Development and Distribution of Pathologic Lesions Are Related to Immune Status and Tissue Deposition of Human Granulocytic Ehrlichiosis Agent±Infected Cells in a Murine Model System

Joseph E. Bunnell, Ellen R. Trigiani, S. R. Srinivas, and J. Stephen Dumler

To evaluate pathology and the role of immune status in a murine model system of human granulocytic ehrlichiosis (HGE), C3H/HeJ, C3H-SCID, and Peromyscus leucopus mice were infected with an HGE agent. All mice remained healthy. Ehrlichemia was not detected after day 14 in P. leucopus and C3H/HeJ mice but increased between days 14 and 90 in C3H-SCID mice. In tissues examined at day 21 and later, infection was rarely detected in immunocompetent mice but was present in all C3H-SCID mice and included pulmonary endothelialitis and hepatic mononuclear cell aggregates with apoptoses. HGE agent was demonstrated in mature and immature myeloid cells in hematopoietic tissues and infrequently in lung and liver lesions with deposition of infected cells. HGE agent infection in immunocompromised mice progresses slowly, has a higher infectious burden and more tissue pathology and is persistent. A murine model for HGE may be useful to assess pathologic lesions, transmission, and persistence.

Materials and Methods

Inoculation. Twelve Mus musculus C3H/HeJ, 12 M. musculus C3H-SCID, and 16 P. leucopus were inoculated intraperitoneally with 0.5 mL of HGE agent-infected equine acid-citrate-dextrose-anticoagulated fresh blood containing 10⁶ infected neutrophils from a horse with clinical signs after experimental infection with the HGE agent BDS strain [4]. Mock-infected mice (8 P. leucopus, 7 C3H/HeJ, and 8 C3H-SCID) were inoculated with fresh uninfected equine blood. Mice were examined daily for fur ruffling, depression in activity, anorexia, hunching posture, tachypnea, lameness, joint swelling, or death.

Serology. Plasma was evaluated by immunofluorescence assay for antibodies by use of Ehrlichia equi antigen, as elsewhere described [5, 6].

Necropsy. Mice were exsanguinated and necropsied on days 21, 45, and 90, and samples of brain, bone marrow (extrusion from femurs for polymerase chain reaction [PCR]; decalcified for histopathology), liver, lung, and spleen were obtained from each. On days 21 and 45, 2 HGE agent±infected mice and 1 mock-infected mouse from each of the 3 mouse groups tested were necropsied;
all remaining mice were necropsied on day 90, and tissues were obtained for histopathologic and PCR analyses. The degree of lung and liver pathology observed on hematoxylin-eosin–stained tissue sections was ranked according to severity, for use in Spearman’s nonparametric rank correlation analysis ($r_S$).

**Results**

**Serology.** At baseline, no plasma contained anti-HGE agent antibodies; at day 21, all plasmas from HGE agent–inoculated C3H/HeJ and *P. leucopus* mice were seropositive, whereas neither control nor C3H-SCID mice had anti-*E. equi* antibodies.

**Blood PCR.** Sixteen of 216 blood samples from infected animals could not be tested because of insufficient quantity or lack of p53 amplification. In immunocompetent mice, ehrlichemia decreased markedly over time. HGE agent DNA was detected in 63% and 8% of C3H/HeJ mice and in 50% and 10% of *P. leucopus* mice at days 7 and 14, respectively, but not thereafter. In contrast, for C3H-SCID mice, ehrlichial DNA was not detected in blood on days 7, 21, or 28, whereas 22% on day 14 and $\geq$50% on days 45 and 90 contained HGE agent DNA. All mock-infected animals were PCR-negative in blood. The kinetics of ehrlichemia were significantly different ($P < 0.001, \chi^2$ test) between immunocompetent (*P. leucopus* and C3H/HeJ) and immunocompromised (C3H-SCID) mice.

**Tissue PCR.** Except for spleen and lung tissues from 2 C3H/HeJ mice and bone marrow from 1 C3H/HeJ mouse on day 45, all tissues from *P. leucopus* and C3H/HeJ mice were free of HGE agent DNA at all time points. Of 57 C3H-SCID mouse tissue samples, 45 (79%) harbored HGE agent DNA at necropsy. None of the mock-infected animal tissues contained HGE agent DNA. Overall, 10 (91%) of 11 C3H-SCID mice had HGE agent DNA detectable by PCR in bone marrow (bone marrow was not available for 1 animal), and 11 (92%) of 12 contained HGE agent DNA in spleen. HGE agent PCR tests were negative in lung tissues from HGE agent–inoculated C3H-SCID mice on day 21; by days 45–90, lung tissues from all 9 infected C3H-SCID mice were PCR-positive. Five (63%) of 8 C3H-SCID mice on day 90 had HGE agent DNA in both brain and liver.

**Pathology.** Tissues from day 45 became autolized and could not be microscopically examined. No immunocompetent *P. leucopus* and C3H/HeJ mice or mock-infected animals had significant pathology.

At day 21, C3H-SCID livers contained infrequent aggregates of mononuclear cells and apoptotic cells. No other lesions were detected in other tissues. Lymph node and splenic tissues were devoid of lymphoid elements. Histologic findings in lung tissues obtained 90 days after inoculation (all PCR-positive) included changes ranging from mild interstitial pneumonitis to necrotizing endocardialitis and vasculitis (figure 1), which were not evident in PCR-negative tissues from C3H-SCID mice on day 21. The severity of lung pathology was closely associated with detection of ehrlichial DNA. The hepatic changes at day 90 included Kupffer cell hyperplasia and increased apoptotic hepatocytes, some of which were associated with mononuclear cell aggregates (figure 1). The spleens and bone marrow of all *P. leucopus*, C3H/HeJ, and C3H-SCID mice contained hematopoietic cells with normal cellularity and maturation.

**Immunohistology.** With the exception of 1 morula in the PCR-negative spleen from a C3H/HeJ mouse on day 21, morulae were not detected in immunocompetent mice on days 21 or 90. In C3H-SCID mice, morulae were observed in 71% and 63% of PCR-positive lung and spleen samples, respectively. However, 6 livers had morulae, including 3 that were PCR-negative; 2 PCR-positive livers did not have morulae. Single ehrlichial morulae were detected in spleen (2 mice), heart (1 mouse), and bone marrow (1 mouse) of 2 C3H-SCID mice examined on day 21. However, morulae were more easily detected by day 90 (figure 2A, 2B), especially in spleen (mean morula quantity, 29.3; range, 0–196), lung (mean, 14.7; range, 0–58), and bone marrow (mean, 10.0; range, 0–22) and also in liver (mean, 1.6; range, 0–4), lymph node (mean, 0.3; range, 0–1), heart (mean, 1.5; range, 0–6), and brain (mean, 0.2; range, 0–1). Morulae were identified predominantly within band neutrophils and also within nonterminally differentiated cells presumably of myeloid lineage (figure 2C). Infected mature and band neutrophils were also found traversing the endothelial barriers within bone marrow to enter into peripheral circulation (figure 2D).

Most foci of pulmonary endocardialitis and hepatic lesions did not contain ehrlichiae. However, on occasion, single or multiple ehrlichia-infected cells could be detected directly within foci with varying degrees of inflammatory cell infiltration, such as in hepatic sinusoids (figure 1). There was a significant correlation between the degree of severity in lung ($\chi = .66, P<
Figure 1. Pathologic changes in C3H/SCID mice 90 days after human granulocytic ehrlichiosis (HGE) agent experimental infection. Foci of pulmonary endothelialitis and vasculitis (A) and hepatic intrasinusoidal mononuclear cell aggregates with associated apoptosis (arrow) (B). Immunohistologic demonstration of HGE agent in foci of pulmonary endothelialitis (C), infiltrating infected cells in hepatic sinusoid not accompanied by inflammatory changes (D), and infected cells sequestered in hepatic sinusoid accompanied by early mononuclear cell infiltrate (E). A and B. Hematoxylin-eosin stain; original magnification, ×252. C–E. Immunoperoxidase stain with anti-HGE agent antibody and hematoxylin counterstain; original magnifications, ×252 (C) and ×400 (D, E).

Discussion

Although both immunocompetent and immunocompromised inbred mice of identical genetic background (C3H) and outbred mice (P. leucopus) are competent for infection with the HGE agent, when infected these animals lack clinical signs that often accompany rickettsial and Borrelia species infections in murine models [10–12]. This in vivo model, designed to assess persistent infection in mice, demonstrates that the kinetics of infection in mice are consistent with intervals required for natural transmission of the HGE agent, that the infection is propagated by release of infected bone marrow cells, and that there is an association between deposition of infected cells in tissues and the development of pathologic lesions that perhaps results from host inflammation and immunity.

Given the high sensitivity of this PCR system, the decreasing ehrlichemia after day 7 is most likely an accurate reflection of the biologic process. A similar window of infectivity for the HGE agent in rodent–Ixodes scapularis laboratory systems has been described [2, 7]. These findings suggest that host factors, including early inflammatory and immune responses, rapidly destroyed or sequestered infected cells from the peripheral blood. This concept is further supported by the inversely correlated antibody production and ehrlichemia and by the large burden of organisms present in the tissues of immunodeficient but not immunocompetent mice.

Bone marrow and spleen, both hematopoietic organs in mice, frequently contained ehrlichial morulae in both mature and immature myeloid cells, especially late in the course of infection of immunocompromised mice. This finding is in keeping with
Figure 2. Immunohistologic stains for human granulocytic ehrlichiosis (HGE) agent BDS strain in tissues of C3H-SCID mice infected 21 (A, B) and 90 (C, D) days after inoculation. A. Spleen has typical HGE agent morulae in mature granulocytes (arrows). B. Bone marrow contains HGE agent morula (arrow) within mature neutrophil. C. HGE agent morula (arrow) within cytoplasm of mononuclear myelopoietic cell in hematopoietic region of spleen. D. Mature neutrophil (arrow) passing into bone marrow sinusoid to enter peripheral circulation. Immunoalkaline phosphatase stain by use of anti-HGE agent antibody and hematoxylin counterstain; original magnification, ×400.

the observation that the HGE agent has a tropism for immature cells of the myeloid lineage that likely represent the site of primary infection in mammals [13, 14]. The finding that the HGE agent may be distributed in various tissues in the absence of easily detected blood infection suggests the possibility of long-term sequestration in organs such as bone marrow, spleen, or lung. Even after the 2 C3H/HeJ mice sacrificed on day 45 had ceased to be ehrlichemic, the HGE agent was detected, by PCR, in spleen, lung, and bone marrow.

Despite the absence of clinical signs in all animals, significant pathologic lesions were observed in C3H-SCID mice. The focal inflammatory lesions in the liver and vasculitic lesions in the lung appear to represent an advanced stage that results after sequestration of infected cells. The relative lack of host tissue injury in the absence of significant inflammatory responses during infection suggests an immunopathologic component. That similar advanced pathologic lesions were not detected in either P. leucopus or C3H/HeJ immunocompetent mice suggests an important role for host immune function in regulating ehrlichia- or host-mediated tissue damage. Because the infectious process in mice is significantly modulated by the immune system, immunologic reactions must be considered to be mostly protective. Such a situation apparently also exists in humans, who usually develop a transient ehrlichemia and eliminate infection as immunity develops [5, 7–9, 13].

Inoculation of CD-1, DBA, C3H/HeN, C3H/HeJ, and outbred P. leucopus mice has resulted in successful infection, but no clinical illness has been demonstrated [1–3]. In established mouse models of HGE, no comprehensive pathologic or immunohistologic evaluation of infected mice has been attempted. Unlike the mouse, the horse model of HGE is characterized by significant and reproducible disease when similar inocula are used, and necropsies demonstrate pathologic and immunohistologic findings similar to those in humans and mice [4, 15]. Although the severe pulmonary vasculitis observed in the C3H-SCID mice has not been observed in other models of granu-
locytic ehrlichiosis or in humans who have died after HGE, it clearly demonstrates the potential for significant tissue damage in this infection. Pathologic lesions in mice result from initial deposition of infected cells that elicit a range of inflammatory reactions and tissue injury that correlates with disease in horses and humans [15]. Thus, although lacking clinical signs, this murine model may have a role in the investigation of HGE pathogenesis. Moreover, given evidence of persistence, the mouse model may be used to study natural maintenance and transmission through small mammal reservoirs.

These experiments, controlled for inoculum dosage and route not achievable by tick-bite inoculation, confirm that mice develop an interval of patent ehrlichemia that lasts 7–21 days. We have conducted ≥4 additional experiments with inbred and outbred mice that confirm this observation (unpublished data). Microscopic evaluations indicated that ehrlichiae predominantly infect cells suggestive of myeloid precursors in hematopoietic organs from which infected maturing neutrophils emerge into the blood. As immunity develops, ehrlichiae are eliminated after inciting locally protective or potentially injurious inflammatory reactions. Poorly restricted ehrlichial propagation yields more significant pathologic injury and presumably disease in humans. Ongoing studies will further elucidate kinetics and pathology during the critical first 3 weeks of infection.

Acknowledgments

We are grateful for the technical advice and assistance provided by Gregory E. Gurri-Glass, Kristin M. Asanovich-Knight, Jennifer J. Walls, Michelle Beach, Mary Martin, Jeffrey J. Floyd, Justin W. Garyu, and Jennifer L. Nargi. We thank Louis A. Magnarelli for providing important P. leucopus control sera and John E. Madigan for providing blood from a horse experimentally infected with the HGE agent BDS strain.

References