

## Brief Report

SIMULTANEOUS HUMAN  
GRANULOCYtic EHRLICHIOSIS  
AND LYME BORRELIOSIS

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**I**NFECTION with the agent of human granulocytic ehrlichiosis occurs in areas in which *Borrelia burgdorferi* and *Babesia microti* are endemic.<sup>1-4</sup> The most likely vector of human granulocytic ehrlichiosis is the deer tick, *Ixodes scapularis*, which is also the vector of Lyme disease and babesiosis.<sup>3,4</sup> Coinfection in humans with both the agent of human granulocytic ehrlichiosis and *B. burgdorferi* can be anticipated because ixodes ticks infected with the two organisms have been identified in several locales.<sup>3-5</sup>

The diagnosis of simultaneous infection with *B. burgdorferi* and the agent of human granulocytic ehrlichiosis is important because the natural history of each of the diseases may change in the presence of the other and because dual infection may affect the choice of antimicrobial therapy. For example, amoxicillin, which is widely used to treat early Lyme disease, is considered to be ineffective for human granulocytic ehrlichiosis.

Establishing proof of coinfection requires the cultivation of both organisms, but cultivation of the agent of human granulocytic ehrlichiosis has become feasible only recently.<sup>6</sup> We now report the case of an acutely ill patient from whom *B. burgdorferi* and the agent of human granulocytic ehrlichiosis were isolated in culture to demonstrate that simultaneous infection with these two agents occurs in humans.

## CASE REPORT

A 47-year-old man from Westchester County, New York, was seen by a physician on August 5, 1996; the patient had symptoms

of fever, headache, myalgia, arthralgia, and generalized weakness. He also reported a stiff neck, cough, mild dizziness, and difficulty in concentrating. He had previously been in good health. The fever, myalgia, and arthralgia had begun around July 19, one month after the removal of a small tick from the patient's right thigh. These symptoms resolved spontaneously after a week but then recurred during the first week of August. The patient was unaware of any rash.

The patient's temperature was 38.3°C. His pulse was 100 beats per minute. Conjunctival injection of the right eye was present and a faint, pink circular rash (9 by 9 cm) suggestive of erythema migrans, with some central clearing and a central papule, was observed on his right flank. There were no other skin lesions.

The results of a complete blood count included a white-cell count of 3800 cells per cubic millimeter (normal range, 4600 to 10,600). Fifty-seven percent were neutrophils, 30 percent lymphocytes, 12 percent monocytes, and 1 percent basophils. The patient had a hemoglobin level of 15.4 g per deciliter and a platelet count of 148,000 per cubic millimeter (normal range, 160,000 to 410,000). There were several mildly abnormal results from liver-function assays, including a lactate dehydrogenase level of 279 U per liter (normal range, 110 to 225), an aspartate aminotransferase level of 44 U per liter (normal range, 4 to 35), an alkaline phosphatase level of 120 U per liter (normal range, 35 to 110), and a  $\gamma$ -glutamyltransferase level of 49 U per liter (normal range, 1 to 45). The electrocardiogram was normal.

After specimens were obtained for the purpose of culturing *B. burgdorferi* and the agent of human granulocytic ehrlichiosis, the patient was given 100 mg of doxycycline twice daily for 14 days. On August 14, nine days after presentation, his condition was markedly improved; he reported only mild fatigue, and the results of a physical examination, a complete blood count, and liver-function tests were all normal. His erythema migrans rash had resolved by the eighth day of treatment. At subsequent clinical evaluations, on August 28, 1996 (23 days after presentation), and March 11, 1997 (7 months after presentation), the patient felt himself to be completely recovered; the results of physical examination, complete blood counts, and liver-function assays remained normal.

## METHODS

## Evaluation for Infection with the Agent of Human Granulocytic Ehrlichiosis

Buffy-coat smears of peripheral blood were prepared with Wright's stain, and 1000 granulocytes were examined under magnification ( $\times 500$  and  $\times 1000$ ) for the presence of human granulocytic ehrlichiosis morulae. Polymerase-chain-reaction (PCR) assays were performed on whole blood, collected in tubes containing EDTA, with primer set 521 and 747 (as described by Pancholi et al.<sup>3</sup>) and primer set GER3 and GER4 (as described by Goodman et al.<sup>6</sup>).

Cells were cultured with the techniques of Goodman et al.<sup>6</sup> To visualize the agent of human granulocytic ehrlichiosis, slides of cultured cells were stained with Wright's stain. In addition, aliquots of cultured HL-60 cells were assayed for the presence of human granulocytic ehrlichiosis by PCR amplification and by an indirect immunofluorescence assay that used serum from another patient who had a high titer of antibodies to the agent of human granulocytic ehrlichiosis.<sup>6</sup> Serum samples were tested for antibodies with an indirect immunofluorescence assay that used homologous and heterologous strains of the agent of human granulocytic ehrlichiosis propagated in an HL-60 cell line.<sup>1,2</sup>

Evaluation for *B. burgdorferi* Infection

A 2-mm skin-punch biopsy was performed approximately 1 cm inside the margin of the erythema migrans lesion and cultured as previously described.<sup>7,8</sup> The motile spirochetes visualized by fluorescence microscopy were confirmed to be *B. burgdorferi* with a PCR assay using primers IS1 and IS2.<sup>7</sup> In addition, PCR analysis for the identification of *B. burgdorferi* was performed directly on the skin-biopsy tissue.<sup>7</sup>

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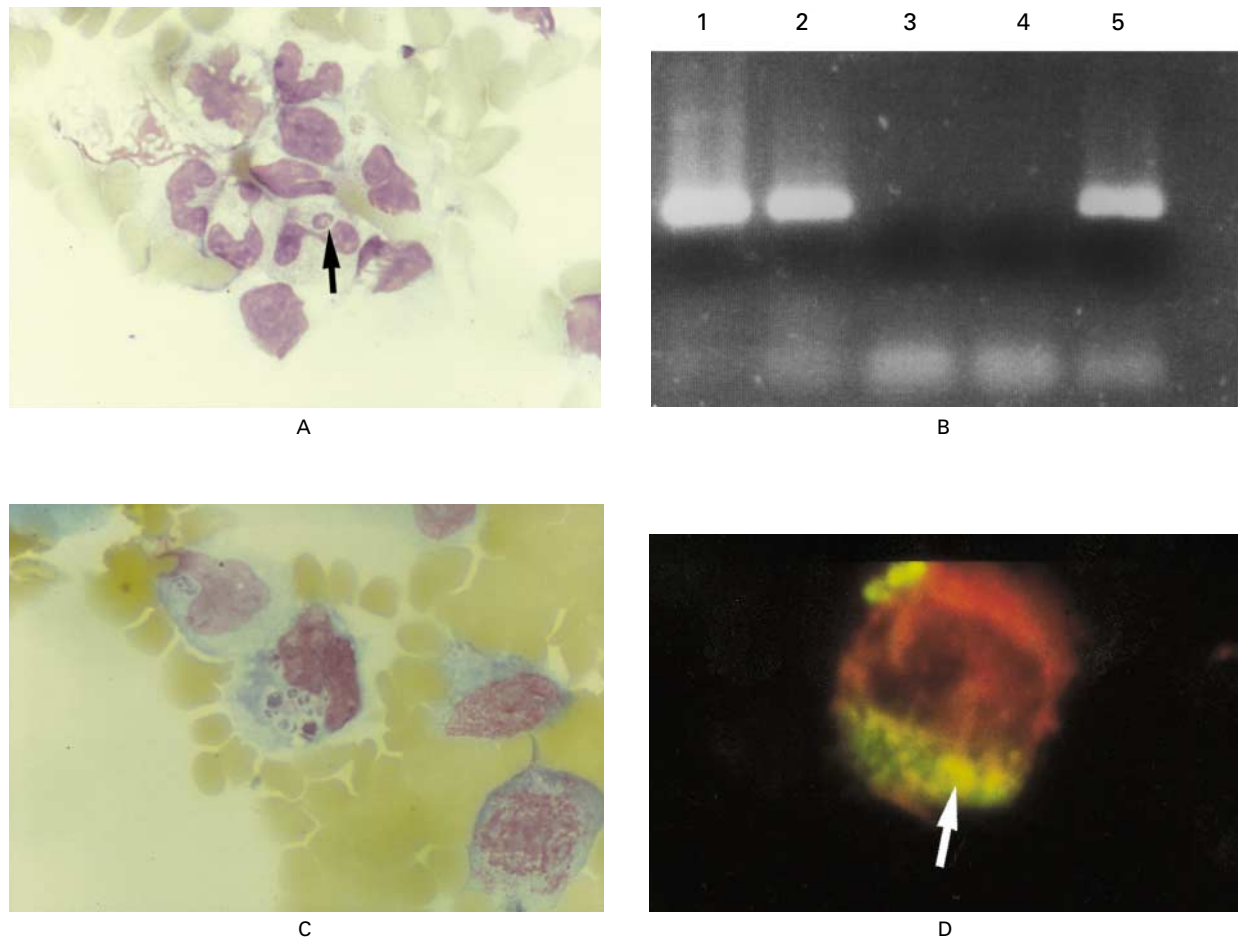
Serum antibodies to *B. burgdorferi* were assayed by an IgM-IgG enzyme-linked immunosorbent assay (EIA Lyme Stat, Bio-Whittaker, Walkersville, Md.) according to the manufacturer's instructions.<sup>7,8</sup> Separate IgM and IgG immunoblot assays for antibodies to *B. burgdorferi* were performed with MarDx test kits (MarDx Diagnostics, Carlsbad, Calif.), according to the manufacturer's instructions, and interpreted with published criteria.<sup>9</sup>

### RESULTS

Examination of the buffy coat at presentation revealed morulae in 0.3 percent of neutrophils (Fig. 1A); the PCR assay of blood detected the presence of DNA of the agent of human granulocytic ehrlichiosis (Fig. 1B). Cultures with HL-60 cells revealed morulae, visualized with Wright's stain, in 1 percent

of the cells on day 3, 52 percent of the cells on day 6, and 79 percent of the cells on day 9 (Fig. 1C). The organism in the cell culture was confirmed to be the agent of human granulocytic ehrlichiosis both by a PCR assay (Fig. 1B) and by immunofluorescence microscopy, which showed specific staining of morulae. Such staining was not observed in uninfected HL-60 cells or in infected cells incubated with serum from a healthy control.

Serum titers of antibodies against the two strains (homologous and heterologous) of the agent of human granulocytic ehrlichiosis were 1:640 (for the antibodies against each of the isolates) on the day of presentation, 1:1280 (antibodies against the ho-



**Figure 1.** Demonstration of Infection with the Agent of Human Granulocytic Ehrlichiosis.

Panel A shows a buffy-coat smear of peripheral blood obtained at presentation, with morulae characteristic of human granulocytic ehrlichiosis (arrow) (Wright's stain,  $\times 1000$ ). Panel B shows the results of a PCR assay and DNA amplification to detect the agent of human granulocytic ehrlichiosis: lane 1, the patient's blood; lane 2, cultured HL-60 cells inoculated with the patient's blood; lanes 3 and 4, blood from an uninfected patient and a master mix lacking target DNA, respectively, as negative controls; and lane 5, 0.1 pg of 16S ribosomal DNA of the agent of human granulocytic ehrlichiosis, as a positive control. Panel C shows morulae characteristic of human granulocytic ehrlichiosis in HL-60 cell culture six days after inoculation with the patient's blood. Approximately 50 percent of the cells appear infected (Wright's stain,  $\times 1000$ ). Panel D shows the results of an indirect immunofluorescence assay using the patient's serum and HL-60 cells infected with the patient's human granulocytic ehrlichiosis isolate (arrow) ( $\times 1000$ ).

mologous isolate) and 1:2560 or more (antibodies against the heterologous isolate) on day 9 of treatment, and 1:1280 (antibodies against each of the isolates) on day 24 (Fig. 1D). The initial serum titers as evaluated with indirect immunofluorescence against *Ehrlichia equi* (1:2560 or more) and *E. chaffeensis* (less than 1:80) (both assays kindly performed by Dr. J. Stephen Dumler) were unchanged at the two subsequent dates of testing.

Motile spirochetes were visualized by fluorescence microscopy two weeks after inoculation of a skin-biopsy specimen into modified Barbour–Stoenner–Kelly medium (Fig. 2A). These were confirmed as *B. burgdorferi* by PCR (Fig. 2B). In addition, direct PCR amplification of the skin-biopsy specimen verified the presence of this organism (Fig. 2B). A serial enzyme-linked immunosorbent assay to measure antibodies to *B. burgdorferi* from serum samples collected on August 5, 14, and 28, 1996, found the following Lyme-index values: 1.39 (positive) for se-

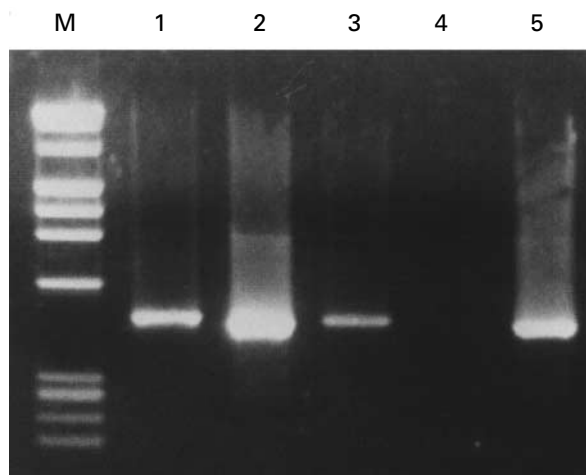
rum collected on August 5, 1.43 (positive) for August 14, and 0.87 (equivocal) for August 28. Immunoblotting of serum collected on the same dates showed the following bands: August 5 — IgM: 93 and 26 kd (negative); IgG: 75 kd (negative); August 14 — IgM: 93, 75, 66, 63, 41, 34, 26, and 24 kd (OspC) (positive); IgG: 75, 63, 41, 35, and 29 kd (negative); and August 28 — IgM: 75 kd (negative); IgG: 75, 41, 35, and 29 kd (negative).

## DISCUSSION

Although there has been serologic evidence that Lyme borreliosis and human granulocytic ehrlichiosis can occur simultaneously in the same patient,<sup>3,10</sup> we now have convincing evidence that this actually does occur. Coinfection with *B. burgdorferi* and the agent of human granulocytic ehrlichiosis was demonstrated by the isolation of both organisms from clinical specimens. Serologic evidence alone<sup>3,10</sup> is insufficient to verify a dual infection, because positive



A



B

**Figure 2.** Demonstration of *Borrelia burgdorferi* Infection.

Panel A shows spirochetes isolated from a biopsy specimen from the erythema migrans lesion on the patient's flank, seen under fluorescence microscopy ( $\times 500$ ). Panel B shows the results of the PCR assay for *B. burgdorferi*: lane M, DNA molecular-size markers; lanes 1 and 2, amplification of DNA from the patient's skin-biopsy specimen and from a culture inoculated with the specimen, respectively; lanes 3 and 5, a skin-biopsy specimen from another patient with culture-confirmed erythema migrans and 500 lysed *B. burgdorferi* cells, respectively, as positive controls; and lane 4, a master mix lacking target DNA, as a negative control.

assay results may be nonspecific<sup>11-14</sup> and may persist after clinical cure<sup>15,16</sup>; such results do not necessarily indicate active infection. Data from patients<sup>17</sup> and preliminary data from animals<sup>18</sup> suggest that infection with the agent of human granulocytic ehrlichiosis may by itself produce false positive results on serologic tests for Lyme disease. PCR may also yield false positive results if the appropriately specific primers are not used or if proper precautions are not taken to prevent contamination.<sup>19</sup>

The patient we studied, however, had an illness with clinical and laboratory features characteristic of both infections. His erythema migrans rash was characteristic of early Lyme borreliosis, and many of his systemic symptoms (fever, headache, myalgia, arthralgia, and weakness) and liver-function abnormalities may occur in both Lyme disease and human granulocytic ehrlichiosis.<sup>1,2,6,8</sup> He also had a cough, which is uncommon in Lyme disease<sup>8</sup> but has been reported in nearly one third of patients with human granulocytic ehrlichiosis.<sup>1</sup> Similarly, he had leukopenia and thrombocytopenia, which are common in human granulocytic ehrlichiosis<sup>1,2</sup> but rare, or perhaps nonexistent, in Lyme borreliosis.<sup>8,20</sup> The patient's peripheral-blood smear showed morulae suggestive of human granulocytic ehrlichiosis. However, such morulae do not appear to be a sensitive index of infection; they were detected in only 4 of 12 other patients with human granulocytic ehrlichiosis in our institution.<sup>2</sup>

The actual frequency of human coinfection with human granulocytic ehrlichiosis and Lyme borreliosis is a point of great interest. Using PCR methods, several investigators who studied adult *I. scapularis* ticks from Wisconsin,<sup>3</sup> Massachusetts (Nantucket Island),<sup>4</sup> and New York (Westchester County),<sup>5</sup> collected between 1982 and 1995, found that between 2.2 percent and 26 percent were coinfecting with *B. burgdorferi* and an agent resembling *E. equi*; 5.5 percent of the nymphal *I. scapularis* ticks at the Westchester County site were infected with both organisms.<sup>5</sup> Humans could therefore be coinfecting through the bite of a single tick that harbored both organisms or, alternatively, through bites from different ticks, each transmitting a separate infection. (It is unclear which happened to our patient.)

Dual infection with *Babesia microti* and *B. burgdorferi* may result in more serious disease than infection with either agent alone.<sup>21</sup> It remains to be seen whether this pattern also holds true for coinfection with the agents of Lyme borreliosis and human granulocytic ehrlichiosis. Since  $\beta$ -lactam antibiotics are ineffective in the treatment of human granulocytic ehrlichiosis, doxycycline should be strongly considered for the treatment of early Lyme borreliosis in patients who can tolerate tetracyclines and who have illnesses with clinical or laboratory features suggestive of human granulocytic ehrlichiosis.

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