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Infectious but Nonpathogenic Isolate of *Borrelia burgdorferi*

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We document for the first time an infectious but nonarthritogenic variant of *Borrelia burgdorferi*. Strain 25015, previously isolated from an *Ixodes dammini* larva collected in upstate New York, was infectious but failed to produce arthritis or carditis in laboratory rats and mice. By contrast, pathogenic strain N40 invariably caused arthritis. This nonarthritogenic variant, with proteins with molecular weights different from those of the standard B31 strain, was frequently isolated from normal joint tissues of experimentally infected rats. Outer surface proteins A and B of strain 25015 have molecular weights of about 32,500 and 35,500, respectively, in contrast to molecular weights of approximately 31,000 and 34,000, respectively, for outer surface proteins A and B of strains B31 and N40. A prominent low-molecular-weight protein of about 23,500 also characterizes strain 25015. Test animals infected for 30 to 60 days had relatively high antibody titers (≥1:1,280). The nonarthritogenic variant will be useful, along with pathogenic strains, in providing comparative insight into the pathogenesis of Lyme borreliosis. Homologous immunoblotting of sera from rats and mice inoculated with both the arthritogenic and nonarthritogenic strains revealed antibody reactivities to proteins of *B. burgdorferi* different from those revealed in the heterologous tests.

The etiologic agent of Lyme borreliosis (30), *Borrelia burgdorferi* (13, 17), causes multisystemic infection with signs and symptoms primarily associated with integumentary, musculoskeletal, cardiac, and nervous systems (29). Disease severity and manifestations are highly variable. Different patterns of disease also occur in different geographic areas. For example, acrodermatitis chronicum atrophicans and lymphadenosis benigna cutis are common in Europe (3, 33) but rare in the United States (19). This diversity in clinical manifestations may, in part, be related to differences among *B. burgdorferi* isolates or host susceptibility. Heterogeneities in molecular weights of structural proteins, antigenicity, and DNA have all been demonstrated among *B. burgdorferi* isolates (7, 20, 24). Proteins of North American isolates tend to be more homogeneous than those of European isolates (7, 34), but a few North American isolates which vary substantially from the typical pattern have been described (1, 2, 11, 18).

Laboratory rats and mice both have been shown to be susceptible to infection and induction of arthritis and carditis by *B. burgdorferi* (8–10, 23), thus allowing evaluation of infectivity and pathogenicity of *B. burgdorferi* strains. Among several *B. burgdorferi* isolates found to be capable of infecting rats experimentally, all were also found to be arthritogenic (9, 10). We report here an atypical variant from upstate New York (2) that is infectious, but not pathogenic, in laboratory rats and mice. We further document that homologous antisera reacted to proteins of *B. burgdorferi* in immunoblot analyses differently from the heterologous antisera.

MATERIALS AND METHODS

Rats, mice, and borreliae. The infectiousness and ability to induce arthritis of two strains of *B. burgdorferi* with distinctly different proteins of different molecular weights were compared in inbred Lewis/SSNCR rats obtained from the Frederick Cancer Research Facility, National Cancer Institute (Frederick, Md.) (10), and in C3H/HeNCrlBR (C3H) mice from Charles River Laboratories, Raleigh, N.C. (8). The N40 strain of *B. burgdorferi* originated from an adult *Ixodes dammini* collected in Westchester County, N.Y., and has been shown to infect and cause arthritis in rats and mice (8, 10). The second strain (25015) was isolated from a fully fed *I. dammini* larva removed from a white-footed mouse captured in Dutchess County, N.Y. (2). This isolate had higher-molecular-weight OspA and OspB proteins (about 32,500 and 35,500, respectively) than the standard B31 and N40 strains (31,000 and 34,000, respectively), had a prominent protein band with a relatively low molecular weight of 23,500 (see Fig. 1), and was infectious in Syrian hamsters.

The two isolates were low passage (three passages or less) and grew in modified Barbour-Stoenner-Kelly (BSK) medium (4). Aliquots of N40 and 25015 spirochetes were frozen in liquid nitrogen until needed, at which time cultures were inoculated with seed stock and incubated at 35°C for approximately 1 week. Three-day-old rats and 3-week-old mice were inoculated intraperitoneally in the lower left abdominal quadrant with 10^6 borreliae of either the N40 or 25015 strains in 0.1 ml of BSK medium. Spirochetes were enumerated in a counting chamber (Hausser Scientific Partnership, Horsham, Pa.) under dark-field microscopy. Immature rats and 3-week-old mice were selected because arthritis invariably occurs in these animals following inoculation of pathogenic strains (8, 10). Rats were examined daily for joint swelling and lameness and were sacrificed with carbon dioxide gas on days 14, 30, or 60 postinoculation. Mice were tested similarly, except they were sacrificed 21 days after inoculation.

Borreliae isolation and SDS-PAGE procedures. Attempts were made to isolate spirochetes in BSK medium from the blood and from the spleen, kidney, bladder, joint, brain, ear,

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TABLE 1. Incidence of arthritis and isolation of *B. burgdorferi* from tissues at intervals after inoculation of 3-day-old rats or 3-week-old mice with strain N40 or 25015

Species	Inoculum	Days after inoculation	No. of tissues positive/total no. tested									
			Arthritis	Bladder	Spleen	Kidney	Liver	Joint	Blood	Brain	Ear	Combined
Rat	N40	30	5/5	5/7	7/7	7/7	3/5	1/5	0/5	5/5	NT[a]	7/7
	25015	14	0/5	1/5	2/5	5/5	0/5	5/5	1/5	0/5	3/5	5/5
		30	0/5	0/5	1/5	1/5	0/4	5/5	0/5	0/5	5/5	5/5
		60	0/6	5/6	0/6	0/6	0/6	4/6	0/6	0/6	0/6	6/6
Mouse	N40	21	4/4	NT	4/4	NT	NT	NT	NT	NT	NT	4/4
	25015	21	0/8	NT	2/7	2/8	NT	NT	NT	NT	NT	3/8

[a] NT, Not tested.

or liver of each test animal (16, 26, 28). Isolates from different tissues were compared with the original inoculum by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7). Gels were stained with Coomassie brilliant blue R-250 after proteins were separated by their molecular weights in a vertical gel unit (SE600; Hoefer Scientific Instruments, San Francisco, Calif.).

ELISA procedures. Serum of cardiac blood from each inoculated rat was tested for detectable antibody by a whole-cell antigen enzyme-linked immunosorbent assay (ELISA) (22), using horseradish peroxidase labeled goat anti-rat immunoglobulins (heavy and light chains) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) diluted 1:300 in phosphate-buffered saline solution. In this ELISA, cutoff optical density values of >0.21, >0.18, and ≥0.13 were considered positive for rat serum dilutions of 1:160, 1:320, and ≥1:640, respectively. The cutoff values were determined by statistically analyzing net optical density values for dilutions of serum representing 15 normal rats.

Western immunoblot analysis. Proteins of *B. burgdorferi* strains N40 and 25015 were separated by SDS-PAGE and transferred to nitrocellulose paper (32). The protein-containing membrane was saturated in blocking solution (10% calf serum in Tris-buffered saline [15 g of Trisma hydrochloride, 2.82 g of Trisma base, and 175.44 g of NaCl brought to 6 liters of distilled water with the pH adjusted to 7.5 with HCl]) and was then exposed for 2 h to a 1:100 dilution of rat or mouse serum. Following two washings in blocking solution, the membrane was submersed for 2 h in alkaline phosphatase-labeled goat conjugate (affinity-purified antibody to rat immunoglobulin G [IgG] [γ]) or alkaline phosphatase-labeled affinity purified goat antibody to mouse IgG (γ) (Kirkegaard and Perry Laboratories) diluted 1:500 in Tris-buffered saline. The membrane was again washed two times in Tris-buffered saline containing 0.05% Tween 20, and protein bands with attached antibody were stained with phosphatase substrate (0.1 M Tris buffer solution [pH 9.5], Nitroblue Tetrazolium concentrate solution, and 5-bromo-4-chloro-3 indolyl phosphate concentrate solution; Kirkegaard and Perry Laboratories).

The biotinylated SDS-PAGE molecular weight protein standards (Bio-Rad, South Richmond, Calif.) of rabbit muscle phosphorylase *b* (97,400), bovine serum albumin (66,200), ovalbumin (42,700), bovine carbonic anhydrase B (31,000), soybean trypsin inhibitor (21,500), and egg white lysozyme (14,400) were used to identify the molecular weights of protein bands that reacted positively in immunoblots. Murine monoclonal antibodies H5332 and H9724 were used to verify molecular weight locations of the OspA and flagellin proteins (6, 7).

Pathology. Brain, heart, lung, spleen, liver, kidney, and joints of all limbs (shoulder, elbow, carpus, knee, tarsus, hip, metacarpus, metatarsus, and phalanges) of rats and mice were fixed in neutral-buffered Formalin (pH 7.2). Tissues, including joints after they were demineralized, were embedded in paraffin, sectioned to thicknesses of 5 to 7 μm, and stained with hematoxylin and eosin. The Dieterle silver method was used to stain spirochetes in selected tissues (14).

RESULTS

Infection and pathology. The N40 and 25015 strains of *B. burgdorferi* both were infectious in 3-day-old rats, but only N40 was pathogenic (Table 1). Borreliae were isolated from one or more tissues of each animal inoculated with either strain. Strain N40 was isolated from all tissues tested. Isolations of strain 25015 were most frequently made from joint and skin tissues. In contrast to strain N40, strain 25015 did not frequently infect the liver or brain. Borreliae reisolated from rats inoculated with N40 or 25015 were indistinguishable from their respective original inoculum by SDS-PAGE (Fig. 1).

The N40 but not the 25015 strain of *B. burgdorferi* was arthritogenic following inoculation of 3-day-old rats (Table 1). All five rats inoculated with strain N40 had histological evidence of acute inflammation involving multiple joints,

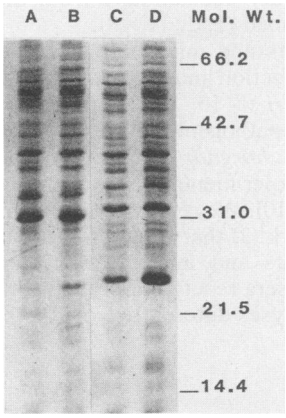

FIG. 1. Coomassie brilliant blue R-250-stained proteins of whole-cell lysates of *B. burgdorferi* N40 and 25015 inoculated into rats and after reisolation. Lane A, N40 prior to inoculation; lane B, N40 reisolated from spleen of rat infected for 30 days; lane C, 25015 prior to inoculation; lane D, 25015 reisolated from spleen of rat infected for 30 days. Molecular weights are in thousands.

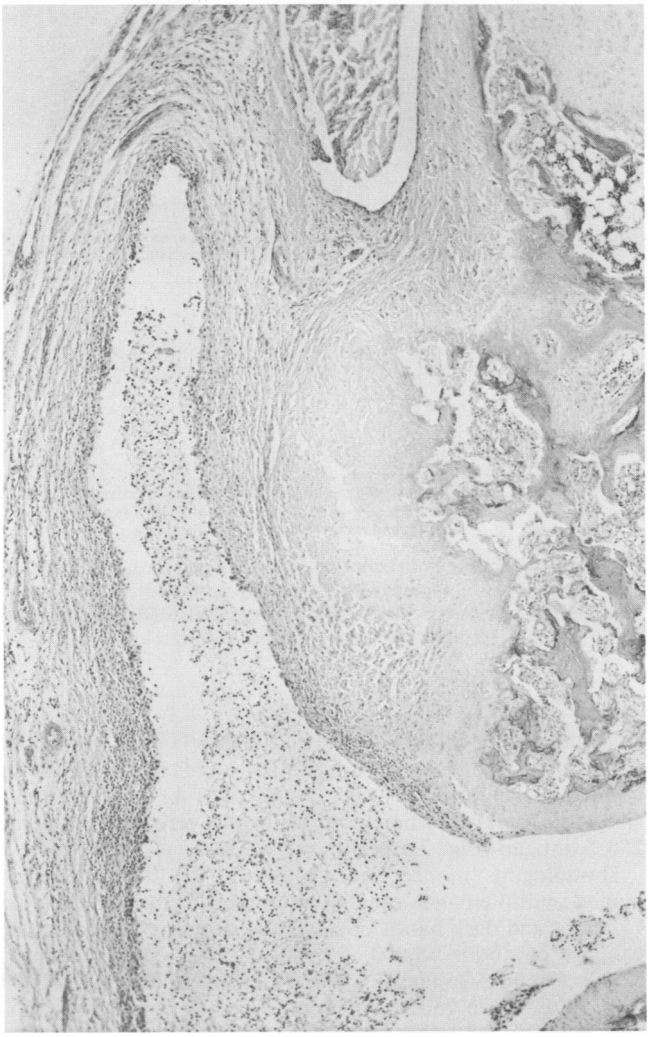

FIG. 2. Arthritis of the tibiotarsal joint of a rat at 30 days after intraperitoneal inoculation of *B. burgdorferi* N40. The synovium is hypercellular due to infiltration with leukocytes, and the joint space contains fibrinopurulent exudate. Hematoxylin-eosin stain was used. Magnification, ×60.

FIG. 3. Spirochete within dermal connective tissue (arrow) of a rat at 14 days after intraperitoneal inoculation of *B. burgdorferi* 25015. The Dieterle silver stain method was used. Magnification, ×965.

tendons, and bursae of one or more limbs at 30 days after inoculation (Fig. 2). There was infiltration of periarticular connective tissue within neutrophils, with exudation of neutrophils and fibrin into synovial spaces. This was accompanied by vascular congestion and edema as described previously (8–10, 23). One of these rats also had carditis. Inflammatory lesions were not observed in rats examined at 14, 30, or 60 days after inoculation with strain 25015, even though borreliae were isolated from joint tissues of 14 of 16 rats and from other tissues in all of the rats (Table 1). Spirochetes were discernible in the connective tissue around joints and in the skin (Fig. 3) of rats examined at 14 days after inoculation with strain 25015.

Likewise, the pathogenicity of strain N40, but not strain 25015, was demonstrated in C3H mice inoculated at 3 weeks of age (Table 1). All four mice inoculated with strain N40 were culture positive and had inflammatory lesions in multiple joints, tendons, bursae, and heart at 21 days after inoculation. Eight mice inoculated with strain 25015 had no lesions at 21 days after inoculation. Borreliae were isolated from the spleens, kidneys, or both tissues of three of these mice.

ELISA. There were positive IgG antibody titers, using N40 antigen, recorded for all six rats infected for 30 days with strain N40 and for 0 of 5, 4 of 13, and 5 of 6 rats infected with strain 25015 for 14, 30, and 60 days, respectively. Significant ELISA titers for homologous and heterologous tests ranged from 1:160 to 1:640.

When rats were inoculated with strain 25015, 1 of 5, 12 of 12, and 6 of 6 serum specimens tested with homologous antigen were positive at 14, 30, and 60 days postinoculation, respectively. Sera from rats inoculated for 30 or 60 days had homologous titers of ≥1:1,280, with 10 of 18 serum specimens having titers of ≥1:10,240. Six of seven N40 antisera from rats infected for 30 days had positive titers ranging from 1:320 to 1:1,280 when 25015 antigen was tested.

Western blot analyses. Sera from rats inoculated with strain N40 for 30 days had light to dark bands with molecular weights of 20,000 and 24,000 and two to four bands with

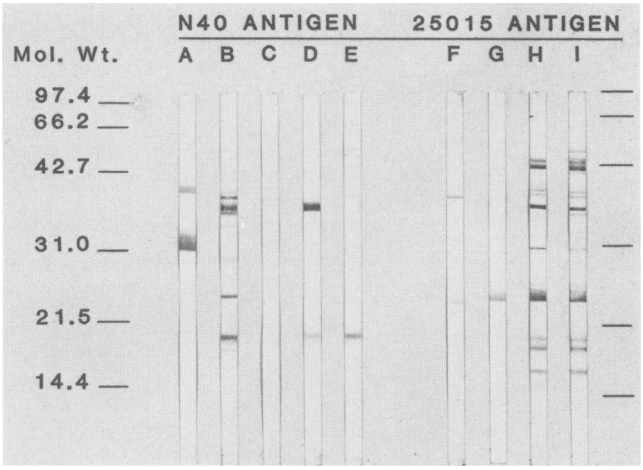

FIG. 4. Representative IgG immunoblots of *B. burgdorferi* N40 and 25015 with homologous and heterologous sera from rats infected for 14, 30, or 60 days and with monoclonal antibodies H5332 and H9724. Lane A, Monoclonal antibodies H5332 and H9724; lanes B and F, 30-day-old N40 antiserum; lanes C and G, 14-day-old 25015 antiserum; lanes D and H, 30-day-old 25015 antiserum; lanes E and I, 60-day-old 25015 antiserum. Molecular weights are in thousands.

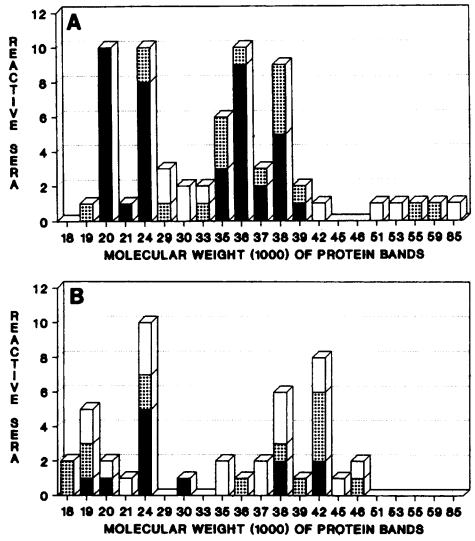

FIG. 5. Frequency distribution of visualized protein bands of sera from 10 rats infected with *B. burgdorferi* N40 for 30 days in immunoblot tests with strain N40 (A) and 25015 (B) antigens. ■, Dark staining; ▨, light staining; □, faint staining.

molecular weights of 35,000 to 39,000 when homologous tests were conducted (Fig. 4 and 5). When 25015 antigen was reacted with N40 antisera, a band that varied from faint to dark also was consistently present at a molecular weight of 24,000, and 8 of 10 serum specimens had a band that varied in intensity with a molecular weight of 42,000 (Fig. 4 and 5). Total numbers of bands in the homologous tests ranged from 5 to 11, while in the heterologous tests 1 to 9 bands were observed. The OspA and flagellin standard monoclonal antibodies that were used in the immunoblots attached to proteins of N40 antigen had molecular weights of 30,000 and 38,000 to 39,000, respectively; the molecular weights were 32,000 and 38,000 to 39,000, respectively, for 25015 antigen.

Progressively more bands appeared in immunoblots with time of infection in rats inoculated with strain 25015. In homologous tests, numbers of bands ranged from 3 to 4, 5 to 12, and 11 to 16 for infection intervals of 14, 30, and 60 days, respectively. In the heterologous tests with N40 antigen, only one serum specimen with two bands reacted at 14 days, two to six bands appeared at 30 days, and three to seven bands appeared after 60 days. All sera from rats infected for 14, 30, and 60 days reacted in homologous tests with protein bands with molecular weights of 24,000 and 42,000 (Fig. 4 and 6B and D). These bands were light to dark. In homologous tests with sera from rats infected for 30 and 60 days, a distinct dark band also consistently stained at a molecular weight of 36,000. When 25015 antisera from rats infected for 30 and 60 days were reacted with N40 antigen, bands often appeared at molecular weights of 20,000, 36,000 to 38,000, and 53,000 (Fig. 4 and 6A and C). The staining intensities of these bands varied from faint to dark.

Sera from mice infected for 21 days reacted strongly in immunoblots (Fig. 7 and 8). As with the rats, more bands were expressed in homologous than heterologous tests. There were 9 to 12 bands stained in N40 homologous reactions compared with 5 to 8 bands when the 25015 antigen was tested with N40 antisera. Light or dark bands in the homologous test consistently appeared at molecular weights of about 20,000, 24,000, 30,000, 35,000, 36,000, 37,000, and 42,000 (Fig. 7 and 8A). Lightly stained bands to proteins with molecular weights of 24,000, 39,000, and 42,000 were consistently detected when N40 antisera were reacted with 25015 antigen (Fig. 7 and 8B).

Homologous mouse antisera to the 25015 antigen displayed multiple bands ranging in number from 15 to 17 (Fig. 7). Light and dark bands consistently occurred at molecular weights of about 18,000, 20,000, 21,000, 24,000, 30,000, 32,000, 34,000, 35,000, 36,000, 38,000, 39,000, and 42,000 (Fig. 8D). When 25015 antisera were exposed to N40 antigen, 6 to 10 bands became apparent (Fig. 7); staining was either light or dark at molecular weights of about 20,000, 24,000, 30,000, and 37,000 (Fig. 8C).

Sera from uninfected rats and mice were negative with N40 and 25015 antigens.

DISCUSSION

In previous studies, inbred and outbred rats and mice were susceptible to infection with *B. burgdorferi* and developed arthritis (8–10, 23). All of the strains shown to be infectious thus far were also arthritogenic in rats (9, 10). Our report of an infectious but nonarthritogenic strain of *B. burgdorferi* is unique and was confirmed in two different host species. This variant possesses protein bands that are distinctly different from those of the standard B31 strain. It offers the opportunity to compare the DNAs and other chemical compositions of strains that cause arthritis with those of one that is apparently nonpathogenic. Future studies could help clarify the pathogenic basis of lesion development.

Strains with protein bands similar to those of strain 25015 have not yet been isolated from humans. Thus, the relationship of this variant to human disease is unknown. Rats and mice infected with 25015 exhibit a strong immunologic response, even though pathogenesis is inapparent. Although sera from humans diagnosed with Lyme borreliosis react with 25015 in ELISA, the specificity of the antigen is insufficient to differentiate between variants (2). Nonethe-

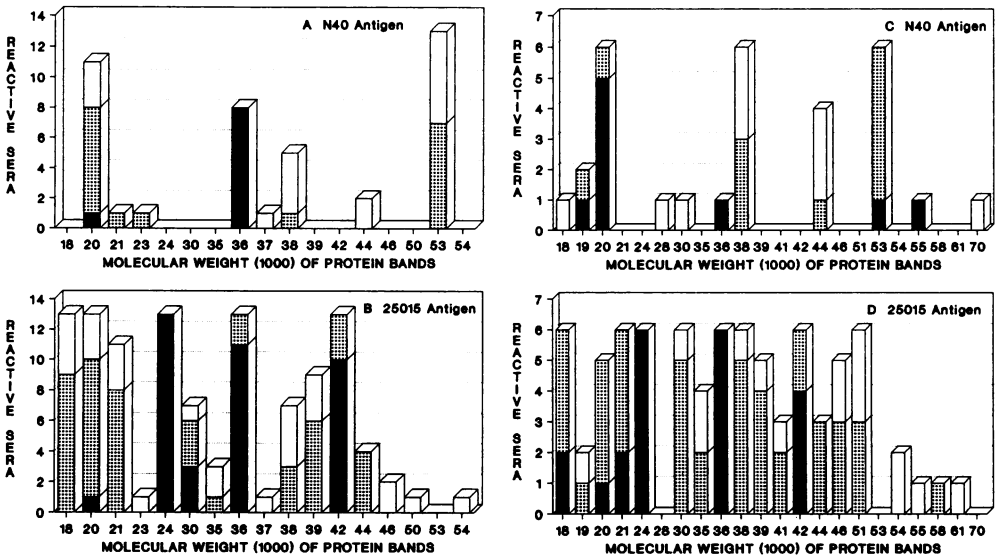

FIG. 6. Frequency distribution of visualized protein bands of sera from rats infected with *B. burgdorferi* 25015 for 30 days (A and B; n = 13 serum specimens tested) or 60 days (C and D; n = 6 serum specimens tested) in immunoblot tests with antigens from strains N40 and 25015. ■, Dark staining; ▨, light staining; □, faint staining.

less, asymptomatic individuals with significant antibody titers to antigens indistinguishable from the B31 strain are documented (31). Possibly, variants similar to strain 25015 are infectious to humans but are nonarthritogenic, as strain 25015 is in rats and mice. Further efforts are needed to determine the prevalence of the 25015 variant and to determine whether humans and domestic animals become infected.

Live spirochetes of the N40 strain are needed to cause arthritis in rats (10). Yet, 25015 organisms were frequently isolated from the joint capsules or closely adhering connective tissues of animals without arthritis. It is unknown whether this diversity of host response results from different antigens or quantities of spirochetes in the tissues or whether the spirochetes invade different tissues. Detailed histological and chemical studies may explain these differences in host responses.

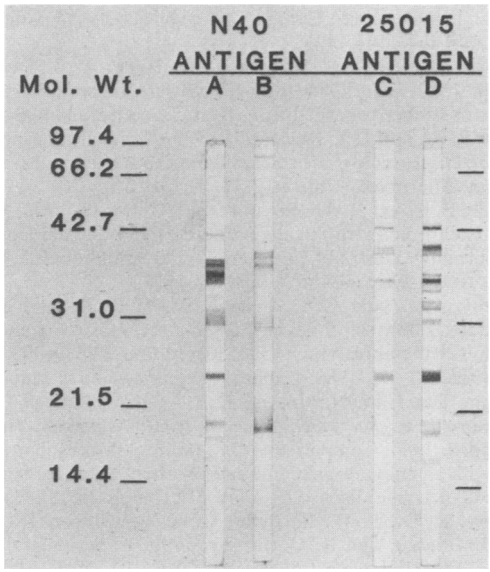

FIG. 7. Representative IgG immunoblots of *B. burgdorferi* N40 and 25015 with homologous and heterologous sera from mice infected for 21 days. Lanes A and C, N40 antiserum; lanes B and D, 25015 antiserum. Molecular weights are in thousands.

We confirmed the findings of an earlier study (23) which showed that serum antibodies to *B. burgdorferi* in rats increased with time of infection. There were rising antibody titers in ELISA and an expansion of immune response in Western blot analyses. The presence of antibodies in sera from rats and mice infected with the nonarthritogenic strain 25015 suggests to us that pathogenicity is not necessarily correlated with antibody production.

More and different antigens were expressed in homologous rat and mouse immunoblots than were expressed in heterologous tests. Serologic specificity has been reported in murine sera exposed to spotted fever group rickettsiae and has been used to serotype antigens (25). Possibly, reciprocal cross testing of borrelial antigens and antisera by immunoblotting procedures could similarly be used to further characterize variants and could also be useful in determining whether antigens of one strain are likely to confer immunity to other isolates.

Immunoblots increasingly are being used to detect antibodies to *B. burgdorferi* in humans (5, 21) and domestic animals (12, 15). Our finding of diverse reactions in homologous and heterologous tests with laboratory mice and rats infected with different variants leads us to suggest that efforts should be made to determine immunoblot patterns of human and domestic animal sera, with available variants being used as the antigen. Previously, sera of dogs and white-footed mice were reported to produce immunoblot patterns that varied when similar but slightly different strains of *B. burgdorferi* were used as the antigen (15, 27). Reactions in serum to antigens of two or more distinctly different *B. burgdorferi* variants may result in significantly different banding patterns.

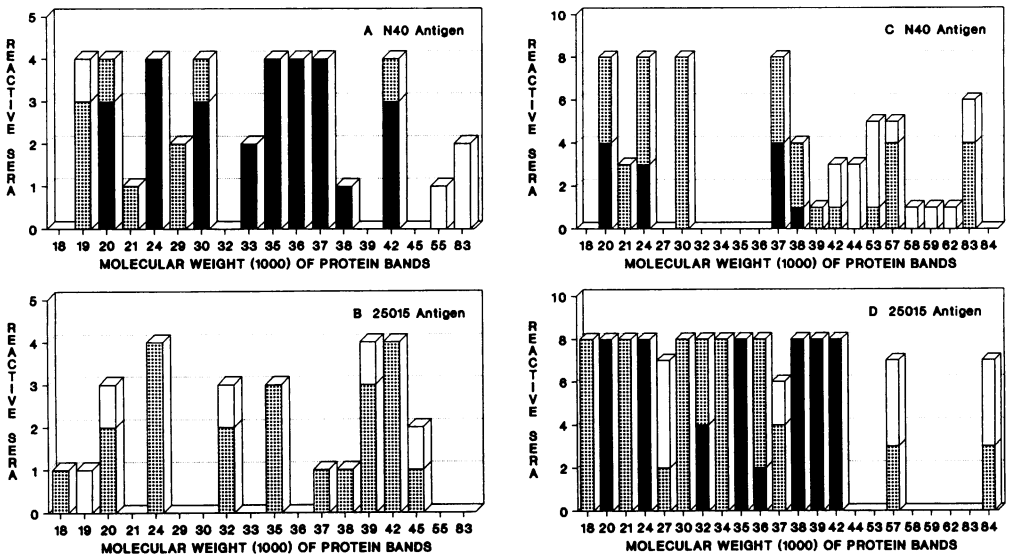

FIG. 8. Frequency distribution of visualized protein bands of sera from mice infected for 21 days with *B. burgdorferi* N40 (A and B; n = 4 serum specimens tested) and 25015 (C and D; n = 8 serum specimens tested) in immunoblot tests with antigens N40 and 25015. ■, Dark staining; ▨, light staining; □, faint staining.

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LITERATURE CITED

1. **Anderson, J. F., L. A. Magnarelli, R. B. Lefebvre, T. G. Andreadis, J. B. McAninch, G.-C. Perng, and R. C. Johnson.** 1989. Antigenically variable *Borrelia burgdorferi* isolated from cottontail rabbits and *Ixodes dammini* in rural and urban areas. J. Clin. Microbiol. **27**:13–20.
2. **Anderson, J. F., L. A. Magnarelli, and J. B. McAninch.** 1988. New *Borrelia burgdorferi* antigenic variant isolated from *Ixodes dammini* from upstate New York. J. Clin. Microbiol. **26**:2209–2212.
3. **Asbrink, E., A. Hovmark, and B. Hederstedt.** 1984. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. Acta Dermatol. Venerol. **64**:506–512.
4. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–525.
5. **Barbour, A. G., W. Burgdorfer, E. Grunwaldt, and A. C. Steere.** 1983. Antibodies of patients with Lyme disease to components of the *Ixodes dammini* spirochete. J. Clin. Invest. **72**:504–515.
6. **Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrumpf, and S. L. Tessier.** 1986. A *Borrelia*-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. **52**:549–554.
7. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae; a molecular analysis of North American and European isolates. J. Infect. Dis. **152**:478–484.
8. **Barthold, S. W., D. S. Beck, G. M. Hansen, G. A. Terwilliger, and K. D. Moody.** 1990. Lyme borreliosis in selected strains and ages of laboratory mice. J. Infect. Dis. **162**:133–138.
9. **Barthold, S. W., K. D. Moody, and D. S. Beck.** 1990. Susceptibility of laboratory rats to isolates of *Borrelia burgdorferi* from different geographic areas. Am. J. Trop. Med. Hyg. **42**:596–600.
10. **Barthold, S. W., K. D. Moody, G. A. Terwilliger, P. H. Duray, R. O. Jacoby, and A. C. Steere.** 1988. Experimental Lyme arthritis in rats infected with *Borrelia burgdorferi*. J. Infect. Dis. **157**:842–846.
11. **Bissett, M. L., and W. Hill.** 1987. Characterization of *Borrelia burgdorferi* strains isolated from *Ixodes pacificus* ticks in California. J. Clin. Microbiol. **25**:2296–2301.
12. **Bosler, E. M., D. P. Cohen, T. L. Schulze, C. Olsen, W. Bernard, and B. Lissman.** 1988. Host response to *Borrelia burgdorferi* in dogs and horses. Ann. N.Y. Acad. Sci. **539**:221–234.
13. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick borne spirochetosis? Science **216**:1317–1319.
14. **Duray, P. H., A. Kusnitz, and J. Ryan.** 1985. Demonstration of the Lyme disease spirochete by a modified Dieterle stain method. Lab. Med. **11**:685–687.
15. **Greene, R. T., R. L. Walker, E. C. Burgess, and J. F. Levine.** 1988. Heterogeneity in immunoblot patterns obtained by using four strains of *Borrelia burgdorferi* and sera from naturally exposed dogs. J. Clin. Microbiol. **26**:2287–2291.
16. **Johnson, R. C., N. Marek, and C. Kodner.** 1984. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin. Microbiol. **20**:1099–1101.
17. **Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. **34**:496–497.
18. **Lane, R. S., and J. A. Pascocello.** 1989. Antigenic characteristics of *Borrelia burgdorferi* isolates from ixodid ticks in California. J. Clin. Microbiol. **27**:2344–2349.
19. **Lavoie, P. E., A. J. Wilson, and D. L. Tuffanelli.** 1986. Acrodermatitis chronica atrophicans with antecedent Lyme disease in a Californian. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **263**:262–265.
20. **LeFebvre, R., and G.-C. Perng.** 1989. Genetic and antigenic characterization of *Borrelia coriaceae*, putative agent of epizootic bovine abortion. J. Clin. Microbiol. **27**:389–393.
21. **Magnarelli, L. A.** 1989. Laboratory diagnosis of Lyme disease. Rheum. Dis. Clin. North Am. **15**:735–745.
22. **Magnarelli, L. A., J. M. Meegan, J. F. Anderson, and W. A Chappell.** 1984. Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. J. Clin. Microbiol. **20**:181–184.
23. **Moody, K. D., S. W. Barthold, G. A. Terwilliger, D. S. Beck, G. M. Hansen, and R. O. Jacoby.** 1990. Experimental chronic Lyme borreliosis in Lewis rats. Am. J. Trop. Med. Hyg. **42**:165–174.
24. **Persing, D. H., S. R. Telford, A. Spielman, and S. W. Barthold.** 1990. Detection of *Borrelia burgdorferi* infection in *Ixodes dammini* ticks with the polymerase chain reaction. J. Clin.

Microbiol. **28**:566–572.

25. **Philip, R. N., E. A. Casper, W. Burgdorfer, R. K. Gerloff, L. E. Hughes, and E. J. Bell.** 1978. Serologic typing of rickettsiae of the spotted fever group by microimmunofluoresence. J. Immunol. **121**:1961–1968.
26. **Schwan, T. G., W. Burgdorfer, M. E. Schrumpf, and R. H. Karstens.** 1988. The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (*Peromyscus leucopus*). J. Clin. Microbiol. **26**:893–895.
27. **Schwan, T. G., K. K. Kime, M. E. Schrumpf, J. E. Coe, and W. J. Simpson.** 1989. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). J. Clin. Microbiol. **57**:3445–3451.
28. **Sinsky, R. J., and J. Piesman.** 1989. Earpunch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. J. Clin. Microbiol. **27**:1723–1727.
29. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733–740.
30. **Steere, A. C., S. E. Malawista, D. R. Snydman, R. E. Ohope, W. A. Andiman, M. R. Ross, and F. M. Steele.** 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. **20**:7–17.
31. **Steere, A. C., E. Taylor, M. L. Wilson, J. F. Levine, and A. Spielman.** 1986. Longitudinal assessment of the clinical and epidemiological features of Lyme disease in a defined population. J. Infect. Dis. **154**:295–300.
32. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
33. **Weber, K.** 1986. Remarks on the infectious disease caused by *Borrelia burgdorferi*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **263**:206–208.
34. **Wilske, B., V. Preac-Mursic, G. Schierz, R. Kuhbeck, A. G. Barbour, and M. Kramer.** 1988. Antigenic variability of *Borrelia burgdorferi*. Ann. N.Y. Acad. Sci. **539**:126–143.