Early and Early Disseminated Phases of Lyme Disease in the Rhesus Monkey: a Model for Infection in Humans

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We demonstrate that Borrelia burgdorferi infection in the rhesus monkey mimics the early and early disseminated phases of human Lyme disease. Clinical, bacteriological, immunological, and pathological signs of infection were investigated during 13 weeks after inoculation of the spirochete. Three animals were given B. burgdorferi (strain JDI) by needle inoculations, six animals were exposed to the bite of B. burgdorferi-infected Ixodes dammini ticks, and three animals were uninfected controls. B. burgdorferi could be recovered from all animals that were given the spirochete. Bacteria were detectable until week 6 postinoculation (p.i.) in blood, until week 8 p.i. in skin biopsies, and at 10 weeks p.i. in the conjunctiva of one of two animals which developed conjunctivitis. Erythema migrans (EM) appeared in one of the three animals infected by needle inoculation and in five of the six animals infected by ticks. Deep dermal perivascular lymphocytic infiltrations (characteristic of human EM) were observed in all animals showing EM clinically. Both EM and conjunctivitis were documented concomitantly with the presence of the spirochete. Lethargy, splenomegaly, and cerebrospinal fluid pleocytosis were also noted in some animals, but the direct connection of these signs with the infection was not shown. The appearance rate of immunoglobulin M and immunoglobulin G antibodies to B. burgdorferi, as well as the antigen spectra recognized, were remarkably similar to those seen in humans. Serum antibodies from infected animals were able to kill B. burgdorferi in vitro in the presence of rhesus complement. The rhesus monkey model appears to be useful for the investigation of the immunology and pathogenesis of Lyme disease and for the development of immunoprophylactic, diagnostic, and chemotherapeutic protocols.

Lyme borreliosis, caused by the spirochete Borrelia burgdorferi, is considered the most frequently reported arthropod-borne infection in Europe and North America (20). In fact, in 1992 it accounted for over 80% of all reported vector-borne infections in the United States (10). The infection, which is transmitted through the bite of ticks of the Ixodes ricinus "complex," has an incidence as high as 100 cases per 100,000 population in hyperendemic foci of the United States (8, 25).

The disease was first identified in November 1975 in association with an arthropathy affecting a group of children in the town of Old Lyme, Conn. (39). Since then, considerable progress has been made in understanding its complexity; Lyme borreliosis is now recognized as a multiple-system disease often heralded by an expanding, usually erythematous, skin lesion. Erythema migrans (EM), as the lesion is called, may be followed by one or more of a variety of neurologic, musculoskeletal, ocular, reticuloendothelial, and cardiac symptoms (39).

Unfortunately, none of the animal models used currently in the experimental analysis of Lyme disease displays this spectrum of signs. Studies with rabbits (6), hamsters (13, 19, 21, 36), rats (3), mice (4, 34), and dogs (28) have contributed much useful information about the biology of B. burgdorferi and about possible mechanisms of protective immunity and drug action. Yet, understanding disease pathogenesis and developing reliable protocols for immunoprophylaxis and chemotherapy require an animal model producing signs that accurately mirror the course of infection in humans. Here we provide evidence that B. burgdorferi infection in the rhesus monkey produces an array of signs that closely mimics the human Lyme disease syndrome.

The signs and symptoms of Lyme borreliosis may be broadly classified as early and late (1, 39). Early infection is characterized by the presence of EM; during the early disseminated phase, the disease may produce acute central nervous system, cardiac, ophthalmic, and/or reticuloendothelial symptoms and sometimes arthritis. The late phase is defined by the chronic expression of early signs and symptoms and usually begins 1 year after disease onset. We identified and investigated clinical, bacteriological, immunological, and pathological signs of infection appearing during the first 13 weeks of infection, a period which should encompass the early and early disseminated phases of the disease in humans. This is the first part of an ongoing study aimed at defining both the early and late phases of Lyme disease in the rhesus monkey.

At the clinical level, we focused on dermal, ocular, reticuloendothelial, cardiovascular, and neurologic signs, as well as on the development of arthropathies. This information was supported by histopathological data whenever possible. Progress of the infection was monitored bacteriologically by in vitro culture of B. burgdorferi from skin biopsies, blood, cerebrospinal fluid (CSF), and urine and by immunohistochemistry of skin and conjunctival biopsy sam-

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ples with \( B. \ burgdorferi \)-specific antibodies. The spirochete species recovered by in vitro culture was identified by using the polymerase chain reaction (PCR) with species-specific DNA oligonucleotide primers. At the immunological level, humoral responses were investigated as a function of time after infection. The time course of appearance of immunoglobulin M (IgM) and IgG antibodies to whole \( B. \ burgdorferi \) antigens was monitored by Western blot (immunoblot). The ability of serum antibodies to kill the spirochete in vitro in the presence of rhesus monkey complement was also assessed. In every facet of this investigation an attempt was made to identify both similarities and differences between manifestations of infection in the rhesus monkey and in humans.

**MATERIALS AND METHODS**

**Study population.** The study population consisted of 12 male, 2-year-old Chinese rhesus monkeys (Macaca mulatta). The average weight of the animals was 3.2 kg (range, 2.6 to 4.0 kg). Three monkeys (group I) were given \( B. \ burgdorferi \) (strain JD1) (31) by needle inoculations. Six animals (group II) were exposed to the bite of \( B. \ burgdorferi \) (strain JD1)-infected nymphal stage \( Ixodes dammini \) ticks. One animal was given a sham needle inoculation (group I control), and two animals were exposed to the bites of uninfected ticks (group II control).

**Animal housing and care.** All infected animals were housed in separate quarters without an infectious disease housing area. Control uninfected monkeys were likewise housed by themselves in a separate room. The perimeters of all room entrance and escape routes were taped with Tangle Trap to prevent escape of potentially escaped ticks and access of other possible vectors from the outside. All monkeys were singly caged, were fed commercial monkey chow (Purina Mills 5037), and had water available ad libitum. Animal care facilities were accredited by the American Association for Accreditation of Laboratory Animal Care and licensed by the U.S. Department of Agriculture. All animals were cared for according to the guidelines prescribed by the National Institutes of Health Guide to Laboratory Animal Care.

**Inoculation protocol.** Animals were separated into two groups according to the route of infection. Three animals (group I: animals K167, J743, and J748) were given an inoculation of \( 4 \times 10^4 \) \( B. \ burgdorferi \) (strain JD1, third passage) organisms in 3.1 ml of RPMI 1640 medium, using needle and syringe. The inoculum was divided and delivered intraperitoneally (2 ml), subcutaneously (1 ml), and intradermally (0.1 ml) at one site located on the lower right abdominal quadrant. Animal J743 from this group was only given intraperitoneal (3 ml) and intradermal (0.1 ml) inoculations (\( 4 \times 10^4 \) \( B. \ burgdorferi \) organisms). The control animal for group I, K204, was given a sham inoculation in the same fashion as the infected animals with RPMI 1640 medium alone. Six animals (group II: animals K205, J677, J200, J797, J415, and J307) were infected by bites of \( B. \ burgdorferi \)-infected \( I. \ dammini \) nymphal ticks placed in capsules located dorsolaterally on the cranial thorax (see “Monkey inoculation by ticks” below). Two animals, J622 and J206, were exposed to the bites of uninfected ticks and served as controls for group II.

**Animal monitoring: sample collections and physical examinations.** Sample collections and physical examinations were performed while the animals were anesthetized with Ketamine HCl (10 mg/kg). Skin biopsies were taken aseptically from the periphery of the inoculation sites, or wherever a lesion arose, with 8-mm dermal biopsy punches after surgical preparation of the skin sites. CSF was taken by cisterna magna puncture after surgical preparation of the overlying skin. Urine was recovered by cystocentesis. Physical examination consisted of visual observation of body systems, auscultation of the thorax, abdominal palpation, and palpation and grading of peripheral lymph nodes and spleen. Electrocardiograms were performed if an abnormal rhythm was noted on auscultation.

Ocular examinations were performed weekly for the first 8 weeks, biweekly for the next 4 weeks, and monthly thereafter. Following external inspection of conjunctiva and adnexa, a slit lamp and dilated fundus examination was performed. Conjunctival biopsies were taken under local anesthesia and with separate sterile instruments for each animal.

Prior to infection, physical examinations and skin biopsies were performed and found to be normal. Routine parasitological fecal exams were performed, and animals with intestinal infestations were treated appropriately 4 weeks prior to infection with \( B. \ burgdorferi \). Complete blood counts, serum chemistries, urinalysis, and physical examinations were performed for each animal prior to inoculation to determine baseline levels and then once weekly after inoculation. CSF was collected once monthly beginning at week 4 postinoculation (p.i.) for culture and cytology. Two tick-inoculated animals (J415 and J307) and one needle-inoculated animal (J748) were monitored with sequential CSF sampling. Animal K204 was monitored as a negative control. Other samples collected on a weekly or biweekly basis included blood for Western blotting and in vitro culture, urine for culture, and skin biopsies for culture and histopathology.

**Source of ticks and tick infection procedure.** All nymphal \( I. \ dammini \) ticks were derived from a colony originating from Great Island, Mass. (12). This colony has been maintained free of spirochetes for ca. 5 generations. Larval ticks were allowed to feed on 7-month-old \( B. \ burgdorferi \) (strain JD1)-infected Swiss outbred mice at 1 month p.i. Replete larvae were collected, held at 21°C and melted to nymphs. Nymphs were allowed to feed on monkeys at >2 months postmolting.

**Monkey inoculation by ticks.** The tick containment capsule consisted of a 60-ml polyethylene bottle that was cut off near the top, leaving the neck of the bottle attached to a flat base, and covered with a screw cap. A circular piece of fine mesh cloth extending about 1 in. (ca. 2.5 cm) beyond the base was glued to the capsule base with liquid plastic (Plasti Dip International, St. Paul, Minn.). A 1/4-in. (ca. 0.6-cm) hole was drilled in the center of the screw cap. A piece of fine mesh cloth was draped over the bottle neck, and the opening was covered by screwing the cap over it. The upper torso of each animal was closely shaven, rinsed with water, dried, and then sponged with Skin Prep (Smith & Nephew Medical, Massillon, Ohio), except in the area where the capsule would be placed. The cloth apron fastened to the capsule base and the skin area where the apron would eventually rest were coated with Skin Bond contact adhesive (Smith & Nephew Medical). The base of the capsule itself was coated with Super Glue, which is nonreactive with skin. The capsule base and cloth apron were adhered to the skin overlying the right scapula of each animal. The capsule was further fastened in place by wrapping a continuous 2-in. (ca. 5-cm)-wide strip of Hypafix adhesive dressing tape (Smith & Nephew Medical) three times around the entire upper torso, the capsule top being forced through a slit in the tape with each passage. The Hypafix tape then was covered in the same way with two layers of 2-in.-wide adhesive tape to help prevent the animal from pulling it loose from its body. Last,
a nylon mesh vest that zippered in the back (Medical Arts, Los Angeles, Calif.) was fitted over the body of each animal. Mouth aspirators constructed from glass transfer pipets were used to separate the ticks into groups of 20 each and to transfer each group from the laboratory to the animal holding room. The pipet tip was inserted into a small hole drilled through the neck and screw cap, and then the ticks were blown into the capsule and the hole was sealed with hot glue. The tick capsule remained on the animal’s body for 8 days, with the ticks put into the capsule on the third day.

B. burgdorferi infection rate of engorged ticks. All engorged ticks were assessed 3 weeks postattachment for residual spirochetes by the direct fluorescence microscopy method of Piesman et al. (32).

In vitro cultivation of B. burgdorferi from stocks and from body fluids and tissue samples. Spirochetes were cultured in BSK II medium (2) at 34 to 35°C. Bacterial counts were made with a dark-field microscope on 5 to 10 calibrated standardized microscope fields (25 x objective), using 5 μl of culture medium; the mean number of bacteria per field was calculated and expressed as spirochetes per milliliter. The spirochetes were mass cultured in 500-ml spinner flasks or in 30-ml petri dishes either in gas-tight incubator boxes that had been continuously gassed with a mixture of 3% CO₂, 5% O₂, and the balance N₂ or in a trisgas incubator with the same gas concentrations. In cultures started with material from serial dilutions of spirochetes down to 0.7 cell per well, 7 of 10 wells became positive, confirming that single-cell inocula could be successfully expanded. For animal infection, cultured material was washed twice with RPMI 1640 medium to remove most of the macromolecular components of the BSK II medium. This was done to help avoid sensitizing the animals to these proteins. Viability of washed spirochetes was confirmed by in vitro culture. Samples of animal tissue, blood, urine, and CSF were placed in 24-well tissue culture plates with 1.0 ml of medium per well and kept for a maximum of 6 weeks in the same gas mixture as described above. For skin biopsy samples, one half of a 4-mm skin biopsy was minced and distributed into two wells. A volume of 50 μl of bodily fluid was dispensed into each of two wells. The wells were examined for spirochetes with an inverted positive-phase-contrast microscope at a magnification of ×400, and presence of spirochetes was confirmed by dark-field microscopy. Part of the material from positive wells was used to confirm the Borrelia species by PCR, and the rest was stored in liquid nitrogen.

Species identification of cultured spirochetes by PCR. The PCR procedure of Goodman et al. (16) was used with some modifications. Oligonucleotide primers of 26 and 28 bp were synthesized and used to amplify a 231-bp fragment of B. burgdorferi chromosomal DNA. Initial trials showed these primers to be specific for B. burgdorferi DNA and thus not able to hybridize to DNA from the closely related species Borrelia hermsii. This is in agreement with the results of Goodman et al. (16), who also tested these primers for absence of hybridization to DNA from human cells, Escherichia coli, Staphylococcus aureus, and Clostridium difficile. Species identification by PCR thus afforded a specificity comparable to that of monoclonal antibodies, plus the possibility of completing species identification of all cultured (frozen) samples simultaneously in 1 day of work. Modifications of the original protocol included (i) employing one of the new generation of thermostable DNA polymerases, i.e., Vent polymerase (New England Biolab, Beverly, Mass.) in lieu of Taq polymerase and (ii) use of a second amplification primer that is 7 bases downstream of the one originally described (15a). These primers were used to confirm the species of the spirochete present in medium from in vitro-cultured blood and skin biopsy samples. A 10-μl aliquot of the culture medium was placed in each reaction tube after all other components had been added. A positive control with pure B. burgdorferi DNA and a negative control consisting of all reaction components except added DNA were included. The mixtures were placed in a Perkin-Elmer Cetus DNA thermal cycler 480 and subjected to denaturation at 95°C for 5 min followed by a 9-min annealing period at 50°C and an extension time of 1 min at 72°C. The subsequent 39 cycles were at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. During the final cycle, an extension time of 5 min at 72°C was used.

Histo- and immunohistochemicals. Each 5-mm skin biopsy was fixed in 10% buffered formalin, placed in tap water for 30 min, and then held in 80% ethyl alcohol until processed with a standard automated paraffin tissue processor. Tissues were then sectioned at 3 to 6 μm and stained with hematoxylin-eosin. For immunolocalisation staining, an alkaline phosphatase-conjugated polyclonal antibody against organisms of the Borrelia genus and later a more specific anti-B. burgdorferi antibody (rendered species specific by adsorption with B. hermsii antigens), both produced in goats, were used as primary antibodies (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). The sections were then stained by using alkaline phosphatase-linked anti-goat immunoglobulin antibodies and appropriate color development reagents (Kirkegaard & Perry Laboratories). Borrelia organisms stained red with a 1:400 dilution of the primary antibody. Positive control tissues included sectioned pellets of cultured organisms. Negative control tissues were from uninfected animals.

Western blotting. B. burgdorferi JD1 (fourth passage) was cultured in BSK II medium at 34°C up to a concentration of 10⁸ cells per ml. Bacteria were pelleted and washed five times by centrifugation (8,000 x g, 20 min) with ice-cold phosphate-buffered saline (PBS), pH 7.2. After the last wash, the pellet was resuspended in an equal volume of sodium dodecyl sulfate-polycrylamide gel electrophoresis (SDS-PAGE) lysis buffer (0.05 M Tris-HCl [pH 7.5], 1% SDS, 10% [vol/vol] glycerol, 5% [vol/vol] 2-mercaptoethanol, 0.002% bromophenol blue) and heated at 100°C for 5 min. The lysate was centrifuged at 12,000 x g for 5 min, and the supernatant was collected, aliquoted in small volumes, and kept at −20°C until used. A volume of lysate containing 10⁸ solubilized bacteria (or 25 μg of protein measured by optical density at 280 nm, with an extinction coefficient of 1.4 ml/cm mg) was dispensed per track of 1-mm-thick 10 to 20% acrylamide gradient minigels, electrophoresed, and transferred onto nitrocellulose essentially as described by Towbin et al. (41). Nitrocellulose strips were blocked with 3% fat-free powdered milk in PBS containing 0.05% Tween 20 (PBS-T) for 2 h at room temperature. The strips were washed three or four times with PBS-T after each of the incubation periods described below. Blocked strips were incubated for 1 h with serum samples diluted 1:100 in PBS-T. Bound antibodies were probed by incubation for 1 h with a 1:200 dilution of biotinylated anti-human IgG (γ chain specific) and IgM (μ chain specific) antibodies (Vector Laboratories, Burlingame, Calif.). Biotinylated antibodies were detected with an avidin–biotinylated horseradish peroxidase complex according to the manufacturer’s instructions (Vector). The reagent 4-chloro-1-naphthol (Sigma Chemical Co., St. Louis, Mo.) was used as a chromogen for color development. Mouse monoclonal antibodies H5332 and H9724...
TABLE 1. Detection of B. burgdorferi by in vitro culture of samples of skin and bodily fluids from rhesus macaques infected with the spirochete by needle inoculation and by exposure to the bite of B. burgdorferi-infected ticks

<table>
<thead>
<tr>
<th>Animal*</th>
<th>Method of inoculation</th>
<th>No. of engorged ticksa</th>
<th>In vitro detectionb of spirochetes from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin biopsy</td>
</tr>
<tr>
<td>J743</td>
<td>Needled</td>
<td>NA</td>
<td>2-6</td>
</tr>
<tr>
<td>J748</td>
<td>Needle</td>
<td>NA</td>
<td>2-4</td>
</tr>
<tr>
<td>K167</td>
<td>Needle</td>
<td>NA</td>
<td>2-6</td>
</tr>
<tr>
<td>J200</td>
<td>Tick bite</td>
<td>2</td>
<td>1-2</td>
</tr>
<tr>
<td>J415</td>
<td>Tick bite</td>
<td>2</td>
<td>1-6</td>
</tr>
<tr>
<td>J307</td>
<td>Tick bite</td>
<td>4</td>
<td>1-3</td>
</tr>
<tr>
<td>J677</td>
<td>Tick bite</td>
<td>10</td>
<td>1-2</td>
</tr>
<tr>
<td>K205</td>
<td>Tick bite</td>
<td>10</td>
<td>1-2</td>
</tr>
<tr>
<td>J979</td>
<td>Tick bite</td>
<td>14</td>
<td>1-4</td>
</tr>
</tbody>
</table>

* The study included three additional control animals, one inoculated by needle with RPMI 1640 medium and two exposed to the bite of uninfected ticks.

b Spirochetes were detected after blood feeding in 41 of 42 ticks. One engorged tick from animal J677 was dead at the time of examination and was not tested. NA, not applicable.

c Samples were collected biweekly; the first samples from needle-inoculated animals were put into culture 2 weeks p.i., and samples from tick-inoculated animals were put into culture 1 week p.i. Numbers indicate the first and last weeks p.i. at which spirochetes were detectable in vitro. -, spirochetes not detected; ND, not determined.

d The needle inoculum contained $4 \times 10^8$ spirochetes in 3.1 ml (2 ml intraperitoneally, 1 ml subcutaneously, and 0.1 ml intradermally). Animal J743 received only intraperitoneal and intradermal inoculations.

(Symbicom, Umeå, Sweden) were used to identify OspA and flagellin antigens, respectively. In these experiments, bound monoclonal antibodies were detected by using biotinylated anti-mouse IgG (heavy plus light chains) antibodies (Vector). The Lyme Miniblot IgG assay kit (Immunetics, Cambridge, Mass.) was used to produce the results shown in Fig. 3G. The manufacturer's instructions were followed verbatim.

Intrathecally produced anti-B. burgdorferi antibody in CSF. For determination of intrathecally produced anti-B. burgdorferi antibody in CSF, the DAKO Lyme Neuroborreliosis Kit (code no. K005; DAKO A/S, Filstroop, Denmark) was used according to the manufacturer’s instructions. This is a sandwich-type enzyme immunoassay for the determination of intrathecally produced IgG and IgM antibodies to B. burgdorferi flagellum protein.

In vitro assay for antibody-dependent complement-mediated lysis of B. burgdorferi. Normal monkey serum was used as a source of complement. Blood samples were collected from normal uninfected anesthetized rhesus macaques by femoral venipuncture and allowed to clot at room temperature for 30 min. Clotted blood was then kept at 4°C for 2 h. The serum was collected after centrifugation of the samples at 800 x g for 20 minutes and stored in small aliquots at -70°C. For the in vitro assay, cultured B. burgdorferi organisms (JD1 strain, fourth passage) were centrifuged at 10,000 x g for 20 min, resuspended in BSK II medium, and counted. The complement-mediated killing assay was carried out in 96-well tissue culture plates. A total of 2.5 x 10⁶ spirochetes in 25 µl of BSK II medium were added to each well containing serially diluted 50-µl serum samples. The plates were incubated at 34°C for 20 min before the addition of 25 µl of normal rhesus monkey serum (source of complement). After 6 h of incubation at 34°C, the total numbers of dead (nonmotile) and live (motile) bacteria were determined by dark-field microscopy. The killing antibody endpoint titer was defined as one-half the value of the serum dilution at which the percentage of spirochetes killed was equal to that killed by rhesus monkey complement alone (10%). Equivalence between nonmotility and death was periodically controlled by reculturing the spirochetes over a 14-day period and verifying that the number of organisms recovered was proportional to the initial percentage that were motile. No organisms were recovered from wells initially rated as containing 100% nonmotile bacteria.

RESULTS

Infection status. B. burgdorferi could be recovered from all animals that were exposed to infected ticks or given the spirochete by injection, as revealed by in vitro culture of skin or blood samples (Table 1) and by immunostaining of skin sections (Table 2). The spirochete species in cultured samples was confirmed to be B. burgdorferi by PCR. Thus, the expected species-specific 230-bp fragment of chromosomal DNA was amplified from all samples containing cultured spirochetes, but not in the absence of template.

Neither the route of infection nor the number of ticks that had fed on a given animal of group II affected the ability to detect the spirochete by in vitro culture (Table 1). Thus, detection of B. burgdorferi in skin was possible 6 weeks p.i. in animals that received needle inoculations (J743 and K167) and in a monkey fed upon by only two ticks (J415). Results in vitro cultures from blood samples were also independent of route of inoculation or number of infecting ticks, but overall, spirochetes were detectable for shorter time periods p.i. than in skin biopsies (Table 1). No blood-borne organisms could be recovered from animal J979 (Table 1).

Spirochetes were not recovered by in vitro culture from either urine or CSF during the first 8 weeks of infection; it should be noted, however, that CSF was sampled from only three animals (Table 1). Overall, spirochetes were detected in blood by in vitro culture until week 6 p.i. (J743, K167, J415; Table 1), in skin biopsies taken from the periphery of the inoculation site until week 6 p.i. by in vitro culture (Table 1) and week 8 p.i. by immunostaining (Table 2), and at week 10 p.i. in the conjunctiva of one animal selectively biopsied (see below).

Physical and microscopic examination findings. (i) Dermatologic. The gross and microscopic dermatologic evaluations are given in Table 2. Erythema scored as E1 was the skin response associated with the feeding of the ticks. This response was present in animals exposed to the bite of both Borrelia-infected and uninfected ticks, but it persisted longer than 1 week p.i. only in infected animals. Erythema scored
as E2 was the response seen by or after week 1, and it appeared as a fine macular rash in the needle-inoculated animals (Fig. 1a). The rash was more intense with defined borders in the form of a ring or circular patch of about 8 to 10 cm in diameter in tick-inoculated animals (Fig. 1b). E2 was seen in one animal of group I and, with various intensities and durations, in five of the six animals of group II but not in control monkeys. Erythema E3 differed from E2 only in that its location was distant from the site of inoculation. This type of secondary erythema was seen in animals J797 (tick inoculated) and K167 (needle inoculated). In the latter animal it was intense at 4 and 6 weeks p.i. (Table 2).

Microscopic evaluation consisted of hematoxylin-eosin- and immunostained sections of skin biopsies. Dermatitis scored as D1 consisted of macrophage and lymphocyte infiltration into the superficial perivascular space immediately adjacent to the basement membrane of the epidermis. This was accompanied by a prominent eosinophil and an occasional basophil response. This response was observed in all animals exposed to the bite of a tick throughout a 6-week period p.i. The dermatitis termed D2 was characteristic of animals infected with *B. burgdorferi*. It consisted of lymphocytic infiltrates of the superficial perivascular areas as seen in D1 and, in addition, of the deep dermal adipose tissues (Fig. 1c) and the perivascular spaces within muscle. The D2 response, which persisted through 8 weeks p.i. in three animals, was usually accompanied by the presence of *Borrelia* organisms, as demonstrated by immunostaining (Fig. 1c). The D3 dermatitis contained all the features of D2 but was more severe; cellular infiltrates, consisting primarily of mononuclear-type cells and lymphocytes, were often confluent in both the superficial and deep dermis. This degree of response was observed in two tick-inoculated animals (J797 and J415) at weeks 1 and 2 p.i.

Cutaneous nerve involvement, termed N (Table 2), was prominent at 4 weeks p.i. in three animals of group II (K205, J677, and J415). Lymphoplasmacytic infiltration of the nerve and vacuolation of nerve sheaths was evident, suggesting cutaneous nerve damage. However, immunostaining procedures did not demonstrate the presence of *B. burgdorferi* within the nerve sheath. Dermal fibrosis, termed F in Table 2, was prominent in two animals of group II (J677 and J797). It consisted of a reparative (anaplastic), sheet-like formation of fibrous connective tissue at the muscle-adipose tissue interface. A mild lymphocytic infiltration was seen in the connective tissue.

(ii) Ocular. All animals were examined prior to inoculation and found to be free of any observable ocular lesions. One needle-inoculated animal, K167, was noted to have mild conjunctival hyperemia when examined 1 week p.i. This persisted until 3 weeks p.i. and then disappeared. All other animals were within normal limits. During the 10th week p.i., two independent observers noted on different examination days that one tick-inoculated animal, J307, had bilateral conjunctivitis, greater in the right eye than in the left eye. At this time, the conjunctiva of K167 appeared normal bilaterally. Animals were examined by slit lamp biomicroscopy, and no evidence of uveitis was found. A conjunctival biopsy from the temporal limbus of both eyes of K167 (group I) and J307 (group II) was performed under topical anesthesia at 10 weeks p.i. The tick-inoculated animal, while manifesting obvious conjunctival injection, had histologic congestion but no inflammatory cell infiltration. No organisms were observed within the conjunctiva by immunohistochemistry. In contrast, needle-inoculated animal K167 showed no clinical signs of conjunctivitis but histologically demonstrated both epithelial hyperplasia with a mild lymphoplasmacytic infiltration of the submucosa and congestion of the vasculature (Fig. 2a). *Borrelia* organisms were present, as assessed by immunohistochemistry (Fig. 2b).

(iii) Reticuloendothelial. Transient splenomegaly was noted in three animals, one of group I (K167) and two of group II (K205 and J307), by weeks 8 (one animal) and 13 (two animals) p.i. Splenic biopsies were not performed. Gross peripheral lymphadenopathy was not observed at any time in any animal.

(iv) Cardiovascular. Animal J677 (tick inoculated) demonstrated transient bradycardia (<60 beats per minute) at weeks 11 and 13 p.i. Electrocardiography demonstrated sinus bradycardia on both occasions.

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**TABLE 2.** Gross and microscopic cutaneous tissue patterns of rhesus macaques infected with *B. burgdorferi* by needle inoculation and by exposure to the bite of *B. burgdorferi*-infected ticks

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>E1, D1</td>
<td>D1</td>
<td>D1</td>
<td>D1</td>
<td>NEG</td>
</tr>
<tr>
<td>J622 (uninfected tick)</td>
<td>E1, D1</td>
<td>D1</td>
<td>D1</td>
<td>D1</td>
<td>NEG</td>
</tr>
<tr>
<td>J206 (uninfected tick)</td>
<td>NEG NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>K204 (culture medium)</td>
<td>NEG NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Needle inoculated</td>
<td>E2, D2, +</td>
<td>E2, D1, +</td>
<td>E3, D1, +</td>
<td>E3, D2, +</td>
<td>D1, −</td>
</tr>
<tr>
<td>K167</td>
<td>NEG, −</td>
<td>NEG, −</td>
<td>D1, −</td>
<td>D1, −</td>
<td>NEG, −</td>
</tr>
<tr>
<td>J743</td>
<td>NEG, −</td>
<td>NEG, −</td>
<td>D1, −</td>
<td>D1, −</td>
<td>NEG, −</td>
</tr>
<tr>
<td>J748</td>
<td>NEG, −</td>
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<td>D2, +</td>
<td>D2, F, +</td>
</tr>
<tr>
<td>K305</td>
<td>E2, D2, +</td>
<td>E1, D2, +</td>
<td>D2, +</td>
<td>D2, +</td>
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</tr>
<tr>
<td>J677</td>
<td>E1, D1, +</td>
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<td>D2, +</td>
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</tr>
<tr>
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<td>NEG, −</td>
</tr>
<tr>
<td>J797</td>
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<td>D1, −</td>
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<tr>
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<td>D1, −</td>
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</tr>
<tr>
<td>J307</td>
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<td>NEG, −</td>
<td>D1, −</td>
<td>D1, −</td>
<td>NEG, −</td>
</tr>
</tbody>
</table>

*NEG, negative. Gross lesion observations: E1, papillary erythema at site of tick attachment; E2, erythema around site of tick attachment or inoculation; E3, erythema peripheral to tick or inoculation site. Microscopic lesion observations: D1, minimal dermatitis, superficial perivascular; D2, mild dermatitis, superficial and deep dermal infiltrate; D3, moderate dermatitis, infiltrate tends to be confluent; F, fibrosis of dermis; N, cutaneous neuritis (lymphocyte infiltrate of nerve). Immunoperoxidase stain for *B. burgdorferi*: +, positive; −, negative.†
FIG. 1. EM at the gross and microscopic levels in rhesus macaques. (a) EM 14 days p.i. in a rhesus macaque needle inoculated with B. burgdorferi. The site of inoculation is indicated by the black India ink spot (A). (b) EM 14 days p.i. in a rhesus macaque given B. burgdorferi by tick bite. Sites of tick attachment are indicated (arrowheads). Bar, 2 cm. (c) Perivascular mononuclear cell infiltrate of the deeper dermis (type D2) in a B. burgdorferi-infected rhesus macaque. v, blood vessel. The arrowheads point to B. burgdorferi organisms. Magnifications were ×400 and ×650 (lower right corner). Staining was with an alkaline phosphatase-labelled B. burgdorferi-specific antibody and nuclear fast red chromogen; counterstaining was with hematoxylin.

(v) Neurologic. Animal K167 (needle inoculated) demonstrated signs of lethargy and depression during weeks 2 and 3 p.i. These signs resolved spontaneously. Animal J200 (tick inoculated) had multiple episodes of seizure activity under anesthesia. No neurologic findings were noted in this animal while it was unanesthetized.

(vi) Musculoskeletal. No clinical signs of arthritis were observed in any of the infected animals during the 13-week period reported.

Clinical laboratory findings. Complete blood counts, serum chemistries, and urinalyses, performed weekly, were normal throughout the 13-week period reported. Serum chemistry analyses included tests for serum electrolytes, glucose, alanine aminotransferase, serum alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, blood urea nitrogen, creatinine, globulin, and albumin.

CSF analysis. CSF was analyzed for the presence of cells, B. burgdorferi spirochetes, and anti-B. burgdorferi antibodies. Cellular infiltration was assessed for the first time at week 4 p.i. in two infected animals of group II (J415 and J307) and one of group I (J748) and in one control animal (K204). Both tick-inoculated animals tested demonstrated severe pleocytosis, which could not have been caused iatrogenically by prior CSF sampling. Absolute leukocyte (WBC) numbers were 4.9 × 10⁴/ml in J415 and 4.8 × 10⁴/ml in J307. Erythrocyte counts were 1,200/ml and 300/ml, respectively. No differential cell counts were performed. The two animals with pleocytosis have had normal CSF since week 4 p.i. The control animal (K204) and the needle-inoculated animal (J748) had normal CSF cytology since initial sampling 4 weeks p.i.

We were unable to demonstrate the presence of B. burgdorferi in CSF by in vitro culture (Table 1). Antibodies to B. burgdorferi antigens were present in CSF samples, as assessed by Western blot analysis, but the antigens recognized did not differ from those bound by antibodies in serum samples collected concomitantly with the CSF (data not shown). Using a specific antibody capture enzyme-linked immunosorbent assay (ELISA), we could not demonstrate intrathecal antibody production.

Analysis of the antibody response to B. burgdorferi. Two characteristics of the serum antibodies elicited in response to B. burgdorferi infection were analyzed: (i) their antigen recognition pattern, as a function of time after infection, and

FIG. 2. Lyme conjunctivitis in rhesus macaques. (a) Conjunctiva from rhesus macaque K167, which had early transient conjunctivitis during weeks 2 to 6. Biopsy was performed at week 10 p.i. Note epithelial hyperplasia (A), congestion of vasculature (B), and lymphocyte submucosal infiltrate. Hematoxylin-eosin stain was used; magnification, ×250. (b) Higher magnification of panel a. Blind biopsy of clinically uninflamed conjunctiva revealed several B. burgdorferi organisms as shown (arrowhead). Immuno-alkaline phosphatase stains B. burgdorferi red; magnification, ×650.
(ii) their ability to kill the spirochete in vitro, by antibody-dependent complement-mediated lysis. The latter effector mechanism is known to kill the spirochete in vivo in rodent models (35) and in vitro with antibodies from infected animal models and humans (23, 26).

**IgM and IgG serum antibody response to B. burgdorferi antigens as a function of time after infection.** By week 1 p.i., animals of groups I and II had detectable IgM antibodies to antigens of Ms 95,000 (one animal), 65,000 to 66,000 (three animals, including infected control J206; four animals), 41,500 (three animals), 39,000 (three animals), 31,000 to 34,000 (OspA-OspB; three animals), 29,500 (all animals, including controls), 25,200 (seven animals), and 18,800 (three animals), as assessed by Western blot analysis (Fig. 3A). The IgG response was directed to a partly different group of antigens of Ms 90,200 (four animals, including uninfected control J206; track 1), 60,000 (nine animals), and 41,500 (flagellin; seven animals). Additional dimly stained antigens of lower Ms were also detectable. Control animals showed antibodies to antigens of Ms 88,800 (J206; Fig. 3B, track 1), 60,000 (J622 and K204; tracks 2 and 9, respectively), and 41,500 (flagellin; J206, J622, and K204). Between weeks 2 and 3 of infection, the IgM response appeared to reach its peak. At that time, both the number and intensity of bands were the highest in the 6-week period investigated (Fig. 3C). Bands were visualized at Ms 88,800 (five animals), 65,000 to 66,000 (four animals), 41,500 (flagellin; nine animals), 39,000 (five animals), 29,500 (seven animals), 25,200 (nine animals), and 18,800 (six animals). From week 1 onwards the IgG antibody response continued to evolve, in both avidity and complexity, and by week 3 p.i. OspA was bound by serum antibodies from all three needle-inoculated animals (Fig. 3D, tracks 10, 11, and 12) but most intensely by K167 (track 12). Anti-OspA antibodies were not detected in serum samples from tick-inoculated animals (tracks 3 to 8). At week 6 p.i., bands of Ms 88,800 (10 animals), 65,000 to 66,000 (nine animals), 41,500 (flagellin; seven animals), 39,000 (nine animals), 36,000 (two animals), 31,000 to 34,000 (OspA-OspB; one animal [K167]), 29,500 (eight animals), 25,200 (nine animals), and 18,800 (nine animals) were evident (Fig. 3F). By this time, however, the IgM response was much diminished (Fig. 3E).

To illustrate the similarities between the specificities of the antibody responses of human and nonhuman primates, serum samples from all infected animals and one uninfected control were analyzed at 11 weeks p.i. with a commercially available Western blot-based diagnostic kit. All of the infected animals satisfied the two positive diagnostic criteria prescribed by the test’s manufacturer, i.e., presence of “p41” and one or more “significant” bands in the “p18” to “p34” region, or presence of two or more “significant” bands in the “p18” to “p53” region (Fig. 3G). Indeed, the similarity between the antigen recognition pattern of the human positive control serum sample supplied by the kit’s manufacturer (chronic infection; Fig. 3G, track III) and the patterns of the infected rhesus macaques is remarkable. In addition, the antigen recognition patterns of three human serum samples obtained from patients in the early or early disseminated phases of Lyme disease are shown in tracks 13 of Fig. 3. Two of these serum samples were obtained from patients at >9 months (Fig. 3A and B) and ≥3 months (Fig. 3E and F) p.i. The third one (Fig. 3C and D) was obtained approximately 1 month p.i. All antigens recognized by rhesus monkey antibodies during the first 6 weeks of infection appeared to be recognized also by the human serum antibodies. Although the full spectrum of antibody specificities observed in long-term chronically infected humans was not elicited in rhesus monkeys during the 13-week investigation period, the trend towards increasing complexity in the antibody response indicates that at some point the diversity observed in humans may be observed also in our model.

**Antibody-dependent complement-mediated killing of B. burgdorferi in vitro.** To investigate whether antibody-dependent complement-mediated killing was operational in the rhesus monkey, we titrated serum antibody capable of mediating killing of B. burgdorferi by complement in vitro. Serum samples from all animals in group I and from three animals in group II were tested. At a time when the IgG antibody response was well developed (week 5 p.i.), animals from both groups had serum antibodies capable of binding complement and mediating killing of the spirochete. The bactericidal antibody titer in monkeys given tick inoculations was 1:80, whereas in animals infected by needle inoculation was 1:320. Representative examples of these results are shown in Fig. 4. Titers were the same at week 3 p.i., when the IgM response is still high but the IgG antibody response is less developed (not shown).

**DISCUSSION**

A useful animal model for an infectious disease of humans should accurately mimic the microbiological, clinical, immunological, and pathological aspects of the infection. Moreover, the animal’s immune system should be amenable to detailed experimental analysis. To date it has not been possible to reproduce the protean nature of Lyme disease in experimental animals. The models reported on in the literature, specifically, rats and mice (3, 4, 34), hamsters (13, 19, 21, 36), dogs (28), and rabbits (6), show a limited number of disease signs, manifested in most cases only in animals with an impaired or undeveloped immune system. In contrast, rhesus macaques displayed not only several of the clinical and pathological signs of human Lyme disease but also important attributes of the human antibody response to infection.

EM, the most often observed sign of infection in humans (60 to 80%) (39), was noted clinically in six of nine infected rhesus monkeys. Microscopically, the tissue patterns observed in skin biopsies were similar to those seen in human Lyme borreliosis, particularly the deep and superficial perivascular lymphocytic infiltrations. These are so characteristic of human EM that they are considered diagnostic by some investigators (5). Dermal fibrosis has been described as a chronic manifestation of human Lyme disease (15, 24). The appearance of dermal fibrosis at 8 weeks p.i. may signal a change in the host response from one characteristic of early Lyme disease, i.e., acute dermatitis, to late, chronic dermatitis.

Both EM and conjunctivitis (detected histochemically) were documented concomitantly with presence of the spirochete in skin and conjunctival biopsies. Conjunctivitis was observed in two animals that were biopsied. One of the two monkeys had red eyes clinically. The second monkey had no redness clinically, but conjunctivitis and B. burgdorferi were detectable histologically. This result indicates that ocular manifestations of Lyme disease may occur in the rhesus monkey more frequently than we have noticed and, by analogy, may be underdiagnosed in humans.

The reticuloendothelial changes noted in three animals, i.e., splenomegaly, disappeared within 2 weeks of their onset. Splenomegaly occurs during the early disseminated phase of human Lyme disease and is characteristically
transient (14). Lymphadenopathy was not observed. However, in a separate group of five *B. burgdorferi*-infected rhesus monkeys currently under investigation, transient peripheral lymphadenopathy was observed in three animals (our unpublished data).

The sinus bradycardia noted on two occasions in one animal is reminiscent of the varying degrees of heart block with secondary bradycardia that have been observed during the early disseminated phase of Lyme disease in humans (39). However, our finding is probably normal, for although conduction abnormalities may have been present in this animal, lengthening of the P-R wave interval was not observed on electrocardiogram.

Neurologic signs such as paresis of oculomotor and facial nerves were not observed. The high WBC count found in the CSF of two of the three animals whose CSF was analyzed is abnormal. Species other than rhesus macaques may show $4 \times 10^3$ to $1 \times 10^4$ WBC per ml in the absence of infection (42), but in our experience rhesus macaques do not show detectable WBC in CSF. Thus, the atypically high WBC count indicates an inflammatory response. Meningeal irritation, a sign of early disseminated Lyme disease in humans, is reflected by an increase in CSF lymphocytes and total protein. Two lines of evidence, albeit negative, indicate that the CSF infiltration may not have been caused by the *B. burgdorferi* infection. First, we were unable to demonstrate the presence of *B. burgdorferi* in CSF by in vitro culture. Second, although antibodies to *B. burgdorferi* antigens were present in CSF samples, the antigens recognized did not differ from those bound by serum antibodies collected concomitantly with the CSF. Furthermore, intrathecal antibody production was not observed. While human neuroborreliosis is not always reliably confirmed by in vitro culture of CSF samples (22), intrathecal antibody production is reputedly the most significant marker for its diagnosis (29). Therefore, at this point we cannot correlate the CSF cellular infiltrates with the *B. burgdorferi* infection. It should be noted, however, that the number of animals with pleocytosis in CSF could be higher than recorded, since only three of nine infected animals were sampled for CSF. Finally, the lymphoplasmacytic infiltration of cutaneous nerves and sheath vacuolation we observed could indicate a port of entry for *B. burgdorferi* into the peripheral nervous system.

Clinical signs of arthritis were not observed in any of the infected animals of group I or II. Nonetheless, we have preliminary evidence that arthritis may indeed occur in the rhesus monkey as a result of *B. burgdorferi* infection. In a separate group of five *B. burgdorferi*-infected rhesus macaques currently under investigation, one animal developed swelling in multiple joints 14 days p.i., *B. burgdorferi* was recovered by in vitro culture of synovial fluid from this animal (our unpublished data). We will continue to monitor animals of groups I and II, in search of chronic signs of arthritis.

Routine clinical laboratory studies of rhesus monkeys showed no abnormal results during the 13-week-period studied. Results of similar studies in humans with Lyme disease are also rarely abnormal (40).

Western blot analysis of serum samples showed that the number of antigens recognized by rhesus monkey antibody increased steadily as a function of time after infection. Such a pattern of increasing complexity in the antibody response is characteristic of Lyme disease in humans (18, 30). Indeed, it has been suggested that its time dependence could be used as an aid to establish duration of infection (30). This contention seems to be borne out by the data from our model, where we can state with certainty the time when infection began.

In most animals the relative molecular weights of the antigens recognized were the same as those described for humans (18, 27, 30), at least as far as it is possible to compare *M*ₜₛ measured in different SDS-PAGE systems. In a recent study (27), antibodies to antigens of *M*ₜₛ of (10⁶) 94, 85, 75, 66, 60, 55, 46, 41, 39, 34, 31, 29, 22, and 17 were regularly detected in a sample of 186 serum specimens from individuals with clinically diagnosed Lyme borreliosis. The Western blot obtained with the commercially available diagnostic kit from Immunetics clearly illustrates the similarity between the antigen recognition patterns of humans and monkeys (Fig. 3G). The most significant marker in terms of frequency of recognition and specificity was the 39,000-*M*ₜₛ antigen, which was recognized by all of the infected and none of the uninfected monkeys by week 6 p.i. (Fig. 3F).

Our in vitro analysis of the main borrelicidal effector mechanism identified to date, antibody-dependent complement-mediated killing, showed that despite the early appearance of diverse antibody specificities to *B. burgdorferi* in rhesus macaques, the borrelicidal titer of these antibodies in tick-inoculated animals was low (1:4) at 3 to 5 weeks p.i. compared with that in hamsters by 3 weeks p.i. determined by using a similar in vitro assay (titers of 1:1,280) (26). Tick-inoculated animals developed a titer of borrelicidal antibodies (1:80) lower than that of needle-inoculated monkeys and lower than that observed in humans with late Lyme disease (about 1:680) (7). Nonetheless, this killing mechanism is available and may be functional in the rhesus monkey. To elicit its full host-protective potential, the bactericidal antibody titer may need boosting by means of an appropriate vaccination protocol administered before *B. burgdorferi* inoculation. That only a low titer of killing antibody was present during the course of infection is consistent with the direct evidence of persistent infection found at week 10 p.i. (*B. burgdorferi* immunostaining in conjunctival biopsy samples) and with the indirect (serological) evidence of infection available at week 11 p.i.

The differences in the borrelicidal antibody titers observed in animals of groups I and II could be due, at least in part, to the fact that serum samples from animals in group I contained easily detectable antibodies to OspA, an outer surface protein, whereas those from animals of group II did not (Fig. 3). Such a difference between the detectabilities of an anti-OspA antibody response in animals infected via the natural route and by injection has already been observed in dogs and in hamsters. Naturally infected animals show no antibody response to OspA detectable by Western blot or ELISA, whereas needle-inoculated animals do produce this antibody in detectable amounts (17, 33). Indeed, in human infections with *B. burgdorferi*, anti-OspA antibodies have been found early after infection only in the form of immune complexes (37). Antibodies detectable by standard diagnostic procedures appear very late in the infection, if at all (11).

Diagnosis of Lyme disease, still an imprecise endeavor (38), will benefit from the availability of the rhesus monkey model. Immunological reagents such as antibodies to human immunoglobulin isotypes cross-react better with their rhesus monkey counterparts than with those of most other nonhuman primate species used in the laboratory (our unpublished data). Thus, recombinant antigens may now be tested for antibody detection longitudinally, with the same reagents used for humans but in a study population positively known to be infected with *B. burgdorferi*. A detailed anatomical
FIG. 3. IgG and IgM antibody responses to B. burgdorferi in rhesus macaques. Western blot analyses of serum samples obtained from 12 rhesus macaques at 1 week (A and B), 3 weeks (C and D), 6 weeks (E and F), and 11 weeks (G) p.i. are shown. Bound antibodies were detected with biotinylated anti-human IgM (κ chain specific) (A, C, and E) and anti-human IgG (γ chain specific) (B, D, and F) followed by biotinylated horseradish peroxidase bound to avidin and by the color reagent 4-chloro-1-naphthol. Animals were infected with B. burgdorferi (strain JD1, third passage) either by tick inoculation (tracks 3 through 8, group II) or by needle inoculation (tracks 10 through 12, group I). Tracks 1 and 2 correspond to control animals exposed to uninfected ticks (J206 and J622, respectively), and track 9 corresponds to a sham needle-inoculated animal (K204). Infected animals were J200 (track 3), J307 (track 4), J415 (track 5), J677 (track 6), J797 (track 7), and K205 (track 8) (group II) and J743 (track 10), K748 (track 11), and K167 (track 12) (group I). Human sera 91-0531 (track 13, panels A and B), 91-0865 (track 13, panels C and D), and 90-211 (track 13, panels E and F) were collected at >9, 1, and >3 months p.i., respectively. Mouse monoclonal antibodies H9724 (track 14, panel F) and H5332 (track 15, panel F) bind B. burgdorferi flagellin and OspA, respectively. Panel D also shows a colloidal gold stain of a blot of B. burgdorferi (JD1, fourth passage) whole extract, which was used in all blots. Polyacrylamide (10 to 20%) gradient gels and Bio-Rad low-molecular-weight standards were used. The Western blot shown in panel G was obtained with the Lyme Miniblot IgG assay kit of Immunetics Inc., following the manufacturers instructions. Tracks with Roman numerals correspond to negative controls with normal human serum (II) and without serum (I). The manufacturer's positive control human serum was used on track III.

map localizing the presence of the spirochete at different times after infection would contribute microbiological evidence of infection in specific bodily fluids and organs. This could be investigated by either PCR or in vitro culture.

In the context of immunoprophylaxis, it is pertinent that rhesus monkeys express what is possibly the main protective immune mechanism to B. burgdorferi infection, i.e., antibody-dependent complement-mediated killing of the spirochete. Testing of vaccines based on molecularly cloned surface antigens such as OspA should now be facilitated by
the availability of an animal model which is phylogenetically closer to the human than other currently available models and which permits 100% infection rates in the presence of a fully developed, unimpaired immune system. Moreover, vaccine efficacy may be evaluated not only by the usual method of contrasting infection rates of immunized animals with that of controls but also by quantifying the rate of expression of pathological signs of infection such as EM. This advantage may also facilitate the development of new and improved chemotherapeutic drugs and drug administration regimens for a malady that already affects over 40,000 people in the United States alone (9, 10).

We conclude that the rhesus monkey is a useful model to investigate methods of diagnosis, immunoprophylaxis, and chemotherapy of Lyme disease, as well as the pathogenesis and immunobiology of the early and early disseminated...
phases of the infection. The time when late (chronic) signs begin to be expressed, which would be around 1 year after infection in humans (39), has not been reached in our study.

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