Pathologic Changes during Acute Q Fever: Influence of the Route of Infection and Inoculum Size in Infected Guinea Pigs

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As assessed by both standard histological staining and immunohistochemistry, intraperitoneal inoculation of C. burnetii in guinea pigs led to pathologic changes mainly in the lungs, whereas intranasal inoculation led to pathologic changes mainly in the lungs. Myocarditis and positive blood cultures were observed only in those animals which received the highest inoculum. We therefore conclude that both the route of infection and the size of the inoculum influence clinical expression in acute Q fever.

Q fever is a worldwide zoonosis caused by Coxiella burnetii, an obligately intracellular organism which multiplies in phagolysosomes of infected cells. In most cases, infection is asymptomatic. The most common acute clinical manifestations are self-limited febrile illnesses, granulomatous hepatitis, and pneumonia (16). Cases of febrile eruptions, myocarditis, pericarditis, and meningocerebral manifestations have been reported. Asymptomatic forms and life-threatening forms such as myocarditis can be observed during the same outbreak (3). Most cases of chronic Q fever are manifested as endocarditis. Clinical manifestations of acute Q fever vary from one geographical area to another. For example, in Nova Scotia in southeast Canada (9), Switzerland (3), and northern Spain (18), the main manifestation of acute Q fever is pneumonia, while in France (16) or southern Spain (18), it is granulomatous hepatitis. It has been hypothesized that the route of infection, as demonstrated in a murine model (10), and the size of the inoculum could influence the clinical presentation of acute cases. However, in mice C. burnetii infection results in either a self-limiting febrile illness or latent infection, whereas in guinea pigs, as in humans, acute Q fever is a life-threatening disease and infectious foci are cleared of microorganisms faster and more effectively (8). A guinea pig model of acute Q fever is therefore probably more relevant to disease in humans. We herein compared the pathological changes observed with infection via the intraperitoneal (i.p.) route (mimicking digestive contamination) and via the respiratory route, in a guinea pig model. The influence of inoculum size was also assessed.

Forty-two male Hartley guinea pigs were inoculated with the Nine Mile I strain of C. burnetii, titrated in shell vials by using an indirect immunofluorescence technique (11). In group A, 16 guinea pigs were inoculated i.p. with $10^5$ IU of C. burnetii. In group B, 13 guinea pigs were inoculated i.p. with $10^6$ IU of C. burnetii. In group C, 13 guinea pigs were inoculated intranasally with $10^3$ IU of C. burnetii. Rectal temperature and weight were recorded every 3 days. Guinea pigs were serially sacrificed during the 1st month and at the end of the 4th month. Blood for serology and culture was obtained at the time of sacrifice. Antibodies to phase I and phase II C. burnetii antigens in the sera of guinea pigs were detected by a microimmunofluorescence test as previously described (17). Blood cultures were performed by using the shell vial assay (13). The heart, the lungs, the liver, the spleen, and the left kidney were excised. Sections of paraffin-embedded tissues were stained with hematoxylin-eosin-saffron. The slides were then coded and read by one of us (H.L.), a pathologist, who was unaware of the study hypothesis. For detection of the presence of C. burnetii in tissues, immunohistochemistry was performed on deparaffinized sections of all organs by using an anti-C. burnetii rabbit polyclonal antibody with an LSAB K 680 kit (Dako, Trappes, France) according to the manufacturer’s instructions.

All animals inoculated i.p. had a febrile response (temperature, $\geq 40^\circ$C) at day 3, whereas none of the animals inoculated intranasally showed a febrile response at $\geq 40^\circ$C (data not shown). Body weight loss, at its highest at day 6 for all guinea pigs, was not significantly different among the three groups (Table 1). The kinetics and titers of antibodies were similar in all groups of guinea pigs (data not shown). Blood cultures were positive only in animals infected with high levels of inoculum and sacrificed at day 3 or 6. Similarly, with human cases of acute Q fever, C. burnetii is isolated in blood culture from only 17% of untreated patients, and isolation is in most cases achieved in samples from patients with no detectable antibodies early in the course of the disease (12).

Except for the kidneys, all organs studied developed specific pathomorphological changes. Hepatic and splenic damage appeared as multifocal granulomas consisting of mononuclear aggregates and composed mainly of macrophages and lymphocytes with a few polymorphonuclear leukocytes (Fig. 1). These granulomas were focal, variable in diameter, and scattered throughout the liver lobules or the portobiliary spaces and the splenic red pulp. Residues of necrotic foci of hepatocytes were found around and/or within some of the inflammatory aggregates. These granulomas were apparent by day 3 and disappeared at day 15 in the spleen but persisted up to 30 days in the liver. In the lungs, the dominant feature consisted of mononuclear cell infiltration in the widening alveolar septa, appearing at day 6. Numerous mononuclear cells and some polymorphonuclear leukocytes were seen in the alveoli. Cellular infiltrates in the lungs were the only pathologic changes observed in guinea pigs sacrificed at 14 weeks. Several foci of myocarditis, consisting mainly of mononuclear cell infiltration interstitially, were found only among animals inoculated with the highest level of inoculum. Interestingly, myocarditis was devoid of vascular inflammation in infected guinea pigs, as has been observed among human acute Q fever sufferers. This tropism of C. burnetii for the myocardium has also been observed with...
FIG. 1. (A) Liver from a guinea pig sacrificed at day 20. An area of granulomatous inflammation is indicated (arrow) at the periphery of a portobiliary space; it is composed largely of macrophages and has lesser numbers of lymphocytes and polymorphonuclear leukocytes. (B) Spleen from a guinea pig sacrificed at day 9. Typical granuloma in the red pulp (arrow) is composed primarily of macrophages. For both panels, hematoxylin-phloxine-saffron stain was used; magnification, ×250.
FIG. 2. Demonstration of *C. burnetii* antigen by immunochemistry in liver (A), spleen (B), lung (C), and myocardium (D). The liver and spleen were from guinea pigs sacrificed at day 6, and the lung and myocardium were from animals sacrificed at day 9. Cytoplasmic vacuoles of macrophages are packed with coarse granular immunopositive material (arrows), especially in the spleen. Magnification, ×400 (A, B, and C) and ×1,000 (D).
human Q fever (3, 16) and is probably underestimated. A dose-response effect in clinical findings has been described for human volunteers (15), for monkeys (6), and from indirect evidence in outbreaks (9) in that the incubation period for the disease ranged from 7 to 30 days, according to the intensity of the exposure. Granulomas observed in most organs were compatible with exposure to vaccinated goats and their unpasteurized dairy products. Am. J. Trop. Med. Hyg. 47:35–40.


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