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Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait

Maria J.C. Gomes-Solecki [a,b,*], Dustin R. Brisson [c], Raymond J. Dattwyler [a,b]

[a] *Department of Microbiology and Immunology, New York Medical College, NYMC, BSB 308, Valhalla, New York, NY 10595, USA*
[b] *Biopeptides, Corp. Valhalla, NY, USA*
[c] *Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA*

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Abstract

Borrelia burgdorferi causes Lyme disease, a potentially debilitating human disease for which no vaccine is currently available. We developed an oral bait delivery system for an anti-*B. burgdorferi* vaccine based in OspA. Mice were immunized orally via gavage and bait feeding. Challenge was performed via *Ixodes scapularis* field nymphs carrying multiple *B. burgdorferi* strains. Vaccination protected 89% of the mice and the systemic immune response was skewed toward IgG2a/2b production. Moreover, this oral vaccine reduced the pathogen in the tick vector by eight-fold. We conclude that this oral vaccine induces a protective systemic immune response against a variety of infectious *B. burgdorferi* strains found in nature and therefore it can eliminate this zoonotic pathogen from its major host reservoirs. Because we observed elimination of the spirochete from the tick vector, a broad delivery of this oral vaccine to wildlife reservoirs is likely to disrupt the transmission cycle of this pathogen.
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1. Introduction

Zoonotic pathogens circulate in several vertebrate reservoir hosts and occasionally cause human disease due to incidental infection. Examples of such diseases are Rocky Mountain spotted fever, Lyme disease and West Nile virus. The pathogens that cause these diseases are transmitted to man via an arthropod vector, such as a tick or a mosquito. The best approach to restrain most vector-borne infectious diseases is through vector control. However, the major method used so far, organochloride pesticides [1], produce unacceptable environmental risks.

One way to prevent human infection is to block transmission of the pathogen in the vertebrate reservoir and/or within the arthropod vector via the development of vaccines to disrupt its zoonotic transmission cycle. Successful vaccination of vertebrate reservoirs should lead to the decrease of the infection prevalence in the arthropod vector, and thus, to a lower probability of pathogen transmission to man. Widespread immunization of vertebrate reservoirs in their natural habitat can only be achieved via the oral route because it is not invasive and is suitable for cost-effective mass vaccination campaigns [2]. Immunizing wildlife against zoonotic pathogens via oral bait has been successfully attempted before to control rabies and plague [3–6]. *Borrelia burgdorferi* cycles in nature between vector ticks of the genus *Ixodes* and a variety of vertebrate reservoir species, such as white-footed mouse, eastern chipmunk and white-tailed deer [7–12]. Lyme disease occurs when *Ixodes scapularis* ticks periodically feed on and transmit the bacteria to man [13]. Here, we set out to develop an oral bait vaccine to control *B. burgdorferi* in wildlife reservoirs and therefore used the natural transmission cycle of this zoonotic pathogen and Lyme disease as a model to test this hypothesis.

* Corresponding author. Tel.: +1 914 594 4920; fax: +1 914 594 4922.
E-mail address: Maria_Gomes-Solecki@nymc.edu
(M.J.C. Gomes-Solecki).

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Outer surface protein A (OspA) inoculated via oral or parenteral administration has been studied as a vaccine against *Lyme borreliosis* [14–21,2,22]. Recently, it has been demonstrated that vaccinating wild mice reservoirs via subcutaneous inoculation reduced the prevalence of *B. burgdorferi* in *I. scapularis* nymphs [23]. However, subcutaneous inoculation of wildlife is not practical. Although OspA vaccination represents an exciting strategy to prevent zoonotic transmission of *B. burgdorferi*, until now, there was no practical system to deliver such vaccines in the wild.

In this study, we demonstrate that our oral vaccine induced a protective systemic (IgG) immune response against multiple wildtype *B. burgdorferi* strains and drastically reduced the pathogen in its tick vector. Thus, this system is an exciting candidate to distribute vaccines in the wild to disrupt the transmission cycle of *B. burgdorferi*, a zoonotic vector-borne pathogen. Here, we describe for the first time, an oral bait vehicle for delivery of vaccines that could be adapted to disrupt the transmission cycle of several other human vector-borne diseases.

2. Methods

2.1. Lyophilization and viability of E. coli expressing B. burgdorferi antigens

B. burgdorferi full length OspA, strain B31, was cloned into the Nde I-Bam HI restriction sites of pET9c and was transformed into *Escherichia coli* BL21 (DE3) pLysS. The parental *E. coli* strain was used as control. After protein induction, cells were harvested, resuspended in 10 ml of TBY containing 24% sucrose [24] and quickly frozen. The antigen was placed in a lyophilizer (Labconco) overnight and stored at $-70\,^{\circ}$C for future use. The viability of the lyophilized bacteria was determined by adding 1 ml of PBS to lyophilized bacteria to get an $OD_{600} = 1$ and plating 1/10 of the suspension onto TBY plates supplemented with proper antibiotic. Protein extraction from lyophilized antigen was done using 200 μl of CELLytic solution (Sigma).

2.2. Oral immunization

B. burgdorferi susceptible 6–8 week female C3H/HeJ inbred mice (Charles River, Boston) were used. Lyophilized, previously induced bacteria, were weighed and resuspended in TBY to an $OD_{600} = 1$ (approximately 10^9 cells/ml). For oral gavage, 250 μl (2.5×10^8 *E. coli* cells in TBY) were inoculated with a ball tipped disposable feeding needle. Mice received the first immunization daily on days 1, 2, 3, 4 and 8, 9, 10, 11. Two weeks later, mice were bled (day 27) and on days 30, 31, 32, 33 received the 1st boost. On day 45, mice were bled for the second time and on days 52, 53, 54, 55 received the second boost. On day 64, mice were bled for the 3rd time. Challenge was performed on day 67. One month later (day 97), mice were euthanized and blood, ear, heart and bladder tissues were obtained to assess for spirochete dissemination. For oral bait, mice received the first round of bait on days 1, 2, 3, 4, the second round on days 23, 24, 25, 26, the third round on days 43, 44, 45, 46 and were bled on days 17, 39 and 60 to compare the anti-OspA immune response with mice immunized by oral gavage. Lyophilized bacteria (10 mg, 50 mg or 100 mg) were mixed with 500 mg of either peanut butter or oatmeal. Mice were placed in individual cages and to train them, the food was removed the night before bait feeding in the first week of the immunization. Next morning, a dose of the bait was provided for ingestion ad libidum. The same dose was provided every day and the mice were allowed to eat for 3 h, after which empty containers were removed from the cage and mouse chow was offered. Water was provided at all times. For the subsequent rounds of bait feeding the food was not removed the night before immunization, the bait was presented the same way and at the same time as the previous round. If given bait and mouse chow at the same time, mice always finished the bait first. All experiments involving animals were performed after obtaining proper IRB approval.

2.3. B. burgdorferi challenge

Needle challenge was performed by intradermal inoculation of 2×10^3 of a *B. burgdorferi* sensu stricto strain cultured from a patient biopsy. This is a highly infectious strain, characterized with an OspC group K, that has not been subjected to further laboratory passages. This strain is used in our laboratory to perform both infections of mice and needle challenges directly from the 1st passage. Tick challenge was performed by placing 9–10 *B. burgdorferi* infected nymphal field ticks on the back of the head of restrained mice that were allowed to feed for 2 h. Three days later (d3 to d5), engorged ticks were collected after naturally falling off, counted and a daily record was kept for each mouse.

2.4. Collection and maintenance of Ixodes scapularis ticks

The field ticks were obtained from *I. scapularis* larva collected from ears of infected white-footed mice trapped at Millbrook, NY in the summer of 2003. Larva were allowed to molt to the nymphal stage in glass flasks, where they were kept at room temperature in a humid environment. The tick rate of infection was checked by darkfield microscopy and by *ospC*-PCR using the following primers: OC6 (+) AAAGAATACATTAAGTGCGATATT; OC623 (−) TTAAGGTTTT TTTTGGACTT TCTGC.

2.5. Determination of vaccine efficacy

Antibody assays: Total IgG and IgG subclass isotyping was performed in immunized mouse sera using either alkaline phosphatase (1:1600) (KPL, Gaithersburg, MD) or horseradish peroxidase secondary antibody (1:50,000) (Bethyl, Montgomery, TX) by ELISA. Because we planed

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