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Human Necrotizing Splenitis Caused by Borrelia burgdorferi

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A 25-year-old Hispanic male presented to the emergency room with complaints of severe left upper quadrant pain. Physicians determined that the patient had an acute inflammatory process with a possible diagnosis of splenic abscess. A splenectomy was performed. Histologic examination of the tissue sections revealed extensive necrosis and inflammation, but no etiologic agent was discernible. Microbiologic cultures of the tissue had negative results. A Dieterle silver stain revealed an overwhelming number of spirochetal bacteria most closely resembling Borrelia spp. The patient's serum was tested for serologic evidence of antibody to *Borrelia burgdorferi* with the following results; by indirect fluorescent antibody 1:32; by enzyme-linked immunosorbent assay for IgM, 1:320; and Western blotting had positive results for the presence of *B. burgdorferi* outer-surface protein antibodies. This is the first human case report of an acute necrotizing splenitis resulting from *B. burgdorferi*. (Key words: *Borrelia burgdorferi*; Lyme disease; Splenitis; Necrotizing) Am J Clin Pathol 1989;91:493–498

AS THE MEDICAL KNOWLEDGE of Lyme disease increases, it is evident that typical signs and symptoms are exceedingly difficult for physicians to detect or patients to recognize. The characteristic dermatologic lesion is known as erythema migrans. It is associated with the bite of the northern deer tick, *Ixodes dammini*. Typical lesions progress annularly outward from the central bite area and resemble a rash at the perimeter. The central area can clear or remain erythematous. Not all patients will have these lesions develop nor will other persons recall the bite of the tick. Untreated Lyme disease can remain latent for a period estimated to last from weeks to years in some patients and may progress toward involvement of multiple organs, including the neurologic and musculoskeletal systems. Systemic involvement has been limited to hepatomegaly in humans with descriptions of elevated liver enzyme levels.[19] The spirochete *Borrelia burgdorferi* has been isolated from skin lesions, blood, synovium, and cerebrospinal fluid of naturally acquired infection,[4,10,18–21] but visceral organ infection has not been reported in humans.

We present the first case description of acute necrotizing splenitis caused by *B. burgdorferi* in a man devoid of any overt clinical signs, symptoms, or history suggestive of or associated with Lyme disease at the time of presentation.

Report of a Case

A 25-year-old Hispanic male presented to the emergency room in late October 1987, complaining of acute left upper quadrant pain radiating to the left shoulder area. The patient reported an abrupt onset of fever 72 hours before admission with nausea and night sweating. The patient denied prior trauma, travel history, recent infections, weight loss, or any similar past episode of pain. The patient consumed two six-packs of beer (ca. 4 L) per weekend and was an intravenous cocaine abuser.

The physical examination revealed a temperature of 38.9 °C. He had sharp, consistent pain upon inspiration or coughing. Needle track marks were observed on the patient's forearms, but no other dermatologic findings were evident, including rashes, cellulitis, or splinter hemorrhages. The spleen was exquisitely tender, and the anterior tip was palpable 7 cm past the midclavicular line. There was no hepatomegaly. No other masses were noted.

Blood was drawn for culture, complete blood count, and serum assays. A computer-assisted tomograph was performed four hours after admission. Splenomegaly and an intrasplenic mass were observed. The patient was treated empirically with intravenous ampicillin, gentamicin, and

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clindamycin 12 hours after admission. A splenectomy was performed 28 hours after admission. The spleen was submitted to the laboratory for histologic and microbiologic analyses. The postsurgical recovery was uneventful. Single antibiotic coverage with cefoxitin was instituted, and the patient was discharged on day 6.

Materials and Methods

Histology

Formalin-fixed, paraffin-embedded splenic tissues were sectioned and stained with the use of the hematoxylin and eosin (H and E) stain and the Brown and Brenn (B and B) tissue Gram's stain. The Ziehl-Neelsen stain, Gomori methenamine silver stain, and Dieterle silver stain were performed at a later time.

Microbiology

Fresh splenic tissue was ground in a tissue grinder and promptly inoculated onto various microbiology media designed for the cultivation and recovery of aerobic and anaerobic bacteria. Media included Trypticase® soy agar with 5% sheep blood, chocolate II agar, MacConkey agar, Columbia CNA agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, MD), anaerobic blood agar, anaerobic KV agar (Scott Laboratories, Inc., West Warwick, RI), and chopped meat glucose medium (Gibco, Lawrence, MA). Gram's stain was performed on the emulsified tissue.

Serology

Serologic assays were performed for evidence of antibody to specific diseases, including the following: infectious mononucleosis screen (Mono-Test®, Wampole Laboratories, Cranbury, NJ); Epstein-Barr virus and human immunodeficiency virus (HIV) (SmithKline Bio-Science Laboratories, Waltham, MA); hepatitis B surface antigen, antibody, and core antibody (Quantum®, Abbott Laboratories, North Chicago, IL); and syphilis (fluorescent treponemal antibody absorption [FTA-ABS], Connecticut State Department of Health Laboratories, Hartford, CT).

Two assays were used to detect *B. burgdorferi* antigen in the patient's serum: (1) an indirect fluorescent antibody (IFA) assay using fluorescein-isothiocyanate (FITC) labeled goat antihuman total immunoglobulins (Sigma Chemical Company, St. Louis, MO); and (2) an enzyme-linked immunosorbent assay (ELISA), both as described previously.[16,17]

Electrophoresis and Immunoblotting

The Laemmli method[12] was modified, as follows. Electrophoretic grade reagents and prestained molecular weight standards (Bio-Rad, Van Nuys, CA) were used. Twenty-five microliters of a 150-μg/mL suspension of sonicated whole cell *B. burgdorferi* strain B31 was added to 175 μL of Laemmli sample buffer and heated at 100 °C for 5 minutes. The Borrelia proteins were loaded onto a 10% (w/v) polyacrylamide gel (30:0.8, acrylamide:bisacrylamide) and electrophoresed in a minislab cell at 30 mA for 90 minutes at 25 °C. Proteins were transferred onto nitrocellulose membranes in a miniblot cell and electrophoresed at 300 mA for one hour at 4 °C. Nonspecific antibody binding sites were blocked by incubation in phosphate-buffered saline (PBS) with 5% (v/v) Tween® 20, 0.05% (v/v) horse serum, and 5% (w/v) powdered milk for one hour[22] at 25 °C. The blots were washed three times in PBS for 5 minutes. The patient's serum was diluted 1:100 in PBS-5% Tween 20 with 5% (w/v) powdered milk and allowed to incubate at 4 °C overnight with gentle agitation. The blots were washed as above. Horseradish peroxidase–conjugated goat antihuman IgG and IgM antibody (Sigma), diluted 1:500 in PBS-5% Tween 20 with 0.05% (v/v) horse serum, was added to the blots and incubated for one hour at 25 °C. The blots were washed as above. Color development of the bands was accomplished by use of 5 ng diaminobenzidine and 5 μL of 30% (v/v) H_2O_2 in 10 mL of PBS. Known positive control sera obtained from patients diagnosed with Lyme disease were used, including samples with a high titer of IgG antibody (>1:40,000) and a low titer of IgM antibody (1:320). A negative serum control was also used.

Results

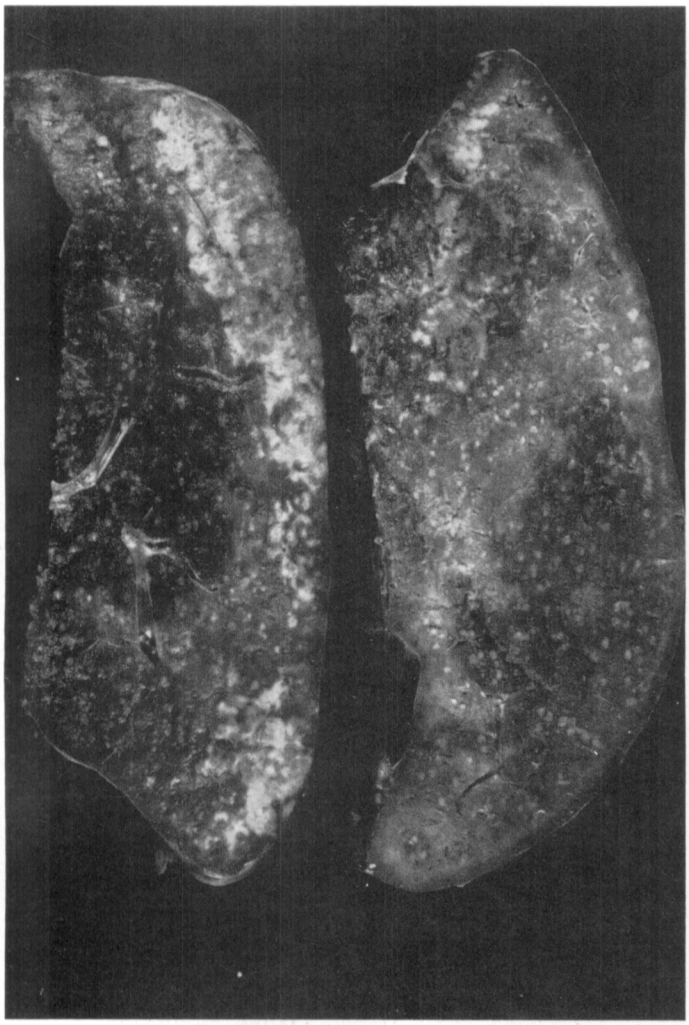
Hematology

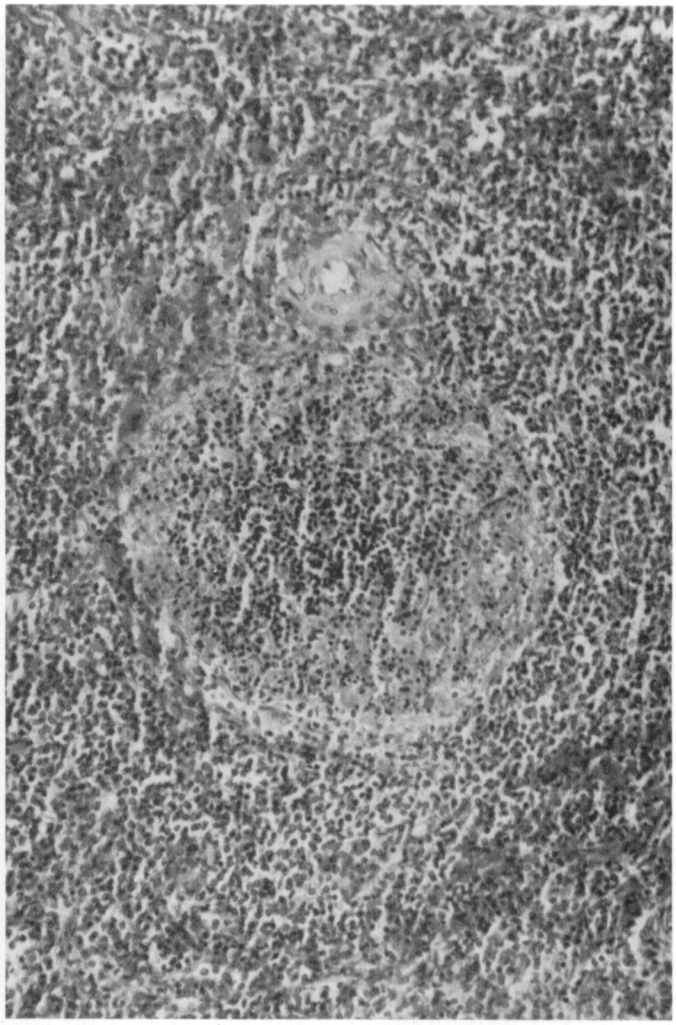
The white blood cell count was 18.1 × 10^9/L (18.1 × 10^3/μL). The blood differential revealed 72 segmented neutrophils, 10 band forms, 13 lymphocytes, 3 monocytes, and 2 eosinophils. The sickle cell disease test had negative results.

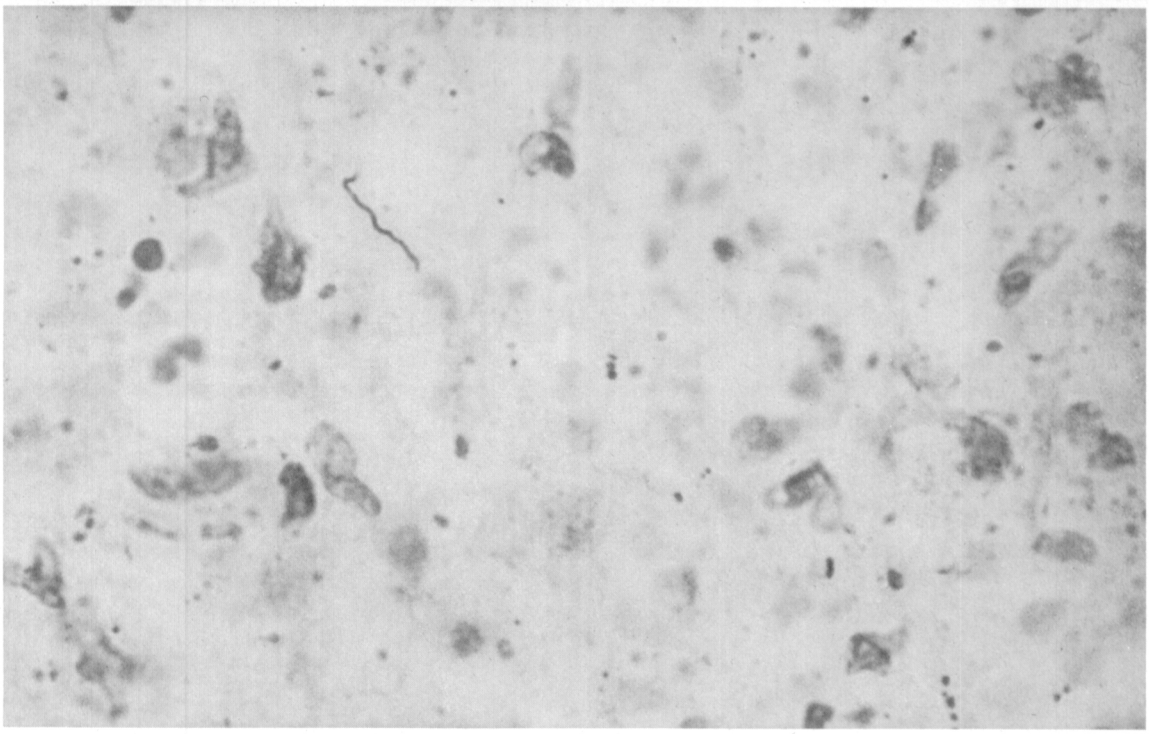
FIG. 1 (*upper, left*). Gross appearance of cut surface of spleen. The areas of acute inflammation and suppuration appear as gray-white discoloration in the subcapsular region.

FIG. 2 (*upper, right*). Splenic follicle showing acute central necrosis. Hematoxylin and eosin (×100).

FIG. 3 (*lower*). *Borrelia burgdorferi.* Two morphologic lengths are exhibited: longer spiriliform morphologic characteristics toward the upper left of the photograph and a short rod with a slight bend toward the lower right of the photograph. Dieterle silver (×1,000).







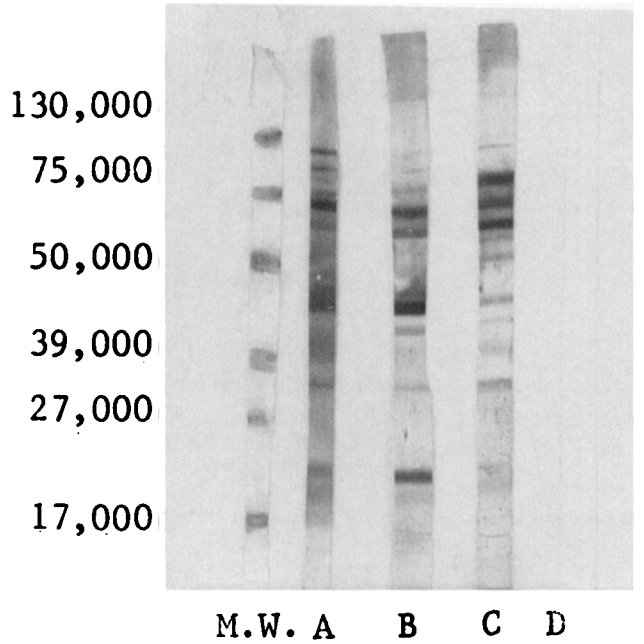


FIG. 4. SDS-PAGE immunoblot to *Borrelia burgdorferi* outer surface proteins. *Lane M. W.* Molecular weight markers. *Lane A.* High titer positive control serum. *Lane B.* Low titer positive control serum. *Lane C.* Patient serum. *Lane D.* Negative control serum.

Gross Pathology

The spleen was submitted in formalin. It weighed 380 g and displayed a smooth, glistening, red–purple capsule. Cut sections of the spleen revealed a soft parenchyma, a dark red to purple color, and prominent follicles (Fig. 1). An ill-defined, mottled, tan–red area in the splenic hilar region was noted along with several irregularly shaped, softened, yellow foci that were subcapsular in location.

Light Microscopy

The H and E stain, and the B and B tissue Gram's stain, showed extensive necrosis and acute inflammation. Many of the splenic follicles showed acute central necrosis (Fig. 2). The histologic results were consistent with an acute nectrotizing splenitis. There was no histologic evidence of sickle cell disease. Acid-fast bacteria were not observed with the Ziehl-Neelsen stain. Fungal hyphae and yeast were not seen with the Gomori methenamine silver stain. The Dieterle silver stain revealed numerous irregular bacteria readily observable in the necrotic areas at 200× magnification. They appeared pleomorphic at 1,000× and varied in lengths. Short organisms were difficult to discern, but longer organisms were spiriliform with coils of irregular amplitude and frequency (Fig. 3). The microscopic morphologic characteristics were consistent with a Borrelia species microorganism.

Microbiology and Serology

The Gram's stain of the ground spleen tissue revealed 1+ polymorphonuclear leukocytes, but no organisms were seen. The blood and splenic tissue cultures had negative results for the presence of aerobic and anaerobic microorganisms. Serologic results were negative in the infectious mononucleosis screen, hepatitis B virus surface antigen and antibody, HIV, and syphilis tests. Hepatitis B virus core antibody was positive. Antibody titers to Epstein-Barr virus antigens were as follows: viral capsid antigen, 1:320; nuclear-associated antigen, 1:10; diffuse early antigen, <1:10; and restricted early antigen, 1:20. These results were interpreted as evidence of a prior but not current infection with Epstein-Barr virus.

Additional serologic tests to confirm past or current *B. burgdorferi* infection revealed a serum dilution of 1:32 by IFA. An ELISA for polyvalent or class-specific IgG antibody had negative results, but a test for class-specific IgM antibody had positive results at a serum dilution of 1:320. The immunoblot analysis for specific antibodies to *B. burgdorferi* was consistent with the control sera, indicating antibody reactivity with spirochetal proteins having approximate molecular weights of 31,000, 41,000, 66,000, and 90,000 daltons (Fig. 4). Fresh tissue was unavailable for immunohistochemistry stains. Attempts at immunogold or monoclonal antibody–conjugated FITC assays were made on formalized tissue without success.

Discussion

Borrelia burgdorferi was discovered in 1981.[5] The reservoirs for *B. burgdorferi* are the white-footed mouse, *Peromyscus leucopus,* and possibly other rodents. Serum specimens from these and other mammals contain immunoglobulins to the Lyme disease agent.[1,17] Natural animal deaths have not been attributed to the spirochete. One study of animal necropsy specimens concluded that the spirochetes concentrate in the spleen.[3] This may indicate infestation without apparent systemic infection or affectation. Successful research efforts with animal models induced spirochetemia and recovery of *B. burgdorferi* from internal organs.[3,9,11] Transplacental transmission of *B. burgdorferi* in humans was documented[6,13] by recovery of the spirochete from fetal spleen and other organs. However, findings for visceral pathogenic involvement were limited to elevated levels of liver enzymes, hepatomegaly, hematuria, and proteinuria.[16]

The unique clinical feature of Lyme disease is the ex-

panding skin lesion. Not all patients manifest typical erythema migrans, and only 30% of patients can associate a tick bite with the lesion.[19] The spirochete has been detected in skin lesions by various staining procedures and cultured from erythema migrans, blood, synovial fluid, and cerebrospinal fluid.[4,10,18-21] The arrival of the spirochete in the patient's spleen may have occurred faster than previously understood, but his splenomegaly and cough were consistent with the less common manifestations described in that report.[19]

Lyme disease diagnoses are most frequent between late June to August,[15] but spirochetes can be isolated from motile stages of the tick year-round, with twice the frequency in the summer as in the winter.[2] Onset of Lyme disease may be protracted in the general human population. The incubation period for exacerbation of the skin lesion is not exact; estimates vary between 3 and 32 days.[19] For a short incubation period, lower serum antibody titers would be consistent with our serologic findings. It has been established by IFA staining that IgM antibody can develop to detectable levels within three weeks after onset of erythema migrans.[14,17,20] The patient's serum showed a low level of antibody by IFA and ELISA. The immunoblot profile was consistent with a report using immunoblots for early Lyme disease diagnosis.[7] The possibility of cross-reacting *Treponema pallidum* antibodies with *B. burgdorferi* antigens[7,16] was disproved by a negative FTA-ABS result.

Histologically, the splenic lesions did not appear to be embolic infarcts because viable areas were observed surrounded by necrotic inflamed tissue. Many vessels were destroyed by the inflammatory process. The necrosis may have been a secondary phenomenon resulting from toxic microbial by-products or the host defense response, yet no foreign materials or bacteria were demonstrable with the H and E or the B and B tissue Gram's stains. The numbers of *B. burgdorferi* seen with the Dieterle stain were overwhelming compared with those seen in five prior postmortem examinations of human splenic tissue (three neonates, two adults) by one of us (P.D.), where the focal involvement was the central nervous system. The necrosis was more extensive than previously observed in human splenic tissue but equivalent to the findings in the animal reservoir. There was no difficulty distinguishing the Borrelia microorganisms from other well-known spirochetes, including *T. pallidum* and Leptospira spp. Habicht and colleagues[8] reported that the lipopolysaccharide of *B. burgdorferi* was responsible for erythema migrans and postulated the induction of interleukin-1 as the agent responsible for the inflammatory changes.

A recent surveillance report of Lyme disease incidence in Connecticut residents[15] indicated that most victims were white. This may be due to the location of the tick vector in rural areas along the Connecticut River or coastline, where the residents are primarily white. There is no evidence that the patient's ethnic background contributed to the disease severity. Surveys by one of us (L.M.) have shown spirochete-infected ticks in Connecticut inland areas. The patient lived in Hartford, denied travel to coastline areas, but recalled ". . . tiny bumps that itched . . ." on his anterior forearm after a visit to an inland state park. He denied having any arthritic aches, pains, or headaches afterward. He was shown photographs of lesions and rashes but could not identify his skin eruption. The patient, a dog owner, viewed a photograph of ticks and readily identified *I. dammini* from *Dermacentor andersoni* as similar to the ticks he removed from his dog sometime in November, a time when *I. dammini* adults are abundant. He admitted to using intravenous cocaine one week before the onset of illness. The specter of intravenous passage of the spirochete through needle-sharing was considered but not pursued. This case seems to be a unique presentation of naturally acquired Lyme disease.

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NEWS AND NOTICES

Notices

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