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Comparison of *Brucella abortus* and *Brucella melitensis* Infections of Mice and Their Effect on Acquired Cellular Resistance

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By using mice infected with strains of *Brucella abortus* and *Brucella melitensis* we examined the histological responses to infection, the relationship of histology to persistence of organisms, and the relation of persistence of organisms to the acquisition of acquired cellular resistance (ACR). Infection with *B. abortus* resulted in well-formed granulomas in the livers, which persisted for more than 30 days. In contrast, infection with *B. melitensis* produced microabscesses in the livers which resolved before 30 days. The clearance of organisms from the tissues was also different. A total of 30 days after infection, large numbers of viable bacteria were recovered from the tissues of *B. abortus*-infected mice whereas bacteria were no longer recoverable from *B. melitensis*-infected animals. ACR to *Listeria monocytogenes*, another intracellular pathogen, persisted for more than 30 days in *B. abortus*-infected mice but waned rapidly in *B. melitensis*-infected animals. This disappearance of ACR due to *B. melitensis* paralleled the clearance of bacteria from the tissues.

In 1912 Fabyan first described epithelioid granulomas in the tissues of guinea pigs experimentally infected with *Brucella abortus* and emphasized the remarkable resemblance of these lesions to the histological changes seen in tuberculosis (5). Subsequent reports have confirmed the granulomatous nature of the tissue response to *B. abortus* in both animals and humans (11). Braude was the first to demonstrate that the pattern of disease in experimental brucellosis varies with the species of brucella producing the infection (1). By using *B. abortus* exclusively, he studied the evolution of the hepatic granuloma, concluding that this tissue response represented a successful defense of the host to the invasion of brucella (2). It was emphasized that this study pertained only to infection with *B. abortus*, and a similar study of the tissue response to *Brucella melitensis* infection has not appeared.

Our failure to detect granulomas in the livers of patients infected with *B. melitensis* (11a, 12) suggested that the pathogenesis of infection due to these closely related organisms and the response of the infected host may be different. Studies described in the present paper were designed to examine these differences.

MATERIALS AND METHODS

Animals. C3H female mice (6 to 8 weeks old) (L. C. Strong Research Foundation, San Diego, Calif.) were used in all studies. Mice were housed under standard conditions and allowed food and water ad libitum.

Liver and spleen histology. Mice were sacrificed on days 5, 10, 15, 20, and 30 after intraperitoneal infection with 5×10^8 colony-forming units (CFU) of either *B. abortus* strain 2308 or 1119 or *B. melitensis* strain EP or Rev-1. The histological results were identical with each species of brucella regardless of the strain used for infection; therefore, only virulent strains 2308 and EP are presented. The livers and spleens were removed aseptically and weighed and fixed in 10% Formalin. After embedding, thin sections were prepared by using a Porter-Blum microtome and stained with hematoxylin and eosin for histological examination. Sections were coded and read as unknowns by two investigators to reduce observer bias.

Bacteria. *Brucella abortus* strain 2308, a virulent smooth strain, and *B. abortus* strain 1119, a smooth vaccine strain, were obtained from the U.S. Department of Agriculture, Ames, Iowa. *B. melitensis* strain EP, a smooth virulent strain from a patient with acute brucellosis, was obtained from K. Ercke, William Beaumont Army Medical Center, El Paso, Tex. *B. melitensis* strain Rev-1, a smooth vaccine strain, was obtained from S. S. Elberg, University of California, Berkeley, Calif. Lyophilized seed cultures were reconstituted with distilled water and maintained on tryptic soy agar slants at 4°C. For individual experiments, cultures were prepared in tryptic soy broth (BBL Microbiology Systems) or Brucella broth (Difco Laboratories) on a shaker at 37°C for 48 h (optical density

0.3 to 0.4 at 540 nm). The actual number of viable bacteria injected was determined by plate counts. *Listeria monocytogenes* strain 19115 was obtained from R. E. Baughn, Baylor College of Medicine, Houston, Tex., and grown in brain heart infusion broth (BBL) for 18 h, distributed in vials, sealed, and then stored at −70°C. To prepare a challenge inoculum, 1 ml was thawed and diluted in phosphate-buffered saline to the appropriate concentration and injected intravenously.

Bacterial assays. The number of viable bacteria per milliliter of liver or spleen homogenate from mice infected intraperitoneally with brucella was determined by the method of Mackaness (8). Intravenous and intraperitoneal inoculations of brucella were compared (data not shown) and there was no difference in the recovery of viable organisms from the tissues; therefore the intraperitoneal route was used. Spleens were homogenized in 5 ml and livers were homogenized in 10 ml of phosphate-buffered saline, and appropriate decimal dilutions were plated in duplicate on well-dried tryptic soy agar plates. Colonies were checked for smoothness and counted after 36 to 72 h of incubation at 37°C. Brain heart infusion broth and agar were used in assays of listeria, and colonies were counted after 19 to 25 h of incubation. Since brucella do not appear before 48 to 72 h and are morphologically distinct from listeria, it was not necessary to add antibiotics to inhibit their growth.

RESULTS

Liver histology after infection with *B. abortus* and *B. melitensis*.

Groups of mice were infected intraperitoneally with 5×10^8 CFU of *B. abortus* strain 2308 or *B. melitensis* strain EP, and at varying times from 5 to 30 days later, the livers and spleens were removed for histological examination. By day 5, mice infected with *B. abortus* had marked splenomegaly and 1- to 3-mm intrahepatic lesions which were located just beneath the hepatic capsule. Microscopically, they consisted of areas of coagulation necrosis surrounded by areas of acute and chronic inflammatory cells (Fig. 1). Also present by day 5 were multiple microscopic inflammatory nodules composed of polymorphonuclear leukocytes, lymphocytes, and plasma cells. By day 10 these inflammatory nodules had evolved into well-formed epithelioid granulomas (Fig. 2). Foci of confluent granulomas with destruction of several contiguous hepatic lobules were present by day 10 in the *B. abortus*-infected livers. On day 30 the granulomas were still present, as were additional early-appearing inflammatory nodules composed of both acute and chronic inflammatory cells, suggesting that *B. abortus* infection was both persistent and active (Fig. 3).

In marked contrast, mice infected with *B. melitensis* did not develop grossly visible lesions at any time. On day 5, microscopic, poorly formed granulomatous nodules composed of neutrophils, lymphocytes, and histiocytes were

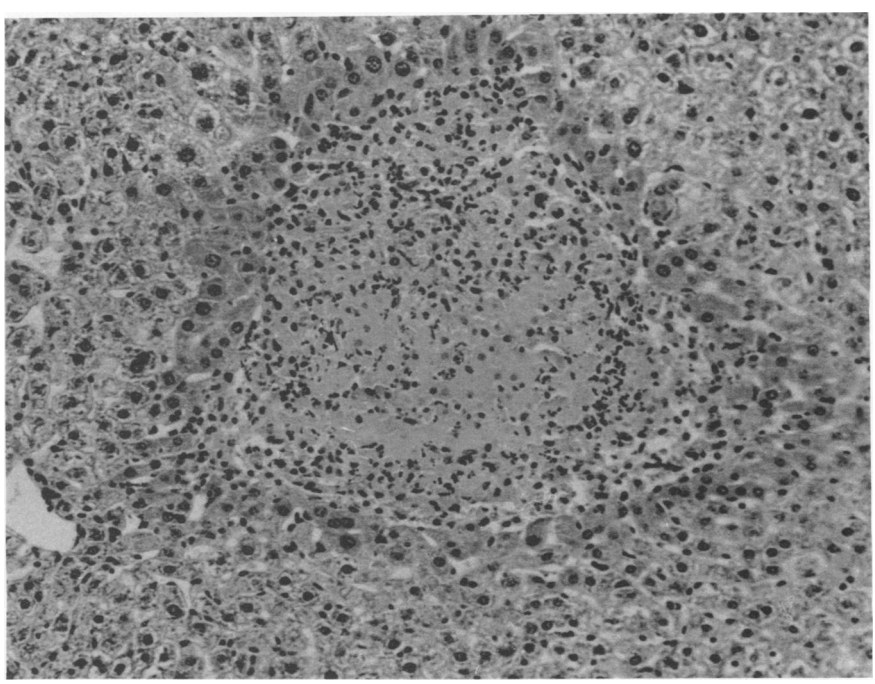

FIG. 1. *Histology of subcapsular lesion due to B. abortus at day 5. Necrotic hepatocytes surrounded by inflammatory cells. ×160.*

present throughout the liver (Fig. 4). By day 10, when well-formed granulomas were present in *B. abortus*-infected mice, the lesions in *B. melitensis* were still composed primarily of acute and chronic inflammatory cells with some hepatocellular necrosis but no well-defined granulomas. By day 30 the microabscesses caused by *B. melitensis* had essentially resolved, and the livers appeared essentially normal.

Spleen histology after infection with *B. abortus* and *B. melitensis*. More pronounced splenomegaly occurred in mice infected with *B. abortus* than with *B. melitensis*. Table 1 lists the mean spleen weights at various times after infection, and it can be seen that by day 30 spleens from *B. abortus*-infected mice were more than three times larger than those from *B. melitensis*-infected animals. Confluent splenic granulomas were extensive by day 10 in the *Brucella abortus*-infected animals. These lesions persisted through day 30. Splenic granulomas also developed in *B. melitensis*-infected mice by day 10, but were less numerous and had essentially resolved by day 30.

Time course of brucella elimination from the liver and spleen. When mice were infected intraperitoneally with 5×10^8 CFU of either *B. abortus* or *B. melitensis*, the number of *B. abortus* in the liver decreased initially at 24 h, remained relatively constant for the next 3 days, and then decreased slowly thereafter with 10^4 organisms remaining at 30 days. In contrast, *B. melitensis* decreased steadily from the time of infection and was no longer detectable by day 30 (Fig. 5).

To insure that differences between these virulent organisms did not merely represent strain differences, these experiments were repeated with *B. abortus* strain 1119 and *B. melitensis* strain Rev-1, two vaccine strains; as with the histological comparisons, similar differences in the rates of clearance of these strains from the liver were observed (Fig. 5).

Figure 6 shows the time course of brucella elimination from the spleens. Both strains of *B. abortus* were eliminated slowly with 10^5 to 10^6 CFU still being present on day 30. *B. melitensis* strains, on the other hand, were eliminated rapidly and were virtually undetectable by day 20. By using the Wilcoxson Rank Sum Test to compare these curves, the differences between *B. abortus* and *B. melitensis* elimination from both livers and spleens were highly significant ($P < 0.001$).

Effect of brucella infections on acquired cellular resistance to *Listeria*. Having estab-

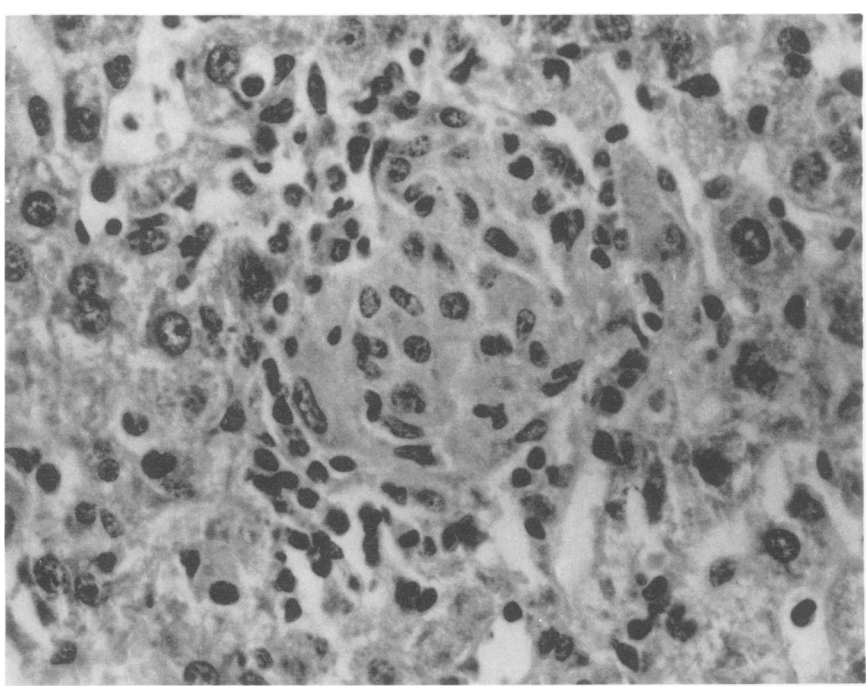

FIG. 2. *Intrahepatic lesion due to B. abortus at day 10. Well-formed epithelioid granuloma in hepatic lobule.* ×400.

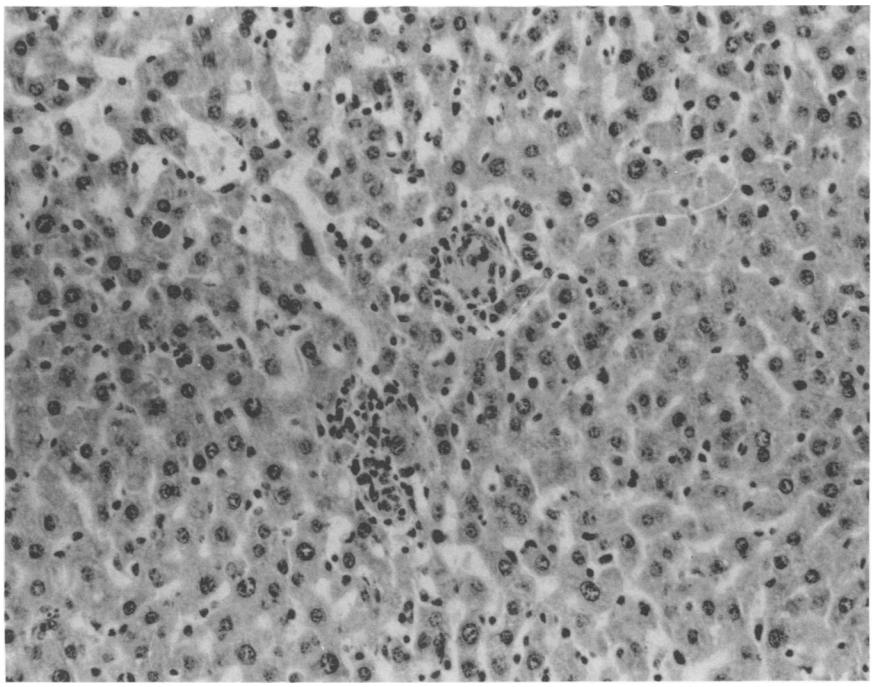

FIG. 3. *Hepatic lesions due to B. abortus at day 30. Persistent granulomas and acute inflammatory foci. ×160.*

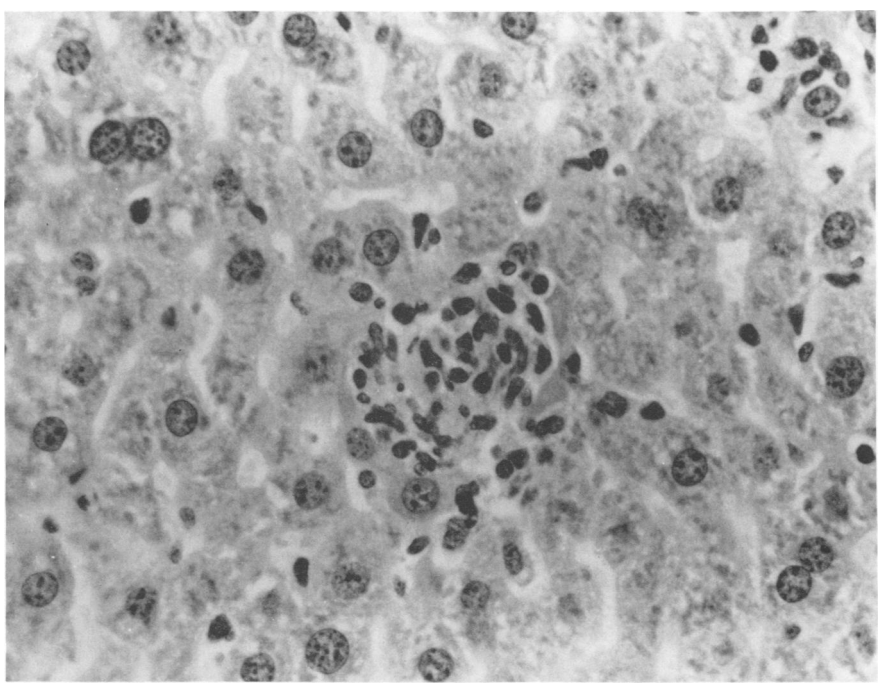

FIG. 4. *Intrahepatic lesion due to B. melitensis at day 5. Poorly formed granuloma with a paucity of epithelioid cells. ×400.*

lished that mice respond differently to *B. abortus* and *B. melitensis*, we then directed attention to the effect that these differences might have on acquired cellular resistance (ACR) to *Listeria monocytogenes*, another intracellular pathogen. Mice were infected intraperitoneally with 5×10^8 CFU of either *B. abortus* strain 2308 or *B. melitensis* strain EP. At 5, 15, 20, and 30 days, groups of 5 mice were challenged intravenously with listeria (10× 50% lethal dose). Mice which had not previously been infected with brucella served as controls. At 24, 48, and 72 h later animals were sacrificed, and their spleens were removed for determination of colony-forming units.

TABLE 1. *Weight of spleens from mice infected with B. abortus and B. melitensis*

Days after infection	Wt (g ± SEM)[a]	
	B. abortus	*B. melitensis*
1	0.28 ± 0.017	0.19 ± 0.015
5	0.25 ± 0.054	0.14 ± 0.008
10	0.70 ± 0.220	0.44 ± 0.012
20	0.46 ± 0.140	0.19 ± 0.013
30	0.71 ± 0.260	0.19 ± 0.004

[a] SEM, Standard error of the mean.

Figure 7 shows the results of several such experiments. On day 5 after infection with either *B. abortus* or *B. melitensis* there was a significant reduction in the number of listeria recovered compared with that of controls ($P < 0.001$). At day 15 this ACR persisted in both groups ($P < 0.001$), but in each case the degree of protection was greater with *B. abortus* compared with *B. melitensis* at 24, 48, and 72 h ($P < 0.02$, $P < 0.01$; $P < 0.01$, respectively). By day 20, *B. melitensis* no longer produced a significant

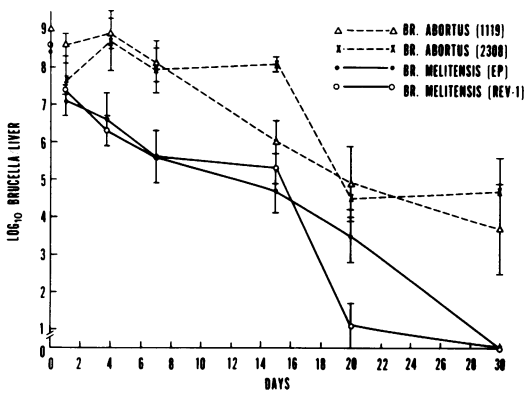

FIG. 5. *Time course of clearance of brucella from the liver. Each point represents the mean of 5 to 10 mice ± standard error of the mean.*

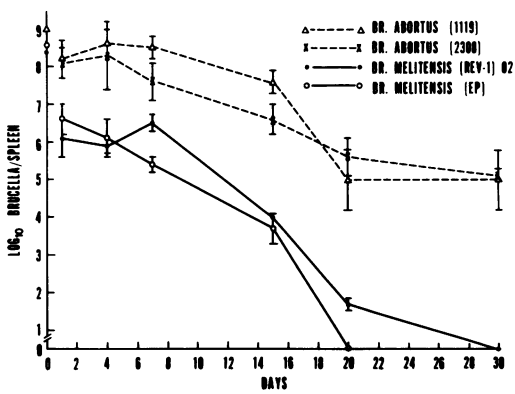

FIG. 6. *Time course of clearance of brucella from the spleen. Each point represents the mean of 5 to 10 mice ± standard error of the mean.*

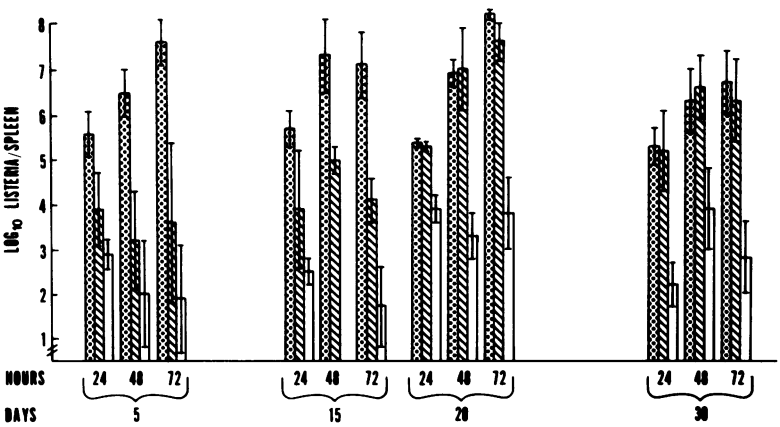

FIG. 7. *Recovery of listeria from the spleens of mice previously infected with brucella. Controls (not previously infected with brucella) are shown by dotted bars; B. melitensis-infected mice are shown as hatched bars; B. abortus-infected mice are shown as open bars. Each result represents the mean of 10 to 20 mice per group ± standard error of the mean.*

degree of ACR ($P > 0.1$), whereas that due to *B. abortus* remained significant at 20 and 30 days ($P < 0.001$).

DISCUSSION

The purpose of the present study was to examine (i) the histological patterns of infection due to *B. abortus* and *B. melitensis* in mice, (ii) the relation between histological patterns and persistence of viable organisms within the tissues, and (iii) the relation of histology and persistence of viable organisms to the appearance of ACR.

We found that infection with *B. abortus* was regularly associated with the development of well-formed microscopic epithelioid granulomas and frequently with subcapsular caseation necrosis similar to that observed in tuberculous granulomas. In contrast, infection with *B. melitensis* was associated with the development of microscopic foci of inflammation composed of acute inflammatory cells and histiocytes, but these lesions evolved into microabscesses with focal areas of hepatic necrosis, not into granulomas, and resolved relatively rapidly.

Differences in pathogenesis were also illustrated by the rates with which these two species of brucella were cleared from the tissues. As has been shown previously (7, 8), *B. abortus* was cleared slowly; even after 30 days, viable organisms were present in large numbers in the livers and spleens. In contrast, *B. melitensis* was eliminated more rapidly from livers and spleens; after 20 days, viable organisms were rarely recovered. Additional mice were injected with a lower inoculum of brucella (6×10^6 CFU) (data not shown) to insure that the differences in hepatic and splenic clearance were not dose dependent, and similar clearance curves were obtained.

As a facultative intracellular parasite, brucella has been prominent in studies of ACR (8, 9). Interestingly, the strains which have been used almost exclusively in such studies have been *B. abortus*. Our studies show that the persistence of ACR during *B. abortus* infection correlated closely with the ability of this organism to stimulate granuloma formation and with the presence of viable organisms in the tissues. In contrast, the rate of clearance of *B. melitensis* was more rapid and the period of enhanced ACR due to this organism was much more transient, as has been observed with infections due to *L. monocytogenes* (4, 6). The period of ACR in previous studies was also similar to that in these studies in which resistance induced by listeria also began to wane by day 15 of infection and virtually disappeared by day 20. It is tempting to speculate that the persistence of antigen within the tissues of *B. abortus* infection results in the continued activation of macrophages by sensitized T-lymphocytes. Our data support previous studies in suggesting that the development of a critical antigenic mass is an essential step in the establishment of cellular immunity (3, 10).

ACKNOWLEDGMENTS

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