HEMATOPATHOLOGICAL CHANGES IN DOGS INFECTED WITH *EHRLICHIA CANIS*

Waner, T.

Israel Institute for Biological Research, P.O. Box 19, Ness Ziona 70400, Israel.

Corresponding author: Dr. Trevor Waner. B.V.Sc., Ph.D., Dipl ECLAM Israel Institute for Biological Research, P.O. Box 19, Ness Ziona, 70400 Israel
Phone 972-8-9381461 Fax 972-8-940124 Email: wanertnt@shani.net

Canine monocytic ehrlichiosis (CME) is a tick-borne disease caused by *Ehrlichia canis* an obligate intracellular rickettsia of the genera Ehrlichia, family Anaplasmatacea; order Rickettsiales (1). E. canis was first identified in Algeria in 1935, and has since gained prominent attention (2). *E. canis* has a worldwide distribution and vertebrate hosts for *E. canis* include members of the Canidae family. The major arthropod vector of *E. canis* is the brown dog-tick, *Rhipicephalus sanguineus.* Experimentally, it has also been transmitted by *Dermacentor variabilis* (American dog-tick) (3). Infection occurs when an infected tick ingests a blood meal and salivary secretions contaminate the feeding site. The incubation period of CME is between 8 to 20 days. During this period the organisms multiply in macrophages of the monocytic phagocytic system spreading throughout the body. The subsequent course of ehrlichiosis has been divided into three phases: acute, subclinical, and chronic phases (3, 2, 4, 1). Infection with *E. canis* causes profound hematological changes (5). The aim of this review is to collate information in order to understand what is known about the effects of *E. canis* on both the circulating blood cells and the hematopoietic system.

A common pattern of the hemopathological effects of *E. canis* can be discerned during all the three phases of CME: In the acute phase typical hematological abnormalities include a characteristic moderate to severe thrombocytopenia, a mild to moderate normochromic normocytic nonregenerative anemia and a mild reduction in the total leukocyte count. The thrombocytopenia is the hallmark of this stage and is characterized by the presence of megaplatelets in the peripheral blood (6). Dog recovering from the acute disease, either those who have not received treatment or those dogs receiving incomplete or inadequate treatment may enter the subclinical phase.

During the subclinical phase the dogs continues to harbor the rickettsia, presumably in the spleen. The dogs appear normal, however mild changes may be detected in their hematological parameters (7). In a singular artificial infection study in beagle dogs comparisons were made between the hematologic profiles of dogs before infection, thorough the acute phase and during the subclinical stage of the disease (8). The most outstanding hematological finding comparing preinfected dogs to dogs in the subclinical phase of CME was a highly statistically significant decline in platelet number in 8 of the 9 dogs examined, to an extent that eight of the dogs were mildly thrombocytopenic (<2000,000 platelets/µl). A concomitant increases in mean platelet volume was seen in all the thrombocytopenic dogs. Other statistically significant hematological parameters included a decline in total leukocyte count (7 out of 9 dogs) and in the absolute neutrophil count (5 out of 9 dogs). A decrease in the packed cell volume (PCV) and hemoglobin concentration was seen in 3 out of 9 dogs. Among the erythrocytic indices a decline in the mean corpuscular volume (MCV) was detected in 5 of the 9 dogs, with an increase in the mean corpuscular hemoglobin concentration (MCHC) in 4 of the dogs tested.(8).

Dogs in the subclinical phase may remain persistent carriers (9). Some dogs for unknown reasons enter the chronic pancytopenic phase of the disease. This phase in its severe form is characterized by bone marrow hypoplasia and impairment of bone marrow production of all blood elements, resulting in a pancytopenia, where dogs suffer from a severe nonregerative anemia, leukopenia and thromobcytopenia. In some dogs the effect may be selective, with only one or two of the blood elements being severely reduced. The prognosis for dogs in the chronic severe form is grave. Dogs in this phase eventually die of secondary infections and/or bleeding. These dogs do not respond to treatment with the antibiotic doxycycline (3, 10).

The effects of *E. canis* on the circulating blood cells are accompanied by bone marrow suppression and/or destruction, occurring to a greater or lesser extent, depending of the stage of the disease and as a result may be associated with deficient production of one or more of the blood elements. In the acute phase, the non-regenerative anemia and mild leukopenia are probably indicative of a malfunction of erythropoiesis and leukopoiesis in that the bone marrow is unable to compensate for the peripheral destruction of erythrocytes and leukocytes. On the other hand the presence of immature megaplatelets, in the light of a severe thrombocytopenia, has been interpreted as a "regenerative" thrombocytopoiesis (6). This interpretation may require reassessment in view of the inability of the bone marrow to compensate for the massive platelet loss. Furthermore, it was found that the increase in mean platelet volume in dogs does not always correlate with increased platelet production (11).

Whether antiplatelet antibodies also act on bone marrow megakarocytes with deleterious effects on thrombopoiesis is unknown (12). The early reduction in platelet numbers first seen

during the incubation period and increasing in intensity during the early days of the acute infection are further evidence for bone marrow dysfunction taking into account the short life span of platelets in the circulation. Even as early as the acute phase, destruction of stem cells or progenitor cells has been proposed to occur as a result of infection *with E. canis* (13).

The anemia seen during the acute phase of CME and the lack of erythropoietic response have attributes similar to that of "anemia of inflammatory disease" where the anemia is classically mild to moderate, normocytic normochromic and nonregenerative (14). In the case of "anemia of inflammatory disease", the anemia results from both a decreased erythrocyte production and a decreased erythrocyte survival. This pattern would fit the picture seen during acute CME taking into account that the incubation period is shorter than the lifespan of the erythrocyte, indicating a direct effect of the Ehrlichia on the circulating erythrocytes during the first few days after infection together with a depressed erythropoiesis. A similar phenomenon has been described for infection with *Anaplasma platys* in dogs (15).

Some effects of *E. canis* on of the peripheral circulating blood elements have firm experimental evidence. Immune-mediated thrombocytopenia is one of the mechanisms causing platelet destruction during the acute phase of the disease (6, 16), and antiplatelet antibodies both unbound and bound have been demonstrated following artificial infection (17). The longevity of the peripheral platelets was found to be reduced and their function compromised during the acute phase of CME (18). The reduction in neutrophil counts during acute CME and the resultant reduction in leukocyte numbers have also been proposed to have an immune-mediated mechanism, although there is no experimental evidence at this time to confirm this theory (1). The chemokines MIP-1α and IL-8, both important mediators of inflammation have been demonstrated to be produced by cells infected with Anaplasma *phagocytophilum* (19). It is possible that these chemokines may play a role in chemotactic recruitment of neutrophils from the peripheral circulation to other tissue sites, thereby causing a reduction in the blood neutrophil counts (20). The pathogenesis of the anemia during acute CME is also unknown, but destruction of erythrocytes was shown to occur during the late incubation period and the acute phase of the disease (21). Immunological mechanism may also be involved in the destruction of erythrocytes (22). Ehrlichial infections have been implicated in diverse pathological conditions that appear to be immune related. These conditions include glomerulonephritis (23, 24), hepatitis (25), uveitis (26), meningoencepahlitis (27) and polyarthritis (28). Furthermore, based on recognized findings associated with *E. canis* infection including polyclonal hypergammaglobulinemia (29), the persistent nature of the infection (8, 9), immunopathological findings (2), the presence of plasma *E. canis* soluble antigenemia (30) and the presence of immune complexes (22). The possibility that bone marrow destruction and/or suppression may also be immune mediated cannot be discounted. Further evidence for immunopathological effects on the bone marrow in the pathogenesis of CME can be found in the milder acute clinical disease course and attenuated effects on all the blood cells in splenectomized dogs artificially infected with *E. canis*, compared with dogs with intact spleens (21). In this study it was proposed that splenectomy may have attenuated the aberrant immune response usually seen in CME with a resultant milder disease and less severe effects on the circulating blood cells.

Cytologic studies of the bone marrow during the acute phase on dogs artificially infected with *E. canis* showed an initial increase in the myeloid to erythoid ratio, 21 to 30 days post-infection with a return to normal after about 2 months post-infection (31). The mean concentration of megakaryocytes in sections of the bone marrow of these dogs showed a gradual increase in their number from the first week post-infection to 2 months post-infection. Histological findings of the bone marrow of dogs in the chronic phase of CME are characterized by a decrease in myeloid and erythroid populations and a decline in the number of megakaryocytes (27). Despite the severe pancytopenia of several months duration no extramedullary hematopoiesis of either the erythroid, myeloid or platelet lineages was evident in the spleen. This fact adds to the assumption that that the effects of Ehrlichia on the bone marrow have a generalized hematopoietic suppressive nature (27), or alternatively that no pluripotential stem cells are available despite the induction for increased cell production that may be expected under these conditions (32).

A striking feature of Ehrlichial infections is the small to negligible number of organisms found in blood or other tissues in contrast to the sometimes severe damage that the infection can incur. In a study of 221 dogs with CME, *E. canis* morulae were detected only in 4% of blood smears (33). A similar experience was demonstrated in human infections with HGE where <10% of granulocytes were seen to be directly infected during the course of the disease (34). In a study to identify characteristic cytoplasmic morulae in peripheral blood leukocytes of humans with *E. chaffeensis* and *E. ewingii*, it was concluded that peripheral blood film examination is diagnostic in a substantial number of Ehrlichia infections, particularly in immunocompromised patients, however the number of infected white blood cells may be less than 0.2%, requiring examination of more than 500 white blood cells (35). Furthermore, there have been no reports of overwhelming bone marrow infections of *E. canis* infection in dogs or other Ehrlichial infections in humans, to justify the signs of myelosuppression seen in these diseases.

The role of cytokines in the pathogenesis of bone marrow suppression during infection with *A. phagocytophilum*, a rickettsia closely related to E. canis, has been proposed (19). In vitro studies using the promyelocytic leukemia cell line HL-60 infected with *A. phagocytophilum* showed a striking increase in the production of CC (monocytic chemotactic protein-1 (MCP-1); macrophage inflammatory protein-1α and -β (MIP-1α and 1-β) and RANTES and CXC (interleukin-8 (IL-8)) chemokines. The increase in these chemokines was significantly higher than in uninfected controls. The kinetics of the chemokine secretion correlated closely with the presence

of Ehrlichial inclusions within the infected cells, reflecting an increasing rickettsial burden. Similarly, cultured bone marrow cells enriched for progenitors also showed increased secretion of MIP-1α, MCP-1, IL-8 and RANTES following infection with *A. phagocytophilum*, when compared to uninfected controls.

MIP-1α, MCP-1 and IL-8 were shown to be capable of suppressing the proliferation of bone marrow progenitors and consequent hematopoiesis both in vivo and in vitro (36). Furthermore, low concentrations of MIP-1α and IL-8 were shown to act synergistically to down-regulate hematopoiesis profoundly (37). In contrast, infected cells did not induce the secretion of the classic proinflammatory cytokines, IL-1, IL-6 or TNF-α (19).

In conclusion, a combination of both peripheral and bone marrow effects appears to dictate the hematological picture seen in all stages of CME, ultimately resulting in the collapse of bone marrow function. The cause and nature of the bone marrow failure in chronic CME still remains unclear. Only after identification of the pathogenesis of bone marrow suppression and/or destruction can prospective treatment modalities be contemplated aimed at reversing the grave prognosis of dogs with pancytopenic CME.

REFERENCES

1. Harrus, S., Waner, T., Mahan, S.M. and Bark, H., Rickettsiales, in Pathogenesis of Bacterial Infections in Animals, C.L. Gyles, et al., Editors. 2004, Blackwell Publishing: Oxford. p. 425-444.
2. Harrus, S., Waner, T., Bark, H., Jongejan, F. and Cornelissen, A.W.: Recent advances in determining the pathogenesis of canine monocytic ehrlichiosis. J Clin Microbiol. 37:2745-2749, 1999.
3. Harrus, S., Waner, T. and Bark, H.: Canine monocytic ehrlichiosis update. Compendium of Continuing Education for the Practicing Veterinarian. 19:431-444, 1997.
4. Waner, T., Keysary, A., Bark, H., Sharabani, E. and Harrus, S.: Canine monocytic ehrlichiosis - an overview. Israel Journal of Veterinary Medicine. 54:103-106, 1999.
5. Harrus, S., Kass, P.H., Klement, E. and Waner, T.: Canine monocytic ehrlichiosis: a retrospective study of 100 cases, and an epidemiological investigation of prognostic indicators for the disease. Vet Rec. 141:360-363, 1997.
6. Waner, T., Harrus, S., Weiss, D.J., Bark, H. and Keysary, A.: Demonstration of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. Vet Immunol Immunopathol. 48:177-182, 1995.
7. Harrus, S., Waner, T., Aizenberg, I. and Bark, H.: Therapeutic effect of doxycycline in experimental subclinical canine monocytic ehrlichiosis: evaluation of a 6-week course. J Clin Microbiol. 36:2140-2142, 1998.
8. Waner, T., Harrus, S., Bark, H., Bogin, E., Avidar, Y. and Keysary, A.: Characterization of the subclinical phase of canine ehrlichiosis in experimentally infected beagle dogs. Vet Parasitol. 69:307-317, 1997.
9. Harrus, S., Waner, T., Aizenberg, I., Foley, J.E., Poland, A.M. and Bark, H.: Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichia canis*. J Clin Microbiol. 36:73-76, 1998.
10. Harrus, S., Kenny, M., Miara, L., Aizenberg, I., Waner, T. and Shaw, S.: Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental *Ehrlichia canis* infection. Antimicrob Agents Chemother. 48:4488-4490, 2004.
11. Weiss, D.J., Platelet production defects, in Schalm's Veterinary Hematology, B.F. Feldman, J.G. Zinkl, and N.C. Jain, Editors. 2000, Lippincott Williams and Wilkins: Philadelphia. p. 469-477.
12. Lachowicz, J.L., Post, G.S., Moroff, S.D. and Mooney, S.C.: Acquired amegakaryocytic thrombocytopenia--four cases and a literature review. J Small Anim Pract. 45:507-514, 2004.
13. Weiss, D.J.: New insights into the physiology and treatment of acquired myelodysplastic syndromes and aplastic pancytopenia. Vet Clin Small Anim. 33:1317-1334, 2003.
14. Waner, T. and Harrus, S., Anemia of inflammatory disease, in Schalm's Veterinary Hematology, B.F. Feldman, J.G. Zinkl, and N.C. Jain, Editors. 2000, Lippincott, Williams and Wilkins: Philadelphia. p. 205-209.
15. Baker, D.C., Gaunt, S.D. and Babin, S.S.: Anemia of inflammation in dogs infected with *Ehrlichia platys*. Am J Vet Res. 49:1014-1016, 1988.
16. Harrus, S., Waner, T., Weiss, D.J., Keysary, A. and Bark, H.: Kinetics of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. Vet Immunol Immunopathol. 51:13-20, 1996.
17. Waner, T., Leykin, I., Shinitsky, M., Sharabani, E., Buch, H., Keysary, A., Bark, H. and Harrus, S.: Detection of platelet-bound antibodies in beagle dogs after artificial infection with *Ehrlichia canis*. Vet Immunol Immunopathol. 77:145-150, 2000.
18. Harrus, S., Waner, T., Eldor, A., Zwang, E. and Bark, H.: Platelet dysfunction associated with experimental acute canine ehrlichiosis. Vet Rec. 139:290-293, 1996.
19. Klein, M.B., Hu, S., Chao, C.C. and Goodman, J.L.: The agent of human granulocytic ehrlichiosis induces the production of myelosuppressing chemokines without induction of proinflammatory cytokines. J Infect Dis. 182:200-205, 2000.

20. Adams, D.H. and Lloyd, A.R.: Chemokines: leucocyte recruitment and activation cytokines. Lancet. 349:490-495, 1997.
21. Harrus, S., Waner, T., Keysary, A., Aroch, I., Voet, H. and Bark, H.: Investigation of splenic functions in canine monocytic ehrlichiosis. Vet Immunol Immunopathol. 62:15-27, 1998.
22. Harrus, S., Day, M.J., Waner, T. and Bark, H.: Presence of immune-complexes, and absence of antinuclear antibodies, in sera of dogs naturally and experimentally infected with *Ehrlichia canis*. Vet Microbiol. 83:343-349, 2001.
23. Troy, G.C., Vulgamott, J.C. and Turnwald, G.H.: Canine ehrlichiosis: a retrospective study of 30 naturally occuring cases. J Am Anim Hosp Assoc. 16:181-187, 1980.
24. Codner, E.C., Caceci, T., Saunders, G.K., Smith, C.A., Robertson, J.L., Martin, R.A. and Troy, G.C.: Investigation of glomerular lesions in dogs with acute experimentally induced *Ehrlichia canis* infection. Am J Vet Res. 53:2286-2291, 1992.
25. Sehdev, A.E. and Dumler, J.S.: Hepatic pathology in human monocytic ehrlichiosis. *Ehrlichia chaffeensis infection*. Am J Clin Pathol. 119:859-865, 2003.
26. Massa, K.L., Gilger, B.C., Miller, T.L. and Davidson, M.G.: Causes of uveitis in dogs: 102 cases (1989-2000). Vet Ophthalmol. 5:93-98, 2002.
27. Hildebrandt, P.K., Huxsoll, D.L., Walker, J.S., Nims, R.M., Taylor, R. and Andrews, M.: Pathology of canine ehrlichiosis (Tropical Canine Pancytopenia). Am J Vet Res. 34:1309-1320, 1963.
28. Breitschwerdt, E.B., The rickettsioses, in Textbook of Veterinary Internal Medicine. Diseases of Dogs and Cats., S.J. Ettinger and B.F. Feldman, Editors. 2000, Saunders: Philadelphia. p. 400-408.
29. Harrus, S., Waner, T., Avidar, Y., Bogin, E., Peh, H. and Bark, H.: Serum protein alterations in canine ehrlichiosis. Vet Parasitol. 66:241-249, 1996.
30. Waner, T., Rosner, M., Harrus, S., Naveh, A., Zass, R. and Keysary, A.: Detection of ehrlichial antigen in plasma of beagle dogs with experimental acute *Ehrlichia canis* infection. Vet Parasitol. 63:331-335, 1996.
31. Reardon, M.J. and Pierce, K.R.: Acute experimental canine ehrlichiosis. II. Sequential reaction of the hemic and lymphoreticular system of selectively immunosuppressed dogs. Vet Pathol. 18:384-395, 1981.
32. Valli, V.E.O., The Hematopoietic System, in Pathology of Domestic Animals, K.V.F. Jubb, P.C. Kennedy, and N. Palmer, Editors. 1993, Academic Press: San Diego. p. 101-.
33. Woody, B.J. and Hoskins, J.D.: Ehrlichial diseases of dogs. Vet Clin North Am Small Anim Pract. 21:75-98, 1991.
34. Bakken, J.S., Krueth, J., Wilson-Nordskog, C., Tilden, R.L., Asanovich, K. and Dumler, J.S.: Clinical and laboratory characteristics of human granulocytic ehrlichiosis. Jama. 275:199-205, 1996.
35. Hamilton, K.S., Standaert, S.M. and Kinney, M.C.: Characteristic peripheral blood findings in human ehrlichiosis. Mod Pathol. 17:512-517, 2004.
36. Broxmeyer, H.E., Sherry, B., Lu, L., Cooper, S., Oh, K.O., Tekamp-Olson, P., Kwon, B.S. and Cerami, A.: Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation in vitro by bone marrow myeloid progenitor cells. Blood. 76:1110-1116, 1990.
37. Broxmeyer, H.E., Sherry, B., Cooper, S., Lu, L., Maze, R., Beckmann, M.P., Cerami, A. and Ralph, P.: Comparative analysis of the human macrophage inflammatory protein family of cytokines (chemokines) on proliferation of human myeloid progenitor cells. Interacting effects involving suppression, synergistic suppression, and blocking of suppression. J Immunol. 150:3448-3458, 1993.