It has been shown that infected ticks feeding upon an OspA-vaccinated host results in the destruction of the majority of spirochetes in the tick. However, it has also been shown that some spirochetes survive within some ticks after a bloodmeal containing OspA antibodies.[6,9,12,46] Presumably, these organisms were not expressing OspA or expressed an OspA variant resistant to the killing antibodies present in the bloodmeal. A relevant question is, what is the disposition of the few spirochetes which do survive in the engorged ticks after feeding upon an OspA-vaccinated host? One would predict that these organisms, for perhaps a significant period of time, do not express OspA in response to the downregulating effects of the bloodmeal. Therefore, do these organisms represent a real or merely a theoretical danger to the individual vaccinated with only OspA? If these organisms gain entry to the vaccinated human host, their ability to quickly adapt and resist the anti-OspA immune response creates a

potentially dangerous scenario. Transmission in presumably very low numbers might establish an undetected asymptomatic infection which later exacerbates as debilitating chronic manifestations of late stage Lyme disease.

THE SIGNIFICANCE OF LATENCY

The issue of the potential development of a latent infection in a previously vaccinated individual or animal has not been vigorously investigated. This is particularly pertinent in view of the recognized capacity for spirochetal pathogens, including *B burgdorferi,* to cause latent infection.[64] Others have recognized this gap in the literature as evidenced by this statement:

> "...it is surprising that so little attention has been paid to the question of asymptomatic infection (as manifested by seroconversion) in experimental test systems of vaccine candidates, given the presumption that latency may occur in human *B burgdorferi* infections." GP Wormser. *Infection* 1996;24:203.

In a published human vaccine trial with OspA plus adjuvant administered to individuals residing in endemic areas of the US, Steere et al[17] reported a vaccine efficacy of 76% (16 confirmed cases) after 3 doses of immunogen. Although no silent seroconverters were found in these vaccinees, 2 persons who received only 2 doses were diagnosed as asymptomatic. A similar study by Sigal et al[18] showed a 92% success rate among individuals who received 3 vaccine doses. However, male subjects ≥ 60 years of age displayed an efficacy of only 75% (L. Sigal et al, Infectious Disease Society of America, San Francisco, CA, 1997, abstract). Absence of infection in the latter study was based upon the lack of evidence of clinically apparent disease in the vaccinated population, although no testing for seroconversion was performed in vaccinated persons who were not presenting evidence of clinical disease. It should be noted that in both the Steere et al[17] and Sigal et al[18] studies, a lesser degree of protection (49% and 68%, respectively) was obtained when only 2 vaccine doses were administered. These data take on added significance when considering that 1) primary vaccinated individuals may not complete the required vaccine series over a 1-year period, and 2) a proportion of those not protected may harbor a latent infection. The question remains as to whether some vaccinated individuals, after exposure to *B burgdorferi,* harbor a low level latent infection. The possibility that a low level infection may not stimulate a measurable antibody response but may exacerbate into clinical Lyme disease at a later time, is suggested by several OspA animal vaccine studies.

In OspA-vaccinated rhesus monkeys, the detection of *B burgdorferi* DNA and antigens in tissues was found at a time when overt symptoms of the disease and Western blot reactivity were absent. These findings suggest the presence of organisms in these tissues although an attempt to "activate" this potential latent infection by administering immunosuppressive drugs was not successful.[12] Although many have argued that the detection of DNA does not indicate the presence of living organisms, a study by Malawista et al[65] has shown a very high correlation between the detection of DNA and positive cultures. Similarly, at the earliest times tested after antibiotic treatment of infected mice the ability to amplify DNA disappeared in concordance with the disappearance of cultivatible spirochetes. The failure to activate a potentially latent infection in the vaccinated monkeys does not unequivocally imply its absence. A parallel can be drawn with latent syphilis in which reactivation is known to occur among latent syphilitics despite the fact that the "triggering" mechanism is not known. During latent infection of rabbits, spirochetes are known to persist despite difficulty in reactivating the infection. In a study by McLeod and Magnuson,[66] symptomatic reactivation was achieved in one rabbit when toxic levels of cortisone were administered. A 33% increase in spirochetemia was observed in drastically immunosuppressed rabbits when compared to latent infected controls and 1 of 7 surviving animals from an original group of 30 developed a darkfield positive skin lesion. Similar doses of cortisone had less of an effect on mice. Of further interest is the fact that infection of mice and rabbits with low numbers of *Treponema pallidum* can establish latency without an antibody response[67] (JN Miller, unpublished studies).

Another question to be considered among OspA-vaccinated persons is whether partial immunity, either from a waning resistance or from an incomplete vaccination regimen, results in an altered disease state upon exposure or a "masking" phenomenon in which infection in the absence of characteristic clinical manifestations such as erythema migrans (EM) occurs. In support of this hypothesis, we found that 4 of 11 OspA-vaccinated rabbits became infected upon challenge with *B burgdorferi* strain B31.[11] In contrast to completely susceptible animals where each site typically develops EM, these infected rabbits developed EM at only 8 of 40 challenged sites while all exhibited disseminated infection. The remaining 7 OspA-immunized rabbits did not develop either EM or disseminated infection. Furthermore, in rabbits exhibiting infection-derived immunity, 2 of 11 exhibited atypical infection while the remaining animals showed complete immunity. The two rabbits from this group became dermally infected following intradermal challenge in the absence of the development of an EM rash and disseminated infection. Further evidence for the potential development of a low level infection in vaccinated animals can be found in a study by Telford et al[6] who showed that 1 of 24 OspA-

vaccinated and heterologously challenged mice, although culture negative, exhibited arthritic changes in the joint.

We believe that these observations support the theory that when states of partial immunity exist, altered forms of disease may arise in some vaccinated and exposed individuals which may make diagnosis more difficult. Because the clinical manifestations may be inconsistent with typical disease, a differential diagnosis that includes Lyme disease, might not be considered given that some physicians may conclude that vaccination reduces or eliminates the chances of acquiring the disease. Furthermore, as suggested by these studies, the infection may be subclinical but could emerge at a later time as more difficult to treat late manifestations.

CAN WE PREDICT A FUTURE VACCINE FAILURE?

Information pointing to the prediction of when spirochetes are likely to evade the immune response of the vaccinated host may currently be available. In two separate studies, a correlation was demonstrated between protective antibody and a specific epitope on OspA, defined by the monoclonal antibody LA-2.[68,69] It was shown in the study by Golde et al[68] that the LA-2 antibody titer is a reliable indicator of immune status following immunization with OspA; vaccinated mice and dogs with a low LA-2 antibody response were susceptible to infection upon challenge. Furthermore, in the human vaccine study conducted by Steere et al,[17] it was reported that patients with breakthrough cases of Lyme disease were found to have significantly lower LA-2 antibody levels at two months following the second injection when compared to a group of vaccinated individuals who had not come down with disease. Interestingly, Padilla et al[61] found that although significant levels of borreliacidal antibodies were elicited in vaccinated individuals and hamsters after two doses, the borreliacidal activity quickly diminished within 180 days. The decline in borreliacidal activity is of concern due to the demonstration by Johnson et al[69] that this activity correlates with protection. The information regarding LA-2 may be useful for determining which individuals are susceptible to *Borrelia burgdorferi* sensu stricto strain B31 type isolates, however, high LA-2 antibody titers may have little or no effect upon variant strains. The frequency of exposure to these variants among a vaccinated population remains to be determined. Thus, the central question still remains as to how many vaccinated patients will develop a classic or altered disease state after exposure, and how this will influence the ability to make an accurate diagnosis.

FUTURE DIRECTIONS TOWARD THE DEVELOPMENT OF A MORE EFFICACIOUS VACCINE

For almost as long as recombinant OspA has been tested as a vaccine candidate, many investigators have recognized the need for an improved vaccine. Recognizing the proven and potential limitations of an OspA vaccine, several investigators have suggested the addition of other components to the vaccine. Such a "cocktail" may include one or more additional recombinant proteins including various OspA serotypes as well as other *B burgdorferi* molecules. Several laboratories have suggested the inclusion of decorin binding protein A (Dbp-A)[31,70,71] which apparently is expressed by *B burgdorferi* while in the vertebrate host.[31] Inclusion of this molecule in a vaccine has been proposed as a means of overcoming the potential danger of spirochetes gaining entry into the OspA-vaccinated host and avoiding antibody-mediated destruction due to the absence of expression in the vertebrate. However, variation in the gene sequence for Dbp-A has been demonstrated in some strains[70] and antibodies against Dbp-A do not protect against some variant types.[31] Thus this heterogeneity among Lyme disease spirochetes emphasizes the probability that no one component will be universally expressed by *B burgdorferi* strains thereby necessitating the inclusion of several components in order to achieve optimal protection against Lyme disease.

In closing, the following quote from the literature summarizes the beliefs of many in the field regarding the OspA vaccine and applies to other potential vaccinogens that will be considered in the future.

> 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. (A Sadziene, AG Barbour. Infection 1996;24:195)

In conclusion, although the OspA vaccine is the most promising candidate thus far, there clearly remains a need for a Lyme disease vaccine that stimulates high levels of long lasting protection against all strains of *Borrelia* responsible for Lyme disease.

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