Heat Shock Protein 70 of the Agent of Human Granulocytic Ehrlichiosis Binds to *Borrelia burgdorferi* Antibodies

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We describe a patient with human granulocytic ehrlichiosis (HGE), a diagnosis confirmed by PCR and immunoblot analysis. Unexpectedly, immunoglobulin G (IgG) directed towards an 80-kDa ehrlichial antigen (without detectable IgM) was present in the patient's serum in the first week of illness. Lyme disease immunoblots were reactive for IgG (but not IgM), a result indicative of prior exposure to the Lyme disease spirochete. Amino-terminal sequencing revealed that the 80-kDa ehrlichial antigen was an HSP-70 homolog similar to *Borrelia burgdorferi* HSP-70. We conclude that antibodies against *B. burgdorferi* HSP-70 may cross-react with the ehrlichial heat shock protein and that this possibility must be considered when serologic test results for HGE and Lyme disease are interpreted.

Human ehrlichioses are emerging zoonotic infections caused by obligate intracellular bacteria of the genus *Ehrlichia*. Two distinct human ehrlichioses occur in the United States: human monocytic ehrlichiosis, primarily a result of infection with *Ehrlichia chaffeensis*, and human granulocytic ehrlichiosis (*E. equi*), caused by the agent of HGE (aoHGE) (15). The aoHGE is closely related to *Ehrlichia equi* and *Ehrlichia phagocytophila*, the respective agents of granulocytic ehrlichiosis in horses and sheep (4). *Ixodes scapularis* ticks have been implicated in the transmission of the aoHGE (12) as well as of *Borrelia burgdorferi*, and it is not surprising that HGE occurs in areas where Lyme disease is common.

The diagnosis of HGE is established by identification of cytoplasmic clusters of organisms (morulae) within neutrophils in patient blood (2). PCR analysis, based on a 16S ribosomal sequence, has also been used to help diagnose infection but is not widely available (4). Immunofluorescence assays (IFA) using *E. equi* as the antigen are currently used to confirm a clinical diagnosis (2, 10). Results of immunoblot assays using *E. equi* or the aoHGE as the substrate suggest that a 44-kDa antigen is most commonly recognized by antibodies in the sera of patients with HGE (6, 8, 11). Moreover, since the aoHGE GroEL, or a fragment thereof, has been shown to be immunoreactive (9), immunoblots are likely to be helpful in diagnosing aoHGE infection, by identifying false-positive reactivity in IFA or enzyme-linked immunosorbent assays (ELISA). For example, some patients' sera contain antibodies that react to both *E. equi* and *E. chaffeensis* in IFA but can be distinguished by immunoblot analysis since the 44-kDa protein is specific for the aoHGE-*E. equi* group. Furthermore, false-positive ELISA results for Lyme disease have been reported to occur for patients with HGE, suggesting that cross-reactive antibodies that bind *B. burgdorferi* and aoHGE may complicate diagnostic testing for both diseases (16, 17). We here show that antibodies that bind the HSP-70 homolog in tests for *B. burgdorferi* and aoHGE (80 kDa) account, at least in part, for this cross-reactivity.

**Patient.** A 70-year-old man with suspected HGE was admitted to Yale-New Haven Hospital with fever, fatigue, and myalgia during the summer. Four days prior to admission, he presented to an emergency room with fever (101°F) and myalgia. An engorged tick (*Ixodes scapularis*) was noted on his right shoulder. No rash was evident. An IgG (but not IgM) ELISA for Lyme disease was positive on the day of admission. The tick was removed and he was treated with amoxicillin for presumed Lyme disease. The symptoms persisted, and 3 days later he was admitted to the hospital for further evaluation. On the first hospital day, morulae were identified within neutrophils on a blood smear and the patient was treated with doxycycline for HGE. Within 3 days, the patient was asymptomatic, discharged home, and placed on antibiotic therapy for 2 weeks. Tests for syphilis (rapid plasma reagin and fluorescent treponemal antibody) were negative.

**PCR.** PCR was performed to confirm HGE and also to determine whether PCR reactivity is altered following treatment. Primers used were Ehr 521 (5'-TGT AGG CGG TTC GGT AAG TTA AAG-3') and Ehr 747 (5'-GCA CTC ATC GTT TAC AGC GTG-3'), which amplify the region of the 16S ribosomal DNA that distinguishes aoHGE from the other ehrlichiae (12). Primers for the β-actin gene (5'-'GGT CAG AAG GAC TCC TAT GCT G-3') and (5'-GGT CTA AAA CAT GAT CTT G-3') were used as controls to ensure the presence of human DNA in the samples. aoHGE DNA was detected in whole blood on the day of the patient's admission and also on the third hospital day (after 3 days of doxycycline). At 6 weeks following hospitalization (and 4 weeks after finishing antibiotic therapy), aoHGE was no longer detected by PCR (Fig. 1A).

**Immunoblotting.** The evolution of the humoral response to aoHGE was then examined by immunoblot analysis with sera obtained at 1, 3, and 6 weeks after the tick bite. At 1 week, the IgM immunoblot was negative. The IgG immunoblot showed reactivity with an 80-kDa aoHGE-antigen at 1 week, and this reactivity was still present at 3 and 6 weeks. IgM primarily directed towards the 44-kDa aoHGE antigen was evident at 3 and 6 weeks (Fig. 1B). IgG to the 44-kDa antigen was also present at 3 and 6 weeks, but the signal was stronger at 6 weeks. At 6 weeks, antibody reactivity against another aoHGE-

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antigen (120 kDa) was also evident. Because the patient had an unexpected IgG response to the 80-kDa protein at 1 week, had a previous Lyme disease ELISA that was positive, and was at risk for Lyme disease because of this location of residence and outdoor activities, we retested his blood in Lyme disease seroassays. The sera yielded a positive Lyme disease ELISA (IgG titer, 1:3,200; IgM titer, negative). A Lyme disease immunoblot identified IgG (but no IgM) antibodies to several B. burgdorferi proteins, including those with molecular masses of 18, 22 (OspC), 41 (flagellin), 58, 68, (HSP-70), and 93 kDa. There was no difference in the bands detected by Lyme disease immunoblot with the sera collected at 1, 3, and 6 weeks (data not shown).

Amino-terminal sequencing. Because the presence of IgG to B. burgdorferi possibly results in false-positive reactivity in aoHGE testing and the heat shock proteins (HSPs) are likely candidates for cross-reactive antibodies, we examined the 80-kDa aoHGE antigen further. The aoHGE isolate (designated NCH-1) initially recovered from an HGE patient (14) was purified from infected HL-60 cells by renografin density gradient centrifugation (5, 7, 8) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The 80-kDa band was isolated from the gel and used for amino-terminal peptide sequencing by high performance liquid chromatography, which revealed that this antigen is a member of the HSP family (Table 1). The aoHGE HSP-70 sequence shows substantial homology to the reported sequence of B. burgdorferi HSP-70 (1).

We conclude that in this patient, antibodies to the B. burgdorferi HSP-70 cross-react with the aoHGE HSP-70 and that this cross-reaction may account for false-positive reactivity in the serodiagnosis of infection. At the time of presentation the patient had a positive IgG Lyme disease ELISA and immunoblot, with IgG being strongly directed against B. burgdorferi OspC, flagellin, and HSP-70. The IgM immunoblot was negative. This result suggests that the patient had previously been exposed to B. burgdorferi, a conclusion drawn by using current diagnostic criteria proposed by the Lyme disease workgroup (3). The initial IgM response to aoHGE was directed towards the 44-kDa proteins, as has been documented for other patients and for mice infected with aoHGE (8). Then the aoHGE-specific IgG response developed and was directed to the 44-kDa antigen, as well as to a 120-kDa protein. These data suggest that antibodies that bind the aoHGE HSP-70 homolog are not necessarily specific for HGE and that they may account, in part, for cross-reactivity between the antibodies of Lyme disease and HGE.

Members of the HSP family are conserved throughout many different species, play an important role in protein synthesis, and help maintain their secondary structure during stress.

![Image](image.png)

**Fig. 1.** (A) PCR detection of aoHGE in patient blood. Lanes 1 to 6, primers specific for granulocytic ehrlichia (347-bp product); lanes 7 to 11, beta-actin primers (control); lanes 1 and 7, patient’s blood on admission; lanes 2 and 8, patient’s blood on day 2; lanes 3 and 9, patient’s blood after treatment and 6 weeks after infection; lane 4, aoHGE in HL-60 culture; lanes 5 and 10, serum of an uninfected human control; lanes 6 and 11, negative control serum with no template; lanes M, molecular size markers. (B) Immunoblots of aoHGE lysates probed with patient sera taken at the following times: 1 week (blots 1 and 4), 3 weeks (blots 2 and 5), and 6 weeks (blots 3 and 6) after the tick bite. Molecular size markers (in kilodalton) are noted at the left.

**Table 1.** Comparison of the amino-terminal sequence of the 80-kDa protein of the aoHGE with the HSP-70 homologs of B. burgdorferi, Escherichia coli, and humans

| Protein          | Amino acid at residue no.* |
|------------------|-----------------------------|
| aoHGE HSP-70     | X X E I — I G I D L G T T N X X |
| B. burgdorferi HSP-70 | M G K I — — I G I D L G T T N S C |
| E. coli HSP-70   | M G K I — — I G I D L G T T N S C |
| Homo sapiens HSP-70 | M A K A A A V G I D L G T T Y S C |

* Amino acid residues are written in the single-letter code. X indicates the amino acid positions in which a characteristic chromatogram was not obtained; —, indicates that gaps were introduced into the sequence.
conditions. HSP homologs of several pathogens, including *Plasmodium falciparum*, *Mycobacterium tuberculosis*, and *Mycobacterium leprae*, have been identified as immunodominant antigens (13, 18). We have identified several other HGE patients whose sera contained both IgM and IgG directed towards HSP-70 and also mice whose sera reflected infection with aoHGE, demonstrating that this antigen is immunogenic (8). However, the response to the chilchial HSP-70 may not always be specific for aoHGE. In contrast, the antibodies to the aoHGE GroEL homolog appear to be specific for HGE (9). Indeed, other tick-borne infections, such as *B. burgdorferi* may induce responses to HSP. These responses may be the explana-
ation for the recent observation by Wormser and his colleagues that HGE patients may have had false-positive sero-logic Lyme disease tests (16, 17). As the individuals who are at risk for Lyme disease are virtually identical to those who are at risk for HGE, a more complete understanding of the antibodies cross-reactive between aoHGE and *B. burgdorferi* will aid in the serodiagnosis of both of these tick-borne diseases.

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