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Variation in Antigenicity and Infectivity of Derivatives of *Borrelia burgdorferi*, Strain B31, Maintained in the Natural, Zoonotic Cycle Compared with Maintenance in Culture

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The original isolate of *Borrelia burgdorferi*, strain B31, can be maintained in vitro indefinitely. A number of studies have demonstrated that there are recognizable changes in the genetic composition of the spirochete after more than 60 passages. We have maintained B31 in the natural zoonotic cycle of transmission of infection between laboratory mice and laboratory-reared *Ixodes* ticks. To determine whether similar changes occur in the natural transmission cycle, we reisolated strain B31 from mouse skin at the fifth zoonotic cycle. This reisolated derivative had the same infectivity as the parent B31 strain, had lost the 8-kb supercoiled plasmid present in B31, and induced a gross serum antibody response indistinguishable from the B31 immune response. Analysis of antigen expression with monoclonal antibodies generated against B31, however, showed differential expression of a subset of antigens between B31 and the reisolated derivative.

The infectivity and pathogenicity of the Lyme disease spirochete *Borrelia burgdorferi* maintained in vitro differ significantly from those of fresh isolates. We and others have compared the differences in immunogenicity, infectivity, and genetic profile between low- and high-passage derivatives. High-passage derivatives of the strains analyzed tend to lose plasmids after extensive culture in vitro, and such derivatives often lose infectivity (18). Antisera from tick-infested animals fail to detect many antigens expressed by high-passage derivatives (7), and a number of specific antigenic differences between low-passage and high-passage derivatives of the same strain, B31, have been reported (12). Data from other laboratories also describe antigenic variation in the spirochete during chronic infection in mice (2, 5, 19), as well as the low frequency of genetic variation in these mice (14).

Strain B31 of *B. burgdorferi* has been maintained in a zoonotic cycle of infection in laboratory mice and a laboratory colony of ticks for a total of five cycles of infection (15). Considering the data about strain variation in vivo and in vitro, we hypothesized that there might be a difference between the original low-passage B31 derivative and the strain of B31 that was present in the infected tick colony, since this laboratory zoonotic cycle had been maintained for five rounds of infection in mice and ticks. We were interested in whether the natural transmission cycle was less permissive of genetic and antigenic variation than chronic infection or in vitro cultivation.

To investigate this question, we isolated *B. burgdorferi* B31 from an ear punch biopsy of an outbred mouse infected in the fifth zoonotic cycle since the initial introduction of strain B31 into laboratory-reared ticks. Here, we present data comparing the parent strain, B31 low (passage 6), with the reisolated strain (B31 Ri-1), as well as the common high-passage laboratory strain of B31 obtained from the American Type Culture Collection, B31 high (passage greater than 60) (12). This study revealed multiple differences between the two low-passage derivatives of B31 and the high-passage strain, as well as more subtle differences between the two low-passage derivatives. The significance of changes in the spirochete under these two different methods of maintenance and the effects of the changes on laboratory studies are discussed.

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MATERIALS AND METHODS

Development and maintenance of the B31-infected *Ixodes scapularis* tick colony. The *I. scapularis* ticks used in these experiments came from a colony that was originally started from adult ticks that were flagged on Great Island, Mass., in 1984 and 1985 (15). The colony was determined to be free of spirochetes by examination of egg batches after oviposition by fluorescent antibody microscopy (16). This colony served as our source for generating infected ticks. Strain B31 (low) of *B. burgdorferi* was obtained from Alan Barbour (University of Texas Health Science Center at San Antonio, San Antonio, Tex.). The lowest passage available, passage 6 of this culture, was inoculated into 3- to 6-week-old male mice from a breeding colony of Institute for Cancer Research (Philadelphia, Pa.) outbred mice maintained at the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colo.

At 4 weeks after infection, larval ticks from the uninfected colony were allowed to feed to repletion on known infected mice. Approximately 500 replete larvae were collected from each mouse and placed in 8-ml mesh-covered glass vials at 50 ticks to a vial. Each vial contained a mixture of plaster of Paris and charcoal in the bottom. The ticks were kept in a bioclimatic chamber at 97% humidity at 21°C and with a photoperiod of 16 h of daylight.

Once larvae molted to nymphs, five nymphs from each of the mice were picked at random. The midgut was dissected and examined by dark-field and fluorescent antibody microscopy (16). The infection rate observed was between 50 and 70% (16). Subsequently, mice were infected by infestation with these nymphs. Ten to 30 nymphs were placed on each mouse and allowed to feed to repletion. An ear punch biopsy was taken from each mouse 4 weeks after repletion. The biopsy was placed in BSKII medium and assayed for infection as described previously (21). Mice that were culture positive were then used to infect the next group of larval ticks. The infection rate in these ticks after molting to nymphs was 70 to 100%. An infected tick colony was maintained by a zoonotic cycle of ticks transmitting the spirochete to mice and then having uninfected ticks feed on the infected mice; this represented one cycle. This colony had been maintained through five cycles at the initiation of this study, and the infection rate in nymphs generated by this protocol is routinely between 90 and 95%.

Assay for infectivity. Relative infectivity was determined by needle inoculation of spirochetes at the indicated dosages into ICR outbred mice. Aliquots of spirochetes were thawed into 8.0-ml cultures in BSK-H medium (Sigma Chemical Co., St. Louis, Mo.) and harvested during log-phase growth. Spirochetes were counted by dark-field microscopy at ×100 magnification on a Zeiss Askioskop microscope (Carl Zeiss, Inc., Thornwood, N.Y.) calibrated with a Petroff-Hauser chamber. Dilutions were prepared for injection intradermally in a volume of 0.1 ml of BSK-H medium. Mice aberrantly injected subcutaneously rather than intradermally were discarded.

Ear punch biopsies were taken as described previously (21) 28 days after inoculation. Serial bleeds were taken at 15-day intervals, starting with prebleeds on the day of inoculation through 60 days after injection.

Pulsed-field gel electrophoresis. The plasmid profiles of the various derivatives of *B. burgdorferi* B31 were analyzed by pulsed-field electrophoresis. Samples of genomic DNA were prepared from spirochetes by pelleting 5×10^8 bacteria, washing them with cold 10 mM Tris–150 mM NaCl (Tris-saline), and resuspending them in 0.5 ml of Tris-saline. An equal volume of 2% low-melting-point agarose (Bio-Rad Laboratories, Richmond, Calif.) in Tris-saline was added, and plugs were cast in sample molds holding a volume of 0.1 ml. Agarose plugs were cooled at 4°C, removed from the molds, and then incubated in lysis buffer (10 mM Tris, 150 mM NaCl, 20 mM EDTA, 0.1% sodium dodecyl sulfate [SDS]) for 2 h with tumbling at room temperature. Plugs were then washed in 0.5× Tris-borate-EDTA with two changes and stored in 0.5× Tris-borate-EDTA at 4°C.

Genomic DNA was separated on a CHEF Mapper (Bio-Rad, Richmond, Calif.) in 0.5× Tris-borate EDTA at a constant temperature of 14°C. The autoalgorithm for separation was set for a range of 5 to 55 kb, that is a gradient of 6 V/cm, an initial switch interval of 0.14 s, a final switch interval of 2.89 s, and an included angle of 120° with a linear ramping factor. Gels were stained with ethidium bromide and were photographed under UV light.

Purification of *B. burgdorferi* genomic DNA and agarose gel electrophoresis. Cultures of *B. burgdorferi* cells were grown in BSK-H medium to late log phase (approximately 8 ml of 10^8 spirochetes per ml). The bacteria were pelleted and washed with phosphate-buffered saline (PBS) and then resuspended in 1 ml of 50 mM Tris (pH 8.0)–50 mM EDTA. Fresh lysozyme solution was prepared at 10 mg/ml in 0.25 M Tris (pH 8.0), and 100 μl was added to the cell suspension, mixed, and placed on ice for 45 min. The cell suspension was then incubated with 200 μl of STEP solution (0.5% SDS, 50 mM Tris [pH 7.5], 100 mM EDTA, 1 mg of proteinase K per ml) and incubated at 50°C for 60 min with occasional mixing. RNase A was added to a final concentration of 200 μg/ml, and the suspension was incubated at 37°C for 30 min. The DNA was extracted twice with Tris-buffered phenol and precipitated with 3 M sodium acetate in ethanol, dried, and resuspended in 10 mM Tris–1 mM EDTA.

Approximately 2 to 4 μg of DNA was subjected to electrophoresis in 0.4% agarose–Tris–acetate–EDTA in a horizontal mini-gel chamber. The gel was run slowly at 10 V of constant voltage overnight. Gels were then stained with ethidium bromide and photographed under UV light.

Protein profiles. The profiles of proteins expressed by the three derivatives of strain B31 were assayed by SDS-polyacrylamide gel electrophoresis (PAGE). Briefly, lysates of spirochete cultures were prepared by harvesting bacteria at late log phase (10^8 spirochetes per ml), washing them in PBS–5 mM $MgCl_2$, and then lysing them in a Dounce homogenizer in a volume of 1 ml of PBS-$MgCl_2$ per 100 μg of bacterial pellet. Lysates were heated at 100°C for 5 min and assayed for protein concentration. Proteins were separated by SDS-PAGE (10% polyacrylamide); 10 to 15 μg of protein was loaded in each well. Gels were stained with Coomassie blue and subsequently destained.

Western blot (immunoblot) assays. The serum antibody responses, as well as monoclonal antibodies of interest, were assayed by Western blotting as previously described (7). Samples were run on SDS-PAGE (10% polyacrylamide) preparatory gels at a concentration of 100 μg of protein per gel. Proteins were electrophoretically transferred to nitrocellulose filters, and filters were incubated in blocking solution overnight. The filters were then loaded into slot blotters (Immunetics, Inc., Cambridge, Mass.) and assayed with the designated source of anti-*B. burgdorferi* antibody at a final dilution of 1:100. The detection system was a colorimetric assay based on alkaline phosphatase conjugated to anti-mouse immunoglobulin G (IgG) plus IgM (Kirkegaard & Perry, Bethesda, Md.).

Monoclonal antibodies. The monoclonal antibodies used in this study were generated from BALB/c or C57Bl/10 mice immune to *B. burgdorferi* B31 as a result of tick transmission of infection. These mice were cured with antibiotics (tetracycline at 1 mg/ml in the drinking water), rested for 6 weeks, and given a booster with a second tick infestation. Spleens were harvested at either 5 or 7 days after infestation and fused to a BALB/c B-cell lymphoma, P3X63Ag8.653, with polyethylene glycol in a standard protocol. Primary hybridomas were screened for reactivity with strain B31 by Western blotting. Hybridomas of interest were cloned by limiting dilution. A total of 10 hybridomas were cloned, and of these, 9 are of the IgM isotype. When these antibodies are used in Western blot assays, an anti-mouse IgM reagent is used as the secondary antibody (9).

RESULTS

To analyze the genetic and immunogenic nature of the derivative of *B. burgdorferi* B31 being maintained in the zoonotic cycle in our laboratory, we studied the test culture from an ear punch biopsy of an outbred mouse infected in the fifth zoonotic cycle of transmission. This passage 0 culture was harvested at log phase, and aliquots were frozen as source material for further study.

Infectivity of the derivatives of B31. The relative infectivity of these different derivatives of strain B31 was determined by the 50% infectious dose (ID_{50}) by intradermal inoculation of spirochetes. As has been reported previously (18), the high-passage B31 isolate is noninfectious (Table 1). Over the course of three experiments, the two low-passage derivatives of B31 had comparable ID_{50}s of between 10^2 and 10^3 spirochetes. Because these low-passage strains tend to aggregate, the linearity of the titration curve is compromised at these low doses. Within the limitations of this test, B31 low and B31 Ri-1 were equally infectious.

TABLE 1. ID_{50} of the three derivatives of *B. burgdorferi* B31 by intradermal inoculation

No. of spirochetes inoculated	No. of mice infected in:						
	Expt 1			Expt 2		Expt 3	
	B31 high	B31 low	B31 Ri-1	B31 low	B31 Ri-1	B31 low	B31 Ri-1
10^8	0 of 4	ND[a]	ND	ND	ND	ND	ND
10^7	0 of 4	ND	ND	ND	ND	ND	ND
10^6	0 of 4	4 of 4	4 of 4	ND	ND	ND	ND
10^5	ND	4 of 4	4 of 4	ND	ND	ND	ND
10^4	ND	4 of 4	4 of 4	4 of 4	4 of 4	4 of 4	4 of 4
10^3	ND	2 of 4	4 of 4	4 of 4	3 of 4	4 of 4	4 of 4
10^2	ND	0 of 4	2 of 4	3 of 4	1 of 4	1 of 4	0 of 4
10^1	ND	ND	ND	0 of 4	0 of 4	0 of 4	0 of 4

[a] ND, not done.

Genetic analysis of the derivatives of strain B31. Pulsed-field gel electrophoresis was performed with the three derivatives of B31 under study to compare the plasmid profiles of these isolates. As has been reported previously, there are multiple plasmids in the range of 8 to 49 kb. These include at least three linear plasmids with sizes of 16, 29, and 49 kb, as well as 8-, 20-, and 28-kb supercoiled, circular plasmids (10, 11, 18, 20). Comparison of the three B31 derivatives showed that high-passage B31 lost a number of genetic elements, including plasmids in the 20- to 40-kb range and the small 8-kb supercoiled plasmid (Fig. 1A). Interestingly, the reisolate of B31 lost genetic elements even when maintained in the natural zoonotic cycle. Results in Fig. 1 show that the reisolate lost the 8-kb supercoiled, circular plasmid compared with the original B31 low isolate.

In our experience, as well as the experience of others (19, 20), pulsed-field gel electrophoresis is inappropriate for analysis of the supercoiled plasmids of *B. burgdorferi* because these are often trapped in the sample wells of the pulsed-field gels. This subset of plasmids is more accurately identified in low-percentage-agarose gels. To confirm the results in Fig. 1A, we prepared genomic DNA from the three derivatives of strain B31 for analysis by this alternative method. Figure 1B shows a more complex banding pattern in the lower-molecular-size range than the pulsed field gel, indicating the supercoiled plasmids were accurately represented. Here, the 8-kb plasmid, which migrates between the 4- and 6-kb molecular size markers as a supercoiled, circular plasmid, was absent in the reisolate. The double bands are likely to be pBBC1 and pBBC2, described by Simpson et al. (20). Given that the lane loaded with B31 Ri-1 was overloaded relative to B31 low, this lack of expression was not likely a result of concentration differences.

These results suggest that nonchromosomal genetic elements of *B. burgdorferi* sensu stricto can be lost during zoonotic propagation, as well as during extensive in vitro culturing. Another possibility is that the 8-kb plasmid was not lost, but rather was located within a different genetic element, although

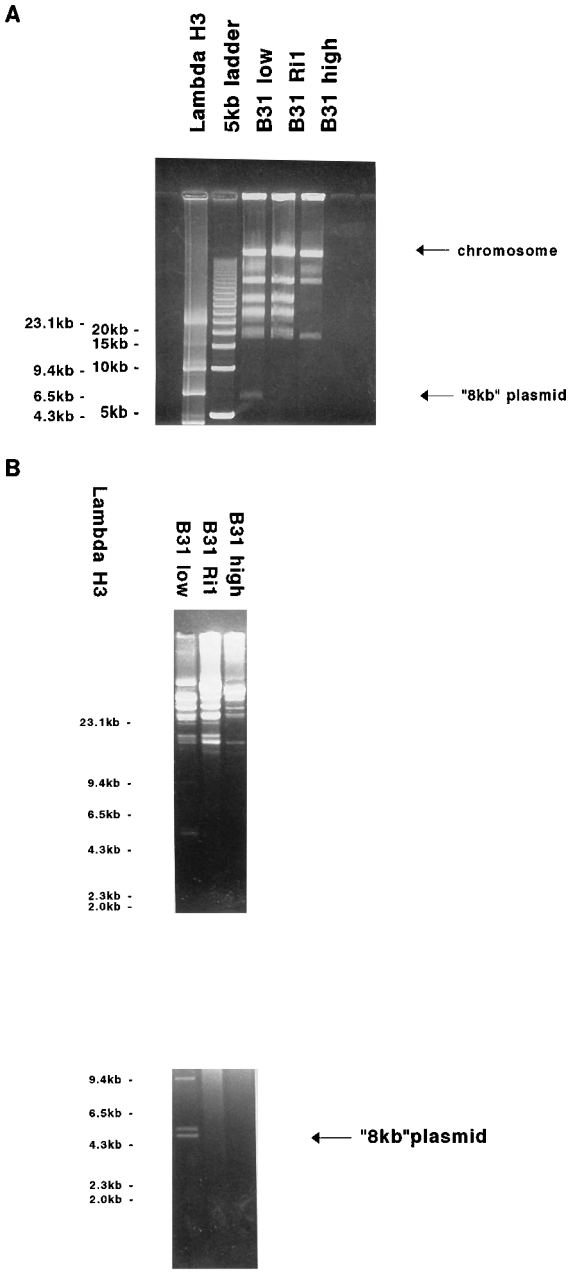


FIG. 1. (A) Pulsed-field gel electrophoresis of whole, genomic DNA prepared from the three derivatives of *B. burgdorferi* B31. Arrows indicate the migration of the chromosomal DNA as well as that of the 8-kb plasmid. (B) Genomic DNA from the three derivatives of *B. burgdorferi* B31 run in 0.4% agarose gels to visualize small, supercoiled plasmids. The top panel shows a photographic exposure that allows analysis of bands in the molecular size range of 20 to 50 kb. The bottom panel is a longer exposure of the same gel to detect the lower-molecular-size DNA species.

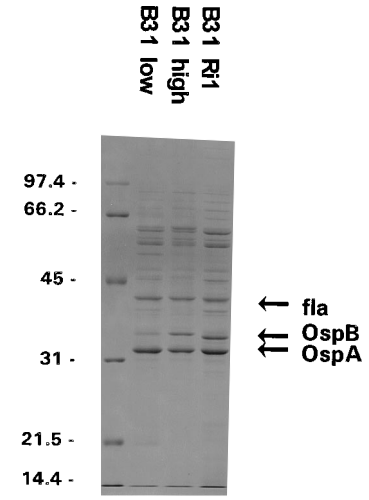


FIG. 2. Coomassie blue staining of SDS-PAGE (10% polyacrylamide) analysis of the three B31 derivatives. Protein concentrations were normalized to the intensity of staining of the flagellin (fla) bands of each sample. Also identified are the outer surface proteins A and B (OspA and OspB, respectively).

transposable elements have not been described for *B. burgdorferi*. The degree of plasmid loss appears to be greater in culture than in the natural transmission cycle, but these analyses were not designed to address this question definitively.

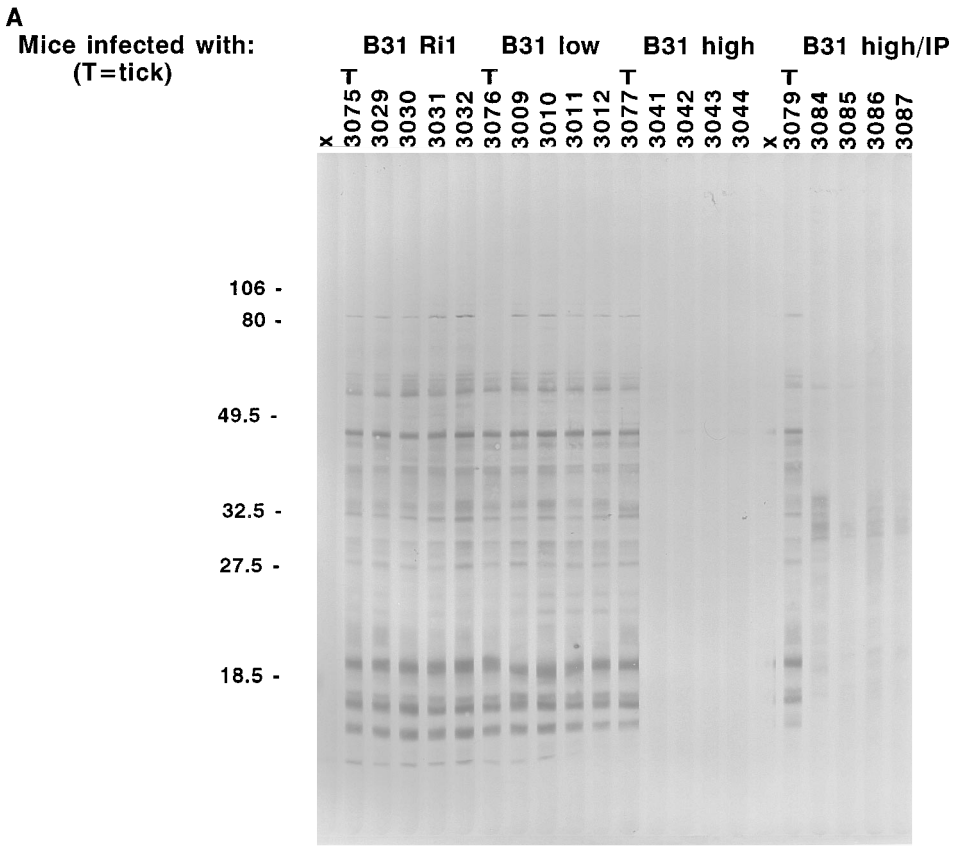
Protein profiles of the three derivatives of B31. Lysates were prepared from the three derivatives of strain B31 (7) and examined by SDS-PAGE (10% polyacrylamide). The protein profiles of these substrains show some detectable variation in the proteins expressed, even though samples were normalized to the intensity of the flagellin band to generate the most comparable profile (Fig. 2). For example, where the flagellin and OspA bands were very comparable, the OspB band in the reisolate appeared to migrate slightly faster than the same band of the other derivatives. Other bands that appeared to be present in some derivatives and absent in others may be variably expressed or may indicate experimental variation.

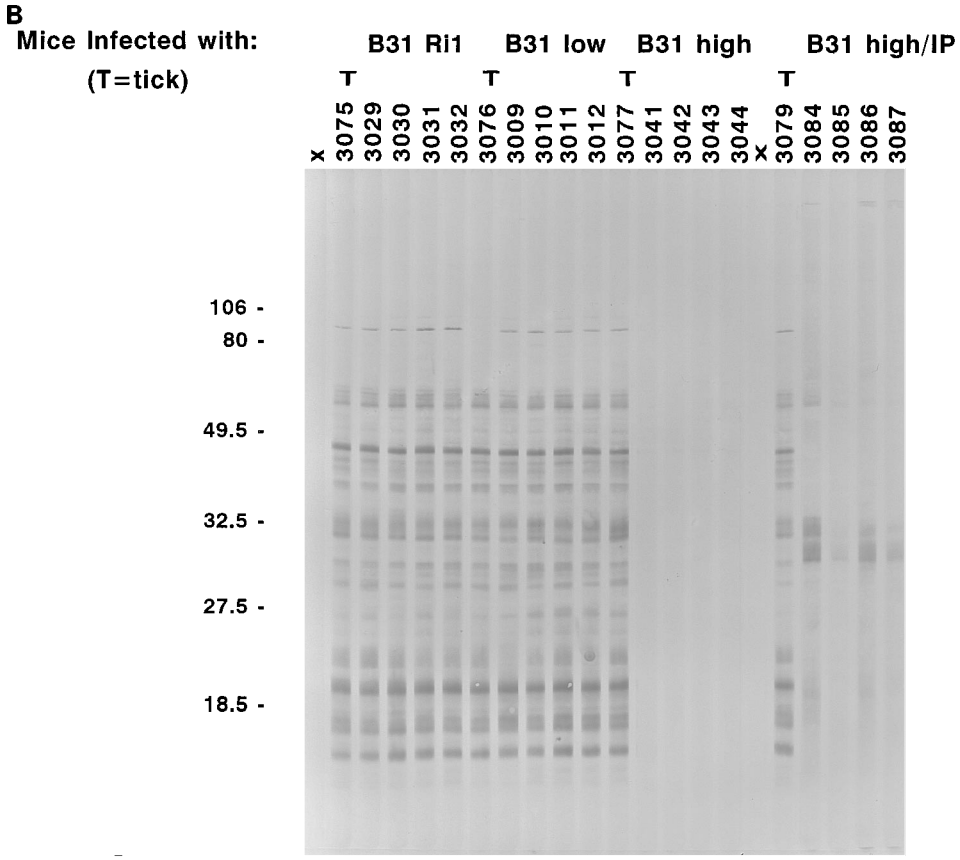
Serum antibody response of mice inoculated intradermally with B31. Serum samples were collected from animals in the ID_{50} experiments to investigate whether the immune responses to strain B31 derivatives varied and to compare them with serum samples from mice infected by tick transmission. We carried out Western blot assays with samples from day 60 after infection with lysate preparations for all three strain subtypes as the antigen.

In the first panel of Fig. 3, we show results with B31 Ri-1 as antigen. These Western blot patterns show that there was no detectable difference between the serum antibody responses to 10^4 spirochetes of B31 Ri-1 (mice 3029 to 3032) and B31 low (mice 3009 to 3012) injected intradermally. In fact, there was no difference in the responses to either of these derivatives transmitted by needle inoculation and the natural tick transmission of strain B31 (lanes marked by T) when serum drawn 60 days after infection was used, as reported previously (2, 13, 17). These serum samples gave very similar signal intensities for most bands; however, one tick-infected animal, 3076, lacked a response to a protein with a molecular mass of approximately 93 kDa. All animals inoculated with low-passage derivatives by needle had this anti-P93 activity.

Surprisingly, we observed that the intradermal inoculation of 10^8 spirochetes of high-passage B31 was not only noninfectious but apparently yielded no serum antibody response 60 days after injection. This is not a result of the inability of these mice to generate a serum antibody response to high-passage B31, because the last group of mice (3084 to 3087), injected intraperitoneally with 10^8 spirochetes of this strain, had serum antibody reactivity specific for OspA (31 kDa) and OspB (32.5 kDa). These responses are minimal or absent from the intradermally inoculated or tick-infected animals, consistent with our previous findings (8).

In the second panel of Fig. 3, we show the same group of





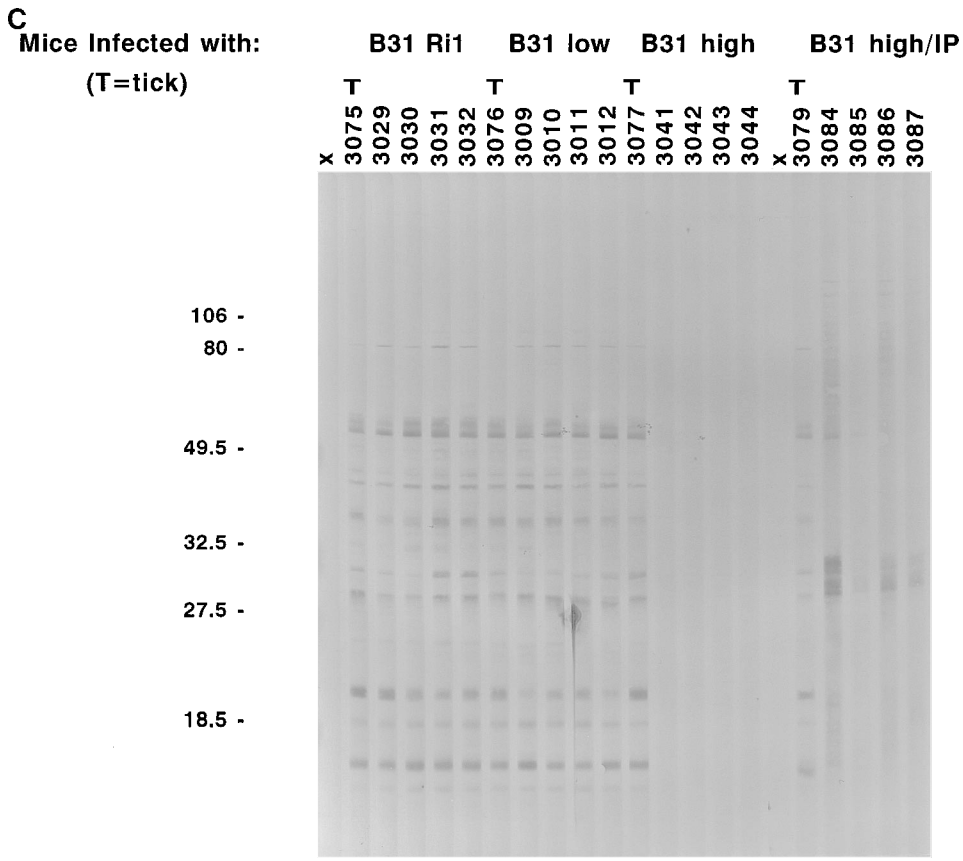


FIG. 3. (A) Serum antibody response of mice injected intradermally with the three derivatives of *B. burgdorferi* B31, mice infested with B31-infected *I. scapularis* nymphs, and mice inoculated intraperitoneally (IP) with high-passage B31, as assayed by Western blotting with lysates of B31 Ri-1 as antigen. The mouse immunization is given above each group. T designates mice infected by tick transmission. Serum samples were from 60 days after infection or inoculation. (B) Serum antibody response of mice injected intradermally with the three derivatives of *B. burgdorferi* B31, mice infested with B31-infected *I. scapularis* nymphs, and mice inoculated intraperitoneally with high-passage B31, as assayed by Western blotting with lysates of B31 low as antigen. Serum samples were from 60 days after infection or inoculation. (C) Serum antibody response of mice injected intradermally with the three derivatives of *B. burgdorferi* B31, mice infested with B31-infected *I. scapularis* nymphs, and mice inoculated intraperitoneally with high-passage B31, as assayed by Western blotting with lysates of B31 high as antigen. Serum samples were from 60 days after infection or inoculation. Lanes marked with an X are blank lanes containing no serum samples, only secondary antibody.

sera used in Western blotting with low-passage B31 as the antigen. The pattern of banding by these serum antibodies was very similar to the pattern seen with B31 Ri-1 lysates. In fact, the intensities of the reactivity with P93 were indistinguishable, including the lack of response from mouse 3076. One apparent difference was the expression of a protein with an apparent molecular mass of 14 kDa in the Ri-1 derivative absent from low-passage B31. This may be a real difference between these derivatives, but the 14-kDa protein is possibly unique to this preparation of the B31 Ri-1 antigen. Again, there was no detectable response of the outbred mice intradermally injected with high-passage B31 to the antigens of low-passage B31 present in this lysate.

For the last panel of Fig. 3, the same serum samples were used to blot lysates from high-passage B31. Sera from outbred mice infected with B31 low or B31 Ri-1, as well as sera from tick-infected animals, have reduced reactivity with B31 high in Western blots, with fewer bands detected than in Western blots with low-passage B31 and B31 Ri-1 as the antigen. As in the previous blots, animals injected intradermally with high-passage B31 had no detectable antibody response, even when the homologous strain was the antigen in the assay.

Reactivity of monoclonal antibodies with strain B31. We screened these lysates with a series of monoclonal antibodies generated from tick-infected animals that have specificity for antigens not previously described (9). Two antibodies reacted with bacterial antigens that were expressed differentially in lysates of low-passage B31 and the reisolate (Fig. 4). The antibody B10B31-103.1 reacted with a 14-kDa protein when B31 low was the antigen and with a 55-kDa protein, as well as the 14-kDa species, when B31 Ri-1 was the source of the lysate. We are investigating whether the specificity for the 55-kDa species was the result of a cross-reactivity between two distinct antigens or whether B31 Ri-1 made a multimer of the 14-kDa protein that migrates at the higher molecular mass. In either case, B31 Ri-1 lysates had an antigen that was reactive with B10B31-103.1 that was not expressed by low-passage B31.

The antibody designated B10B31-64.4, specific for a 35-kDa protein, reacted only with the B31 Ri-1 lysate in this figure, but in other experiments, we have detected this protein in low-passage B31 with this antibody. The last antibody of note, B10B31-221.11, reacted with a 17-kDa species in the lysate from B31 low. As shown in the second panel of Fig. 3, the same antibody reacted with a 34-kDa protein expressed by B31 Ri-1. It is possible that this was a dimer of the same protein expressed by B31 low. We are presently determining whether these are differential forms of the same protein or are different, cross-reactive antigens.

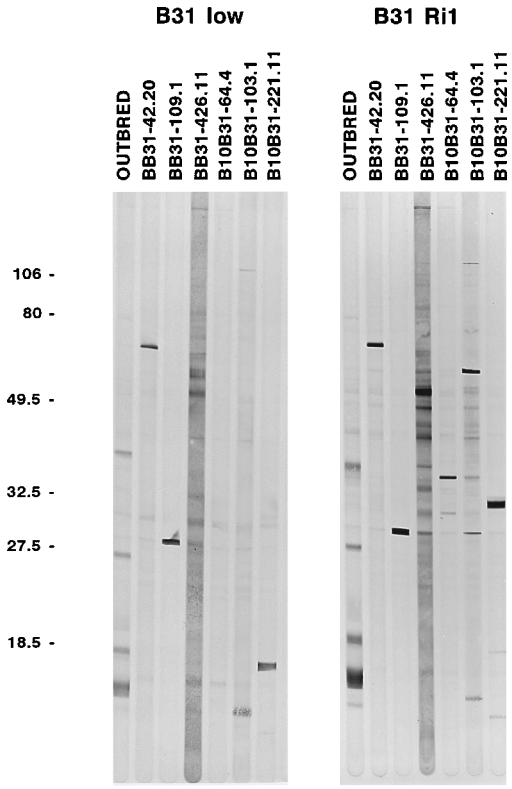


FIG. 4. Western blot of two derivatives of *B. burgdorferi* B31 assayed with monoclonal antibodies generated from mice immune to B31 as a result of tick transmission of infection. The blots were probed with an anti-IgM secondary antibody, because all of these monoclonal antibodies are of the IgM isotype.

DISCUSSION

Our analysis of the murine immune response to the Lyme disease spirochete has used the model of natural transmission of infection by infestation with infected *Ixodes* ticks. This has given us the unique ability to account for the role of the vector in the response to the pathogen. The ability to more accurately test the usefulness of various bacterial antigens as diagnostic or prophylactic reagents is greatly enhanced by this laboratory model. One assumption of this approach is that the spirochete is genetically and antigenically stable in the natural zoonotic cycle of transmission, which is not the case when *B. burgdorferi* is maintained in vitro. It was important, therefore, to determine if this assumption was true by comparative analysis of reisolated B31 with the original inoculating strain, low-passage B31.

Whereas most analyses revealed no detectable difference between the reisolate B31 Ri-1 and B31 low, some analyses detected clear differences. Specifically, the reisolate appeared to no longer maintain the 8-kb supercoiled plasmid present in the parent strain. Conservatively, we believe that B31 Ri-1 does not maintain the 8-kb plasmid in a stoichiometric ratio to the chromosome. In the crude analysis reported here, the concentrations of DNA from all derivatives of B31 were equivalent when the concentrations of the chromosome or the 49-kb linear plasmid were compared, for instance, supporting the interpretation that the 8-kb plasmid was lost.

This result indicates that the genes encoded by the 8-kb plasmid were not critical to infectivity, at least for strain B31, since both of these derivatives had equivalent levels of infectivity. The high-passage B31 strain also lost this plasmid, as well as many other linear and circular plasmids. As shown previously for strain Sh-2-82 (18) and in this report for high-passage B31, the in vitro strain is noninfectious and is antigenically distinct from the parent strain. Dunn et al. (6) have sequenced the entire 8-kb plasmid of strain Ip21, classified originally as *B. burgdorferi* sensu stricto (1), but recently reclassified as *Borrelia afzelii* (4). Interestingly, there are nine open reading frames encoded by the plasmid, one of which is potentially a lipoprotein. Probes specific for the sequences of the Ip21 8-kb plasmid reported by Dunn and colleagues do not hybridize with any derivative of B31 (5a). If B31 Ri-1 has lost this plasmid, we may have a way of analyzing the role of the genes encoded by this plasmid since we have a mutant strain negative for these genes.

The serum antibody response to these three derivatives of B31 also yielded interesting results. It is clear that at the level of using whole serum for immunoblotting, there are no detectable differences in the responses to tick infection with either intradermal inoculation of B31 low or intradermal inoculation of B31 Ri-1. This result is in agreement with those of a number of investigators who have reported that intradermal inoculation of low doses of spirochetes (10^4 to 10^5) yields a unique antibody response (2, 13, 17). However, intradermal inoculation of B31 high resulted in no infection, as predicted, but also elicited no detectable serum antibody response. This was very surprising considering that the dose of B31 high inoculated into these animals was orders of magnitude greater than those of the other B31 derivatives that induced a good immune response. When this high-passage, noninfectious derivative was inoculated intraperitoneally at equivalent doses, a qualitatively different but readily detectable serum antibody response resulted in the absence of infection.

These data indicate the remarkably different microenvironment that exists in the initial site of infection in Lyme disease, specifically the skin. When the avirulent form of the spirochete is inoculated into the skin, even at large doses, it is effectively sequestered from the B-lymphocyte response. The response to this inoculum in the skin is very different from the response to the same inoculum administered intraperitoneally, the latter inducing a serum antibody response. In the skin microenvironment, there may be some mechanism of clearance of the bacterium by phagocytic cells, for instance, distinct from cell populations that act as antigen presenting cells and that initiate a subsequent T-cell and eventually B-cell response upon intraperitoneal inoculation. Alternatively, virulent forms of the bacterium are able to spread systemically such that they can be isolated from an ear biopsy and stimulate a vigorous serum antibody response. This illuminates the possibility that vaccination strategies designed to target immunity at the primary site of infection, the skin, may allow a protective immune response and efficient clearing of spirochetes in the absence of a systemic antibody response, as has been suggested for arboviruses (3). The advantage of such a strategy is elimination of any potential pathology induced by the immune response to the vaccine and subsequent exposure to the pathogen.

Finally, an analysis of these various derivatives of B31 by Western blotting with monoclonal antibodies as probes showed that individual proteins may be alternatively expressed. We described two antibodies that detect species with different molecular masses in the two infectious, low-passage B31 strains. One of these target antigens, the 17-kDa species detected by B10B31-221.11 in reisolated B31, may be related to the 34-kDa species detected in B31 low. Furthermore, a 14-kDa species detected by the B10B31-103.1 monoclonal antibody reacts with species with multiple molecular masses in the reisolate. The complexity of proteins expressed by these spirochetes as as-

sayed by migration in SDS-PAGE is apparently extensive, and it will require an extensive panel of monoclonal antibodies to analyze this complexity in these *B. burgdorferi* strains.

This report presents data showing that the maintenance of a well-characterized strain of *B. burgdorferi* in a laboratory zoonotic cycle allows genetic and antigenic variation to occur. By the analyses shown here, that variation is limited compared with in vitro cultivation of the same strain. These results emphasize the need to consider the source and history of isolates of *B. burgdorferi* when interpreting experimental results with such isolates. Although the laboratory model of propagation of strain B31 exclusively in a zoonotic cycle does not perfectly recreate the transmission of *B. burgdorferi* in nature, these results predict that genetic and antigenic drift is possible and may be ongoing.

This study was initiated to analyze *B. burgdorferi* B31 that had been maintained in the zoonotic cycle for 5 cycles. The purpose of this study was to compare the reisolate with the isolate used to initiate the laboratory colony of infected *Ixodes* ticks and to determine what was presently in those ticks. In order to more accurately and completely analyze genetic and antigenic drift in a laboratory zoonotic cycle, we have generated clones of low-passage B31 and initiated the infectious cycle. Spirochetes will be reisolated at each stage of infection from both the vector and the host. When a number of cycles of infection are complete, we will then be able to compare a comprehensive series of reisolates for genetic and antigenic variation without the caveat of the starting material being derived from a potentially mixed population.

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