AN OVERVIEW OF RESEARCH ON EHRLICHIOSIS

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Ehrlichiosis is a disease caused by a group of rickettsiae, known as ehrlichiae, which parasitize circulating leukocytes of man and a variety of domestic and wild animals. A characteristic morphologic feature of ehrlichiae is their occurrence in membrane-bound vacuoles in the cytoplasm of leukocytes, forming inclusions that contain variable numbers of organisms. The tick is the apparent vector of ehrlichiae, but such evidence is not available for all species.

Organized research efforts on ehrlichiae started in 1968 when *Ehrlichia canis* was identified as the cause of a fatal disease of hundreds of military working dogs in Vietnam. The advancement of knowledge of the biological properties of *E. canis* led to the recognition of its close antigenic and morphologic relationship with *Rickettsia sennetsu*, the etiologic agent of human sennetsu fever in Western Japan. Hence, *R. sennetsu* was included in the genus *Ehrlichia* under the name *Ehrlichia sennetsu*. The availability of the latter agent facilitated the identification and isolation of *Ehrlichia risticii*, the causative agent of equine monocytic ehrlichiosis (EME, Syn., Potomac horse fever). Accordingly, the current major species of the genus *Ehrlichia* include blood monocytic invaders (i.e., *E. canis*, *E. sennetsu* and *E. risticii*) and granulocytic invaders (i.e., *E. equi* and *E. phagocytophila*).

With the exception of *E. canis*, all other species can infect a variety of unnatural hosts under experimental conditions. Recently, however, an *E. canis*-like agent has been incriminated as a human pathogen based on parasitologic and serologic evidence.

Although *E. canis*, the type species of the genus *Ehrlichia*, has been known since 1935 (47), it was not until 1968 that its full pathogenic potential for the dog was first recognized. It was in Vietnam during 1968-1970 that a severe epizootic of canine ehrlichiosis occurred among the U.S. military dogs, resulting in hundreds of cases of mortality and morbidity among these animals. This severe and often fatal form of the disease is known as tropical canine pancytopenia (24). Since that time, numerous cases of ehrlichiosis have been reported from the United States and many other parts of the world. While the most common pattern of disease occurrence was that of a sporadic nature, some epizootics of canine ehrlichiosis have also been reported (24). An organized and intensive research effort which followed at the military and university levels, resulted in the development of methods for *in vitro* cultivation of *E. canis* and serologic identification of infected dogs (32,41). Over the past decade, the use of these new means of disease detection made it possible to recognize ehrlichiosis as one of the most important canine infectious diseases in the United
With the acquisition of more knowledge of the biological properties of *E. canis*, a close relationship was recognized between the latter agent and a human pathogen called *Rickettsia sennetsu* (40). The latter rickettsia is the causative agent of human sennetsu fever. Previous immunologic studies failed to demonstrate an antigenic relationship between *R. sennetsu* and other rickettsiae of medical importance. Hence, the agent was classified as a species incertae sedis. Recently, a close antigenic relationship between *R. sennetsu* and *E. canis* was demonstrated (40). The similarity between the two agents was further substantiated on the basis of their common morphologic properties and their predilection for blood monocytes. On the basis of these findings, *R. sennetsu* was included in the genus *Ehrlichia* under the name *Ehrlichia sennetsu* in the 9th edition of *Bergey's Manual of Systematic Bacteriology* (38).

The availability of *E. sennetsu* and corresponding serodiagnostic reagents in our laboratory has facilitated the identification and subsequent isolation of the causative agent of equine monocytic ehrlichiosis (synonym, Potomac horse fever) (18). The newly isolated causative agent of equine monocytic ehrlichiosis, *Ehrlichia risticii*, was found to be antigenically closely related to *E. sennetsu* and, to a lesser degree, to *E. canis* (18,19). None of the sera against 12 major rickettsiae reacted serologically with *E. risticii*, thereby confirming the close relationship of the new agent to members of the genus *Ehrlichia* (18,19).

**Canine Ehrlichiosis:** Canine ehrlichiosis, caused by *Ehrlichia canis*, is a worldwide disease transmitted by the tick *Rhipicephalus sanguineus*. In the tick, transstadial transmission of the agent has been readily demonstrated (47). The disease varies from a mild, febrile illness, to a severe and often fatal syndrome termed tropical canine pancytopenia (TCP) characterized by pancytopenia, particularly severe thrombocytopenia. Epistaxis is a pathognomonic feature for German shepherd dogs.

Among various prominent pathological manifestations in fatal canine ehrlichiosis is the extensive invasion of parenchymal organs, and perivascular cuffing, by plasma cells, particularly of the lungs, meninges, kidneys, and spleen, suggesting an immunopathological etiology. Such an etiology was further substantiated by the finding that lymphocytes of infected dogs exert a cytotoxic effect upon autologous monocytes (22). This monocytotoxicity was shown to bear a temporal relationship with the disease-associated thrombocytopenia. Further indication that the thrombocytopenia is immunologically mediated, was provided by evidence from in vitro studies that sera of *E. canis*-infected dogs induced inhibition of normal platelet migration (22,23). Scanning electron microscopy indicated that platelet migration was interfered with by inhibiting pseudopod formation. Affected platelets became rounded and showed evidence of clumping and leakage (23).

As a follow-up to these studies, a soluble factor, termed platelet migration inhibition factor (PMIF), was isolated from the serum of dogs with acute ehrlichiosis (1). The synthesis of PMIF, was found to be lymphocyte-dependent and the factor can be produced in vitro using lymphocytes derived from the blood of *E. canis*-infected dogs. Canine lymphocytes derived from ehrlichia-free dogs can be induced by *E. canis*-infected canine monocytes to produce significant levels of PMIF under in vitro conditions. The concentration of PMIF in the plasma of affected dogs is directly related to the virulence of the infecting *E. canis* isolate. The PMIF was preliminarily characterized as a heat-stable glycoprotein of 160-190 kD (1).

**Human Ehrlichiosis:** The first human case of ehrlichiosis caused by an *E. canis*-like microorganism was reported in 1987 (29). Retrospective studies (6,15,17,45) based on medical histories, clinical signs and symptoms, hematologic abnormalities, exposure to tick bites, and differential serodiagnosis, identified more than 70 additional putative human cases of ehrlichiosis in the U.S. The preliminary diagnosis in the majority of these cases was that of Rocky Mountain spotted fever (RMSF). However, the negative serology to *R. rickettsii* and other pathogens causing similar clinical disease, ruled them out as potential etiologic agents. The four-fold rise or fall in serum antibody titers to *E. canis* in these patients, however, provide confirmatory evidence of ehrlichiosis. More recently, simultaneous outbreaks of ehrlichiosis and Lyme disease in members of an Army reserve unit exposed to ticks was reported (15). Finally, the authors recently participated in the study of ehrlichiosis involving an 8-year-old Caucasian girl. Six weeks prior to onset of the disease, the patient was bitten by a tick while hiking with her father in the woods near San Antonio, Texas (May-June, 1988). She was admitted into Brook Army Medical Center hospital with signs of recurrent fever, headache, back pain, and general malaise. Hematologically, she experienced leukopenia (1,100/mm3) and thrombocytopenia (100,00/mm3). She was initially treated with cyclosporins and sulfas compounds without noticeable improvement. At the time of patency, she was serologically positive for *E. canis* at a titer of 1:40 and negative for *R. rickettsii*, *Borellia burgdorferi*, and all other potential causative agents. Upon intravenous administration of tetracycline, there was a rapid clinical improvement, and her temperature returned to normal. Three weeks after recovery, her anti-*E. canis* antibody titer rose to 1:2560.

Based on the child’s medical history, which included exposure to tick bites, typical hematologic abnormalities, response to tetracycline therapy and a six-fold rise in titer to *E. canis* after recovery, doctors concluded that she was affected by ehrlichiosis.