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Appearance of Cellular and Humoral Immunity in Guinea Pigs After Infection with *Coxiella burnetii* Administered in Small-Particle Aerosols

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The development of humoral and cell-mediated immune responses was studied in guinea pigs infected with *Coxiella burnetii* administered in small-particle aerosols. Direct macrophage migration inhibition was observed in cultured peritoneal exudate cells as early as 3 days after exposure. Maximum inhibition of macrophages cultured with phase I or II antigen occurred 14 to 21 days postexposure and persisted through 35 days. This inhibitory action was no longer detectable at 42 days. Serum antibody to the phase II antigen of *C. burnetii* was detected at 14 days, and serum antibody to phase I antigen was detected at 21 days, 18 days after the cell-mediated immune response.

Previous in vitro studies indicated that homologous immune serum to phase I and II *Coxiella burnetii* not only enhanced the phagocytic uptake of rickettsiae (14–17), but also potentiated their destruction within phagocytes (15–17). Although immune serum has not been shown to have a direct rickettsicidal action on *C. burnetii*, its passive transfer to nonimmune animals modifies the infection (1).

The mechanism(s) involved in acquired immunity to *C. burnetii* have not been studied extensively. Recent studies indicate that cell-mediated immunity (CMI) may play a role in *C. burnetii* infection. Peritoneal macrophages from guinea pigs previously immunized with phase I antigen are capable of killing phase I rickettsiae in the absence of homologous immune serum (16, 17), and peripheral lymphocytes from humans who, for up to 8 years earlier, had been exposed to *C. burnetii* demonstrate marked lymphocyte transformation in vitro to both phase I and II *C. burnetii* antigens, even in the absence of circulating antibody (13). Therefore, it appears that CMI may be required for protection against Q fever infection.

In the present study, guinea pigs were infected with *C. burnetii* in small-particle aerosols to simulate a natural respiratory exposure. Measurements of cellular and humoral parameters of immune response and its temporal development in infected animals were examined.

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MATERIALS AND METHODS

Preparation of rickettsial stock suspension. The third egg passage of the Henzerling strain of *C. burnetii* in phase I was grown in chicken embryo cells as previously described (17). The infectivity of the rickettsial suspension was estimated to be $10^{9.5}$ mouse median infectious doses after administration by the intraperitoneal route.

Guinea pigs. Outbred male Hartley strain guinea pigs, weighing approximately 350 to 450 g, were obtained from Buckberg Lab Animals, Tompkins Cove, N.Y. All animals were provided water and commercial guinea pig chow ad libitum.

Aerosol dissemination and animal exposure procedures. A total of 100 animals were exposed to 10^4 mouse median infectious doses of *C. burnetii* presented as small-particle aerosols. Aerosols composed of rickettsiae were generated with a Collison atomizer (19) into a Henderson apparatus (22) that was modified to include an exposure box suitable for simultaneous whole body exposure of eight guinea pigs. The rickettsial suspension was diluted in Earle medium 199 (GIBCO, Grand Island, N.Y.) prior to dissemination, and the concentration of rickettsiae was estimated during dissemination with samples collected in all-glass impingers (12) filled with Earle medium 199. Animals were exposed to the aerosols for 10 min periods. The dose of rickettsiae inhaled (10^4 mouse median infectious doses) by guinea pigs was computed on the basis of the estimated mouse median infectious doses per liter of aerosol multiplied by the minimal respiratory volume of the guinea pig (0.11 liters/min; 7) and the duration of exposure (min). Thirty-two guinea pigs that had been exposed to aerosols of sterile Earle medium 199 served as controls. The rectal temperatures and weights of 10 guinea pigs from each infected and control group were determined daily.

Inhibition of macrophage migration. Peritoneal

exudate cells were collected 4 days after intraperitoneal injection of guinea pigs with 25 ml of sterile mineral oil (Marcol no. 90, Humble Oil and Refining Co., Houston, Tex.). The peritoneal exudate cells were harvested and processed according to the method of Harrington and Stastny (8). An agarose droplet method (8) was employed to detect the presence of direct macrophage migration inhibition. All cultures were maintained in Earle medium 199 supplemented with 15% fetal calf serum. Twenty-seven replicate agarose droplets containing exudate cells were prepared for each sample. Subsets of 9 droplets each were overlaid with 0.2 ml of Earle medium 199 supplemented with 15% fetal calf serum alone or with 0.2 ml of medium containing either 2×10^7 formalin-killed particulate phase I from the third egg passage of the Henzerling strain or 4×10^6 phase II rickettsiae from the 88th egg passage of the Nine-Mile strain. Cultures were incubated at 37°C for 24 h in 5% CO_2 and 95% humidified air. Exudate cells from noninfected guinea pigs were included as a control for antigen toxicity.

To estimate migration inhibition, agarose-cell droplets were examined with an inverted microscope fitted with a 0.5-mm grid in the ocular; the number of squares traversed by the margin of migrating cells was recorded. Inhibition of macrophage migration was calculated as follows.

$$\% \text{ Migration inhibition} = 100 - \frac{\text{mean area of migration with antigen}}{\text{mean area of migration without antigen}} \times 100$$

Serological assays. Blood was collected from animals at weekly intervals, and serum titrations were performed with the indirect immunofluorescent antibody technique of Bozeman and Elisberg (4) to evaluate antibody activity against phase I and II *C. burnetii*.

Necropsies and histopathological examinations. Four experimental and two control guinea pigs were necropsied at selected intervals for 29 days after infection. Tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

RESULTS

Clinical signs. Mean daily weight and temperature readings for 10 infected and 10 control animals are shown in Fig. 1. The onset of fever occurred 6 days after exposure, with maximum readings on days 8 to 11; other overt clinical signs, such as roughened fur, lethargy, excessive thirst, and coryza, were noted 5 to 7 days postinfection, and they persisted for 3 to 5 days. Infected animals failed to gain weight before termination of fever; subsequently, weight gain for the convalescent group paralleled that of the control group. Temperatures returned to baseline values by day 13. Control animals showed gradual increases in body weight and maintained constant body temperatures of 38 to 39°C throughout the period of observation.

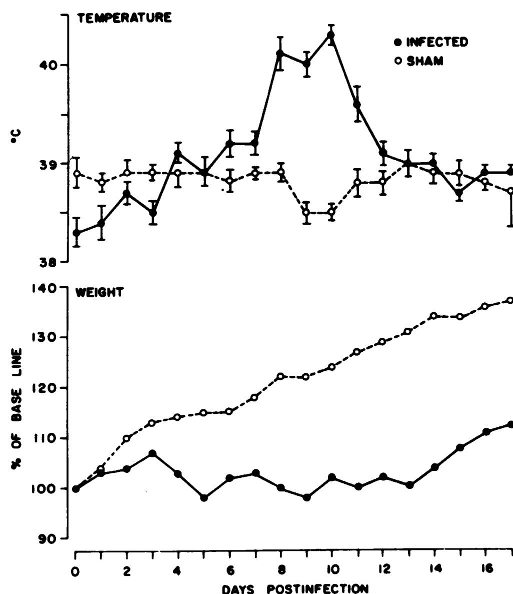

FIG. 1. *Daily weight and temperature readings. Each point represents the mean ± standard error of the mean for 10 infected or sham-treated guinea pigs.*

Pathology. Interstitial pneumonia, detected after 5 days, became most pronounced and characterized by an abundant exudation of fibrin, neutrophils, macrophages and lymphocytes in 9 to 11 days. Maximum development of lung lesions coincided with maximum temperature. Thirteen days after exposure, pneumonia with areas of consolidation still constituted the predominant lesion, with the infiltrate containing macrophages and lymphocytes. At 20 days, pneumonitis became less pronounced; at 29 days, lung lesions were minimal, although macrophages and lymphocytes were still present. Granulomas of the liver and spleen were apparent at 9 days. Granulomatous splenitis was absent by 20 days, but hepatitis persisted for 29 days. Minimal lymphoreticular myocarditis and epicarditis were first observed on day 9.

Inhibition of macrophage migration. As early as 3 days after exposure, migration of macrophages was inhibited when peritoneal exudate cells from infected guinea pigs were cultured in phase I, but not phase II, antigen (Fig. 2). Macrophage inhibition was most pronounced at 14 and 21 days in the presence of either phase I or II antigen and persisted at demonstrable levels until day 35. Reactivity, however, was no longer detectable 42 days post-

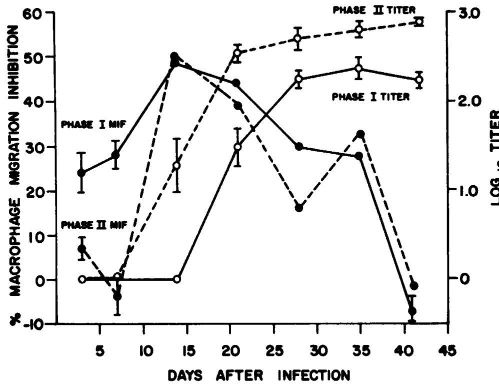

FIG. 2. *Indirect fluorescent antibody titers and the percent macrophage migration inhibition of samples from guinea pigs infected with Coxiella burnetii. Each point represents the mean ± the standard error of the mean for four to eight animals.*

exposure. Simultaneous tests with macrophages from control guinea pigs and phase I or II *C. burnetii* antigen were found to be ±5% of that observed for antigen-free cultures. Attempts to detect local induction of CMI in the respiratory tract at each sample time were unsuccessful, possibly due to the paucity of lymphoid cells.

Antibody response in infected guinea pigs. Indirect fluorescent antibody titers for sera from guinea pigs infected with *C. burnetii* are shown in Fig. 2. Antibody against phase II antigen, initially detected on day 14, was still present when the experiment was terminated at 42 days. Phase I antibody developing later than phase II antibody appeared at 21 days; maximum titers were achieved by day 28 and persisted throughout the 42-day observation period. Control guinea pigs developed no antibodies to either antigen.

DISCUSSION

These studies demonstrate that specifically sensitized populations of cells are present in guinea pigs soon after their exposure to a small-particle aerosol of *C. burnetii*. Macrophage migration inhibition was detected as early as 3 days postexposure, prior to overt clinical signs and well before any measurable humoral response. Macrophage migration inhibition was most marked at 14 days postexposure, shortly after maximum damage to lungs and other tissues was observed. This antigen-specific responsiveness of exudate cells was still present on day 35 but was no longer detected at 42 days. These data are in agreement with that of Heggers et al. (10), who reported the antigen-induced inhibition of migration of peritoneal macrophages obtained from guinea pigs on days 14 through 40 after infection with *C. burnetii* by the intraperitoneal route, but they performed no assays prior to 14 days.

Histologically, the most pronounced lesions were severe interstitial pneumonia 9 to 11 days postexposure, granulomatous hepatitis, splenitis and minimal lymphoreticular myocarditis, and epicarditis. Lesions in infected guinea pigs were similar to those reported in man (21). Our aerosol-infected animals developed more severe lung lesions and less severe liver lesions than those reported to occur in guinea pigs infected with *C. burnetii* by the intraperitoneal route (9). Physical signs of infection, fever and weight loss, disappeared before phase I antibodies were detected. Although only phase II antibodies were detected at this time, host defense mechanisms were actively reversing the disease process.

The appearance of migration inhibition as early as 3 days after exposure is not unusual. Kostiala and McGregor (18) reported that in rats infected with *Listeria monocytogenes*, localization of migration inhibition factor-producing cells in the peritoneal cavity coincided with the arrival on day 5 of circulating lymphocytes capable of protecting normal recipients against a *Listeria* challenge. Zinkernagel et al. (26) found that CMI activity appeared in the peritoneal cavity of mice 3 days after initiating a primary infection with *Listeria*.

Interactions between sensitized lymphocytes and released lymphokines, such as the migration inhibition factor and macrophages, and their role in host defense have not been elucidated. Bloom and Bennett (3) suggest that the migration inhibition factor may participate in macrophage activation. Other investigators (6, 20, 23, 24) have shown that phagocytic, bacteriostatic, and even bactericidal activities of macrophages are enhanced in the presence of sensitized lymphocytes or their soluble products. In our previous study with guinea pigs immunized with killed phase I *C. burnetii* (15, 16), migration inhibition developed at the same time the peritoneal macrophages became capable of killing phase I rickettsiae in vitro in the absence of immune serum. In studies by Hinrichs and Jerrells (11), intracellular growth of *C. burnetii* in cultures of normal guinea pig peritoneal macrophages was inhibited by immune lymphocytes, supernatant fluid from immune lymphocytes cultured with phase I antigen, the migration inhibition factor-rich fraction of such cultures, or with products of normal lymphocytes after stimulation with concanavalin A.

Unlike the CMI response, antibody production is slow to develop after vaccination or infection with *C. burnetii*; antibody directed against phase II antigens can be detected within 14 to 21 days and against phase I antigen by 34 to 35 days (5, 25). In the present study, antibody against *C. burnetii* antigens was absent 3 to 7 days postexposure when activity of migration inhibition factor was demonstrated, indicating that CMI is active and functioning well before humoral antibody is present. Other evidence also suggests that early protection against Q fever does not correlate with humoral antibody response; Benenson (2) found that, although 50% of volunteers vaccinated with phase I *Coxiella* developed no detectable antibody, all were resistant to aerosol challenge with virulent phase I rickettsiae. However, persistence of antibody after detectable CMI activity suggests that both humoral and cellular elements are involved in resistance to *C. burnetii* infection. This paper does not prove that both humoral and CMI cooperate in the response to *C. burnetii*, but it does present the kinetics of the responses that would support that view.

We were unsuccessful in detecting local induction of CMI in the respiratory tract during infection. This may have been due to the lack of lymphoid cells in bronchoalveolar wash fluids. In continuing studies we are studying the role of other lymphokines in *C. burnetii* infections.

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