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 (ca. 4 L) per weekend and was an intravenous cocaine abuser. 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.

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 megaline in humans with descriptions of elevated liver enzyme levels. The spirochete Borrelia burgdorferi has been isolated from skin lesions, blood, synovium, and cerebrospinal fluid of naturally acquired infection, 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 per weekend and was an intravenous cocaine abuser. 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.

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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 Biotechnology 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 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. 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) H2O2 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 \times 10^9/L (18.1 \times 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 suppurative discoloration in the subcapsular region. 

FIG. 2 (upper, right). Splenic follicle showing acute central necrosis. Hematoxylin and eosin (X100).

FIG. 3 (lower). Borrelia burgdorferi. Two morphologic lengths are exhibited: longer spiriform 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 (X1,000).
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 D 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 necrotizing 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 200X magnification. They appeared pleomorphic at 1,000X and varied in lengths. Short organisms were difficult to discern, but longer organisms were spirilliform 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.117 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 spirochtemia and recovery of B. burgdorferi from internal organs.3,9,11 Transplacental transmission of B. burgdorferi in humans was documented6,13 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.6

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.\textsuperscript{19} The spirochete has been detected in skin lesions by various staining procedures and cultured from erythema migrans, blood, synovial fluid, and cerebrospinal fluid.\textsuperscript{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.\textsuperscript{19}

Lyme disease diagnoses are most frequent between late June to August,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{7} The possibility of cross-reacting \textit{Treponema pallidum} antibodies with \textit{B. burgdorferi} antigens\textsuperscript{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 \textit{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 \textit{T. pallidum} and Leptospira spp. Habicht and colleagues\textsuperscript{8} reported that the lipopolysaccharide of \textit{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\textsuperscript{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 \textit{I. dammini} from Dermacentor andersoni as similar to the ticks he removed from his dog sometime in November, a time when \textit{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.

\textbf{References}

NEWS AND NOTICES

Notices

ASCP/CAP CALL FOR SCIENTIFIC ABSTRACTS

The American Society of Clinical Pathologists (ASCP) and the College of American Pathologists (CAP) will sponsor an exchange of information and ideas in anatomic and clinical pathology at the ASCP/CAP 1989 Fall Meeting in Washington, D.C., October 28–November 3, 1989. Laboratory professionals are invited to submit scientific abstracts for presentation in a poster format or as papers (residents only). The postmark deadline for submissions is April 24, 1989.

The two residents presenting the best papers will win cash awards of $500 as part of the Pathology Resident Award competition. On the basis of abstracts, ten papers will be selected for presentation, five each in anatomic and clinical pathology. The final award winner will be selected on the basis of both content and presentation of papers. Residents must be currently enrolled in a pathology program to participate.

All scientific abstracts accepted by the ASCP Abstract review Committee will be published and indexed in the American Journal of Clinical Pathology. The criteria used in the selection of abstracts includes the timeliness of subject matter, the author’s cognizance of the subject and evidence that the “scientific method” was used in the investigation (i.e., state the problem, propose the hypothesis, select the appropriate methods to test it, and observe/interpret the results).

For complete information and an abstract submission form, call the ASCP toll-free: 800-621-4142 (in Illinois call: 312-738-4890); or write: Manager for Scientific Assembly, American Society of Clinical Pathologists, 2100 West Harrison Street, Chicago, Illinois 60612-3798.

Continuing Education Opportunities

American Society of Clinical Pathologists/College of American Pathologists

October 28–November 3, 1989 Joint National Meeting (Washington, DC)
March 24–25, 1990 Joint National Meeting (San Francisco, CA)

ASCP Continuing Education Courses

April 10–13, 1989 Review of Current Methods in Blood Banking and Transfusion Medicine (Hartford, CT)
April 23–29, 1989 Pathology Update—Spring 1989 (Pasadena, CA)
April 24–28, 1989 Surgical Pathology of the Gastrointestinal Tract (San Antonio, TX)
April 30–May 4, 1989 Fine-Needle Aspiration Cytology—Principles and Practice (Seattle, WA)
May 19–21, 1989 Lymph Node Pathology: A Systematic Approach (Williamsburg, VA)
May 22–26, 1989 Gynecologic Pathology (Washington, DC)
May 30–June 2, 1989 Diagnostic Cytology of the Female Genital Tract (San Diego, CA)
July 7–9, 1989 Weekend of Pathology (Seattle, WA)

For further information regarding the above programs contact: ASCP Customer Service, 2100 W. Harrison Street, Chicago, Illinois 60612, or call (800) 621-4142 or (312) 738-4890 (in Illinois)

Harvard Medical School

June 26–30, 1989 Advances in Cytology (Ritz Carlton Hotel, Boston, MA)

For further information contact: Harvard Medical School, Department of Continuing Education, P.O. Box 825, Boston, MA 02117, or call (617) 732-1525.

The University of Utah, Department of Pathology, Divisions of Clinical Microbiology and Immunology

July 16–22, 1989 1989—7th Annual Summer Update in Clinical Microbiology and Immunology (Jackson, WY)

For further information contact: John M. Matsen, M.D., Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah 84132, or call (801) 381-7480.