Resistance to Lyme disease in decorin-deficient mice

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Microbial adhesion to the host tissue represents an early, critical step in the pathogenesis of most infectious diseases. *Borrelia burgdorferi*, the causative agent of Lyme disease (LD), expresses two surface-exposed decorin-binding adhesins, DbpA and DbpB. A decorin-deficient (*Dcn*$^{-/-}$) mouse was recently developed and found to have a relatively mild phenotype. We have now examined the process of experimental LD in *Dcn*$^{-/-}$ mice using both needle inoculation and tick transmission of spirochetes. When exposed to low doses of the infective agent, *Dcn*$^{-/-}$ mice had fewer *Borrelia*-positive cultures from most tissues analyzed than did *Dcn*$^{+/+}$ or *Dcn*$^{+/-}$ mice. When the infection dose was increased, similar differences were not observed in most tissues but were seen in bacterial colonization of joints and the extent of *Borrelia*-induced arthritis. Quantitative PCR demonstrated that joints harvested from *Dcn*$^{-/-}$ mice had diminished *Borrelia* numbers compared with issues harvested from *Dcn*$^{+/+}$ controls. Histological examination also revealed a low incidence and severity of arthritis in *Dcn*$^{-/-}$ mice. Conversely, no differences in the numbers of *Borrelia*-positive skin cultures were observed among the different genotypes regardless of the infection dose. These differences, which were observed regardless of genetic background of the mice (BALB/c or C3H/HeN) or method of infection, demonstrate the importance of decorin in the pathogenesis of LD.

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Introduction

Borrelia burgdorferi sensu lato is the causative agent of Lyme disease (LD). It is transmitted by infected ticks that deposit a small number of organisms in the dermis of a host animal during feeding, leading to a localized infection (1). The initial skin infection is usually accompanied by a local rash (erythema migrans) and can include other manifestations such as fever, muscle pain, and headaches (2–4). Bacterial dissemination and colonization of many different organs may follow. Late stages of LD can include neurological, ocular, cutaneous, and cardiac disease in addition to arthritis (1–3). Early antibiotic therapy will usually resolve *B. burgdorferi* infection. However, symptoms sometimes may reappear, and up to 10% of patients with Lyme arthritis are classified as treatment resistant (1, 5, 6).

Microbial adhesion to and colonization of host tissues is an early, critical event in most infection processes (7). Pathogenic bacteria often express several adhesins that can participate in parallel and independent attachment mechanisms that result in tissue colonization. In LD, bacterial adhesion to host tissue may be important in determining both the fate of the initial challenge organisms in the dermis and their ability to disseminate. Spirochetes deposited in the dermis are found associated with collagen fibers in the extracellular matrix (ECM). However, *B. burgdorferi* do not attach directly to collagen but to decorin, a small leucine-rich proteoglycan (SLRP) that is associated with and "decorates" collagen fibers (8, 9). Decorin is distributed throughout the mammalian body and could be a target for *B. burgdorferi* adherence during dissemination, as these spirochetes express two decorin-binding proteins, DbpA and DbpA. However, alternate adhesion mechanisms also may be used by the spirochetes depending on the tissue target and stage of dissemination (10, 11). In addition to the two decorin-binding microbial surface component–recognizing adhesive matrix molecules (MSCRAMMs), *Borrelia* also express a fibronectin-binding MSCRAMM, BBK32, and a putative glycosaminoglycan-binding MSCRAMM (12–14). *B. burgdorferi* have been shown to attach to a variety of mammalian cells in vitro, and the organisms can bind to the integrins $\alpha_M\beta_2$, $\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$ (8, 9, 15–19).

Recently, a decorin-deficient (*Dcn⁻/⁻*) mouse was developed and characterized (20). The phenotype of this mouse was relatively mild and appeared to be restricted to skin laxity and fragility, a consequence of irregular collagen fibers in the dermis (20). This result was somewhat surprising given that decorin can have profound effects in vitro. The proteoglycan can regulate the proliferation of cells, the activity of TGF-β and the complement system (C1q), as well as the adherence of mammalian cells to fibronectin (21–27). The availability of a *Dcn⁻/⁻* mouse provided an opportunity to assess the importance of this proteoglycan in *B. burgdorferi* dissemination to various organs and tissue colonization in vivo. It has been previously shown that the method of *B. burgdorferi* transmission may affect the progression of LD and that different strains of mice can vary in the development and presentation of LD (28, 29). Therefore, we examined the role of decorin in the development of LD by infecting *Dcn⁻/⁻* mice that have been backcrossed onto genetic backgrounds that exhibit either severe (C3H/Hen) or relatively mild arthritis (BALB/c). Although the ID$_{50}$ for both strains of mice is similar, spirochete dissemination and arthritis develop at different rates and intensities (28–30). Additionally, we examined the effect of decorin during different infection processes by comparing disease development in mice that had been infected using either cultured organisms administered by needle or arthropod-adapted spirochetes transmitted by ticks (31).

Methods

Mice. Pathogen-free (MTV⁻) *Dcn⁻/⁻* mice (BL/Swiss × 129Sv) (20) were backcrossed for five and ten generations into BALB/c or C3H/HeN backgrounds (Harlan Sprague Dawley, Indianapolis, Indiana, USA). Mice were genotyped for the decorin allele by PCR analysis of mouse tail DNA as described previously (20). BALB/c and C3H/Hen mice used in all experiments were backcrossed to the fifth generation unless otherwise specified. The animals were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care in accordance with current regulations and standards of the United States Department of Agriculture, Department of Health and Human Services, and NIH. All animal procedures were approved by the Institutional Animal Care and Use Committee of Texas A&M University Health Science Center Institute of Biosciences and Technology. Female *Dcn⁻/⁻*, *Dcn⁺/⁻*, and *Dcn⁺/⁺* mice ranging in age from 8 to 10 weeks were used at the start of the experiments.

Borrelia infections. *Borrelia burgdorferi* sensu stricto, strain B31, was obtained at in vitro passage 5 from S.J. Norris (The University of Texas Medical School at Houston, Houston, Texas, USA) (32) and inoculated into BSK-II medium supplemented with antibiotics (50 μg/ml rifampicin and 100 μg/ml phosphomycin) at 34°C as described previously (33). *Borrelia* used for infections in all experiments described were cultured from aliquots of the same passage 5 frozen stock. Bacterial cultures contained in screw-cap tubes were incu-

bated in a CO$_2$-enriched atmosphere using a GasPak chamber (Becton Dickinson Microbiology Systems, Sparks, Maryland, USA) containing BBL GasPak Plus envelopes and a GasPak anaerobic indicator (Becton Dickinson, Cockeysville, Maryland, USA) until the cells reached a density between 5×10^7 and 1×10^8/ml. *Borrelia* were counted using dark-field microscopy and a Petroff-Hausser chamber. Tick- and needle-inoculation procedures were used during the course of these experiments. *Borrelia* (10^1–10^4) were needle-inoculated intradermally into shaved dorsal skin at the base of the tail (100 μl/mouse). For tick transmission, *B. burgdorferi* strain B31-infected *Ixodes scapularis* nymphs were allowed to feed to repletion. Mice were lightly anesthetized with Metofane (methoxyflurane; Pitman-Moore, Mundelein, Illinois, USA) during the tick attachment period. Immediately after tick placement, mice were individually housed in raised wire-bottom cages (Lab Products, Maywood, New Jersey, USA) containing approximately 20 ml of water at their base for the duration of the infection period (4–5 days). All cages were monitored three times daily during the first 3 days and four times daily during days 4 and 5, when replete ticks dropped from the mice (34).

Detection of Borrelia in replete ticks. Replete ticks from each mouse were collected and stored in a humid chamber for 12 days. At the end of this incubation period, each tick was sterilized by incubating in 3% hydrogen peroxide (3 minutes) followed by a 10- to 15-minute wash in 70% ethanol. Ticks were crushed individually using flat-end tweezers in BSK II-filled tubes (6 ml). Tick-inoculated media was cultured for 2 weeks as already described here and were examined for the presence of *Borrelia*.

Culturing of blood and tissues. At various time points after infection (days 3, 7, and 14) blood samples were collected from the mice and cultured for the presence of *Borrelia*. Tick-inoculated mice were maintained for 28 days after infection, and blood was cultured for the presence of *Borrelia* up to postinfection day 21. Their tails were sterilized with a propidium-iodine swab (Professional Disposables Inc., Orangeburg, New York, USA) and bled under a laminar flow biosafety cabinet. Blood dilutions ranging from 1:100 to 1:10,000 (final volume of 5 ml) were made with BSK II and incubated at 34°C. The cultures were checked 2 and 3 weeks later for the presence of viable *Borrelia,* using dark-field microscopy. Similarly, after sacrifice, joints, heart, bladder, and ear samples were aseptically removed and cultured for the presence of *Borrelia* (days 14 and 28 for needle- and tick-inoculated mice, respectively). Ear biopsy samples were collected by using a 3-mm Baker biopsy punch (Baker Norton Pharmaceuticals, Miami, Florida, USA). Care was taken to remove all skin from isolated joints. All instruments were sterilized between dissections (Dry Sterilizer IS-400; Inotech Biosystems International, Lansing, Michigan, USA).

Arthritis assessment. Evidence of arthritis was determined by histopathological examination of formalin-

fixed hind tibiotarsal joint samples. Tissues for histological examination were embedded in paraffin and stained with hematoxylin and eosin. All sections were examined without knowledge of the infection or genotype status of the mice. A positive/negative assessment of arthritis was made initially. To determine the severity of arthritis, joints were scored according to the levels of neutrophil infiltrate as follows: 0, no arthritis; 1, minimal or rare (≤ 10% tissue involvement); 2, mild (10–20%); frequent (20–50%); and 4, severe (>50%) (35).

Serum analysis (ELISA). Assays were performed on Corning Easy Wash modified flat-bottom 96-well plates (Corning-Costar Corp., Cambridge, Massachusetts, USA) as described previously (35). Briefly, the titer of the serum samples was determined and tested for reactivity against *Borrelia* using antibodies directed against each murine antibody class/subclass (IgG1, IgG2a, IgG2b, IgG3, IgE, IgA, and IgM) as described previously (35).

DNA preparation for PCR quantitation. A rear ankle joint (devoid of skin) and one ear were harvested from $Dcn^{-/-}$ and $Dcn^{+/+}$ C3H/HeN mice 4 weeks after infection. Individual tissues were incubated in 0.1% collagenase A at 37°C overnight before addition of an equal volume of 0.2 mg/ml proteinase K (Sigma Chemical Co., St. Louis, Missouri, USA) as described previously (36). After an overnight incubation at 55°C, DNA was recovered by phenol-chloroform extraction and ethanol precipitation (36). After digestion with DNase-free RNase (Sigma Chemical Co.) at 1 mg/ml, samples were again extracted and DNA was recovered by precipitation. This precipitate was resuspended in 1.5 ml of water, and the DNA content was determined by measuring the absorbance at 260 nm (36).

Quantitation of Borrelia. PCR analyses were performed in a fluorescence temperature cycler (LightCycler LC24; Idaho Technology, Idaho Falls, Idaho, USA). Amplification was performed on 200 ng of sample DNA in a 10 μl final volume containing 50 mM Tris (pH 8.3), 3 mM MgCl$_2$, and 4.5 μg of BSA, 200 μM deoxynucleoside triphosphates, a 1:30,000 dilution of SYBR Green I (Molecular Probes Inc., Eugene, Oregon, USA), 5 μM of each primer, 0.5 U of *Taq* polymerase (Life Technologies Inc., Gaithersburg, Maryland, USA), and 110 ng of TaqStart antibody (CLONTECH Laboratories Inc., Palo Alto, California, USA). Amplification was performed for 40 cycles, with each cycle consisting of heating at 20°C per second to 95°C with a 1-second hold, cooling at 20°C per second to 60°C with a 1-second hold, and heating at 1°C per second to 84°C. This procedure continuously monitored the cycle-by-cycle accumulation of fluorescently labeled product. The cycle at which the product was first detected was used as an indicator of the relative starting copy number present in each sample. Copy numbers for the mouse *nidogen* gene and *B. burgdorferi recA* were calculated by using the LightCycle software, and *recA* values were corrected by normalization based on the *nidogen* gene copy number. The oligonucleotide primers used to detect murine *nidogen* were nidoF (5′-CCA GCC ACA GAA TCA CAT CC-

3′) and nidoR (5′-GGA CAT ACT CTG CTG CCA TC-3′). The oligonucleotide primers used to detect *B. burgdorferi recA* were nTM17.F (5′-GTG GAT CTA TTG TAT TAG ATG AGG CTC TCG-3′) and nTM17.R (5′-GCC AAA GTT CTG CAA CAT TAA CAC CTA AAG-3′) (36, 37).

Results

Experimental Borrelia infection. The involvement of decorin in the establishment and dissemination of *B. burgdorferi* in mice was examined using $Dcn^{-/-}$, $Dcn^{+/-}$, and $Dcn^{+/+}$ mice. Because the genetic background of an infected mouse influences the severity of arthritis, the $Dcn^{-/-}$ mice were backcrossed to BALB/c and C3H/HeN animals. C3H/HeN mice are reported to develop severe arthritis when infected with *B. burgdorferi*, whereas BALB/c mice develop a relatively mild arthritis that is influenced by the dose of *B. burgdorferi* administered (28, 29, 38).

In initial experiments, BALB/c mice of each genotype were needle inoculated with 10^3 or 10^4 *B. burgdorferi* B31. Blood was collected at days 3, 7, and 14 days after infection and cultured to detect the presence of *Borrelia* (35). No spirochetes were detected in the blood 3 days after infection. $Dcn^{-/-}$ mice infected with either 10^3 or 10^4 *B. burgdorferi* consistently had a lower percentage of *Borrelia*-positive blood cultures 7 days after infection compared with $Dcn^{+/+}$ and $Dcn^{+/-}$ mice at both 1:100 and 1:1,000 blood dilutions in three separate experiments (Table 1). These differences were not statistically significant in individual experiments. However, if the data for experiments 1 and 2 were combined (both used an infectious dose of 10^4 spirochetes), the difference in *Borrelia*-positive blood cultures between $Dcn^{-/-}$ (2/14) and $Dcn^{+/+}$ (12/20) was significant for the 1:100 dilution ($P < 0.05$; Fisher's exact test). Spirochetes were not detected in blood dilutions of ≥ 1:10,000. Blood samples collected 14 days after infection had a low incidence of *Borrelia*-positive cultures (<20%), and no significant difference was observed between the different *Dcn* genotypes (data not shown).

Table 1
Blood cultured for the presence of *B. burgdorferi* at 7 days after infection

Genotype	n[A]	%Positive cultures[B]	
		1:100[C]	1:1,000[C]
Experiment 1			
$Dcn^{+/+}$ (10^4)[D]	10	60 (6/10)	30 (3/10)
$Dcn^{+/-}$ (10^4)[D]	10	60 (6/10)	10 (1/10)
$Dcn^{-/-}$ (10^4)[D]	6	16 (1/6)	0 (0/6)
Experiment 2			
$Dcn^{+/+}$ (10^4)[D]	10	60 (6/10)	20 (2/10)
$Dcn^{+/-}$ (10^4)[D]	12	50 (6/12)	25 (3/12)
$Dcn^{-/-}$ (10^4)[D]	8	12.5 (1/8)	12.5 (1/8)
Experiment 3			
$Dcn^{+/+}$ (10^3)[D]	5	100 (5/5)	100 (5/5)
$Dcn^{+/-}$ (10^3)[D]	5	80 (4/5)	60 (3/5)
$Dcn^{-/-}$ (10^3)[D]	5	40 (2/5)	40 (2/5)

[A]Number of mice of each genotype used. [B]Blood was collected aseptically and cultured in BSK II medium for 2 weeks and examined for the presence of *B. burgdorferi*. [C]Blood dilution tested for the presence of *B. burgdorferi*. [D]*B. burgdorferi* infection dose.
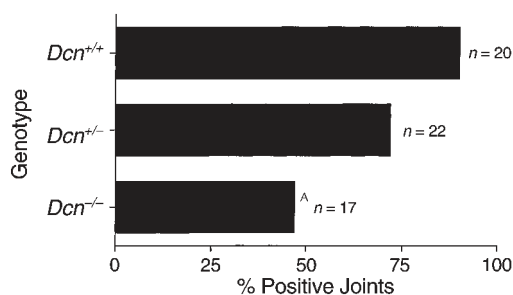
Figure 1
Percent *Borrelia*-positive joint cultures. BALB/c $Dcn^{-/-}$ (n = 17), $Dcn^{+/-}$ (n = 22), and $Dcn^{+/+}$ mice (n = 20) were infected with 10^4 *B. burgdorferi*. Joints were harvested 2 weeks post infection and inoculated into BSK II medium and examined for the presence of spirochetes 14 days later. [A]P < 0.01; Fisher's exact test compared with wild-type controls.

To examine whether any differences in the dissemination of *Borrelia* could be observed in these mice, the animals were sacrificed 14 days after infection, and ear biopsy samples, heart, bladder, and one hind tibiotarsal joint were harvested and cultured for the presence of spirochetes (35). *Borrelia* recovery from joints was significantly reduced in $Dcn^{-/-}$ mice (Figure 1), whereas essentially all cultures of other tissues examined were *Borrelia*-positive (data not shown). Only 47% of joints obtained from $Dcn^{-/-}$ mice were *Borrelia*-positive compared with 90% of joints obtained from $Dcn^{+/+}$ mice (P < 0.01; Fisher's exact test). $Dcn^{+/-}$ mice had an intermediate number of *Borrelia*-positive joints (72%). Histological examination of hematoxylin and eosin–stained, blind-coded sections of the contralateral hind tibiotarsal joint also revealed reduction in both arthritis incidence and severity in animals lacking decorin (Table 2). The average arthritis rating was only 1.11 in $Dcn^{-/-}$ mice but increased to 1.78 and 2.35 in $Dcn^{+/-}$ and $Dcn^{+/+}$ mice, respectively. Severely arthritic joints (scored as 4) were found in all three genotypes. These data, and the reduced number of positive blood cultures in $Dcn^{-/-}$ mice, suggested that fewer *Borrelia* survived the initial infection and/or fewer *Borrelia* were able to colonize the joints after dissemination from the skin.

One possible explanation for these results is that the high inoculum dose used ($\geq 10^3$ *Borrelia* per mouse) might mask any discernible differences in colonization patterns for tissues other than the joints from the different genotypes. Because spirochetal persistence in tissues has been related to inoculum dose in BALB/c mice (31), we infected the three genotypes of mice with low doses of *B. burgdorferi* (10^1 or 10^2 *Borrelia*) and examined mice for spirochete dissemination 14 days after infection. All tissues examined from mice infected with 10^1 spirochetes were *Borrelia* negative by culture (data not shown). However, an infectious dose of 10^2 *Borrelia* revealed differences in colonization in most tissues examined. The most notable differences between genotypes were observed in joint and heart cultures; only 22% of joint and heart cultures harvested from $Dcn^{-/-}$ mice were *Borrelia*-positive (Figure 2), a marked but not statistically significant reduction at the sample size

examined. Tissues from $Dcn^{+/-}$ mice had an intermediate level of *Borrelia*-positive cultures. Bladders were also colonized in a decorin-dependent manner with 33%, 45%, and 67% of the cultures scoring positive for *Borrelia* from the $Dcn^{-/-}$, $Dcn^{+/-}$, and $Dcn^{+/+}$ mice, respectively. However, ear biopsy specimens cultured from infected mice of the various genotypes revealed no differences (Figure 2). The arthritic response by decorin genotype was also examined in mice that received 10^2 *Borrelia*. Histological analysis revealed that $Dcn^{-/-}$ mice had an arthritis incidence of 55%, whereas $Dcn^{+/+}$ and $Dcn^{+/-}$ mice had an arthritis incidence of 100% and 44%, respectively (data not shown). The mean arthritis rating among the $Dcn^{-/-}$ and $Dcn^{+/-}$ mice (1.3 and 1.22, respectively) was also less than that observed for $Dcn^{+/+}$ mice (1.87) (data not shown). These results demonstrated a reduced colonization of joint, bladder, and heart, but not blood or skin in $Dcn^{-/-}$ mice compared with $Dcn^{+/+}$ controls when the infectious dose was low.

Quantification of Borrelia in tissues. To examine whether the resistance to experimental Lyme arthritis observed in $Dcn^{-/-}$ BALB/c mice was also manifested in the C3H/HeN background, we infected $Dcn^{-/-}$ and $Dcn^{+/+}$ C3H/HeN mice with 200 *Borrelia* intradermally and quantified the numbers of *Borrelia* in different tissue samples. DNA was purified from ears and joints at 4 weeks after infection, and the numbers of *B. burgdorferi* organisms present were determined by quantitative PCR (36). All samples were blind-coded before analysis. The results of a representative experiment are shown in Figure 3. Although *Borrelia* numbers were similar in ear samples, $Dcn^{-/-}$ C3H/HeN mice had significantly less *Borrelia* in joints than did $Dcn^{+/+}$ controls. Differences were also apparent in the arthritis incidence and severity. $Dcn^{-/-}$ mice had 55% arthritis incidence and a mean arthritis score of 1.08 compared with 100% incidence and a 2.3 mean arthritis score in $Dcn^{+/+}$ mice (P < 0.05; Fisher's exact test and Student's *t* test for percent incidence and severity, respectively). Figure 4 illustrates differences in hematoxylin and eosin–stained representative joint samples from $Dcn^{-/-}$ and $Dcn^{+/+}$ mice (Figure

Table 2
Arthritis development in BALB/c mice after infection with *Borrelia burgdorferi*[A] at 14 days after infection

Genotype	Mean arthritis rating	Arthritis frequency (%)
$Dcn^{+/+}$ (0,1,1,2,2,2,2,2,2,2,2, 3,3,3,3,3,3,3,4,4)[B]	2.35	19/20 (95%)
$Dcn^{+/-}$ (0,0,0,1,1,2,2,2,2,2,2,2, 2,2,2,3,3,3,3,4)[B]	1.78[C]	16/19 (84%)[C]
$Dcn^{-/-}$ (0,0,0,0,0,0,0,0,0,0,1,1,1, 2,2,2,3,4,4)[B]	1.11[D]	9/18 (50%)[E]

[A]$Dcn^{-/-}$ mice were infected with 10^4 or *B. burgdorferi* strain B31 (passage 5) intradermally at the base of the tail. [B]Individual arthritis rating for each mouse. [C]Not significant. [D]P < 0.001 versus $Dcn^{+/+}$ group; Student's *t* test. [E]P < 0.001 versus $Dcn^{+/+}$ group; Fisher's exact test.
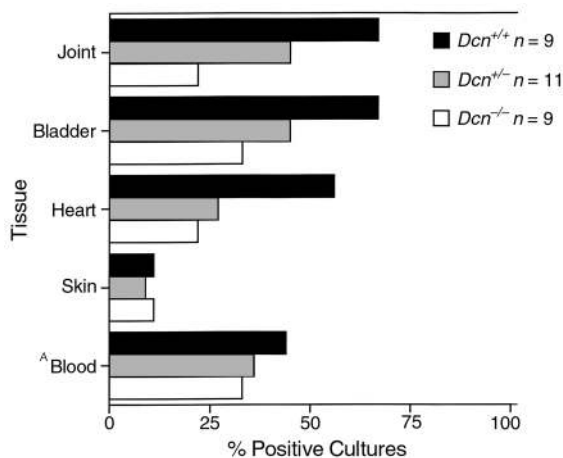
Figure 2

Percent *Borrelia*-positive cultures. BALB/c *Dcn*⁻/⁻ (*n* = 9), *Dcn*⁺/⁻ (*n* = 11), and *Dcn*⁺/⁺ (*n* = 9) mice were infected with 10² *B. burgdorferi*. Tissues were aseptically removed 2 weeks after infection, inoculated into BSK II medium, and examined for the presence of spirochetes 14 days later. ᴬBlood was cultured at postinfection day 7. Differences between genotypes were not statistically significant.

4, a and b, respectively). Joints harvested from *Dcn*⁺/⁺ mice had noticeably greater numbers of infiltrating cells, particularly neutrophils, compared with joints obtained from *Dcn*⁻/⁻ mice.

Tick inoculations. Lyme disease progression and development in mice, as well as the nature of the immune response generated against *B. burgdorferi*, differs between culture grown *B. burgdorferi* cells injected by needle- and tick-transmitted organisms (39, 40). We therefore also examined spirochete dissemination throughout the host and arthritis formation in *Dcn*⁻/⁻ BALB/c mice after infection of animals by the natural vector. *Borrelia*-infected *Ixodes scapularis* nymphs (three or five nymphs per mouse) were allowed to feed to repletion on *Dcn*⁻/⁻, *Dcn*⁺/⁻, and *Dcn*⁺/⁺ mice, and blood samples were collected at postinfection days 7, 14, and 21 (34, 39). The animals were sacrificed 28 days after infection, and samples from ear, heart, bladder and joints were examined for the presence of *B. burgdorferi* (Table 3). To determine the number of *Borrelia*-positive ticks feeding on individual mice, ticks were cultured for the presence of *Borrelia* after the feeding period. Tick attrition during the course of the experiment, combined with *Borrelia*-negative ticks, reduced the mean number of infected ticks per mouse in both experiments to 2.8–3.5 and 1.7 (Table 3, experiments 1 and 2, respectively).

The number of *Borrelia*-positive ticks allowed to feed on individual mice of the different genotypes affected dissemination patterns of the bacteria in a manner similar to that observed in the high- and low-dose needle inoculation experiments. Similar to low-dose needle inoculations, *Dcn*⁻/⁻ mice infested with less than two *B. burgdorferi*–infected ticks had fewer *Borrelia*-positive heart, bladder (54% positive), and joint cultures (38% positive) compared with *Dcn*⁺/⁺ controls (80% *Borrelia*-positive cultures for all tissues examined) (Table 3,

experiment 2). Reduced numbers of *Borrelia*-positive cultures from ear biopsies collected from *Dcn*⁻/⁻ mice compared with *Dcn*⁺/⁺ mice were observed when mice were tick inoculated but not needle inoculated. The reason for this difference is currently unknown. When the infectious dose was increased by allowing a larger number of infected ticks to feed on the mice, this difference was not generally observed (Table 3, experiment 1). However, the joints again represent an exception. Regardless of the number of infected ticks allowed to feed, *Dcn*⁻/⁻ mice always had fewer *Borrelia*-positive joint cultures than did *Dcn*⁺/⁺ controls (Table 3). However, in contrast to needle-inoculated mice, the differences in arthritis scores were not significant. We observed differences in *Borrelia*-positive blood cultures for both high- and low-dose tick inoculations 21 days after infection (Table 3). When 2.8–3.5 infected ticks were allowed to feed on the mice, 33% of the blood cultures from *Dcn*⁻/⁻ mice were *Borrelia*-positive at 21 days after infection compared with 70% and 50% positive in blood cultured from *Dcn*⁺/⁺ and *Dcn*⁺/⁻ mice, respectively. When fewer ticks were allowed to feed to repletion, 38% compared with 63% of blood cultures (1:100 dilution) were *Borrelia*-positive from *Dcn*⁻/⁻ and *Dcn*⁺/⁺ mice, respectively. Blood collected from the different genotypes earlier during the infection process did not show any considerable difference with respect to *Borrelia*-positive cultures (Table 3, experiment 2). Unless indicated, differences in *Borrelia*-positive cultures were not statistically significant (Table 3).

Immune responses in decorin-deficient mice. Decorin has been previously shown to affect a number of different mediators of immune responses, including TGF-β, in addition to affecting the complement system. What role decorin plays in regulating immune responses is not known. This raised the possibility that the observed increased resistance to *Borrelia* infection in *Dcn*⁻/⁻ mice might be linked to decorin-related changes in immuni-
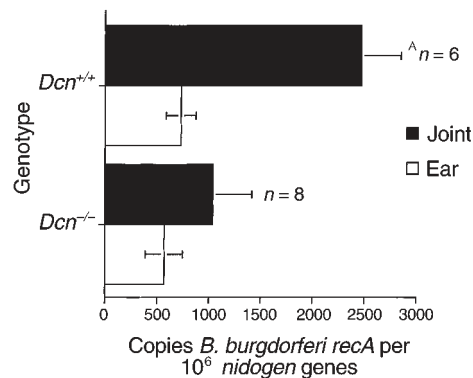

Figure 3

Detection of *B. burgdorferi* DNA. *Borrelia* was quantitated from ear and joint samples collected from *Dcn*⁻/⁻ (*n* = 8) and *Dcn*⁺/⁺ (*n* = 6) C3H/HeN mice. Mice were infected with 200 *B. burgdorferi*. The indicated tissues were harvested 4 weeks after infection and assessed for *B. burgdorferi*-specific DNA by quantitative PCR of the *B. burgdorferi recA* gene. Results were normalized to those of the host *nidogen* gene. The data are expressed as mean ± SEM. ᴬ*P* < 0.05; Student's *t* test.
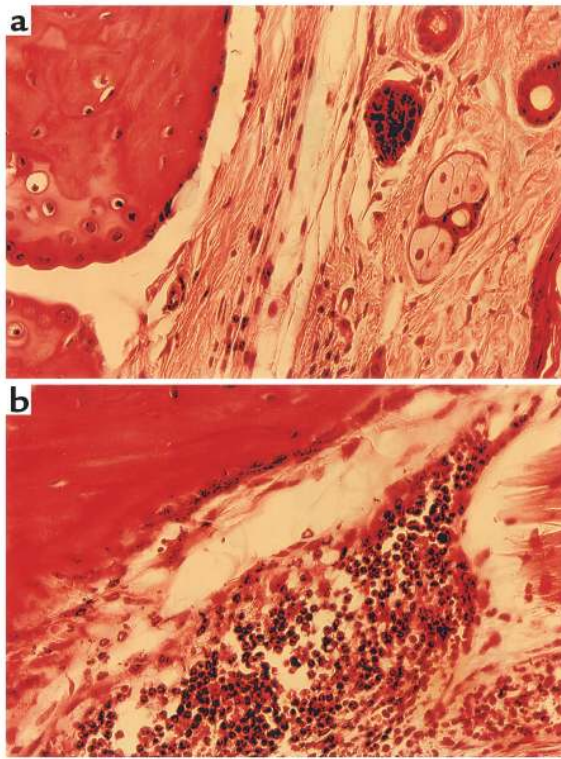
Figure 4
Hematoxylin and eosin–stained sections of hind tibiotarsal joints. C3H/HeN $Dcn^{-/-}$ (**a**) and $Dcn^{+/+}$ (**b**) mice were infected with 200 borreliae. Mice were sacrificed and the joints harvested for histological analysis at 28 days after infection.

ty (21, 24–27, 41). To address this, both cellular and humoral responses in $Dcn^{-/-}$ and $Dcn^{+/+}$ mice were examined. The serum from tick-inoculated mice (described in Table 3, experiment 2) was screened by ELISA for antibodies reactive to whole *B. burgdorferi* (Figure 5). Only IgG1, IgG2a, and IgG2b *Borrelia*-reactive antibodies were detected, and no differences between $Dcn^{-/-}$ and $Dcn^{+/+}$ mice were observed. In addition, serum from needle-inoculated mice screened for DbpA reactivity revealed no differences among genotypes tested ($Dcn^{-/-}$, $Dcn^{+/-}$, or $Dcn^{+/+}$) (data not shown). Cellular responses in $Dcn^{-/-}$ were also unaffected. Immunization with DbpA elicited

a similar delayed-type hypersensitivity response in $Dcn^{-/-}$ and $Dcn^{+/+}$ after challenge with DbpA (data not shown). Therefore, we concluded that the $Dcn^{-/-}$ background did not noticeably affect the development of either humoral or cellular immune responses.

Discussion

Bacterial adhesion to the host tissue is considered an early, critical step in the development of infection. Many pathogenic organisms have acquired several parallel and apparently independent mechanisms of adherence, probably reflecting the importance of this step in the disease process. Elimination of individual adhesins through genetic manipulations, nevertheless, can result in bacterial mutants with reduced virulence (42, 43). Heterologous expression of adhesins from pathogenic bacteria in less-virulent strains has also been used as a strategy to demonstrate a role of individual adhesins as virulence factors (42). Here we have taken a reverse approach by examining the infectious disease process in mice that are deficient in an adhesion ligand.

The adherence potential of *B. burgdorferi* is incompletely understood. So far, two decorin-binding (8) and one fibronectin-binding (13) MSCRAMM have been identified, as well as candidate adhesins for the observed adhesion of *B. burgdorferi* to β_3 integrins and to glycosaminoglycans (12, 14, 16, 44–46). The $Dcn^{-/-}$ mice used in this study have a surprisingly mild phenotype considering the potent effects of decorin in vitro. Decorin is a member of the SLRP family of structurally and apparently functionally related molecules (41, 47). Some members of this family may have similar roles in vivo, resulting in a certain degree of redundancy. Furthermore, the inactivation of one SLRP gene in mice can result in upregulation of the expression of other SLRP genes and/or enhanced tissue retention of these molecules (48). The SLRP-binding spectra of the *B. burgdorferi* MSCRAMMs DbpA and DbpB have not been examined beyond work demonstrating that DbpA, in addition to binding decorin, also bound to the related proteoglycan, biglycan (E. Brown et al., unpublished observations). In view of the multiple adhesion mechanisms apparently available to the spirochete, it is remarkable that we have demonstrated dif-

Table 3
Examination of tick-inoculated mice for *Borrelia* dissemination and arthritis development

Genotype	n^A	% Positive blood cultures^B d7	d14	d21	% Positive tissues Ear	Heart	Bladder	Arthritis assessment Joint	Arthritis score
Experiment 1 $Dcn^{+/+}$ (2.8)^C	10	90/ND/ND	80/ND/ND	70/ND/ND	100	100	100	80	1.9
Experiment 1 $Dcn^{+/-}$ (3.5)^C	10	70/ND/ND	80/ND/ND	50/ND/ND	80	80	80	40	1.2
Experiment 1 $Dcn^{-/-}$ (3.3)^C	9	100/ND/ND	90/ND/ND	33/ND/ND	100	100	77	33^D	1.6
Experiment 2 $Dcn^{+/+}$ (1.7)^C	10	60/40/30	40/10/0	63/27/ND	80	80	80	80	1.9
Experiment 2 $Dcn^{-/-}$ (1.7)^C	13	61/38/0	42/8/0	38/7/ND	54	54	54	38	1.5

^{A}Number of mice of each genotype used. ^{B}Blood was collected as described previously at 7, 14, and 21 days after infection (1:100/1,000/10,000 dilutions). ^{C}Average number of *Borrelia*-positive ticks per mouse in each group. Five (experiment 1) or three (experiment 2) *Ixodes scapularis* nymphs were allowed to attach to each mouse. At the end of the feeding period (days 4–5), all remaining nymphs per mouse were collected and stored for 12 days, at which time individual ticks were inoculated into BSK II media and cultured for the presence of spirochetes. $^D P < 0.05$ compared with $Dcn^{+/+}$ control (experiment 1); χ^2 test. $P < 0.07$; Fisher's exact test (not significant). ND, not determined.

ferences in *Borrelia* infection in mice in which only one adhesion ligand was removed. Decorin binding, therefore, is a major mechanism by which *B. burgdorferi* disseminates. To our knowledge, this is the first report demonstrating in vivo the importance of an MSCRAMM ligand in an infectious disease process.

Most tissues examined from the $Dcn^{-/-}$ mice had fewer *Borrelia*-positive cultures compared with $Dcn^{+/+}$ mice when the infection doses were low. In most cases, the differences were eliminated when the infection dose was increased. The joints were an exception in that this tissue from $Dcn^{-/-}$ mice had fewer *Borrelia*-positive cultures and reduced *Borrelia* numbers regardless of the infection dose. Furthermore, the joints were consistently less arthritic in $Dcn^{-/-}$ mice compared with the $Dcn^{+/+}$ controls regardless of the genetic background of the mice. In general, the number of *Borrelia*-positive cultures were intermediate in $Dcn^{+/-}$ mice. This could reflect presumed intermediate levels of decorin in heterozygous mice based on decorin mRNA analysis (20). This difference suggested that decorin is a crucial substrate for the colonization of the joints by *B. burgdorferi*. One explanation for the apparent decorin-dependent colonization of joints observed in these studies is the very high content of the proteoglycan in cartilage. Fibronectin, another potential substrate for *B. burgdorferi* adhesins, is normally low in this tissue. Conversely, no differences in *Borrelia*-positive cultures were generally observed from skin biopsies regardless of genotype or infection dose, although $Dcn^{-/-}$ mice had fewer *Borrelia*-positive skin biopsies compared with $Dcn^{+/+}$ mice when low-dose tick transmission was used. However, these results cannot be taken to indicate that Dbp-dependent adhesion mechanisms did not contribute to the ability of the spirochetes to colonize skin, as ligands for the Dbp's other than decorin could be of importance. In fact, fluorescence microscopy of embryonic skin fibroblasts derived from $Dcn^{-/-}$ mice and probed with DbpA demonstrated the presence of additional ligands for the MSCRAMM (E. Brown and M. Höök, unpublished observations).

Substantial differences have been observed in the presentation of LD in mice depending on whether the spirochetes were culture grown and injected by needle or were tick transmitted (39, 40). However, $Dcn^{-/-}$ mice were resistant to LD regardless of the inoculation strategy used, and similar effects between the infection dose and tissue colonization by *Borrelia* were observed in mice infected by either inoculation procedure.

We have so far assumed that the observed resistance to LD in $Dcn^{-/-}$ mice was due to the proteoglycan's role as a ligand to the Dbps and as a substrate to tissue adherence of *B. burgdorferi*. There are several observations that are consistent with this assumption: first, there is a tissue-specific effect that to some extent reflects the abundance of available decorin; second, heterozygous mice often showed intermediate colonization numbers, suggesting that the amounts of decorin available for *Borrelia* adherence could be limit-

ed; and third, with a high-dose inoculum the differences in positive cultures disappeared for most tissues, presumably because the spirochetes used alternative adhesion substrates. However, at this point we cannot exclude alternative explanations to the observed resistance to experimental LD in $Dcn^{-/-}$ mice. We considered the possibility that $Dcn^{-/-}$ mice might have an enhanced host defense against bacterial infections. The results presented here demonstrated that $Dcn^{-/-}$ mice could mount both humoral and cellular immune responses indistinguishable from $Dcn^{+/+}$ controls. Furthermore, $Dcn^{-/-}$ BALB/c mice were found in preliminary experiments to be at least as susceptible as $Dcn^{+/+}$ mice to *Staphylococcus aureus*–induced septic arthritis (E. Brown and M. Höök, unpublished observations). Thus, $Dcn^{-/-}$ mice did not appear to be dramatically different from $Dcn^{+/+}$ mice in their general host defense potential.

In summary, we have demonstrated that $Dcn^{-/-}$ mice were more resistant to LD, which suggested that decorin
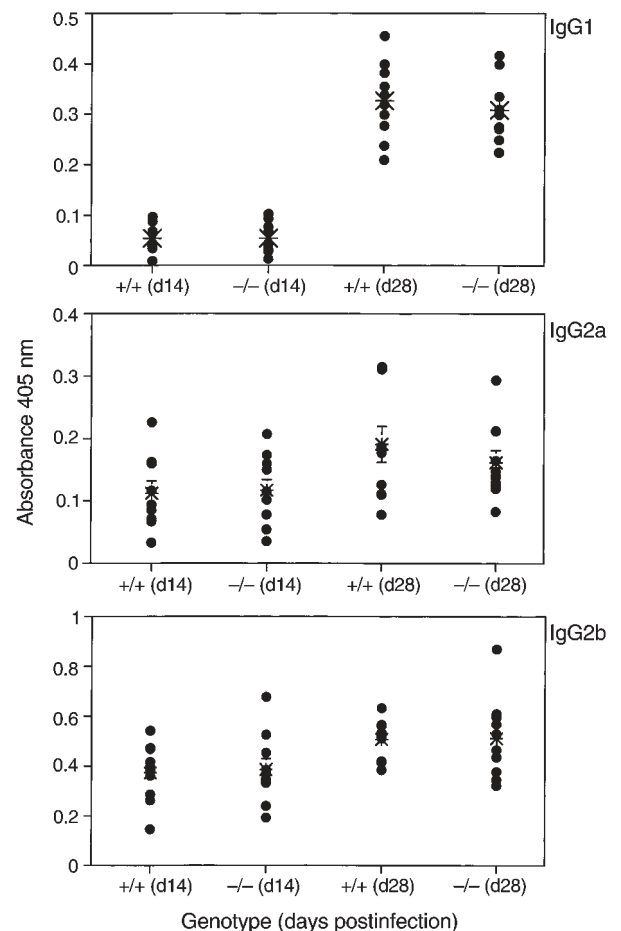

Figure 5
Antibody response to *B. burgdorferi* from tick-inoculated mice. Serum from infected mice described in Table 3 was tested for reactivity against *B. burgdorferi* 14 and 28 days after infection. Each data point represents the mean absorbance from triplicate wells from individual mice at 405 nm minus the substrate control. The mean absorbance value for each group is represented by a large asterisk. There was no statistical differences between the $Dcn^{-/-}$ and the $Dcn^{+/+}$ mice.

could be a limiting factor as a substrate for the adherence of *B. burgdorferi*. However, this resistance could be overcome for most tissues examined by increasing the infection dose, suggesting that additional adhesion ligands could participate in the disease process in vivo.

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