Deployment of a Reservoir-Targeted Vaccine Against Borrelia burgdorferi Reduces the Prevalence of Babesia microti Coinfection in Ixodes scapularis Ticks

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In the Northeast and upper Midwest of the United States, Babesia microti and Borrelia burgdorferi use Ixodes scapularis ticks as vector and Peromyscus leucopus mice as major reservoir host. We previously established, in a 5-year field trial, that a reservoir-targeted outer surface protein A vaccine reduces the prevalence of B. burgdorferi-infected ticks. We accessed ticks and mouse blood samples collected during the trial, extracted total DNA, and amplified the B. microti 18S rRNA gene. Vaccine deployment reduced the prevalence of ticks coinfected with B. microti and that of mice infected with B. microti. Breaking the enzootic cycle of B. burgdorferi may reduce the incidence of babesiosis.

Keywords. Babesia microti; babesiosis; Borrelia burgdorferi; coinfection; Lyme disease; Peromyscus leucopus; tick; vaccine.

In the United States, nearly all cases of human babesiosis are caused by the hemoparasite Babesia microti and develop following the bite of an infected Ixodes scapularis tick [1]. Common symptoms include fatigue, fever, chills, sweats, headache, myalgia, and anorexia. In the elderly and the immunocompromised, the illness can be severe. Complications include severe anemia, acute respiratory distress syndrome, and renal insufficiency or failure. Despite antimicrobial therapy, babesiosis can be fatal. No vaccine is available. Current prophylaxis, which is limited to endemic area avoidance and tick exposure reduction, has been ineffective in containing the emergence of babesiosis [2].

Babesia microti is maintained in its enzootic cycle by alternate transmission between I. scapularis ticks and reservoir hosts such as white-footed mice (Peromyscus leucopus), short-tailed shrews, and eastern chipmunks [3, 4]. Larval ticks, which are free of B. microti because the parasite does not colonize the ovaries of adult female ticks, may acquire B. microti when taking a blood meal from reservoir hosts. Nymphs that result from the molt of infected larvae harbor B. microti and infect reservoir hosts upon which they feed. If nymphs become infected when feeding, adult ticks that result from the molt are infected. Thus, the enzootic cycle of B. microti resembles that of Borrelia burgdorferi, the causative agent of Lyme disease [4].

Ixodes scapularis nymphs are the primary vector for transmission of B. microti to humans [1], which are incidental hosts. Adult female ticks also transmit B. microti to humans. Given that I. scapularis nymphs and adults can transmit B. microti and B. burgdorferi simultaneously [4], a diagnosis of babesiosis should prompt testing for Lyme disease [5]. Conversely, babesiosis should be considered in Lyme disease patients for whom symptoms are severe, not typical of Lyme disease, or poorly responsive to standard antibiotic therapy [5].

Babesiosis is reported from areas that have long been endemic for Lyme disease but not from those in which B. burgdorferi recently spread [4]. The delayed spread of babesiosis has been attributed to the poor transmission of B. microti from reservoir host to tick and to the facilitation of this transmission by B. burgdorferi [6]. To assess the importance of B. burgdorferi in maintaining B. microti in their shared enzootic cycle, we accessed I. scapularis ticks and P. leucopus blood samples collected during a field trial of an oral reservoir-targeted vaccine against B. burgdorferi [7].

METHODS

Ethics Statement

Experiments were in compliance with the regulations of the Institutional Biosafety Committees (IBC) and Institutional Animal Care and Use Committees (IACUC) of the Cary Institute of Ecosystem Studies (IACUC 07-021, 10-0111), the University of Tennessee Health Science Center (IBC 08-313/12-416; IACUC 1741/13-010/13-012/14-007), and Tufts Medical Center (IBC 2013-TIA61; IACUC B2014-56).

Vaccine Deployment

The field study was conducted at the Cary Institute of Ecosystem Studies and has been described elsewhere [7].
Briefly, vaccination plots and control plots had comparable basic vegetative structure and small mammal community. Each plot consisted of an 8-by-8 array of Sherman live traps, which were distributed every 15 m. Plots were separated by at least 500 m, ensuring biological and statistical independence. From mid-May to mid-September, in the late afternoon of each workday, every trap was set with 1 single bait. Baits for vaccination plots consisted of crimped oats admixed with 200 mg of lyophilized, outer surface protein A (OspA)-expressing Escherichia coli, whereas baits for control sites contained no E. coli. From May to June, ticks were collected by drag sampling. Ticks available for the present study (see Supplementary Table 1) were those collected in 2008 and 2011 at 2 control sites (Ctrl1, Ctrl2) and 2 vaccination sites (NY1, NY2). Ticks were tested for Bb and Bm by amplifying the flaB gene and the 18S rRNA gene, respectively. (A) Graphs depict the proportions (%) of ticks infected with Bb or Bm among those collected at the control sites (n = 62 in 2008, n = 102 in 2011) and the vaccination sites (n = 80 in 2008, n = 105 in 2011). Whether deployment of the OspA vaccine had an effect on either pathogen was tested by use of binomial logistic regression. The term “site [vaccine] * time [2011]” differed from the term “site [control] * time [2008]” for B. burgdorferi (P = .003) but not for B. microti (P = .145). (B) Graphs depict the proportions (%) of ticks infected with a single pathogen or both pathogens among those collected at the control and vaccination sites (see A). Whether deployment of the OspA vaccine had an effect on single infection and/or coinfection was tested by use of multinomial logistic regression (see Supplementary Table 2). The term “site [vaccine] * time [2011]” differed from the term “site [control] * time [2008]” for singe infection with B. burgdorferi (P = .024) and for coinfection (P = .013) but not for single infection with B. microti (P = .69). ns, not significant (P > .05).

**Figure 1.** A reservoir-targeted outer surface protein A (OspA) vaccine reduces the prevalence of Ixodes scapularis ticks coinfected with *Borrelia burgdorferi* (Bb) and *Babesia microti* (Bm). From mid-May to mid-September, crimped oat baits were placed at control sites (labeled as “control”) and vaccination sites (labeled as “vaccine”) [7]. Baits for vaccination sites contained lyophilized OspA-expressing Escherichia coli, whereas baits for control sites contained no E. coli. From May to June, ticks were collected by drag sampling. Ticks available for the present study (see Supplementary Table 1) were those collected in 2008 and 2011 at 2 control sites (Ctrl1, Ctrl2) and 2 vaccination sites (NY1, NY2). Ticks were tested for Bb and Bm by amplifying the flaB gene and the 18S rRNA gene, respectively. (A) Graphs depict the proportions (%) of ticks infected with Bb or Bm among those collected at the control sites (n = 62 in 2008, n = 102 in 2011) and the vaccination sites (n = 80 in 2008, n = 105 in 2011). Whether deployment of the OspA vaccine had an effect on either pathogen was tested by use of binomial logistic regression. The term “site [vaccine] * time [2011]” differed from the term “site [control] * time [2008]” for B. burgdorferi (P = .003) but not for B. microti (P = .145). (B) Graphs depict the proportions (%) of ticks infected with a single pathogen or both pathogens among those collected at the control and vaccination sites (see A). Whether deployment of the OspA vaccine had an effect on single infection and/or coinfection was tested by use of multinomial logistic regression (see Supplementary Table 2). The term “site [vaccine] * time [2011]” differed from the term “site [control] * time [2008]” for singe infection with B. burgdorferi (P = .024) and for coinfection (P = .013) but not for single infection with B. microti (P = .69). ns, not significant (P > .05).
**Statistical Analysis**

Differences in the proportion of infected ticks or infected mice were tested for significance by use of logistic regression. Differences in pathogen burden (log-transformed data) were tested for significance by use of two-way analysis of variance (ANOVA) followed by Fisher’s LSD test or by unpaired t test with Welch’s correction. A difference was declared significant when \( P \leq 0.05 \). Multinomial logistic regression was run using SPSS v.28.0.1.1; all other analyses were run using GraphPad Prism v.9.3.1.

**RESULTS**

Most nymphs that had been tested for *B. burgdorferi* during our field trial [7] were available for *B. microti* testing (see Supplementary Table 1). As expected, OspA vaccination reduced the prevalence of *B. burgdorferi*-infected nymphs (Figure 1A; binomial logistic regression, \( P = 0.003 \) for site*time) but failed to reduce the prevalence of *B. microti*-infected nymphs (\( P = 0.145 \)). We next classified nymphs as infected with *B. burgdorferi*, *B. microti*, or both (Figure 1B). OspA vaccination reduced the prevalence of nymphs infected with only *B. burgdorferi* (multinomial logistic regression, \( P = 0.024 \)) as well as the prevalence of coinfected nymphs (\( P = 0.013 \)) but had no effect on the prevalence of nymphs infected with only *B. microti* (\( P = 0.69 \)). At the end of the 4-year period, when compared with the start of this period (see Supplementary Material), the odds of finding a nymph infected with only *B. burgdorferi* were 3.5 times lower at a vaccination site than at a control site (odds ratio \( [OR] = 0.28 \)) (Supplementary Table 2). The odds of finding a coinfected nymph were 7.5 times lower at a vaccination site than at a control site (\( OR = 0.13 \)) (Supplementary Table 2). We conclude that our reservoir-targeted vaccine is effective at clearing *B. microti* from coinfected nymphs and that this effect does not come at the cost of a greater prevalence of nymphs infected with only *B. microti*.

Coinfection of *P. leucopus* mice with *B. burgdorferi* and *B. microti* facilitates the transmission of *B. microti* to feeding larvae as revealed by the greater prevalence of *B. microti* in nymphs generated by the molt of these larvae [6]. To test whether *B. microti* burden is greater in nymphs that are coinfected with *B. burgdorferi*, we turned to nymphs collected at the control sites in 2008 and 2011. *Babesia microti* burden, as assessed by the mean 18S rRNA gene copy number, did not differ between coinfected ticks and ticks infected with only *B. microti* (Supplementary Figure 1A). Likewise, *B. burgdorferi* burden, as assessed by the mean flaB gene copy number, did not differ between coinfected ticks and ticks infected with only *B. burgdorferi* (Supplementary Figure 1C). To test whether OspA vaccination lowers the pathogen burden of ticks, we analyzed ticks collected at the control and vaccination sites. Having established that *B. burgdorferi* coinfection has no impact on *B. microti* burden and vice versa, we classified ticks as infected with *B. microti* (Supplementary Figure 1B) or...
DISCUSSION

Nearly all cases of babesiosis are reported from the Northeast and the upper Midwest of the United States, particularly from areas that have long been endemic for Lyme disease [2, 4]. In these areas, Lyme disease has greater incidence than babesiosis, a difference attributed to the greater prevalence of B. burgdorferi-infected I. scapularis nymphs [8]. Some of the nymphs that harbor B. burgdorferi also harbor B. microti. The prevalence of coinfected nymphs is greater than predicted by independent assortment of the 2 pathogens [4, 9], stressing the importance of coinfected nymphs to the rising incidence of babesiosis. In the past 2 decades, considerable efforts have been invested in developing a reservoir-targeted vaccine that would reduce the incidence of Lyme disease [7, 10, 11]. We previously reported that deployment of one such vaccine reduces the prevalence of B. burgdorferi-infected nymphs [7]. We now report that this OspA-based vaccine reduces the prevalence of nymphs coinfect with B. microti.

OspA antibodies interrupt the enzootic cycle of B. burgdorferi by preventing transmission of B. burgdorferi from nymph to host and from host to larva [12, 13]. How can OspA antibodies interfere with B. microti? OspA antibodies are unlikely to be detrimental to B. microti in feeding nymphs because OspA antibodies ingested with a blood meal are confined to the midgut, whereas B. microti sporogony is confined to the salivary glands [1]. Accordingly, OspA vaccination of humans will not protect them from B. microti present in feeding nymphs. Keeping in mind that we studied host-seeking (unfed) nymphs, we postulate that OspA antibodies interfere with B. microti in feeding larvae. As larvae feed on a coinfected host, B. burgdorferi spirochetes and B. microti-infected red blood cells accumulate in their midgut. If the host has been immunized with OspA, OspA antibodies contained in the blood meal block the acquisition of spirochetes by larvae in a host complement-dependent manner [14]. We speculate that complement activation in the gut lumen of larvae that feed on OspA-immunized coinfect hosts leads to the lysis of red blood cells, including those containing B. microti, thereby explaining why our vaccine is effective against coinfected nymphs but spares nymphs that are infected with only B. microti. Red blood cell lysis would occur early because spirochetes begin expressing OspA in the larva midgut within 24 hours postattachment [13]. Early red blood cell lysis would halt the metamorphosis of B. microti gametocytes and the ensuing generation of gametes, processes that are initiated in red blood cells while in the tick midgut and are completed only when ticks are replete [15]. Experiments are needed to establish that complement-mediated red blood cell lysis can halt sexual reproduction of B. microti.

Reservoir hosts that ingest an OspA-based vaccine are not protected from B. burgdorferi infection because spirochetes express OspA in the tick midgut but not in their salivary glands [12, 13]. Accordingly, reduction in the prevalence of B. microti-infected mice by our vaccine is best explained by a reduction in the prevalence of coinfect larvae before their molt to coinfect nymphs. By reducing the prevalence of nymphs that are infected with only B. burgdorferi [7], our vaccine may also hinder the maintenance of B. microti in its enzootic cycle because (1) white-footed mice that are chronically infected with B. microti may become infected with B. burgdorferi and (2) such coinfection may create an immunological conflict that favors B. microti in mice [4].

As the geographic range of B. microti continues to expand into areas already endemic for B. burgdorferi, the incidence of coinfections in ticks and humans will rise further. In this study, we provide evidence that a reservoir-targeted OspA vaccine is effective at reducing the prevalence of coinfection with B. burgdorferi and B. microti in their shared tick vector and the prevalence of B. microti infection in their major reservoir host. Initially designed to reduce the risk of Lyme disease, our reservoir-targeted vaccine may also reduce the risk of concurrent babesiosis.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.
Notes

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Potential conflicts of interest. MGS has stocks and patents that relate to the use of a reservoir-targeted OspA-based vaccine.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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