Route of Infection Determines the Clinical Manifestations of Acute Q Fever

Thomas J. Marrie, Andreas Stein, David Janigan, and Didier Raoult

In Nova Scotia the main manifestation of acute Q fever is pneumonia, while in France it is granulomatous hepatitis. To test the hypothesis that the route of infection is the major determinant of the manifestations of acute Q fever, 10 groups of 10- to 12-g female BALB/c mice (4 animals/group) were used. Five groups were inoculated intraperitoneally (ip) and 5 intranasally (inl) with Coxiella burnetii. Both routes of infection resulted in pneumonia. However, the inl route resulted in greater airway changes (on a numeric scale with 0 being no changes): 2.05 ± 2.20 versus 0.60 ± 0.83 (P < .002). The ip route resulted only in hepatosplenomegaly. It was concluded that the route of infection is one determinant of the manifestations of acute Q fever.

Human Q fever, due to the obligate intracellular pathogen, Coxiella burnetii, may be acute or chronic [1]. Acute Q fever can have a variety of clinical presentations, but those most commonly recognized are self-limited fever, pneumonia, and granulomatous hepatitis [1, 2]. In Nova Scotia we have observed pneumonia as the only manifestation of acute Q fever [3, 4], while in France the majority (61.9% of 323) of acute Q fever cases were hepatitis [5]. In France only 45% of acute Q fever cases had pneumonia [5].

Recently the heterogeneity of C. burnetii strains isolated from patients with acute or chronic forms of this infection has been recognized [6], and the recognition of cases of chronic Q fever in immunocompromised hosts has emphasized the importance of host factors in this disease [7]. We sought to determine whether these diverse manifestations of Q fever are due to properties of the infecting strain, the route of infection (respiratory versus gastrointestinal), the inoculum size, or properties of the host.

Materials and Methods

C. burnetii strains. Strains MPZ (human placenta), NSCI (cat uterus), and Q229 (brachial artery clot from a patient with Q fever endocarditis) were isolated by J. C. Williams (US Army Medical Research Institute, Fort Detrick, MD) from specimens submitted from Nova Scotia. Strains Nine Mile phase I and II were provided by one of us (D.R.).

All isolates except Nine Mile phase I were grown in L929 cells and passaged until at least 80% of the cell monolayer showed a cytopathic effect. The monolayers were harvested using sterile glass beads and, following centrifugation, were stored in 1-mL vials at −80°C. Before use, the vials were thawed, and 0.5% trypsin was added for 30 min at 37°C to lyse any remaining L929 cells. The trypsin was then heat-inactivated (65°C for 1 h). Nine Mile phase I was purified by renografin density-gradient centrifugation [8]. All isolates except Nine Mile phase I were in phase II at the time of mouse inoculation.

Animals. Female 10- to 12-g BALB/c mice were obtained from Charles River (Saint-Aubin-Les-Elbeuf, France). Animals (4/group) were kept by groups in cages and given water and food ad libitum. Uninfected controls and infected animals were housed in separate rooms.

Experimental design. Mice were inoculated intraperitoneally (ip) or intranasally (inl) with 0.5 mL of a 1:10 dilution of the stock material of each strain. Gimenez staining of this material indicated that the inoculum was ~106 cells. In all, there were 10 groups of 4 animals each. Group 1A animals received strain MPZ ip and group 1B animals received this strain inl. Group 2 received Nine Mile phase II; group 3, NSCI; group 4, Q229; and group 5, Nine Mile phase I. Control animals (4/group) received ip or inl 0.5 mL of L929 cells with tissue culture medium containing inactivated trypsin.

On day 10, the animals were sacrificed. Blood was obtained for determination of antibodies to C. burnetii phase I and II antigens. Pieces of spleen, liver, and lungs were placed in formalin for histology and immunohistochemistry. An impression smear was made on a glass slide from the cut surfaces of both lungs from each animal for staining by an immunofluorescence technique [3].

Histology. After dehydration, tissues were embedded in paraffin at 53°C. Sections (4 μm thick) were cut. One section was stained with hematoxylin-eosin, and the others were used for immunoperoxidase staining. After quenching of endogenous peroxidase, sections were sequentially incubated with a 1:300 dilution of rabbit anti–C. burnetii phase II (titer 1:64 by indirect immunofluorescence assay [IFA]) obtained from J. C. Williams, biotinylated link antibody, and avidin-biotin-peroxidase complex rabbit IgG (Vectastain; Vector Laboratories, Burlingame, CA). The same procedure was followed to stain a second slide, except that wild rabbit antiserum with a monoclonal anti–phase I and II C. burnetii antibodies [8, 9].
Table 1. Histologic changes in mice infected intraperitoneally (A) or intranasally (B) with Coxiella burnetii.

<table>
<thead>
<tr>
<th></th>
<th>Parenchyma score</th>
<th>Airways score</th>
<th>P</th>
<th>Parenchyma score</th>
<th>Airways score</th>
<th>P</th>
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<tbody>
<tr>
<td>Lungs</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>A</td>
<td>17</td>
<td>1.67 ± 1.62</td>
<td>NS</td>
<td>0.60 ± 0.83</td>
<td>&lt;.001</td>
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<tr>
<td>B</td>
<td>18</td>
<td>1.50 ± 1.42</td>
<td></td>
<td>2.65 ± 2.20</td>
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<thead>
<tr>
<th></th>
<th>Cell aggregates score</th>
<th>Increase in Kupffer cells score</th>
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<tr>
<td>Liver</td>
<td></td>
<td></td>
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<tr>
<td>A</td>
<td>17</td>
<td>1.94 ± 0.83</td>
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<tr>
<td>B</td>
<td>18</td>
<td>0.56 ± 0.68</td>
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<tr>
<th></th>
<th>No. with total size increase</th>
<th>No. with follicle size increase</th>
<th>No. with T zone increase</th>
<th>P</th>
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<tr>
<td>Spleen</td>
<td></td>
<td></td>
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<tr>
<td>A</td>
<td>16</td>
<td>15</td>
<td>&lt;.001</td>
<td>14</td>
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<tr>
<td>B</td>
<td>15</td>
<td>0</td>
<td>14</td>
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NOTE. Scores are mean ± SD. See Materials and Methods for explanation of scoring system.

Antibody determination. Antibodies to phase I and II C. burnetii whole cell antigens were determined in mouse sera by a microimmunofluorescence test using antigens of Nine Mile strain as described [10]. The starting dilution for the serum samples was 1:8, and samples were titered to end point.

Histopathology. Hematoxylin-eosin-stained slides of lung, liver, and spleen were coded and read by one of us (D.J.), a pathologist, who was unaware of the study hypothesis. The changes were assessed semiquantitatively as follows: 0 = not present, 1 = present but hard to find, 2 = easy to detect but limited in amount and distribution, 3 = diffusely present. The changes assessed in each organ were as follows: lung, cellular infiltrates or exudates (or both) in (1) parenchyma (both alveolar spaces and interstitial planes) and (2) airways, lumens, and walls; liver, cellular infiltrates with or without cell necrosis in (1) hepatocytes and (2) portal zones; spleen, cellular increases and therefore enlargement of (1) follicular or B cell zones and (2) interfollicular and T cell zones.

Statistical analysis. The difference between means was assessed using the two-tailed t test for paired data. Differences between proportions were assessed using Fisher’s exact test.

Results

All infected mice became ill as manifested by ruffled fur and inactivity. Two mice in group IA died. All mice that received C. burnetii ip had hepatosplenomegaly. The animals inoculated inl had grossly normal livers and spleens. Microorganisms were seen by IFA in all lung specimens from animals infected via the ip route and in none from animals infected inl.

All animals infected ip had an antibody response (titers 1:50–1:800) to phase II antigen. The animals infected inl had no detectable antibody response (titer <1:8). Animals with many organisms in the lungs (by IFA) had lower antibody titers than those that showed only a few organisms.

Table 1 summarizes the histologic changes in the lungs, liver, and spleen. Changes in the lung parenchyma were similar following inl and ip challenges. These changes were predominantly interstitial alveolitis. The changes in the airways, however, were significantly more pronounced among animals inoculated inl and consisted of interstitial infiltrates of mononuclear cells in the walls of airways, associated with swelling or proliferation (or both) of epithelial lining cells, and desquamation of cells (figure 1).

On a semiquantitative basis, the ip route resulted in significantly greater changes than the inl route in the liver. These changes consisted of mononuclear cell aggregates scattered throughout the liver lobules. In some of these aggregates, residues of necrotic hepatocytes were found. Another change was an increase in the number and size of Kupffer cells. No significant changes were found in portal areas.

The interfollicular T zones of the spleens were notably enlarged among 14 of the 16 animals inoculated ip. The increased cells were lymphocytes, mononuclear cells, and possibly hematopoietic cells, all normally present in the spleen. None of the animals inoculated inl had such changes. Changes in follicle size were less consistent.

On immunoperoxidase staining, many C. burnetii antigen–positive cells were seen in the livers and spleens of the animals inoculated ip. Most of these tissues showed greater positivity with phase I antiserum. The lungs of animals in groups 3 and 4 had a few C. burnetii–containing cells. The animals in groups 1, 2,
and 5 showed no antigen-containing cells in their lungs by this technique, even though all lungs from group A animals were positive by direct IFA. All tissues examined from animals inoculated inl were negative by immunoperoxidase staining. The uninfected control mice had normal tissues histologically and negative impression lung smears for C. burnetii by direct IFA.

Discussion

In this study, all 5 strains of ip-administered C. burnetii induced hepatosplenomegaly and caused interstitial pneumonia. In contrast, inl inoculation did not cause hepatosplenomegaly but did cause pneumonia. While both infection routes resulted in interstitial pneumonia, the inl route caused more changes to the airways. Challenge by either route (inl or ip) with trypsin-disrupted L929 cells in tissue culture medium (trypsin was inactivated prior to use) did not cause any changes in the airways. Microorganisms were seen in the lungs of mice challenged ip but not inl. Likewise, ip-infected mice mounted a humoral immune response, while inl-infected mice did not. It is possible that the mucosal priming of inl-infected mice...
resulted in clearance of the organisms from the lungs. In this regard, it is noteworthy that C. burnetii has never been demonstrated in the livers of patients with Q fever granulomatous hepatitis [2]. Certainly, in our model, numerous organisms were seen in the liver following ip infection.

During studies of C. burnetii vaccines, Williams et al. [11] found that when control mice were given an aerosol challenge of 10 LD$_{50}$ of C. burnetii phase I Henzlerling strain, 9 of 10 died and the spleen contained $5 \times 10^9$ infectious organisms (mean) and weighed 94 mg (mean). When challenged ip, 8 of 10 mice died; the spleen weighed 790 mg (mean) and contained $5 \times 10^8$ infectious organisms (mean). Baumann et al. [12] inoculated 2 strains of mice, C57BL/6(CH-2$^d$ and BALB/cJ(H-2$^d$), with 3.85 $\times 10^6$ ifu of C. burnetii Nine Mile phase I via the ip route. They found no difference in the distribution of lesions or manifestations of infection between the 2 strains of mice. Hepatosplenomegaly developed 3 days after infection and was prominent between 8 and 60 days after infection. They found no significant lesions in the lungs. We were able to demonstrate C. burnetii in the lungs of ip-infected animals by direct IFA but not in all animals by immunoperoxidase staining; this discrepancy is probably due to the increased sensitivity of the former test.

Franti et al. [13] found that male mice were more severely affected (as measured by splenomegaly) than female mice by the Hopland strain (C. burnetii isolated from a female deer at Hopland in northern California and from a female coyote in northern California in 1966). However, both sexes showed the same response to C. burnetii Henzlerling (isolated from human blood).

The inference from our study is that the route of infection may account for the predominance of pneumonia as the major manifestation of Q fever in Nova Scotia [3, 4] and for granulomatous hepatitis as its major manifestation in France [5]. Dupont et al. [5] speculated that ingestion of raw milk in rural France might account for the predominance of Q fever hepatitis in that country. They cited the epidemiology of Q fever hepatitis in France as support for the statement that 50% of the hepatitis cases occurred in persons with a country lifestyle who lived in animal breeding areas, whereas only 20.8% of the pneumonia cases occurred in these areas [5]. Other epidemiologic studies suggested that ingestion of raw, presumably contaminated milk was a risk factor for acquisition of Q fever in humans [14]. Investigations have shown that Q fever patients in Nova Scotia did not ingest raw milk [4].

There is agreement that inhalation of contaminated aerosols is the primary method by which Q fever infection occurs in humans. A dose-response effect has been demonstrated for the aerosol route in human volunteers [15] and from indirect evidence in outbreaks [4], in that the incubation period ranged from 7 to 30 days according to the intensity of the exposure. Animal data indicate that infection via the aerosol route can result in appearance of organisms in the spleen [11].

From the work of Baumgärtner et al. [12] and the data reported here, it is evident that intraperitoneal injection of C. burnetii can result in hepatitis, splenitis, and pneumonia. Thus, it is probable that the route of infection determines what will be the predominant manifestation of Q fever—pneumonia or hepatitis. This model has several drawbacks as a test of the route of infection: The ip route results in some ingestion of organisms and does not mimic perfectly the aerosol route, nor does ip injection mimic perfectly the gastrointestinal route. Furthermore, there is likely repeated exposure among those who ingest contaminated raw milk. A better model would be percutaneous placement of a gastric tube and repeated gastrointestinal challenge with C. burnetii.

References