Reinfection with *Ehrlichia chaffeensis* in a Liver Transplant Recipient

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Human monocytic ehrlichiosis is an emerging infection caused by *Ehrlichia chaffeensis*, but reinfection with this agent has not been described. We report a case of reinfection with *E. chaffeensis* after a 2-year interval in a 56-year-old liver transplant recipient with frequent tick attachments.

Human monocytic ehrlichiosis (HME) is a tickborne zoonosis that is endemic in the central and southern United States. It is caused by *Ehrlichia chaffeensis*, an intracellular pathogen of peripheral blood mononuclear cells [1]. The clinical manifestations of HME include fever, headache, and malaise. Most patients report a history of tick exposure [2].

A closely related species, *Ehrlichia canis*, causes persistent infection in dogs, with progression to fatal pancytopenia [3]. In experimentally infected dogs, reinfection with both identical and different strains of *E. canis* has been demonstrated [4]. These observations raise concern that persistent or recurrent *Ehrlichia* infection may also occur in humans. To date, 1 case of persistent infection with *E. chaffeensis* in a human has been reported [5]. In addition, 1 case of reinfection with a related species, *Anaplasma phagocytophila* (previously referred to as the agent of human granulocytic ehrlichiosis), has been reported [6].

Single episodes of HME have been reported in immunosuppressed patients; however, persistent or recurrent infections have not previously been recognized in this group of patients [7, 8]. We recently cared for a liver transplant recipient who experienced 2 distinct episodes of HME caused by *E. chaffeensis* that were separated by 2 years. We used molecular testing techniques to show that the second episode represented reinfection with a different strain of *E. chaffeensis*, rather than persistent infection with the initial strain.

**Methods.** Amplification of *Ehrlichia* DNA from whole blood was performed as described by Buller et al. [9], using PCR with primers that amplify a segment of the 16S rRNA gene from all pathogenic members of the *Ehrlichia* genus (ECA and HE3) and primers specific for *E. chaffeensis* (HE1 and HE3) [10].

Molecular typing of *Ehrlichia* DNA from 2 episodes in a single patient was performed by PCR and nucleotide sequencing of the *E. chaffeensis* variable-length PCR target (VLPT) and 120-kDa protein genes, as described elsewhere [11, 12]. The VLPT gene of *E. chaffeensis* strains can differ in numerous ways, including (1) the number and sequence types of 90-bp repeat units present within the gene, (2) single-nucleotide substitutions at 4 specific locations, (3) the presence or absence of an aspartic acid codon deletion at a site upstream from the repeat units, and (4) the presence or absence of a 9-bp deletion downstream from the putative stop codon [11]. The 120-kDa protein gene in strains of *E. chaffeensis* varies in the number of repeat units.

Serum samples collected during and ~1 month after the first episode and 2, 3, 8, 9, 10, 11, and 13 months after the second episode of HME in our patient were tested for IgG and IgM antibodies that were reactive with *E. chaffeensis* by the Viral and Rickettsial Zoonoses Branch of the Centers for Disease Control and Prevention (Atlanta), using an indirect immunofluorescence assay (IFA) [13]. Western blot analysis was performed on selected specimens, using purified antigens derived from the *E. chaffeensis* Arkansas strain, as described elsewhere [14].

Informed consent was obtained from the patient who participated in this research project. The guidelines for human experimentation of the US Department of Health and Human Services and/or the authors’ institutions were followed in conducting the clinical research.

**Episode 1.** A 56-year-old white man who underwent liver transplantation in 1992 presented to an emergency department in June 1997 with fever, malaise, headache, arthralgias, myalgias, and nausea. His medications included cyclosporine (250 mg orally twice daily), prednisone (5 mg orally once daily), and trimethoprim-sulfamethoxazole (1 double-strength tablet
[160 mg of trimethoprim and 800 mg of sulfamethoxazole] twice daily). He lived in a rural area in Missouri and owned several dogs. The patient and his dogs had recently sustained multiple bites by ticks thought to be *Amblyomma americanum* (the lone star tick) because of white spots noted on the dorsal surfaces.

Physical examination findings were normal, except for a temperature of 39.1°C. The total WBC count was 1.8 x 10^9 cells/L; hemoglobin level, 980 g/L; platelet count, 85 x 10^9 cells/L; and absolute lymphocyte count, 0.3 x 10^9 cells/L. The aspartate aminotransferase (AST) level was 76 IU/L, and alanine aminotransferase (ALT) and bilirubin levels were normal. The results of tests for various infectious agents, including cytomegalovirus, were negative. The patient was treated for tickborne infection with doxycycline (100 mg orally twice daily), and became afebrile within 24 h of the first dose, with resolution of symptoms. The patient was discharged on day 4 after administration with instructions to complete the 14-day course of doxycycline therapy, and he recovered completely.

**Episode 2.** The patient presented again in May 1999 and complained of 2 days of fever (temperature as high as 39°C), arthralgias, and headache; these were similar to the symptoms of the previous episode of HME. He otherwise had been generally well during the interim. The patient was taking cyclosporine (175 mg twice daily), prednisone (2.5 mg once daily), and trimethoprim-sulfamethoxazole (1 double-strength tablet once daily). He again reported frequent tick exposure. The interim well during the interim. The patient was taking cyclosporine (175 mg twice daily), prednisone (2.5 mg once daily), and trimethoprim-sulfamethoxazole (1 double-strength tablet once daily). He again reported frequent tick exposure. The findings of physical examination were unremarkable, except for a temperature of 38.4°C. The total WBC count was 1.8 x 10^9 cells/L; hemoglobin level, 1100 g/dL; platelet count, 129 x 10^9 cells/L; and absolute lymphocyte count, 0.3 x 10^9 cells/L. Electrolyte, AST, ALT, and bilirubin levels were within normal limits, and the results of examination of the CSF were normal. The patient was treated with ceftriaxone, ampicillin, and metronidazole, and urine samples yielded negative results, except for small numbers of coagulase-negative staphylococci in the urine and CSF, which were thought to be skin contaminants. Results of cultures and PCR testing of blood for cytomegalovirus were negative. The fever subsided, and the patient felt much better 24 h after doxycycline treatment was initiated. Administration of doxycycline continued for 14 days, and the patient recovered completely.

**Results.** Primers HE1 and HE3 amplified appropriately sized DNA fragments of the 16S rRNA gene of *E. chaffeensis* recovered from blood samples obtained on day 7 of the first episode and day 3 of the second episode of HME. Sequence analysis of the VLPT gene PCR products amplified from whole blood during both episodes revealed 5 repeat units in each amplicon; however, the nucleotide sequences differed in several other respects (table 1). The sequence type profile for the repeat units was 1, 2, 3, 4, 5 for the sample from the first episode and 1, 2, 3, 4, 4 for the sample from the second episode. In addition, the VLPT gene differed in 3 of 4 single-nucleotide substitution sites and in the presence or absence of an aspartic acid codon deletion.

Variation was also detected in the 120-kDa antigen gene PCR products amplified from the 2 episodes. The amplicon size for the sample from the first episode was consistent with the presence of 3 repeat units, and the amplicon size for the sample from the second episode was consistent with the presence of 4 repeat units [12].

A serum sample collected on day 8 of the first episode tested negative for antibodies reactive with *E. chaffeensis*. Serum obtained on day 22 of the same episode contained anti-*E. chaffeensis* IgM antibodies (titer of 64), with no detectable anti-*E. chaffeensis* IgG antibodies (screening dilution of 1:32). IgG antibody titers of ≥512 (range, 512–2048) were repeatedly detected during the first year after the second episode but decreased to 128 by 13 months after the episode; IgM antibodies were also detected (titers of 32–64) during this interval.

Western blot analysis was performed on serum samples obtained on days 8 and 22 of episode 1 and months 3, 8, and 11 after episode 2. The samples obtained after the second episode showed a reaction pattern distinct from that seen in samples from the first episode, with more prominent bands of <30 kDa and no prominent 70-kDa band (figure 1).

**Discussion.** We report 2 episodes of symptomatic *E. chaffeensis* infection that occurred 2 years apart in a liver transplant recipient. If detailed serologic and molecular testing techniques had not been used, it could be suggested that the second episode represented recrudescence of a persistent infection. However, Western blot analysis of the patient’s antibody responses and

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<th>Table 1. Molecular typing of <em>Ehrlichia chaffeensis</em> variants from 2 episodes of ehrlichiosis in a liver transplant recipient.</th>
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<td>Gene, variable</td>
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<td>Aspartic acid codon deletion</td>
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<td>Gap of 9 bp</td>
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<td>120-kDa protein gene, no. of repeat units</td>
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Figure 1. Western blot analysis of serum samples from a patient who experienced 2 episodes of human monocytic ehrlichiosis. The Western blots for each date were performed using uninfected DH82 cells (dog macrophage cell line [negative control]; shown in the first lane) and DH82 cells infected with purified *Ehrlichia chaffeensis* Arkansas strain (Ech; shown in the second lane). The blots performed with serum samples obtained after the second episode (on 26 August 1999, 20 January 2000, and 3 April 2000) show the development of numerous bands not present in blots performed with serum samples obtained after the first episode (on 23 June 1997 and 9 July 1997). Dates are given in the figure as month/day/year.

molecular typing of *Ehrlichia* DNA obtained during each illness indicated that the 2 episodes represented successive infections with different strains of *E. chaffeensis*.

The conclusion that the different antibody responses seen in Western blots performed on serum samples drawn during the 2 episodes of HME in our patient indicate separate infections is based on observations of animals that were experimentally infected with a single strain of *E. canis*, in which Western blot band patterns in individual animals were stable over extended periods of time [15]. The applicability of these observations to human infection is uncertain, but no comparable human data are available. The conclusion that the differences in the VLPT and 120-kDa protein genes indicate separate infections is based on the numerous differences detected and the observation made elsewhere [8] that the number of repeat units in the VLPT gene was stable from the time at which *E. chaffeensis* was detected in patient blood to the time at which the organism was isolated in cell culture and through 8 passages in cell culture.

Our patient was receiving maintenance immunosuppressive therapy following liver transplantation, which may have attenuated the immune response to *Ehrlichia* infection. Experience with other intracellular human pathogens and with *Ehrlichia* infection in other mammals suggests that primary infection with *Ehrlichia* species might not confer enduring immunity, even in healthy hosts [4]. It is notable that, during the first episode, our patient developed an IgM antibody response but did not have demonstrable titers of anti-*E. chaffeensis* IgG antibodies on day 22 of illness. In a previous study, antibodies were detectable by IFA in all 18 patients with ehrlichiosis who were tested during the third week of illness by use of an *E. canis* antigen [13]. Interestingly, our patient had a dramatic, sustained IgG response after the second episode.

During 1999–2000, we diagnosed ehrlichiosis in 58 patients from Missouri and Illinois. Of these, 13 (22%) were immunosuppressed as a result of a variety of causes. Our experience suggests that many of these patients, including those who are immunosuppressed, return to lifestyles that involve frequent exposure to ticks. It is important to instruct these patients, particularly those with comorbidities or those who are of advanced age and have higher risks of complications and death [2], that they may be susceptible to multiple episodes of ehrlichiosis and should continue to take precautions to reduce exposure to ticks.

**Acknowledgment**

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**References**