BRIEF REPORT: FATAL SERONEGATIVE EHRLICHIOSIS IN A PATIENT WITH HIV INFECTION

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HUMAN infection with *Ehrlichia chaffeensis*, a recently identified bacterium included in the family Rickettsiaceae,[1,2] results in an acute, generally self-limited, febrile illness associated with cytopenia and hepatic-enzyme abnormalities. Several hundred cases of *E. chaffeensis* infection have been recognized since 1986, although ehrlichiosis has not been described in patients infected with the human immunodeficiency virus (HIV). We report the course of a fatal seronegative ehrlichial infection in a woman with advanced HIV infection. The infection was diagnosed by light and electron microscopy, immunohistochemical techniques, and the polymerase chain reaction. The potential for successful antimicrobial treatment, combined with the possible absence of serologic markers in immunocompromised persons with HIV infection, makes this disease an important diagnostic consideration in the appropriate clinical setting.

CASE REPORT

A 41-year-old HIV-seropositive woman with a history of intravenous drug use was admitted to a San Francisco hospital in June 1992, with a six-day history of fever and malaise. Approximately two weeks before admission, she had traveled by bus and motor home from her residence in a rural part of north central Arkansas to visit friends in San Francisco. Three days after her arrival, fever (temperature of up to 40°C), lethargy, anorexia, and diarrhea developed. She had not had any of these symptoms when she left Arkansas in late May, and neither of her two traveling companions described similar symptoms. She described a history of frequent "bug bites," although she reported no specific exposure to ticks. Antibody to HIV had initially been detected in July 1990, and an absolute CD4 lymphocyte count of 64 cells per cubic millimeter was documented in January 1991. A blood count approximately one week before the patient's departure from Arkansas showed a hemoglobin level of 10.7 g per deciliter, a leukocyte count of 3500 per cubic millimeter, and platelet count of 120,000 per cubic millimeter. Documented previous infections included intestinal strongyloidiasis in July 1990 and recurrent oropharyngeal candidiasis. At the time of admission, the patient was taking zidovudine, dapsone, fluconazole, and nystatin.

Physical examination revealed a thin, acutely ill woman with rigors. She had a temperature of 38.5°C, a pulse rate of 108 beats per minute, a respiratory rate of 22 per minute, and a blood pressure of 100/70 mm Hg. Oropharyngeal candidiasis was present, and crackles and dullness to percussion were detected over the right lung base. There was mild cervical adenopathy, but no hepatosplenomegaly. The neurologic examination was normal, and dermatologic evaluation revealed no petechiae, bruises, or rashes.

The hemoglobin level was 11.1 g per deciliter, the mean corpuscular volume was 106 μm^3, and the leukocyte count was 1700 per cubic millimeter, with 64 percent neutrophils, 17 percent bands, 14 percent lymphocytes, and 5 percent monocytes. The platelet count was 25,000 per cubic millimeter. The prothrombin time was 12.5 seconds (control, <13.5), and the partial-thromboplastin time was 43.5 seconds (control, <40.6). The serum lactate dehydrogenase level was 1215 units per liter, and the aspartate aminotransferase level was 140 units per liter. The serum sodium, potassium, chloride, bicarbonate, urea nitrogen, glucose, alanine aminotransferase, alkaline phosphatase, bilirubin, and albumin concentrations were within normal limits. Arterial-blood gas analysis performed while the patient was breathing room air revealed a partial pressure of oxygen of 69 mm Hg, a partial pressure of carbon dioxide of 27 mm Hg, and a pH of 7.44. A chest radiograph showed diffuse, bilateral interstitial infiltrates, with no identifiable adenopathy or effusions.

A presumptive diagnosis of *Pneumocystis carinii* pneumonia and zidovudine-induced pancytopenia was made. Treatment with a combination of trimethoprim and sulfamethoxazole was begun, with ceftazidime and tobramycin added to cover possible bacterial sepsis. Sputum induction on hospital day 3 and bronchoalveolar lavage on day 5 revealed no evidence of pneumocystis, mycobacteria, fungi, or viral inclusions, and multiple blood cultures revealed no bacterial growth. Serum samples were negative for antibodies to *Coccidioides immitis* and *Toxoplasma gondii* and for cryptococcal antigen. A serum Venereal Disease Research Laboratory test for syphilis was negative. An examination of the peripheral-blood buffy coat was negative for *Histoplasma capsulatum*. The serum was weakly positive for hepatitis B surface antigen but negative for anti–hepatitis B core IgM and IgG and anti–hepatitis A IgM. A CD4 lymphocyte count on hospital day 5 was 0. Erythromycin was added to the treatment regimen on day 5, and acyclovir and ceftriaxone on day 6. The patient's condition continued to deteriorate, and a bone marrow biopsy was performed on the eighth hospital day because of worsening pancytopenia (leukocyte count, 400 per cubic millimeter, a hemoglobin level of 8.3 g per deciliter, and a platelet count of 18,000 per cubic millimeter). On the same day, the patient became increasingly lethargic and somnolent, with mild epistaxis and spontaneous vaginal bleeding. Amphotericin B was added to the antimicrobial regimen. On day 10, small intraleukocytic inclusions suggestive of microorganisms were identified in the bone marrow, although bone marrow cultures were negative for viruses, mycobacteria, chlamydiae, and fungi. On the same day, the patient described worsening shortness of breath. A chest radiograph showed progressive interstitial and alveolar infiltrates, primarily in the lower lung fields, and bilateral pleural effusions (Fig. 1). The patient's respiratory status deteriorated rapidly, and she died several hours later. An autopsy limited to the chest and abdomen was performed.

RESULTS

Formalin-fixed, paraffin-embedded sections of bone marrow obtained by biopsy and tissue obtained at autopsy were examined by light microscopy after standard histologic staining and immunoperoxidase staining with biotinylated antibody to *E. chaffeensis*.[3,4] Intracytoplasmic ehrlichial inclusions (morulae) were detected in the bone marrow sample with hematoxylin and eosin, Giemsa's, and periodic acid–Schiff stains (Fig. 2A), although the minute organisms were dif-

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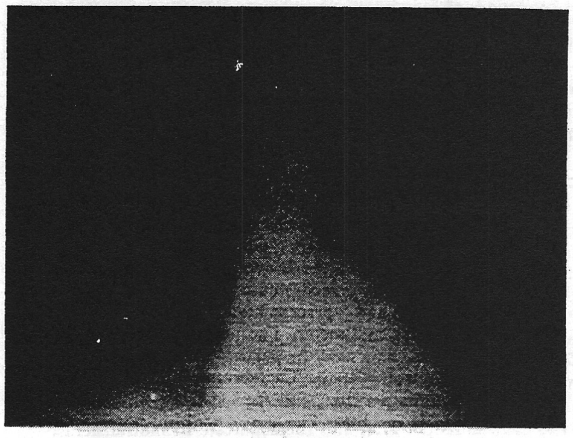


Figure 1. Chest Film Obtained Shortly before the Patient's Death on Hospital Day 10, Showing Diffuse, Predominantly Basilar Interstitial and Alveolar Infiltrates.

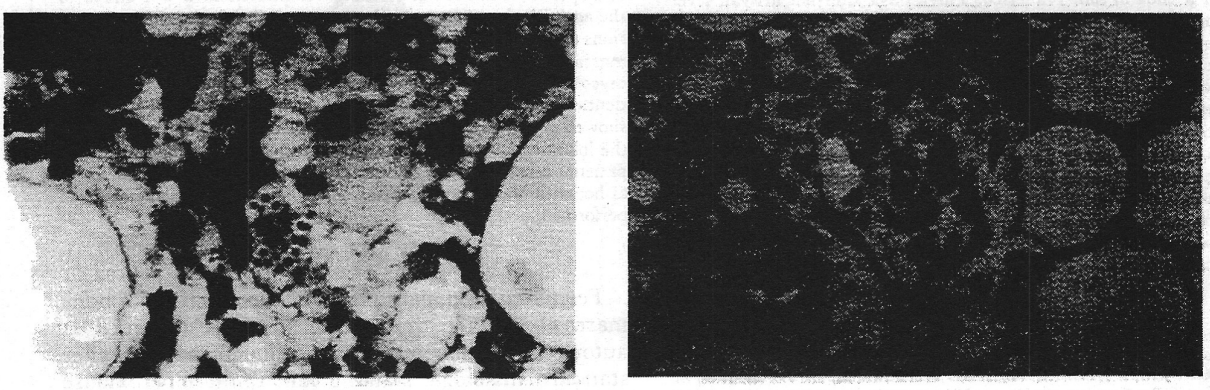
ficult to resolve on casual microscopical inspection. Immunoperoxidase staining provided clear accentuation of ehrlichiae in bone marrow (Fig. 2B) and multiple other tissues, including the spleen (Fig. 3A), lymph nodes, liver, and lung. The density of organisms in these organs was high as compared with the densities in previously published accounts of human ehrlichiosis.[3,4] In Wright-stained preparations of the bone marrow aspirate, morulae were detected in approximately 10 percent of all leukocytes, predominantly histiocytes, and appeared as round inclusions averaging 2 to 3 μm in width and varying in color from violet to deep blue. Infected cells typically contained one or two morulae, although some leukocytes had as many as 15 distinct inclusions. On electron microscopy, the inclusions appeared as intracytoplasmic vacuoles containing multiple, closely clustered, round-to-ovoid bodies, ultrastructurally compatible with ehrlichia[5] (Fig. 4).

The patient's lungs had extensive alveolar hemorrhage, microvascular congestion, and hemorrhage and edema of interlobular septa. There was moderate hyperplasia of type II cells, although inflammatory infiltrates were scarce or absent in the pulmonary parenchyma. The spleen had an unusual lesion: circumferential lymphoid depletion of periarteriolar lymphoid sheaths, with replacement by a prominent histiocytic infiltration and patchy necrosis (Fig. 3B). A similar lymphocytic depletion and patchy necrosis lined by histiocytes was present in multiple lymph nodes. There was subtle microvesicular steatosis of the liver, but no evidence of inflammation or necrosis.

E. chaffeensis DNA was detected in blood samples by the polymerase chain reaction performed at the Centers for Disease Control and Prevention (CDC) with species-specific primers derived from the 16S ribosomal RNA gene of *E. chaffeensis*.[6] Serum samples obtained one day before death and two days post mortem were negative for antibodies to *E. chaffeensis* (CDC) and *E. canis* (State of California Microbial Diseases Laboratory, Berkeley) by an indirect fluorescent-antibody test.[7] Antibodies against *Rickettsia rickettsii*, *R. prowazekii*, and *Coxiella burnetii* were not detected. By microimmunofluorescence, IgG antibody to *Chlamydia trachomatis* was present at a titer of 1:512, but without detectable IgM antibody. Close reexamination of the antemortem preparation of the peripheral-blood buffy coat revealed rare intracytoplasmic morulae within monocytes and neutrophils. Ehrlichiae were not seen on light-microscopical reinspection of the induced-sputum and bronchial-lavage specimens.

Discussion

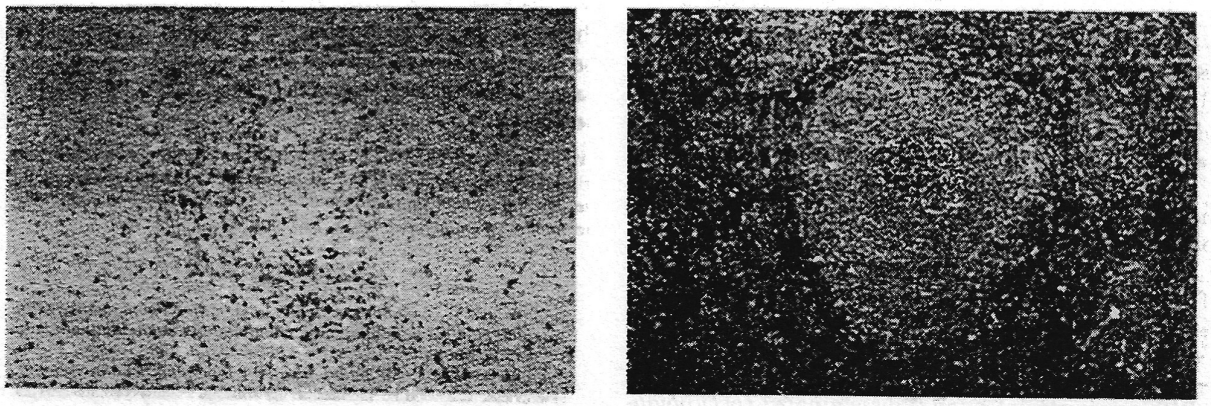
Ehrlichiae are small, pleomorphic gram-negative organisms long recognized as important pathogens of animals. These obligate intracellular parasites typically infect phagocytic leukocytes of a variety of mammalian hosts, including horses, cattle, sheep, and



A B

Figure 2. Bone Marrow Obtained by Biopsy on Hospital Day 8, Showing Numerous Intracytoplasmic Ehrlichiae.

The bone marrow was normocellular and showed mixed hematopoiesis, with normal numbers of megakaryocytes (Panel A). Ehrlichial inclusions were confined to leukocytes, predominantly histiocytes (periodic acid–Schiff with hematoxylin counterstain, ×425). In Panel B, immunoperoxidase staining more clearly shows the widespread *E. chaffeensis* morulae (×350).



A B

Figure 3. Splenic Periarteriolar Lesions Showing Localized Positive Staining for *E. chaffeensis* (Panel A) and Lymphoid Depletion of Periarteriolar Lymphoid Sheaths and Replacement with Histiocytic Aggregates (Panel B).

The micronodular aggregates shown in Panel B contained karyorrhectic foci and frequently surrounded small islands of intact lymphocytes. (Panel A, immunoperoxidase, ×45; Panel B, hematoxylin and eosin, ×45.)

domestic and wild dogs. The natural transmission of ehrlichia species, when known, involves tick vectors.[5,8,9] Until recently, human ehrlichial disease had been described only in Japan and Malaysia, where *E. sennetsu* produces a mononucleosis-like illness known as sennetsu fever.[8,9] The first confirmed case of human ehrlichiosis in the Western Hemisphere occurred in 1986[10]; by 1992 nearly 300 cases had been identified, predominantly in the midwestern and southeastern regions of the United States (CDC: unpublished data). The demonstration of anti-ehrlichial IgG antibody represents the principal diagnostic method for human ehrlichiosis,[7] although microscopical visualization of the characteristic mulberry-like intraleukocytic inclusions in the peripheral blood,[10-12] bone marrow,[11,13] or cerebrospinal fluid[12] of severely ill patients occasionally provides the initial clue to infection.

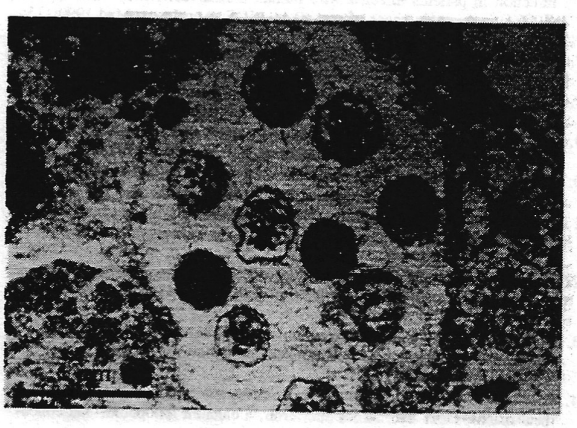


Figure 4. Electron Photomicrograph of an Ehrlichial Inclusion within a Bone Marrow Mononuclear Cell.

The intracytoplasmic vacuole contains multiple individual microorganisms, each measuring approximately 0.2 to 0.5 μm in width.

The clinical, laboratory, and epidemiologic findings in our patient corroborate the diagnosis of human ehrlichiosis. These include the presentation of an acute febrile illness accompanied by leukopenia, rapidly progressive thrombocytopenia, elevated serum aminotransferase levels, and isolated prolongation of the partial-thromboplastin time[8,9,12,14]; a late spring–early summer presentation[9,14]; and residence in rural Arkansas, the location of the first reported case of human ehrlichiosis in this hemisphere[10] and the type species isolate of *E. chaffeensis*.[2] The small intracytoplasmic inclusions identified by light microscopy in peripheral-blood and bone marrow leukocytes resembled ehrlichia morulae morphologically.[10-13] Systemic infection with *E. chaffeensis* was subsequently confirmed by electron microscopy, immunohistochemical techniques, and the polymerase chain reaction.

Importantly, the initial manifestations of ehrlichia infection in this patient mimicked the clinical features of more common forms of opportunistic infections and drug reactions in patients with HIV infection. Findings on physical examination, chest radiography, and arterial-blood gas testing were consistent with a diagnosis of *P. carinii* pneumonia, and the initial pancytopenia was attributed to treatment with zidovudine. Further diagnostic investigations and empirical therapies were directed toward the types of viral, fungal, and bacterial organisms that often affect patients with HIV infection. However, the regimen of broad-spectrum antimicrobial agents used in this patient did not include the treatment of choice for human ehrlichiosis: tetracycline.[15] Although ehrlichiae were subsequently detected on bone marrow biopsy, this diagnostic information was not obtained soon enough to alter treatment.

Several of the manifestations of *E. chaffeensis* infection in this patient were unusual, including the absence of detectable anti-ehrlichial antibodies, the fatal outcome, and the distinct pulmonary and splenic le-

sions. The CDC case definition of human ehrlichiosis requires a fourfold or greater change in antibody titer to ehrlichia in appropriately paired serum samples, with a minimal titer of 1:64.[7-9] In immunocompetent patients with ehrlichiosis, IgG antibody titers typically appear during the first three weeks of illness, and most patients have detectable levels by the end of the fourth week.[7] In our patient, 16 days elapsed between the onset of symptoms and death, and the evaluation of serum obtained during the third week of illness revealed no detectable antibody. The absence of detectable diagnostic antibodies has been described in several HIV-associated diseases, including chronic B19 parvovirus infection,[16] disseminated coccidioidomycosis,[17] disseminated histoplasmosis,[18] and central nervous system toxoplasmosis.[19]

Fatalities associated with human ehrlichiosis are uncommon,[3,14,20] representing less than 3 percent of known cases through 1991 (CDC: unpublished data). Pulmonary hemorrhage, the probable immediate cause of death in this patient, is also an infrequently described complication of ehrlichiosis,[3] although pulmonary infiltrates and marked respiratory insufficiency have been reported in several patients with severe *E. chaffeensis* infection.[11,12,14] Pulmonary hemorrhage has been observed in as many as 60 to 70 percent of fatal infections with *E. canis* in dogs.[21] Granulomas or granulomatous inflammation is a relatively frequent bone marrow finding in patients with *E. chaffeensis* infection.[4] However, our patient had no distinct bone marrow granulomas, and the white-pulp histiocytic aggregates in the spleen represent at best poorly formed granulomas. Interestingly, nearly identical splenic lesions have been described in an animal model of *E. risticii* infection.[22]

Intact T-lymphocyte function is an important host defense in rickettsial diseases.[23] Anti-ehrlichial activity has been demonstrated with the T-cell lymphokine interferon-γ, which stimulates refractoriness to infection and the eradication of intracellular ehrlichiae in macrophages.[24] The rapid and severe progression of ehrlichial infection in this patient suggests that *E. chaffeensis* infection should be included in the expanding spectrum of potentially life-threatening opportunistic infections in persons with advanced HIV disease. Although human ehrlichiosis is relatively uncommon, this disease should be considered as a cause of acute febrile illness and rapidly progressive cytopenia, particularly thrombocytopenia, in HIV-infected persons residing or traveling in areas in which *E. chaffeensis* infection may be endemic. Serologic evaluation for anti-ehrlichial antibodies remains the principal method of diagnosis of human ehrlichiosis; however, the possibility of delayed or impaired antibody synthesis and rapid clinical deterioration in persons with HIV infection necessitates a high index of suspicion when one is examining hematologic and surgical specimens to establish a working diagnosis and initiate appropriate treatment during the acute phase of the illness.

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