Immune Serum from Rabbits Infected with *Borrelia burgdorferi* B31 Confers Complete Passive Protection Against Homologous Challenge

Celeste Chong-Cerrillo, PhD; Xiao-Yang Wu†, MD; Yi-Ping Wang†, MD; David R. Blanco†‡, PhD; Michael A. Lovett†‡, MD, PhD; and James N. Miller, PhD†*

ABSTRACT

Further support for a role of humoral immunity in experimental Lyme disease is shown in this study by the demonstration that passive immunization with infection-derived immune rabbit serum protects rabbits against challenge with *Borrelia burgdorferi*. Animals administered immune rabbit serum both before and after intradermal challenge with virulent *B burgdorferi* strain B31 and were protected against development of erythema migrans (EM) lesions, as well as skin and visceral infection. In contrast, animals that were administered immune serum only after challenge were not protected, although EM lesion development was observed to be altered as compared to controls. Serum antibody levels against outer surface protein (Osp)A, OspC, decorin binding protein (Dbp)A, and DbpB from passively immunized rabbits were determined by ELISA. While rabbits receiving immune sera before and after challenge were protected against infection, their antibody levels against OspA were relatively low and antibody levels against OspC, DbpA, and DbpB were comparable to basal sera. These findings demonstrate that in the rabbit model of Lyme disease, passive humoral immunity can confer complete protection only if administered before challenge. The data also suggest that immune rabbit serum antibody directed against antigens other than OspA, OspC, DbpA, and DbpB may contribute to this protection.

Key words: post-challenge, immune serum, erythema migrans, normal rabbit serum

INTRODUCTION

Lyme disease is a worldwide tick-transmitted infection caused by a group of related spirochetes collectively termed *Borrelia burgdorferi* sensu lato, which includes *B burgdorferi* sensu stricto, *B afzelii*, and *B garinii*. Lyme disease is the most common vector-borne disease in the United States[1] and is transmitted to humans by the bite of an infected *Ixodes* or *Amblyomma* tick. In most patients, Lyme disease is characterized by the initial appearance of a rash-like skin lesion, termed erythema migrans (EM).[2,3] Early and late clinical manifestations include arthritis, neurological manifestations, lymphadenopathy, and carditis, which reflects dissemination to visceral targets.[3-12] Although Lyme disease is rarely fatal, it can be debilitating.

Implicit in the development of an effective vaccine against Lyme disease is a thorough understanding of the pathogenesis and host immune response operative during host-spirochete interaction. The animal model most frequently used in the study of Lyme disease has been the rodent. The rodent represents a permissive host for *B burgdorferi* as chronic infection is a salient feature, similar to what is seen in human infection. The rabbit model of Lyme disease also has unique features relevant to the immunobiology of human *B burgdorferi* infection. It is the only animal model, besides the rhesus monkey,[13] that reproducibly produces EM indistinguishable from that of human disease after intradermal inoculation of *B burgdorferi*, sensu stricto.[14] Most importantly, untreated skin and visceral infection is ultimately cleared, in contrast to the monkey[13] and rodent[7,15-18] models, resulting in complete immunity against reinfection with up to 2×10^7 organisms.[14] This protection against challenge with cultivated virulent organisms is more than three orders of

*From the *Department of Pathology, University of California, Irvine; †Department of Microbiology and Immunology; and ‡Division of Infectious Diseases, Department of Medicine, University of California, Los Angeles, School of Medicine, Los Angeles, California.*

Address correspondence to James N. Miller, PhD, Department of Microbiology and Immunology, UCLA School of Medicine, Center for Health Sciences, 43-239, 10833 Le Conte Avenue, Los Angeles, CA 90095-1747 [e-mail: jmiller@microimmun.medsch.ucla.edu].

magnitude greater than that afforded by immunization of rabbits with outer surface protein A (OspA),[14] a protein currently used for human vaccination.

In this study, we used serum from rabbits that have developed complete protective immunity following infection to determine whether this protective immunity can be transferred by passive immunization to naive rabbits. In addition, we sought to correlate passive immunity following challenge with serum antibodies directed against the OspA, OspC, decorin binding protein (Dbp)A, and DbpB antigens of *B burgdorferi*.

MATERIALS AND METHODS

Animals

Adult, male, New Zealand white rabbits ages 6-9 months (Irish Farms, Norco, CA) were housed individually in a temperature-controlled environment ranging from 18-21°C. Prior to intradermal (ID) inoculation with *B burgdorferi*, the backs of the rabbits were clipped closely with an electric clipper fitted with a size 40 blade to expose the skin (Oster Professional Products, McMinnville, TN).

Bacterial Strains and Preparation of Challenge Inoculum

Virulent *Borrelia burgdorferi* sensu stricto, strain B31, was isolated from infected rabbit tissue, grown in BSK II medium[19] to maximum density, then passed twice more in fresh BSK II medium. After the final passage (passage 2), the organisms were centrifuged at 8,000 × *g* for 10 minutes and washed three times in heat-inactivated (56°C, 30 minutes) normal rabbit serum (NRS) diluted 1:1 with phosphate buffered saline (PBS), pH 7.2 (NRS-PBS). Inoculum for challenge was resuspended in NRS-PBS after the final wash to a final concentration containing 1×10^7 *B burgdorferi* per mL.

Immune and Nonimmune Sera

As a source of immune serum, 25 rabbits were initially infected ID with 6×10^6 *B burgdorferi*, B31, allowed to sit for 24 weeks, and then challenged ID with 1×10^7 *B burgdorferi*, B31. The animals were bled two weeks later, at a time when they were shown, by culture of their skin and viscera, to be immune. We have established previously that after 24 weeks, rabbits resolve both local and disseminated infection and develop immunity to reinfection.[14] Normal rabbit serum (NRS) was obtained from 10 normal rabbits. Individual serum samples were stored at -80°C until ready for use. At the time of initial serum injection, equal amounts of individual IRS or NRS were pooled, heat-inactivated at 56°C for 30 minutes, and filter-sterilized. As determined by enzyme-linked immunosorbent assay (ELISA), the pooled IRS had anti-*B burgdorferi* and anti-OspA titers of 1:16000, an anti-OspC titer of 1:1000, an anti-DbpA titer of 1:2000, and an anti-DbpB titer of 1:1,000. The pooled NRS had baseline antibody reactivity of <1:125 to *B burgdorferi*, OspC, DbpA, and DbpB and <1:250 to OspA. Both pooled sera were aliquoted and stored at -80°C until ready for use.

Passive Immunization and Challenge of Rabbits

Groups of 5 rabbits, each weighing approximately 2.3 kg, were injected intravenously (IV) at various time points via the marginal ear vein with 3 mL per kg body weight of either heat-inactivated, undiluted pooled IRS or NRS, or sterile PBS, pH 7.2. Sera or PBS given to rabbits before and after challenge (IRS "Before," NRS "Before," and PBS groups) were administered at -18, 0, +24, +48, +96, and +216 hours relative to challenge; sera given to rabbits only after challenge (IRS "After" and NRS "After" groups) were administered at +24, +48, +96, and +216 hours relative to challenge. As a control for *B burgdorferi* infection and EM development, one group of rabbits was given neither sera nor PBS (naive group).

Each of the rabbits was challenged ID at the 0 hour time point with 0.1 mL of the challenge inoculum at each of six sites for a total of 6×10^6 *B burgdorferi*.

Skin Biopsy and Tissue Collection and Culture

Skin punch biopsies were obtained from all rabbits at the time of EM development in the control, naive rabbits (day 8). Rabbits were anesthetized by intramuscular injection with 45 mg Ketaset (Fort Dodge Laboratories, Fort Dodge, IA) and 8.8 mg Xylazine (Lloyd Laboratories, Shenandoah, IA) per kg body weight. A 4 to 5 mm sterile punch biopsy (Baker and Cummings, Lakewood, NJ) was taken adjacent to the inoculation site from the clipped back of each rabbit. Each biopsy specimen was immediately minced and cultured in 5 mL of BSK II medium containing 100 µg phosphomycin per mL and 50 µg rifampin per mL (Sigma, St. Louis, MO).[20]

At 3 weeks post-challenge (pc), rabbits were bled from the central ear artery. Skin punch biopsies, popliteal lymph nodes, joint tissue surrounding the patella, and spinal cord were aseptically removed immediately after each rabbit was sacrificed by lethal IV injection of 100 mg sodium pentobarbital per kg body weight. Portions of each tissue were minced and cultured in BSK II medium containing 100 µg phosphomycin per mL and 50 µg rifampin per mL.

Erythema migrans skin biopsy cultures were incubated aerobically at 34°C for a period of 7 weeks while skin biopsies and viscera obtained at the time of death were

Table 1. Results of intradermal challenge with B burgdorferi *B31 in passively immunized rabbits.*

Group (n=5)	EM Lesions # Positive Sites/Total Injected (# Positive Rabbits/Total)	*B burgdorferi* Culture (# Positive Animals/Total)				
		Day 8 PC§	Week 3 PC			
		Skin	Skin	PLN‡	Joint Tissue	Spinal Cord
IRS Before*	0/30 (0/5)	0/5	0/5	0/5	0/5	0/5
IRS After†	30/30 (5/5)	5/5	5/5	5/5	4/5	1/5
NRS Before*	30/30 (5/5)	5/5	5/5	5/5	5/5	2/5
NRS After†	30/30 (5/5)	5/5	5/5	5/5	5/5	5/5
PBS	30/30 (5/5)	5/5	5/5	3/5	5/5	3/5
Naive	30/30 (5/5)	5/5	5/5	5/5	4/5	3/5

**Serum was administered at -18, 0, +24, +48, +96, and +216 hours relative to challenge.*
†Serum was administered at +24, +48, +96, and +216 hours relative to challenge.
‡PLN, Popliteal lymph nodes.
§pc, post-challenge.

incubated under the same conditions for 15 weeks. The presence or absence of *B burgdorferi* was determined periodically by darkfield microscopy. Cultures were considered negative when no spirochetes were observed during the above indicated observation periods.

ELISA

For determination of serum antibody levels against *B burgdorferi*, OspA, OspC, DbpA, and DbpB, each of the rabbits was first prebled (basal specimen) from the central ear artery and then bled after administration of sera or PBS. Rabbits were bled at the 0 (Before groups only), +24-, +96-, and +216-hour time points in the same order that they received sera or PBS. The serum samples were stored at -80°C until assayed.

Flat-bottomed 96-well immunoassay plates (Immulon 4; Dynatech Laboratories, Inc., Chantilly, VA) were precoated overnight at room temperature with 100 µL containing 1 mg/mL of either sonicated whole *B burgdorferi* B31, passage 3, recombinant OspA (kindly provided by SmithKline Beecham Pharmaceuticals, Rixensart, Belgium), recombinant OspC (kindly provided by Steven M. Callister and Steven Lovrich, Gundersen Lutheran Medical Center, La Crosse, WI), recombinant DbpA, or recombinant DbpB (rDbps kindly provided by Magnus Höök, Texas A&M University, Houston, TX) in PBS, pH 7.2. The plates were washed three times with PBS containing 0.05% Tween 20 (PBS/Tween) and blocked with BLOTTO (5% nonfat dry milk in PBS). After three washes, serum samples serially diluted two-fold in PBS, starting at 1:125 for anti-*B burgdorferi*, OspC, DbpA, and DbpB ELISAs or 1:250 for anti-OspA ELISAs, were added to the wells and incubated at room temperature for 2 hours. Plates were washed three times and then incubated with donkey antiserum against rabbit Ig conjugated to horseradish peroxidase (1:5000) (Amersham Corp., Arlington Heights, IL) at room temperature for 1 hour. The plates were then washed three times and incubated with 2, 2'-azinobis (3-ethyl benzthiazoline sulfonic acid) peroxidase substrate system (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) at room temperature in the dark for 30 minutes. The reaction was stopped with 1% sodium dodecyl sulfate (SDS) and the absorbance of each well was measured at 405 nm with an automated ELISA plate reader (Titertek Multiscan MCC/340; Flow Laboratories, Baar, Switzerland). Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.

RESULTS

Passive Protection Against *B burgdorferi* Challenge

To determine the protective capability of infection-derived immune rabbit serum against experimental *B burgdorferi* infection, naive rabbits were passively immunized and challenged. Rabbits administered IRS both before and after challenge failed to develop EM lesions at any site and all sites were shown to be culture-negative (Table 1). In contrast, animals receiving IRS only after challenge developed culture-positive EM lesions (Table 1). As expected, rabbits receiving NRS either before or after *B burgdorferi* challenge, as well as rabbits receiving only PBS, had culture-positive EM lesions comparable to those of the naive controls. The time of appearance of the EM lesions also differed between groups of rabbits.

Table 2. Serum antibody responses to B burgdorferi *following passive immunization and challenge.*

Group (n=5)	GMT* Serum anti-*B burgdorferi* Ig Time Post-Challenge					
	Basal	0 Hrs.†	24 Hrs.	96 Hrs.	216 Hrs.	3 Wks.
IRS§ Before	125	3200±1095	2800±1095	4000	4000	800±274
IRS After	<125	N/A‡	2000	2800±1095	3200±1095	1800±1304
NRS After	150±56	N/A	125	125	400±137	8000±4899

**Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses ± standard deviations of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.*

†Serum samples obtained before challenge.

‡N/A, serum samples not obtained.

*§IRS anti-*B burgdorferi *titer - 1:16000*

Control rabbits (PBS and naive groups) were the first to develop EM lesions (day 7 pc), followed by rabbits receiving NRS before challenge (NRS Before group; day 7-8 pc), and then by rabbits receiving NRS after challenge (NRS After group; day 9 pc). The duration and diameter of the lesions did not differ significantly between these groups of animals. Rabbits receiving IRS only after challenge (IRS After group), while developing lesions at all sites, did not develop EM lesions until day 13 or 14 p.c. and the duration of the lesions was shorter than the other control groups.

To determine whether passive immunization with infection-derived immune rabbit serum protected against skin and disseminated infection, all rabbits were sacrificed 3 weeks post-challenge and skin and sites of dissemination evaluated for evidence of *B burgdorferi* infection. As shown in Table 1, rabbits receiving IRS both before and after challenge (IRS before group) were completely protected against challenge as evidenced by the absence of infection in the skin and at disseminated sites including the popliteal lymph nodes, joint tissue, and spinal cord. In contrast, rabbits given IRS only after challenge (IRS after group), as well as all control groups, showed both skin and disseminated infection.

Serum anti-*B burgdorferi* Antibody Titers at Intervals After Passive Immunization

Serum samples from the IRS before, IRS after, and NRS after groups were obtained at the 0 (IRS Before group only), +24-, +96-, and +216-hour time points after administration of sera and at 3 weeks post-challenge for the determination of *B burgdorferi*-specific antibodies by ELISA. As shown in Table 2, the levels of serum anti-*B burgdorferi* Ig did not differ significantly between rabbits receiving IRS both before and after challenge and rabbits receiving IRS only after challenge. As expected, the anti-*B burgdorferi* antibody titer of rabbits receiving NRS at the earlier time points was comparable to that of the basal level measured for the NRS pool. At 3-weeks post-challenge, rabbits that received IRS and were free from local and disseminated infection (IRS Before group), had a five-fold lower *B burgdorferi*-specific antibody titer compared to the titer obtained at the 216 hours post-challenge time point. Rabbits in the IRS After group also showed a decrease in the anti-*B burgdorferi* antibody titer at 3 weeks post-challange compared to the 216-hour time point; however, these animals showed both local and disseminated infection. By comparison, rabbits in the NRS After group had a 20-fold increase in anti-*B burgdorferi* Ig titers between the 216-hour and 3-week post-challenge time points consistent with active infection.

Comparison Between Status of Immunity and Anti-OspA, Anti-OspC, Anti-DbpA, and Anti-DbpB Antibody Titers

Several investigators have indicated that antibody against OspA, OspC, and DbpA in mouse immune serum may play a pivotal role in protection against challenge with *B burgdorferi*.[21-25] To determine whether antibodies against these specific proteins play a similar role in protection against challenge in the rabbit model, serum antibody levels against OspA, OspC, DbpA, and also DbpB were determined by ELISA following passive immunization.

During the course of immune serum administration, the antibody titers against OspA in rabbits completely immune to challenge were found to be relatively low ranging from 1:800 ±274 to 1:2,400±894. In this same group, the anti-OspC titers of ≤1:125 were comparable to those obtained for basal sera (Table 3). Further, the anti-OspA and anti-OspC Ig titers did not differ significantly between rabbits from the IRS Before group and rabbits from the IRS After group despite the fact that only rabbits in the Before group were completely protected against challenge (Table 3).

Table 3. Comparison of immune status of passively immunized rabbits and anti-OspA and anti-OspC antibody titers.

Group (n=5)	Rabbit Immune Status	GMT* Serum Anti-OspA Ig (Time Post-Challenge) Basal	0 Hrs.†	24 Hrs.	96 Hrs.	216 Hrs.	3 Wks.
IRS§ Before	Immune	<250	2000	1200±447	2400±894	2000	800±274
IRS After	Non-immune	<250	N/A‡	800±274	2400±894	2000	500
NRS After	Non-immune	<250	N/A	<250	250	688±375	1600±1342

Group (n=5)	Rabbit Immune Status	GMT* Serum Anti-OspC Ig (Time Post-Challenge) Basal	0 Hrs.†	24 Hrs.	96 Hrs.	216 Hrs.	3 Wks.
IRS¶ Before	Immune	<125	125	<125	125	125	<125
IRS After	Non-immune	<125	N/A‡	<125	<125	125	125
NRS After	Non-immune	<125	N/A	<125	<125	150±56	475±335

**Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses ± standard deviations of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.*

†Serum samples obtained before challenge.

‡N/A, serum samples not obtained.

§IRS anti-OspA titer - 1:16000

¶IRS anti-OspC titer - 1:1000

Similarly, the serum antibody titers against DbpA and DbpB were relatively low in each of the passively immunized rabbits (Table 4). During the first 24-hour course of serum administration, no significant differences in the anti-DbpA and anti-DbpB titers were observed among the passively protected and unprotected groups of animals; the titers were comparable to those of the basal sera (<1:125) (Table 4). However, at the 3-week post-challenge time point, rabbits from the IRS After and NRS After groups developed significantly higher antibody levels against DbpA and DbpB as compared to the IRS Before group. These titers ranged from 1:1100±548-1:6400±2191. In contrast, rabbits from the passively protected IRS Before group had no significant increases in their anti-DbpA or anti-DbpB titers compared to their basal sera levels throughout the entire 3-week observation period.

DISCUSSION

In this study, we have shown that the high degree of infection-derived immunity that develops in the rabbit Lyme disease model can be transferred to naive recipients by passive immunization with serum, indicating that antibody is a major contributor to this protective response. The ability of passively administered immune serum to protect against challenge with cultured homologous *B burgdorferi* has also been demonstrated in the murine model.[21,25-28] The murine model is known to be a permissive host for *B burgdorferi* infection, which may relate, in part, to the inability of the murine immune response to clear the infection. By comparison, the immune response in the rabbit results in clearance of infection and complete immunity to reinfection, which may be caused by differences in antigen presentation.

Rabbits receiving immune serum were completely protected against development of EM, dermal infection, and visceral infection for at least 3 weeks after challenge. Complete protection against infection was achieved only when immune serum was administered before challenge (Table 1, IRS Before group). Once infection had been established, passively administered immune serum could not inhibit the establishment of infection and these animals eventually developed EM and dermal and visceral infection (Table 1, IRS After group). A similar observation has been reported for the murine and hamster models of passive immunity where immune serum given after challenge did not eliminate infection.[21,28,29] However, EM appearance in rabbits given immune serum after challenge was significantly delayed as compared to control animals. In addition, these animals showed significantly lower anti-*B burgdorferi* antibody titers at 3 weeks post-challenge as compared to controls. Taken together, these observations suggest that immune serum given only after challenge, while not completely protective, reduced the number of virulent organisms. The reasons for the inability of immune serum to completely protect when given after challenge are not known, but may relate to what has been termed "host-adap-

Table 4. Comparison between the status of immunity of passively immunized rabbits and anti-DbpA and anti-DbpB antibody titers.

Group (n=5)	Rabbit Immune Status	GMT* Serum Anti-DbpA Ig (Time Post-Challenge) Basal	0 Hrs.†	24 Hrs.	96 Hrs.	216 Hrs.	3 Wks.
IRS§ Before	Immune	<125	250	250	300±112	300±112	150±56
IRS After	Non-immune	<125	N/A‡	250	250	300±112	1100±548
NRS After	Non-immune	<125	N/A	<125	<125	125	4400±2191

Group (n=5)	Rabbit Immune Status	GMT* Serum Anti-DbpB Ig (Time Post-Challenge) Basal	0 Hrs.	24 Hrs.	96 Hrs.	216 Hrs.	3 Wks.
IRS¶ Before	Immune	<125	175±68	175±68	225±163	275±205	113±81
IRS After	Non-immune	<125	N/A‡	250	400±137	450±112	2600±1342
NRS After	Non-immune	<125	N/A	<125	<125	<125	6400±2191

**Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses ± standard deviations of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.*
†Serum samples obtained before challenge.
‡N/A, serum samples not obtained.
§IRS anti-DbpA titer - 1:2000
¶IRS anti-DbpB titer - 1:1000

tation." Organisms that have adapted to the host environment are known to upregulate some proteins and express de novo other proteins not found with either cultivated or tick adapted organisms. This property together with the known host-adapted downregulation of other proteins, including the major surface protein OspA, could conceivably make host-adapted organisms less susceptible to the protective effects of immune serum.

B burgdorferi-specific ELISA titers were also consistent with the response to challenge of the passively immunized rabbits. Active infection in the rabbits receiving NRS showed a 20-fold increase in anti-*B burgdorferi* antibody titers between the 216-hour (9-day) and 3-week post-challenge time points. By comparison, rabbits immune to challenge (IRS Before group) had a five-fold lower anti-*B burgdorferi* antibody titer at 3 weeks post-challenge as compared to the titer at 216 hours post-challenge, which is consistent with the clearance of spirochetes. As mentioned above, rabbits receiving IRS after challenge (IRS After group), which had delays in EM appearance but were shown to be infected, did not show an increase in titer but rather had a 2-fold decrease in titer between these same time points, again suggesting that passive transfer of immune serum given after challenge may have reduced the number of virulent organisms.

Anti-OspA antibody titers in protected rabbits (Before group) were relatively low during the course of passive immunization and following challenge (≤1:2400±894). We have previously reported that rabbits immunized with purified OspA and exhibiting antibody levels of 1:40000 were not completely protected against *B burgdorferi* challenge.[30] Barthold and colleagues[21,27] have also reported that active infection of mice induces a stronger protective humoral immune response than immunization with OspA, and that no correlation exists between the protective capability of immune serum and OspA antibody. Taken together, these findings support a conclusion that antibodies against OspA do not contribute to the protective response of passively transferred immune rabbit serum. The further observation that OspA antibody titers in infected rabbits from the IRS After group, were relatively low following challenge (≤1:2400±894) and did not increase comparable to the antibody titers against whole organisms, is consistent with the downregulation of OspA expression in rabbit host-adapted organisms similar to what has been observed in rodents.

Among passively immunized rabbits that were completely protected, titers of anti-OspC antibody at each time point following immunization and challenge were relatively low (≤1:125) and comparable to the normal sera titers (<1:125 to 1:475±335). Thus, the very low levels of OspC antibody detected suggest that OspC antibodies in immune rabbit serum used for passive transfer were not a major contributor to the protective immunity conferred. Alternatively, we cannot rule out at this time that this very low level of anti-OspC antibody was not a factor, although, several observations by other investigators showing that

immunization with OspC stimulates incomplete or no protection in mice using a homologous as well as heterologous challenge[22,28,31,32] indicate that OspC antibodies are not always protective.

The serum antibody titers against DbpA (peak titer; 1:300±112) and DbpB (peak titer; 275±205) among completely protected rabbits were also relatively low at the time of challenge (1:250 and 1:175±68, respectively) and comparable to the normal sera titers (<1:125). In addtion, these titers did not differ significantly from the basal sera levels (<1:125) during the entire 3-week observation period. These data, together with the observations by other investigators that active immunization of mice with DbpA does not result in complete protection against challenge with cultured or host-adapted *B burgdorferi*,[23,24,33,34] suggest that DbpA antibodies do not play a primary role in the protective immunity afforded by infection-derived immune rabbit serum.

In summary, we have demonstrated the ability of infection-derived immune rabbit serum to completely protect against homologous *B burgdorferi* challenge using cultured spirochetes. Our findings further suggest that antibodies present in infection-derived immune rabbit serum directed against target antigens other than OspA, and possibly OspC, DbpA, and DbpB are involved with this protection. The identification of antigens that are the targets for passive protection should provide molecules for new vaccine candidates.

ACKNOWLEDGMENTS

This work was supported by NIH grant AI-37312 to J.N. Miller and NIH grant AI-29733 to M.A. Lovett. We thank Dr. Ellen Shang for helpful suggestions to this study.

REFERENCES

1. Morbidity and Mortality Weekly Report. Lyme disease-United States, 1996. MMWR 1997;46:531-535.

2. Steere AC. Lyme disease. N Engl J Med 1989;321:586-596.

3. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. Ann Intern Med 1987;107:725-731.

4. Ackerman R, Rehse-Kupper B, Gollmer E, Schmidt R. Chronic neurologic manifestations of erythema migrans borreliosis. Ann NY Acad Sci 1988;539:16-23.

5. Garcia-Monco JC, Benach JL. The pathogenesis of Lyme disease. Rheum Dis Clin North Am 1989;15:711-726.

6. Logigan EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. N Engl J Med 1990;323:1438-1444.

7. Pachner AR, Duray P, Steere AC. Central nervous system manifestations of Lyme disease. Arch Neurol 1989;46:790-795.

8. Pachner AR, Steere AC. The triad of neurologic manifestations of Lyme disease: meningitis, cranial neuritis, and radiculoneuritis. Neurology 1985;35:47-53.

9. Schmidli J, Hunziker T, Moesli P, Schaad UB. Cultivation of Borrelia burgdorferi from joint fluid 3 months after treatment of facial palsy due to Lyme borreliosis. J Infect Dis 1988;158:905-906.

10. Stanek G, Klein J, Bittner R, Glogar D. Isolation of Borrelia burgdorferi from the myocardium of a patient with longstanding cardiomyopathy. N Engl J Med 1990;322:249-252.

11. Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease. N Engl J Med 1983;308:733-740.

12. Steere AC, Malawista SE, Hardin JA, Ruddy S, Askenase PW, Andiman WA. Erythema chronicum migrans and Lyme arthritis: the enlarging clinical spectrum. Ann Intern Med 1977;86:685-698.

13. 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.

14. Foley DM, Gayek RJ, Skare JT, et al. Rabbit model of Lyme borreliosis: erythema migrans, infection-derived immunity, and identification of Borrelia burgdorferi proteins associated with virulence and protective immunity. J Clin Invest 1995;96:965-975.

15. Barthold SW, de Souza MS, Janotka JL, Smith AL, Persing DH. Chronic Lyme borreliosis in the laboratory mouse. Am J Pathol 1993;143:959-971.

16. Goodman JL, Jurkovich P, Kodner C, Johnson RC. Persistent cardiac and urinary tract infections with Borrelia burgdorferi in experimentally infected Syrian hamsters. J Clin Microbiol 1991;29:894-896.

17. Preac-Mursic V, Patsouris E, Wilske B, Reinhardt S, Gross B, Mahraein P. Persistence of Borrelia burgdorferi and histopathological alterations in experimentally infected animals: a comparison with histopathological finding in human Lyme disease. Infection 1990;18:332-341.

18. Sonnesyn SW, Manivel JC, Johnson RC, Goodman JL. A guinea pig model for Lyme disease. Infect Immun 1993;61:4777-4784.

19. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 1984;57:521-525.

20. Schwan TG, Burgdorfer W, Schrumpf ME, Karstens RH. The urinary bladder: a consistent source of Borrelia burgdorferi in experimentally infected white-footed mice (Peromyscus leucopus). J Clin Microbiol 1998;26:893-895.

21. Barthold SW, deSouza M, Feng S. Serum-mediated resolution of Lyme arthritis in mice. Lab Invest 1996;74:57-67.

22. Bockenstedt LK, Hodzic E, Feng S, et al. Borrelia burgdorferi strain-specific Osp C-mediated immunity in mice. Infect Immun 1997;65:4661-4667.

23. Cassatt DR, Patel NK, Ulbrandt ND, Hanson MS. DbpA, but not OspA, is expressed by Borrelia burgdorferi during spirochetemia and is a target for protective antibodies. Infect Immun 1998;66:5379-5387.

24. Hanson MS, Cassatt DR, Guo BP, et al. Active and passive immunity against Borrelia burgdorferi decorin binding protein A (DbpA) protects against infection. Infect Immun 1998;66:2143-2153.

25. Schaible UE, Wallich R, Kramer MD, et al. Immune sera to individual Borrelia burgdorferi isolates or recombinant OspA thereof protect SCID mice against infection with homologous strains but only partially or not at all against those of different OspA/OspB genotype. Vaccine 1993;11:1049-1054.

26. Barthold SW. Specificity of infection-induced immunity among Borrelia burgdorferi sensu lato species. Infect Immun 1999;67:36-42.

27. Barthold SW, Bockenstedt LK. Passive immunizing activity of sera from mice infected with Borrelia burgdorferi. Infect Immun 1993;61:4696-4702.

28. Barthold SW, Feng S, Bockenstedt LK, Fikrig E, Feen K. Protective and arthritis-resolving activity in sera of mice infected with Borrelia burgdorferi. Clin Infect Dis 1997;25(1 Suppl):9S-17S.

29. Johnson RC, Kodner C, Russell M, Duray PH. Experimental infection of the hamster with Borrelia burgdorferi. Ann NY Acad Sci 1988;539:258-263.

30. Foley DM, Wang YP, Wu XY, Blanco DR, Lovett MA, Miller JN. Acquired resistance to Borrelia burgdorferi infection in the rabbit. Comparison between outer surface protein A vaccine- and infection-derived immunity. J Clin Invest 1997;99:2030-2035.

31. Cox DL, Akins DR, Bourell KW, Lahdenne P, Norgard MV, Radolf JD. Limited surface exposure of Borrelia burgdorferi outer surface lipoproteins. Proc Natl Acad Sci USA 1996;93:7973-7978.

32. Probert WS, Crawford M, Cadiz RB, LeFebvre RB. Immunization with outer surface protein (Osp) A, but not OspC, provides cross-protection of mice challenged with North American isolates of Borrelia burgdorferi. J Infect Dis 1997;175:400-405.

33. Feng S, Hodzic E, Stevenson B, Barthold SW. Humoral immunity to Borrelia burgdorferi N40 decorin binding proteins during infection of laboratory mice. Infect Immun 1998;66:2827-2835.

34. Hagman KE, Lahdenne P, Popova TG, et al. Decorin-binding protein of Borrelia burgdorferi is encoded within a two-gene operon and is protective in the murine model of Lyme borreliosis. Infect Immun 1998;66:2674-2683.