EHRLICHIA CHAFFEENSIS: A PREVALENT, LIFE-THREATENING, EMERGING PATHOGEN

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ABSTRACT

Ehrlichia chaffeensis are small, obligately intracellular, endosomal bacteria with tropism for macrophages. Persistent infection in reservoir white-tailed deer is transmitted by lone star ticks. Flu-like illness can progress to severe multisystem disease with toxic shock-like syndrome, meningitis, or ARDS. The case-fatality rate is 2.7%. Leukopenia and thrombocytopenia are diagnostically useful. Granulomas are associated with control of the infection. Ehrlichial proteins and glycoproteins have been sequenced and expressed for diagnostic serology and vaccine development. Mouse models (mild disease and persistent infection with *E. muris* and fatal monocytotropic ehrlichiosis with a Japanese tick isolate) revealed that CD4 and CD8 T type 1 lymphocyte responses, IFN-γ, TNF-α, and antibodies play roles in protective immunity, while a weak CD4 T-helper response, overproduction of TNF-α, and very high IL-10 are associated with toxic shock-like mortality. Protection against fatal ehrlichiosis was achieved by prior infection with low virulence *E. muris*. Acute clinical diagnosis is difficult except by PCR. Response to doxycycline is dramatic.

INTRODUCTION

The Pathogen

Ehrlichia are small (0.5 μm), obligately intracellular bacteria that reside as microcolonies in a vacuole, particularly within monocytes/macrophages or polymorphonuclear leukocytes. The family *Anaplasmataceae* contains four genera, *Ehrlichia, Anaplasma, Wolbachia*, and *Neorickettsia* (1). The former two are transmitted by ticks from persistently infected ruminant, cervid, and rodent hosts. *Ehrlichia* possess an unusual gram-negative cell wall that lacks lipopolysaccharide but contains glycoproteins and a family of 28 kDa proteins encoded by a

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locus of 21–25 non-identical members (2,3). *Ehrlichia chaffeensis*, the agent of human monocytotropic ehrlichiosis, contains 22 proteins of 28 kDa each with three hydrophilic, surface-exposed hypervariable domains, a surface-exposed 75–145 kDa glycoprotein with 2 to 5 tandem repeat units and a 200 kDa glycoprotein (3–5). *Ehrlichia chaffeensis* has two ultrastructural forms: a larger reticulate cell and a smaller dense-core cell, both of which divide by binary fission, but only the latter of which expresses the tandem-repeat containing glycoprotein (6,7). The *Anaplasmataceae* share a common ancestor with *Rickettsia*, *Orientia*, and mitochondria. *Ehrlichia* including *E. canis* (canine monocytotropic ehrlichiosis), *E. ruminantium* (heartwater), and *E. ewingii* (canine granulocytotropic ehrlichiosis) have been investigated by veterinary scientists for many decades, but only came to medical attention in 1987.

Ecologic Cycle of *E. chaffeensis* and Epidemiology of HME

Ehrlichia chaffeensis has evolved substantial divergence in North America with three evolutionary lineages having genetic and antigenic differences (8). Currently the major vertebrate mammalian host is the white-tailed deer (*Odocoileus virginianus*), which becomes subclinically persistently infected after inoculation by a feeding *Amblyomma americanum* (lone star tick) vector and serves as a reservoir for uninfected *A. americanum* ticks (9). The ticks maintain the ehrlichiae transstadially but not transovarially.

Naturally infected dogs, coyotes, and a goat have also been observed, and *E. chaffeensis* has also been detected in *Dermacentor variabilis* and *Ixodes pacificus* ticks. Although cases of HME have been reported in 47 states, the vast majority have originated in states where lone star ticks and white-tailed deer are abundant (from New Jersey to Kansas and southward), and travel-associated cases account for at least some of the widespread geographic distribution (10). Infections are seasonal with two-thirds of cases occurring in May-July. Exposure to infected ticks likely accounts for predominance among rural and suburban males although host risk factors for severity may also play a role in the higher incidence in older persons and males. Active, prospective surveillance for HME over a three year period with strict laboratory diagnostic criteria revealed an annual incidence of at least 3.2 cases per 100,000 population (with one particularly motivated primary care family physician establishing diagnoses at a 30-fold higher rate) in Cape Girardeau, Missouri (11).

Clinical Manifestations of HME

HME is a moderate-to-severe disease with 41–63% of patients requiring hospitalization and a case-fatality rate of 2.7% (10,12). The initial presentation is that of a non-specific flu-like illness with fever, headache, and myalgia. Nausea, dizziness, cough, pharyngitis, regional lymphadenopathy, abdominal tenderness, rash, photophobia, confusion, and stiff neck occur in a substantial minority of cases (11–13). Ehrlichial meningoencephalitis and adult respiratory distress syndrome are life-threatening manifestations in severe cases (14,15).

In immunocompetent patients, granulomas are observed in association with control of the infection, and in fatal cases there is a disparity of the prominence of inflammation with the paucity of ehrlichial organisms in the tissues (16). In immunocompromised patients such as those with acquired immunodeficiency syndrome, *E. chaffeensis* behaves as an opportunistic infection with an overwhelming bacterial burden. The median duration of hospitalization is one week and of illness, three weeks. The clinical laboratory data in HME frequently reveal leukopenia, neutropenia, lymphocytopenia followed by γ/δ T lymphocytosis, thrombocytopenia, and elevated serum hepatic transaminases.

Pathogenesis and Immunity

Infection with *E. chaffeensis* is highly associated with tick bite, and the occurrence of regional lymphadenopathy suggests ehrlichial spread from the dermal site of inoculation via lymphatic vessels (13). Hematogenous spread subsequently delivers ehrlichiae to cells of the mononuclear phagocytic system throughout the body including bone marrow, spleen, lymph node, hepatic sinusoids, and perivascular infiltrates of varying intensity in the meninges, brain, lung, kidney, gastrointestinal tract, and heart. Associated lesions include hepatocellular necrosis and apoptosis, cholestasis, meningoencephalitis, interstitial pneumonia/ARDS, and splenic and lymph node necrosis (17–19). There is evidence that the tandem repeat-containing surface glycoprotein is an adhesin; entry is achieved via endocytosis (7). Ehrlichiae inhibit fusion of lysosomes with the endosome in which growth occurs. Ehrlichiae cause direct cytopathic effect *in vitro*, but except in immunocompromised patients do not occur in sufficient quantity to account for the life-threatening clinical manifestations.

Evolution of *E. chaffeensis* over eons as a persistent subclinical infection in the macrophages of deer and coyotes has resulted in the development of mutual mechanisms of survival and avoidance of injury

to the bacteria or its vertebrate host. In a human macrophage-like cell line, gamma interferon (IFN-γ) stimulates macrophages to kill *E. chaffeensis* via sequestration of iron required for ehrlichial growth (20). Conversely, establishment of *E. chaffeensis* infection of the macrophage before its activation by IFN-γ disrupts the ehrlichicidal signal transduction pathway, causing an increased delivery of iron via transferrin to the ehrlichial vacuole (21,22).

Critical evaluation of mechanisms of pathogenesis and immunity required development of appropriate animal models. Although *E. chaffeensis* infection of mice is transient and subclinical, infection of SCID mice has demonstrated that antibodies to an epitope located on the first hypervariable domain of p28-19 are protective (23–25). Infection of major histocompatability class II gene knockout mice and toll-like receptor (TLR) 4 gene knockout mice results in persistent infection, suggesting a critical role for CD4 T cells and initial TLR4-dependent activation of innate immunity (26). Nevertheless, genomic analysis of *E. chaffeensis* reveals that the genes for synthesis of lipopolysaccharide, a known ligand for TLR4, are absent (2).

Development of murine models using other very closely related *Ehrlichia* species has enabled substantially more progress in elucidating mechanisms of both immunity and immunopathogenesis. Infection of mice with *E. muris* accurately recapitulates persistent ehrlichial infection of its normal vertebrate host (e.g., white-tailed deer infected with *E. chaffeensis*) (27). Persistent infection in the murine model resulted in peak bacterial burden on day 9, marked reduction in ehrlichial content in organs after 30 days, and persistence of infection to the end of the study (150 days). Ehrlichiae infected mainly macrophages in the splenic marginal zones and red pulp, pulmonary interstitium, hepatic sinusoids, occasionally hepatocytes, and monocytes marginated to vessel walls. Histopathologic lesions comprised perivascular and interstitial lymphohistiocytic infiltrates in the lung and liver and marginal zone and red pulp of the spleen, occasional hepatocellular apoptosis, Kupffer cell hyperplasia, erythrophagocytosis, and hypercellular bone marrow with myeloid hyperplasia. These lesions were prominent by day 9 and declined substantially after day 30. Antibodies to conformational epitopes were detected on day 9 with appearance of antibodies to non-conformational antigens of 200, 180, 100, 73/75, 45, and 28 kDa on day 30. Very high titer (2560–10,240) IFA antibodies and increasing intensity of immunoblot reactivity of serum antibodies continued through day 150. Control of the infection as determined by sharp reduction in bacterial burden was associated with a marked increase in antibody titer and the appearance of granulomas on day

20–30, but low level infection and inflammatory lesions continued until the end of the study.

The *E. muris* model enabled critical investigations of the mechanisms of protective immunity against ehrlichiae. CD8 T lymphocytes were especially important as 81% of C3H MHC Class I gene knockout mice died compared with no deaths in wild type C3H mice (27a). Furthermore, the additive effect of CD4 and CD8 T lymphocyte subsets was revealed by 80% fatalities among mice depleted of both CD4 and CD8 T lymphocytes compared with only 44% fatalities with CD4 T cell depletion only. A possible mechanism of immunity is the newly documented specific immune CD8 cytotoxic T lymphocyte activity against *E. muris*-infected macrophages. The synergistic importance of the cytokines, IFN-γ and tumor-necrosis factor (TNF)-α was demonstrated by the occurrence of 75% fatalities in mice depleted of both cytokines compared with minimal mortality in *E. muris*-infected mice when each cytokine was depleted individually. The Fc-dependent role of antibodies in immunity was revealed by protection of C57BL/6-SCID mice by passive transfer of antibodies to *E. muris*, but not Fab fragments. Thus, ehrlichial immunity is mediated by an orchestrated immune response involving CD8 and CD4 T lymphocytes, secretion of IFN-γ and TNF-α, and antibodies against *E. muris*.

Another organism that is closely related to *E. chaffeensis* was isolated from Japanese ticks. Mice inoculated intraperitonially with this unnamed *Ehrlichia* species (strain HF565) die with extensive hepatic cell injury and death including prominent apoptosis in the spleen and liver, interstitial pneumonia, bone marrow necrosis, leukopenia, thrombocytopenia, widespread infection of macrophages, focal infection of hepatocytes and pulmonary endothelium (28–30), overproduction of TNF-α by CD8 T lymphocytes, and decreased production of IFN-γ by CD4 Th1 lymphocytes (31). This toxic shock-like syndrome with marked increase in hepatic enzymes, hypoglycemia, and weight loss is an excellent model of fatal HME. However, neutralization of TNF-α failed to protect infected mice from death, and mortality in these mice was associated with IL-12 downregulation and overproduction of IL-10. Further studies showed that, at least in part, severe ehrlichiosis is attributable to early systemic overproduction of TNF-α, resulting in an overactivated immunological state and a later systemic increase in IL-10 resulting in an immunosuppressive state.

The challenge of developing a method for stimulating protective immunity against this highly virulent *Ehrlichia* infection was achieved with crossprotection of mice by initial infection with *E. muris* before challenge with strain HF565 (31). Protective immunity was

associated with the presence of well formed granulomas (similar to humans), increased quantities of CD4 and CD8 type 1 T lymphocytes producing IFN-γ, a high titer of IgG2a antibodies, a low quantity of CD8 T lymphocytes producing TNF-α, and a low serum TNF-α. Optimal adoptive transfer of immunity against strain HF565 required polyclonal anti-*Ehrlichia* antibodies and memory IFN-γ producing *Ehrlichia*-specific CD4 and CD8 type 1 cells. Ironically, these organisms lacking lipopolysaccharride can stimulate the immune system to an extremely immunopathologic state, a state of tranquil persistence, or bacterial clearance.

Diagnosis

The clinical diagnosis of HME without laboratory assistance is nearly impossible as the signs and symptoms are protean and similar to numerous other infectious diseases. The initial clinical clues are thrombocytopenia and/or leukopenia, mildly elevated hepatic transaminases, and a history of potential tick exposure. Search for monocytes with vacuoles containing *E. chaffeensis* in peripheral blood is usually unrewarding except in immunocompromised patients. Blood in the acute stage contains *E. chaffeensis* detectable by polymerase chain reaction in a reference molecular diagnostics laboratory, but antibodies are usually not present until convalescence. Most patients are treated empirically with doxycycline on the basis of clinical suspicion, recover without effective anti-ehrlichial treatment, or die without an accurate diagnosis. Many states require reporting of cases to the public health department, but low reporting rates (possibly owing to misdiagnosis or successful empiric treatment without laboratory confirmation) have left the likely misconception that HME is a rare infection.

Treatment

Doxycycline is the drug of choice. No other class of drugs has been demonstrated to be effective.

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DISCUSSION

DuPont, Houston: David, thank you very much. You're obviously the leader nationally and internationally on ehrlichiosis and rickettsial diseases, but I would like a little more clinical information. I don't think many of us are diagnosing this condition. Should we be considering it in a clinical diagnosis because of the epidemiologic history tick

exposure and being outdoors, and should the condition be considered in patients with pneumonia, meningitis, toxic shock, ARDS? Who is it that we should worry about, and how available is the diagnostic test? Is it one of the tests you send out to California and two weeks later you get something back? Give clinical information.

Walker: I directed a CDC sponsored, active, prospective, epidemiological study that was done in Cape Girardeau County, Missouri and the physician that I worked with the closest was a family doctor, who practiced nearly exclusively outpatient medicine. That's really where you want to diagnose these cases, long before they get into the ICU. If you obtain a CBC on the patients, approximately 60% of them will have thrombocytopenia or leucopenia. And that's the biggest clue. They also have elevated ASTs and ALTs, but hepatic enzymes not something everybody orders on someone who comes into a clinic. The opportunity for tick exposure is something to inquire about, and it's very important. And a lot of these patients are appearing all over the country, not only in areas where the white tail deer and lone star ticks are prevalent. So there appears to be a substantial number of travel-associated cases. I believe that there are a lot of cases, they are just being picked up and treated empirically as if it might be Rocky Mountain spotted fever by the local physicians. The diagnostic knee-jerk reaction with all rickettsial diseases is to send off serology, but serology is virtually useless for the diagnosis of rickettsial or ehrlichial diseases at the time one is making a therapeutic decision. It's going to be negative most of the time during the acute stage and is confirmatory of the diagnosis mainly in convalescence. Empiric treatment is the appropriate clinical approach. PCR is a very good diagnostic method for ehrlichiosis in the acute state because the organisms are really circulating in the blood. PCR is not so good for rickettsioses, but you have someplace to send it to. Of course, you can send the test sample to me.

Billings, Baton Rouge: David, I enjoyed that, and as I know you know, my being from Louisiana the license plates say Sportsman's Paradise. We have a lot of patients who come in with myalgia, fever, and a little bit of cough. My patients, who are cancer patients, continue, until the very end, hunting and fishing and swashing around in the swamp. So should anyone who comes in with these symptoms be started on doxycyline? One of the quinolones or cephalosporins would be my first choice. I don't have a feel of how I should know when I should treat for ehrlichia unless the patient comes in and says, "I pulled this tick off."

Walker: There are documented cases in Mississippi, and so I suspect that there are cases in Louisiana also. Louisiana is similar to West Virginia, where few cases are reported of many diseases that states around them report. You wonder what the difference is, whether it's the public health laboratory or the reporting system, or the ecology. Is there really something different about the ecology? I think that someone should study ehrlichiosis in Louisiana and find out what the prevalence is there. I would predict that if a good study were performed, you would find that there are a lot of patients with ehrlichiosis. Patients that are immunocompromised because they are on cancer chemotherapy are at a great risk for having rapid downhill course and dying.

Hughes, Atlanta: Thank you very much, David; that presentation was very nicely done. A comment and a question. As you know, many, and in fact most, it seems, of the emerging and re-emerging infectious diseases of recent years have fallen into the vector-borne and zoonotic diseases category. And, I was intrigued by two comments – one in response to Bert's question suggesting the important role that the alert clinician plays in surveillance for these diseases. It's really critical for clinicians to have that high index of suspicion. Then, your comment in the beginning about the vets knowing about these diseases long before we did. I don't know if there are any veterinary members of the Association, but I'll bet there aren't too many. We need them, and this is the question I'd ask you because we are spending a fair amount of time thinking about better ways in

which we can connect with the veterinary world and learn and maybe anticipate some of these challenges a little bit more effectively. Any suggestions?

Walker: I work closely with veterinarians and always have. I began with Fred Murphy, who is my mentor, but I will also tell you that I've made some bad mistakes. A very close and dear collaborator Ed Breitschwerdt begged me to work with him in ehrlichiosis beginning back in 1984 and 1985. I arrogantly told him that I didn't have time to work on these veterinary diseases, that I was working on diseases that killed human beings and that they were more important to me than companion animals. If I had taken his advice, I would have probably been 5 years ahead of where I am now in the study of ehrlichia. I am now working closely with veterinarians at Texas A&M and LSU.

Goodenberger, St. Louis: I just wanted to comment on the clinical presentation, because we see a fair amount in St. Louis. I think it's telling that old maps of Rocky Mountain spotted fever distribution show Rocky Mountain spotted fever in Missouri, and there really isn't any. I'm sure all those cases were ehrlichia. About 20% of these patients have a rash that is usually more central than peripheral. I agree that the hemologic abnormalities are very impressive and as important to diagnosis as are the liver abnormalities. Another thing is that, like rickettsial diseases, they typically have a severe frontal headache in the early stages. So if it's tick season, and you're in an area where ehrlichia occurs, if they have an illness that reminds you of the early stage of Rocky Mountain spotted fever, ehrlichia is a reasonable consideration.

Walker: Thank you for your comment. I would like to ask you a question. Would you agree with my opinion that it's almost impossible to diagnose ehrlichiosis with certainty clinically?

Goodenberger: Yes, absolutely. However, our housestaff are so sensitized, that if somebody comes in with the features described, they start them on doxycyline and send the PCR over to St. Louis Children's Hospital where it's done; the turnaround time is about a day for the test.

Walker: So the vector is the lone star tick. When I was living in North Carolina, I saw only one lone star tick. The last time I went back there I picked about 25 of them off my body. There is an absolute explosion of the lone star tick population from New Jersey to Kansas and all points south. It's something that has changed. Of course, the reason is that the deer population is exploding as well.

Goodenberger: The size of the deer population is a huge problem in suburban St. Louis. The other thing I was going to mention — similar to Rocky Mountain spotted fever, only about half or two thirds of the patients will give you a history of tick removal.