Murine Model of Infection by Tropheyma whipplei

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We developed an animal model reproducing several aspects of Whipple’s disease. Immunocompetent mice were persistently infected with Tropheyma whipplei, its etiological agent, and developed liver granulomas. SCID mice were infected similarly but did not develop tissue lesions. The delayed clearance of T. whipplei suggests a protective role for innate immunity.

Whipple’s disease (WD) is a systemic bacterial infection characterized by fever, polyarthritits, lymphadenopathy, intestinal manifestations, and, occasionally, cardiac or ocular manifestations. Its diagnosis is based on the identification of infiltrating large foamy macrophages in duodenal biopsy specimens (10). In 2000, our group successfully cultured the agent of WD, officially named Tropheyma whipplei (11), and two strains were sequenced in 2003 (1, 12). WD is related to bacterial persistence and tissue lesions. Such animal models require down-modulation of the IL-12 pathway (5). This is feasible in intestinal WD in which infiltrating macrophages exhibit an alternative transcription activation phenotype (4). The purpose of our study was to develop a murine model that would emulate human infection. For the first time, we describe T. whipplei infection in mice with bacterial persistence and tissue lesions. Such animal models will facilitate the investigation of WD pathogenesis.

T. whipplei organisms (strain Twist-Marseille) were cultured on MRC5 cell monolayers as described previously (5). Female 6-week-old immunocompetent mice (BALB/c mice) and CB-17 mice with severe combined immunodeficiency (SCID mice) were obtained from Charles River Labs (L’Arbresle, France). Mice were infected intravenously with 3 × 105 organisms or phosphate-buffered saline as a control. Animals were examined daily. Five infected mice and one control mouse of each group were sacrificed 4, 10, 20, 50, and 70 days after inoculation. Spleens, livers, hearts, brains, and gastrointestinal tracts were excised, and a part of each organ was stored at −80°C. The other part was fixed in Bouin’s solution and embedded in paraﬃn. Sections of paraﬃn-embedded tissues (5 μm) were stained with hematoxylin-eosin. Granulomas were deﬁned as a compact aggregate of at least ﬁve macrophages. For immunohistologic detection of T. whipplei, paraﬃn-embedded tissue sections were deparaﬃnized in xylene and rehy-

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the classical aggregation of foamy macrophages in intestinal tissue, the liver, lymph nodes, and spleen exhibit noncaseating, epithelioid cell granulomas. Diffuse granulomatous disease may characterize WD without obvious enteric symptoms or signs of disease (13, 15). The granulomatous lesions observed in our study were similar to the histologic lesions observed in sarcoidosis-like forms of WD (3). This is likely related to the early evolution of T. whipplei infection. Indeed, sarcoid-like reaction is an early manifestation of WD, and in mice, hepatic granulomas were observed 4 days after infection.

Surprisingly, SCID mice controlled T. whipplei infection as did BALB/c mice. We did not observe morbidity or mortality throughout the study. T. whipplei organisms were detected in the liver but not in the spleen, heart, brain, or intestinal tract. The bacterial burden was similar to that found in BALB/c mice at day 4; it decreased thereafter and was undetectable at day 70 (Fig. 1B), demonstrating that SCID mice are able to eliminate T. whipplei in a time frame similar to that for BALB/c mice. The infection of SCID mice was not associated with granulomas in the liver. That SCID mice and immunocompetent mice clear T. whipplei infection in similar ways suggests that T. whipplei does not behave as an opportunistic infectious agent. Because WD is characterized by a deficient IL-12/IFN-γ pathway (5, 8), one would expect that the T-cell deficiency of SCID mice and the concomitant impaired Th1 immune response would result in an increased susceptibility to T. whipplei infection. Yet, that is not what we observed in this study. It is possible that the low pathogenicity of T. whipplei accounts for its ability to control infection in the absence of adaptive immunity. Our findings also suggest, however, that IFN-γ produced by NK cells may be sufficient to control T. whipplei infection in mice.

In conclusion, the mouse model of T. whipplei infection reproduces several features of the human disease, including bacterial persistence and granulomatous lesions. The key differences are the self-limited nature of the infection, the granulomatous response and anatomical distribution of lesions, and the overall bacterial burden per granulomatous lesion. However, this model will enable more-comprehensive investigations of the pathophysiology of WD.

FIG. 1. T. whipplei burden and granuloma formation. (A) Infected BALB/c mice were killed at day 4. T. whipplei organisms were detected both in macrophages, which composed the granuloma (white circle) in liver parenchyma (arrowhead), and in spindle lining the hepatic sinusoids (arrows), which are presumably Kupffer cells. Magnification, ×250. (B) Infected BALB/c mice and SCID mice were killed at different times. The infection of liver was determined by immunohistologic determination, and the results are the means ± standard deviations for five mice per time point. (C) An inflammatory granuloma (white circle) in the liver of a mouse infected with T. whipplei is shown (hematoxylin-cosin). Magnification, ×250.

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