Characterization of the Protective Antibody Response to *Borrelia burgdorferi* in Experimentally Infected LSH Hamsters

J. L. SCHMITZ,^{1,2} R. F. SCHELL,^{1,2,3*} S. D. LOVRICH,² S. M. CALLISTER,^{3,4} AND J. E. COE⁵

Wisconsin State Laboratory of Hygiene^{1*} and Departments of Medical Microbiology and Immunology² and Bacteriology,³ University of Wisconsin, Madison, Wisconsin 53706; Gundersen Medical Foundation, LaCrosse, Wisconsin 54601⁴; and National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana 59840⁵

Received 18 December 1990/Accepted 15 March 1991

We show that serum obtained from normal hamsters infected with *Borrelia burgdorferi* can confer complete protection on irradiated recipients challenged with the Lyme spirochete. Borreliacidal activity was detected 7 days after infection, peaked at weeks 3 to 5, and thereafter decreased. Relatively high borreliacidal activity was detected in immune serum at weeks 3 and 5 of infection. The borreliacidal activity did not correlate with antibody used for the serodiagnosis of Lyme disease, which remained elevated throughout experimental infection. Our results also demonstrated that blocking antibody and antigenic variation in *B. burgdorferi* did not account for the decreasing titer of protective antibody. These findings indicate that protection against reinfection gradually wanes.

We showed previously that serum from uncompromised hamsters infected with *Borrelia burgdorferi* could prevent induction of Lyme arthritis in recipient irradiated hamsters challenged with the Lyme spirochete (27). The irradiated hamsters failed to develop swelling of the hind paws and showed no histologic evidence of arthritis, and spirochetes were not recovered from their tissues. In contrast, irradiated hamsters receiving normal hamster serum or saline developed swelling of the hind paws and had severe histologic changes in the tibiotarsal and knee joints. In addition, spirochetes were cultured from their tissues. This study clearly demonstrated that humoral immunity is important in protection against infection with *B. burgdorferi*.

Other studies (13, 17, 26) have also demonstrated an important role for antibody in protection against infection with *B. burgdorferi*. Johnson et al. (17) conferred protection on hamsters with rabbit anti-*B. burgdorferi* serum. Similarly, Schaible et al. (26) protected mice with severe combined immunodeficiency by passive immunization with immune serum and a monoclonal antibody to outer surface protein A (OspA) of *B. burgdorferi*. In addition, Fikrig et al. (13) passively immunized mice with rabbit or mouse serum and actively immunized mice with *Escherichia coli* expressing recombinant OspA. Collectively, these studies (13, 17, 26, 27) have firmly established that antibody is involved in protection against infection with *B. burgdorferi*.

Although infection with virulent *B. burgdorferi* (27) or vaccination with dead *B. burgdorferi* (18, 19) or its components (13) induces a protective humoral response, the kinetics and duration of the protective immune response are unknown. To address this issue, serum was collected from uncompromised normal hamsters infected with virulent *B. burgdorferi* for 1, 3, 5, 7, 10, 52, and 64 weeks. The immune serum and its dilutions were assayed for protection by passively immunizing irradiated hamsters and challenging with *B. burgdorferi*. We have shown previously (27) that the irradiated hamster model of Lyme arthritis is a rapid assay system to determine the efficacy of immune serum obtained from immunocompetent hamsters infected with the Lyme

spirochete. Two parameters can be examined within 10 days of passive immunization: severity of hind paw swelling and isolation of *B. burgdorferi* from tissues. In the present investigation, we used this model to characterize the kinetics and duration of the protective antibody response to *B. burgdorferi* in experimentally infected normal LSH hamsters.

MATERIALS AND METHODS

Animals. Inbred LSH/Ss Lak hamsters 6 to 8 weeks old were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Hamsters weighing 60 to 100 g were housed three or four per cage at an ambient temperature of 21°C.

Organism. *B. burgdorferi* 297 was obtained from Russell C. Johnson (University of Minnesota, Minneapolis). The strain was originally isolated from human spinal fluid (33) and has been maintained by passage in modified Barbour-Stoenner-Kelly medium (BSK) (6) and hamsters (17-21, 28).

The hamster-passed spirochetes were grown in BSK at 35°C for 5 days. The suspension of *B. burgdorferi* was adjusted with fresh BSK to contain approximately 10⁷ organisms per ml. Samples of 1 ml were then dispensed in vials, which were sealed and stored in liquid nitrogen until use.

Preparation of *B. burgdorferi* for infection of hamsters. A frozen vial containing a suspension of *B. burgdorferi* was thawed and used to inoculate fresh BSK. The culture was grown for 5 days at 31°C and diluted with BSK to contain 5 × 10⁶ organisms per ml. Quantitation of spirochetes was performed by using dark-field microscopy. Hamsters were infected subcutaneously in each hind paw with 0.2 ml of this suspension.

Irradiation of hamsters. Groups of hamsters were exposed to 600 rads of gamma radiation with a cobalt 60 irradiator (Picker Corp., Cleveland, Ohio). Hamsters survived this level of radiation without reconstitution with normal bone marrow cells. The cellularity of the bone marrow, which is normally greater than 90%, is reduced 1 week after irradiation to 25%. Cellularity and the ability of irradiated hamsters to respond with antibody production against *B. burgdorferi*

* Corresponding author.

are restored by weeks 3 and 1, respectively, after irradiation (15, 28).

Preparation of hamster serum. Groups of five or more LSH hamsters were injected subcutaneously in each hind paw with 0.2 ml of BSK containing 10^6 viable *B. burgdorferi*. At 1, 3, 5, 7, 10, 52, and 64 weeks after infection, hamsters were mildly anesthetized by inhalation of ether contained in a nose and mouth cup and bled by intracardiac puncture. The blood was allowed to clot, and the serum was separated by centrifugation at $500 \times g$, pooled, divided into 1-ml amounts, and frozen at -20°C until use. Concomitantly, pooled normal serum was obtained from noninfected normal hamsters.

Passive transfer of resistance. Three or four irradiated hamsters per group were injected intravenously in the sublingual vein with 0.4 ml of saline or normal or immune serum at 3-day intervals (days -3, 0, +3, and +6) for 9 days. In some experiments with undiluted serum, hamsters received fewer injections. Dilutions of immune serum (1:5, 1:10, 1:15, 1:20, 1:25, 1:30, and 1:40) were also administered to hamsters. Three days after the first injection (day 0) hamsters were irradiated and injected in each hind paw with 10^6 *B. burgdorferi*.

Assessment of arthritis. Swelling of the hind paws of irradiated hamsters was used to evaluate the inflammatory response to infection. The volume of each hind paw was measured with a plethysmograph (Buxco Electronics, Sharon, Conn.) on days 1 to 11 after challenge with *B. burgdorferi*. Measurements were obtained by lightly anesthetizing the hamsters and carefully dipping a hind paw into a column of mercury up to the ankle and recording the amount (milliliters) of mercury displaced. If mercury remained on the paws of hamsters, it was carefully removed with a mercury collector. The mean plethysmograph values were obtained from three or four hamsters (six or eight paws) and were used as an index of the severity of arthritic swelling. Mercury displacement was standardized with a volume calibrator. In some experiments the severity of hind paw swelling was measured with a micrometer.

Isolation of *B. burgdorferi* from tissues. Thirteen days after infection, hamsters were killed by inhalation of carbon dioxide. The spleen and urinary bladder were removed aseptically and homogenized separately with 1 ml of BSK in a sterile petri dish. The tissue suspensions were then inoculated into 4 ml of BSK and incubated at 31°C . Cultures were monitored weekly for spirochetes by using dark-field microscopy.

Determination of levels of antibody to *B. burgdorferi*. Anti-*B. burgdorferi* antibody levels in hamster sera were determined with an enzyme-linked immunosorbent assay (ELISA; Whittaker Bioproducts, Inc.) (35). The test was performed as specified by the manufacturer, except that pooled serum from infected or control hamsters was diluted 1:32 and rabbit anti-hamster immunoglobulin G (IgG) was used. Briefly, microtiter wells coated with a sonic extract of *B. burgdorferi* were washed and incubated with 100 μl of pooled serum for 15 min. Wells were washed, and 100 μl of alkaline phosphatase-conjugated rabbit anti-hamster IgG (light and heavy chains; Jackson ImmunoResearch Laboratories, West Grove, Pa.) diluted 1:10,000 was added. After 15 min, the wells were washed and 100 μl of substrate (phenolphthalein monophosphate) was added. The reaction was stopped with 200 μl of trisbasic sodium phosphate, and the A_{550} was read on a Bio-Tek EL-308 enzyme immunoassay reader (Bio-Tek Instruments, Inc., Burlington, Vt.). Determinations were performed in duplicate, and the results were averaged.

Quantitation of immunoglobulins. A radial gel diffusion assay was used to quantitate levels of IgG1, IgG2, and IgM in sera from normal and *B. burgdorferi*-infected hamsters. Rabbit antisera to Syrian hamster IgG1, IgG2, and IgM were used in these assays and prepared as previously described (7-9). Purified hamster immunoglobulin preparations were used as standards.

In vitro immobilization of *B. burgdorferi*. Normal and immune (3, 10, and 52 weeks after infection) hamster sera were heat inactivated at 56°C for 45 min and serially diluted in BSK. A 3-day culture of *B. burgdorferi* was diluted to approximately 6×10^6 organisms per ml, and 100- μl aliquots were added to 1.5-ml microcentrifuge vials containing 100 μl of the heat-inactivated, diluted antisera. Sterile guinea pig serum (50 μl) with a complement activity of 220 50% hemolytic complement units per ml was added, yielding final antiserum dilutions of 1:160, 1:320, 1:640, 1:1,280, and 1:2,460. A second culture of *B. burgdorferi* was incubated for 30 min with 10- and 52-week immune sera at final dilutions of 1:320 and 1:160, respectively. These spirochetes were then added to another set of vials containing 3-week immune serum, yielding the dilutions described above.

All assays were mixed by hand and incubated at 31°C for 6 h. After incubation, 10- μl aliquots were removed in triplicate and the total numbers of live and dead spirochetes were determined. Twenty-five random fields of each 10- μl aliquot were read at $\times 400$ with dark-field microscopy. Live spirochetes were defined as those that were motile.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblotting. A culture of *B. burgdorferi* was grown at 31°C for 3 to 5 days. The spirochetes were centrifuged at $10,000 \times g$ and washed two times in sterile phosphate-buffered saline (PBS). The protein concentration of the resulting suspension was determined with a commercially available kit (Bio-Rad Inc., Richmond, Calif.). The spirochete suspension was boiled in sample buffer for 5 min; then 150 μg of total protein was loaded onto a 10% polyacrylamide gel (4% polyacrylamide stacking gel without comb). Two gels were run simultaneously in a Hoefer SE600 electrophoresis unit (Hoefer Scientific Instruments, San Francisco, Calif.) at 55 mA for 3 h with the buffer system of Laemmli (22). After electrophoresis, proteins were transferred (Hoefer Scientific Instruments) to nitrocellulose for 3 h at 300 mA under conditions similar to those of Towbin et al. (34). The nitrocellulose was cut into strips, which were blocked for 20 min at 22°C in PBS-0.3% Tween 20. Strips were incubated with pooled hamster sera diluted 1:60 or monoclonal antibodies to OspA (H5332) or the flagellin protein (H9724) for 1 h at 22°C . The monoclonal antibodies were obtained from Alan Barbour, University of Texas, San Antonio. Strips were washed three times with PBS-0.05% Tween 20. Peroxidase-labeled anti-hamster IgG (heavy and light chains; Kirkegard and Perry Labs Inc., Gaithersburg, Md.) diluted 1:750 in PBS-0.05% Tween 20 or anti-mouse IgG (Organon Teknika Corp., Westchester, Pa.) diluted 1:10,000 was then added to strips and incubated for 30 min at 22°C . Strips were washed as above and developed with the TMB Membrane Peroxidase Substrate System (Kirkegard and Perry). The developed strips were washed with distilled water and photographed.

Statistical analysis. The plethysmograph values obtained from irradiated hamsters were tested by analysis of variance. The Fisher least-significant-difference test (32) was used to examine pairs of means when a significant *F* ratio indicated

FIG. 1. Antibody responses detected by ELISA in hamsters infected with *B. burgdorferi* for 1, 3, 5, 7, 10, and 52 weeks (■) and in noninfected hamsters (○).

reliable mean differences. The alpha level was set at 0.05 before the experiments were started.

RESULTS

Antibody response to *B. burgdorferi*. Pooled serum from normal hamsters infected with *B. burgdorferi* for 1, 3, 5, 7, 10, and 52 weeks was tested for antibody to *B. burgdorferi* by using an ELISA (Fig. 1). *B. burgdorferi* specific antibody was first detected 1 week after infection, peaked at weeks 5 and 7, and remained elevated to week 52. No antibody activity was detected in pooled serum from normal, noninfected hamsters.

Immunoblots were also performed (Fig. 2). At week 1, serum contained antibodies to proteins of approximately 31, 34, 39, and 46 kDa. By week 5, the antibody response also recognized proteins of approximately 15, 17, 19, 22, 52, 63, 72, 85, 102, and 105 kDa. These proteins remained detectable 10 and 52 weeks after infection (data not shown).

FIG. 2. Immunoblots of hamster sera from noninfected hamsters (lane 1) and hamsters infected for the following (lanes): 2, 1 week; 3, 3 weeks; 4, 5 weeks; 5, 7 weeks; 6, 10 weeks. Lane 7 is a blot reacted with monoclonal antibodies to OspA (31 kDa) and flagellin (41 kDa) proteins.

TABLE 1. Effect of immune serum and its dilutions on induction of arthritis in recipient hamsters challenged with *B. burgdorferi*

Serum and dilution	Arthritis ^a in hamsters at the following times (weeks) after infection:					
	1	3	5	7	10	52
Immune						
Undiluted	0/3	0/4	0/4	0/4	0/4	0/4
1:5	3/4	0/4	0/4	0/4	0/4	3/3
1:10	4/4	0/4	0/4	0/4	0/4	4/4
1:15		0/3	0/4	1/3	2/4	
1:20		0/3	0/3	4/4	4/4	
1:25		0/4				
1:30		1/3				
1:35		3/4				
Normal (undiluted)	4/4	4/4	4/4	4/4	4/4	4/4

^a Data presented as number of hamsters with inflamed hind paws/number of hamsters per group. Swelling of paws was considered significant when a plethysmograph value greater than or equal to 0.60 was obtained. Normal and noninfected irradiated hamsters consistently (100%) gave a plethysmograph value of less than or equal to 0.45.

Development, quantitation, and duration of borreliacidal antibody by transfer of humoral immunity. Hamsters in 29 groups of three or four hamsters each were injected with undiluted or dilutions of serum obtained from hamsters at various intervals after infection. Hamsters in six groups of four hamsters each received normal serum. Subsequently, all recipients were challenged with *B. burgdorferi*.

In all hamsters receiving undiluted immune serum (1, 3, 5, 7, 10, and 52 weeks), swelling of the hind paws was prevented (Table 1). Diluting 1-week serum 5-fold, 3-week serum 30-fold, and 7- and 10-week sera 15-fold abrogated the ability of the sera to prevent the induction of arthritis. A similar response was observed when recovery of *B. burgdorferi* from tissues of these passively immunized hamsters was used to assess borreliacidal activity (Table 2). Week 1 serum lost borreliacidal activity when diluted fivefold. Peak activity was detected with week 3 and 5 sera, which could be diluted 20-fold. Thereafter, borreliacidal activity waned.

TABLE 2. Effect of immune serum and its dilutions on recovery of *B. burgdorferi* from tissues^a of recipient hamsters challenged with *B. burgdorferi*

Serum and dilution	Recovery of <i>B. burgdorferi</i> ^b at the following times (weeks) after infection:					
	1	3	5	7	10	52
Immune						
Undiluted	0/3	0/4	0/4	0/4	0/4	0/4
1:5	2/4	0/4	0/4	0/4	0/4	3/3
1:10	4/4	0/4	0/4	1/4	3/4	4/4
1:15		1/3	0/4	3/3	3/4	
1:20		0/3	0/3	4/4	3/4	
1:25		3/4				
1:30		3/3				
1:35		4/4				
Normal (undiluted)	4/4	4/4	4/4	4/4	4/4	4/4

^a Spleen and urinary bladder tissues were cultured in BSK for 3 weeks at 31°C. If one or both tissues grew *B. burgdorferi*, the hamster was considered infected.

^b Data presented as number of hamsters demonstrating growth of *B. burgdorferi*/number of hamsters per group.

TABLE 3. Concentration of immunoglobulins in hamsters infected with *B. burgdorferi*

Serum ^a and week after infection	Immunoglobulin level (mg/ml)		
	IgG1	IgG2	IgM
IHS			
1	0.30	2.18	1.04
3	0.42	2.96	1.74
5	0.43	3.55	1.39
7	0.15	2.18	1.04
10	0.30	2.67	0.54
52	3.16	2.38	0.54
NHS	0.85	2.57	0.73

^a IHS, immune hamster serum; NHS, normal hamster serum.

When these experiments were repeated, similar results were obtained.

Quantitation of IgG1, IgG2, and IgM in serum from infected hamsters. The amount of IgM was elevated at week 1, peaked (1.74 mg/ml) at week 3, and thereafter decreased (Table 3). The level of IgG2 increased at week 3, peaked (3.55 mg/ml) at week 5, and then decreased. The amount of IgG1 was decreased at all intervals after infection, except week 52, compared with the amount in the control serum (0.85 mg/ml).

Failure to detect blocking antibodies. At 3 and 10 weeks, serum titers for immobilization activity were determined (Table 4) to ascertain whether the decrease in borreliacidal activity after the third week of infection was due to blocking antibodies. Table 4 shows that immobilization activity was lost in 3- and 10-week sera diluted 1:1,280 and 1:640, respectively. When viable (100% motile) *B. burgdorferi* organisms were incubated with 10-week heat-inactivated serum diluted 1:320 and then incubated with 3-week heat-inactivated serum and complement, no decrease in immobilization titer of 3-week serum was detected. In fact, a slight increase in immobilization was detected with spirochetes treated with 3- and 10-week sera diluted 1:320. No decrease in immobilization titer of the 3-week serum was detected when this assay was repeated with 52-week serum (data not shown).

Antigenic variation of *B. burgdorferi*. *B. burgdorferi* was

TABLE 4. Ability of serum obtained from hamsters infected for 10 weeks to prevent immobilization of *B. burgdorferi* by serum from hamsters infected for 3 weeks

Dilution of serum	No. of mobile spirochetes ^a at the following times (weeks) after infection		
	3	10	3+10 ^b
1:320	10 ± 1	267 ± 12	0
1:640	183 ± 6	395 ± 10	164 ± 14
1:1,280	400 ± 6	388 ± 7	327 ± 11
1:2,560	399 ± 6	ND ^c	394 ± 11
NHS ^d	404 ± 7		

^a Data are presented as the number of motile spirochetes/25 high-power fields ± the standard error.

^b Spirochetes incubated with heat-inactivated serum obtained from hamsters infected for 10 weeks were then added to 3-week immune serum containing complement.

^c ND, not determined.

^d NHS, normal hamster serum.

FIG. 3. Swelling of hind paws of hamsters passively immunized with normal (—) or immune (---) serum and challenged with the original isolate (squares) or the 10-week (circles) or 64-week (triangles) isolate of *B. burgdorferi*.

isolated from the spleens of nonirradiated infected hamsters 10 and 64 weeks after infection with *B. burgdorferi*. These isolates were used as challenge strains to determine whether changes in protective epitopes occurred during the course of infection. The two isolates were grown once in BSK and injected into irradiated hamsters passively immunized with serum obtained from normal hamsters infected for 1 week with the original *B. burgdorferi* isolate. Figure 3 shows that 1-week serum prevented induction of arthritis by the 10- and 64-week isolates compared with that in the normal serum controls.

DISCUSSION

The results of this investigation confirmed our previous findings (27) that serum obtained from uncompromised, normal hamsters infected with *B. burgdorferi* can confer complete protection on irradiated recipients challenged with the Lyme spirochete. Relatively high levels of borreliacidal antibody were produced during early infection and gradually decreased. The borreliacidal activity did not correlate with antibody detected with the ELISA, which is the most commonly used test for the serodiagnosis of Lyme disease (2, 5, 11, 14, 23, 25). These findings suggest that protection against reinfection gradually wanes.

The humoral protective response developed rapidly in *B. burgdorferi*-infected hamsters. Undiluted immune serum from hamsters infected for 7 days conferred complete protection on recipients against challenge with *B. burgdorferi*. By weeks 3 and 5, immune serum possessed considerable borreliacidal activity even when diluted 20-fold. Thereafter, the protective capacity of immune serum gradually declined, although borreliacidal activity was still detected 52 weeks after infection. Levels of ELISA antibody also peaked 5 weeks after infection; however, in contrast to protective antibody, they remained elevated throughout the course of infection. Similarly, immunoblots of infected hamster sera demonstrated that the antibody response continued to expand against borreliacidal antigens during the course of infection without loss of activity to proteins during the period of decreased borreliacidal activity. Similar results have been

shown in syphilis (4), where diagnostic antibodies and protective antibodies do not correlate.

These results are important. The decline in protective levels of antibody might allow reinfection with *B. burgdorferi* (16, 36). Although infected normal hamsters possessed protective antibody at week 52 of infection, a complete loss of activity may occur with increasing time after infection. Johnson et al. (18, 19) also showed that protection significantly waned 90 days after vaccination with a whole-cell preparation of *B. burgdorferi*. These results suggest that a more transient protective antibody response occurs under natural conditions. The inoculum of *B. burgdorferi* acquired from an infected *Ixodes dammini* tick would be considerably less than the 2×10^6 viable *B. burgdorferi* we used to infect hamsters and the 50 to 100 μ g (dry weight) Johnson et al. (18, 19) used for vaccination.

The mechanism(s) by which protective antibody decreases after infection with *B. burgdorferi* is not clear. However, several explanations could be proposed. First, the presence of a small number of protective epitopes on *B. burgdorferi* may be responsible. As the number of spirochetes decreases during the course of infection, there would be a corresponding decrease in the amount of protective antigen(s) stimulating the production of borreliacidal antibodies. We have shown previously (15) that by weeks 3 and 5 after infection of normal hamsters the number of spirochetes in the synovium, periarticular soft tissues and perineurovascular areas is greatly diminished. In addition, the acute inflammatory reaction in the hind paws of hamster is replaced by a mild chronic synovitis. It is during this period that the protective antibody response begins to decrease.

A second explanation for the decrease in protective antibody may be the development of blocking antibodies. It is known that hamster IgG1 has a strong affinity for hamster macrophages (24) and does not fix complement (10). IgG1 also has been shown to prevent phagocytosis of sheep erythrocytes coated with hamster IgG2 (24). IgG1 could act as a blocking antibody by preventing complement-mediated killing of IgG2-coated *B. burgdorferi*. This explanation, however, is not supported by the present findings. When spirochetes were incubated with 10-week serum possessing low borreliacidal activity, the serum failed to abrogate or reduce the immobilization titer of 3-week serum possessing maximum borreliacidal activity. In addition, serum obtained 52 weeks after infection, which had a markedly increased level (3.16 mg/ml) of IgG1, also failed to decrease the borreliacidal titer of 3-week immune serum.

Another explanation for the decreasing protective antibody levels could be antigenic variation in the protective epitopes of *B. burgdorferi*. Antigenic variation is known to enable *Borrelia hermsii* to survive in infected individuals (1, 3). Antigenic and plasmid profile changes have been noted during cultivation of *B. burgdorferi* (29, 30); however, no evidence of a functional role for antigenic variation has been reported. Our results suggest that *B. burgdorferi* does not use antigenic variation as a means of surviving in infected hamsters. One-week immune serum protected hamsters from developing arthritis after challenge with isolates recovered from hamsters 10 and 64 weeks after infection. One would expect to see changes in protective epitopes during this interval of infection. We cannot, however, exclude the possibility that a single passage of these isolates in BSK resulted in reversion of the new epitope back to the protective epitope of the original infecting isolate. The simultaneous reversion of these isolates to the original epitope seems unlikely though. When we performed sodium dodecyl

sulfate-polyacrylamide gel electrophoretic analysis of the isolates, no changes in protein profiles were detected among the isolates.

A fourth explanation may be that the protective response is due mainly to the production of IgM. The rapid onset and gradual decline in protection suggest a classic IgM response. This proposal is supported by the finding of elevated total IgM levels during the first 5 to 7 weeks of infection. However, we also observed that IgG2 levels peaked at week 3 of infection and then declined. Taken together, these findings would suggest that the early protective response is due mainly to IgM, with IgG2 contributing at week 3 of infection. Although Schwan et al. (31) did not evaluate the role of immunoglobulins in protection, they also detected a rapid IgM response, followed by sustained levels of IgG in white-footed mice.

The inability of hamsters to eliminate *B. burgdorferi*, despite the development of an effective antibody response, is an enigma. We (15) and Duray and Johnson (12) have shown that spirochetes are sequestered in host tissues that may not be readily accessible to protective antibody. The ability of *B. burgdorferi* to hide in immunologically privileged sites may account for the waning of protective antibody. Once the level of antibody has decreased, the host is susceptible to reinfection and the remitting course of Lyme disease. One might argue that the host can make a rapid antibody response and thus eliminate indigenous or exogenous spirochetes. However, Johnson et al. (20) demonstrated that immune serum administered after infection failed to protect hamsters from challenge with *B. burgdorferi*. This suggests that the Lyme spirochete possesses mechanisms to protect it from an effective antibody response. These might include an amorphous slime layer (3) or shedding of protective epitopes. The 31-kDa protein (OspA) is a candidate because of its location in the envelope of *B. burgdorferi* (3). Constant or transient shedding of OspA might neutralize protective antibody.

In conclusion, we have shown that antibody-mediated immunity is involved in protection against *B. burgdorferi*; however, the protective antibody response wanes. Additional work needs to be performed to determine whether the kinetics of the protective antibody response accurately reflects immune status and is a prognostic indicator of effective therapy. Since the presence of borreliacidal antibody correlates with resolution of active disease, isolation of these protective immunoglobulins would be helpful in defining which antigen(s), in addition to OspA (13), induce protection. Identification of protective antigens is important for the development of a vaccine for Lyme disease.

ACKNOWLEDGMENTS

We thank Ruth West and Lisa Friess for their expert technical assistance. This work was supported by Public Health Service grant AI-22199 from the National Institute of Allergy and Infectious Diseases.

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