Effects of gamma radiation and azathioprine on *Brucella abortus* infection in BALB/c mice

Philip H. Elzer, MS; Gail E. Rowe, BS; Fred M. Enright, DVM, PhD; Alexander J. Winter, DVM, PhD

SUMMARY

Sublethal irradiation of BALB/c mice 4 hours prior to inoculation with $5 \times 10^4$ virulent *Brucella abortus*, caused significant ($P < 0.01$) reductions in bacterial numbers in comparison with numbers in unirradiated controls. Numbers of brucellae in the spleen were significantly lower by 5 days after inoculation and decreased thereafter, so that at 2 and 3 weeks after inoculation, there were up to 1,000-fold fewer organisms in the spleen of irradiated mice. The number of brucellae in the spleen increased in irradiated mice thereafter. The course of events in the liver was similar, but developed more slowly, and peak differences in bacterial numbers were about 1 log less. These phenomena were not attributable to differences in implantation of brucellae in the liver or spleen, nor to an abnormal distribution of organisms in other organs of irradiated mice. Irradiation of mice during the plateau phase of infection also resulted in significant ($P < 0.05$) reductions in bacterial counts in the spleen during the succeeding 4 weeks. Macrophage activation in the spleen, measured by a *Listeria monocytogenes*-killing assay, was significantly ($P < 0.01$) increased by irradiation alone at 1 week after inoculation and at that time was significantly ($P < 0.01$) greater in *B. abortus*-infected, irradiated mice than in *B. abortus*-infected controls. Histologic, cytologic, and immunologic studies revealed that the decrease in numbers of organisms between 1 and 2 weeks after inoculation in irradiated mice occurred at a time when their immune response to *B. abortus* was suppressed and when numbers of neutrophils and monocytes infiltrating the spleen were significantly ($P < 0.01$) diminished. The increase in numbers of *B. abortus* in organs of irradiated mice that began after the third week coincided with recovery of the immune response and an increase in numbers of neutrophils and monocytes in the infected organs. The course of *B. abortus* infection was not substantially altered during the first 11 days after inoculation in mice infected at the height of a profound monocytopenia and neutropenia induced by azathioprine, a drug that by itself failed to activate macrophages. We hypothesized that, in irradiated mice, a rapid radiation-induced activation of resident macrophages to a brucellacidal state was coupled with an absence of newly formed monocytes in which virulent strains of *B. abortus* could establish persistent infection, and that as susceptible monocytes emerged in mice recovering from the effects of irradiation, chronic infection became established.

In 1975, Cheers and Waller\(^1\) made the seemingly paradoxical observation that CBA mice irradiated prior to inoculation with *Brucella abortus* strain 19 had lower numbers of organisms in their spleens than did control mice during the first week of infection. We confirmed this observation in the course of other experiments on mice being used as models for the study of brucellosis, and conducted the studies reported here to characterize more fully the effects of irradiation on infection of mice with *B. abortus*. Experiments were performed to establish (1) the magnitude and duration of this effect when irradiation was performed prior to inoculation or during the plateau phase, (2) the organ distribution of brucellae in irradiated mice, and (3) the consequences of irradiation on the inflammatory and immune responses to *B. abortus*. In addition, treatment with azathioprine was studied to determine the effect of a profound depletion of phagocytes on the establishment of *B. abortus* infection.

Materials and Methods

**Mice**—BALB/c ByJ female mice, 9 weeks old, were obtained from the Jackson Laboratory\(^a\) and held for 1 week before use.

**Bacteria**—Stock cultures of virulent *B. abortus* strain 2308 containing approximately $1 \times 10^9$ organisms/mL of brucella broth\(^b\) were stored at $-70\,\text{C}$. For inoculation, aliquots were thawed and diluted to the desired concentration in phosphate-buffered saline solution (PBSS). Numbers of organisms in the inoculum were confirmed retrospectively by plate counts.\(^2\)

A virulent strain of *Listeria monocytogenes*\(^c\) was grown in tryptic soy broth to an optical density corresponding to about $5 \times 10^8$ cells/mL. The bacteria were then frozen

\(^{a}\) Jackson Laboratory, Bar Harbor, Me.

\(^{b}\) BBL Microbiology Systems, Cockeysville, Md.

\(^{c}\) Obtained from Dr. D. D. McGregor, College of Veterinary Medicine, Ithaca, NY.

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in aliquots and stored at −70 C. Inoculum doses were prepared as described previously.

Irradiation—Mice were placed individually in plexiglass boxes on a rotating carousel. They were exposed, from a cesium 137 source, to 16 rads of gamma radiation/min, for 31.25 minutes, for a total dose of 500 rads of whole body irradiation.

Azathioprine (AZA) treatment—An aqueous suspension of AZA was converted to its sodium salt by adding an equivalent molar quantity of NaOH. The salt solution was lyophilized, reconstituted in PBSS to a concentration of 10 mg/ml, adjusted to pH 9.5 with concentrated HCl, and filter-sterilized. Mice were injected SC with 100 mg of AZA solution/kg of body weight/d.

Delayed-type hypersensitivity (DTH) reactions—Brucella abortus soluble antigen (BASA) was prepared as described by Kaneene et al, and 20 μg of BASA in 20 μl of PBSS was injected into the right hind foot pad. Twenty microliters of PBSS was injected into the opposite foot pad. Foot pad swellings were measured 48 hours later with a Hauptner dial caliper. All readings were made by the same person.

Blood samples and leukocyte counts—Blood samples were obtained retroorbitally. Sera to be tested for antibodies were frozen at −20 C until used. Leukocyte counts were made on samples of blood diluted 1:20 with 0.2% glacial acetic acid. Blood films were stained and differential counts were made on 100 cells.

Enzyme-linked immunosorbent assay—Antibodies were measured by use of an indirect plate ELISA. The antigen was a synthetic complex of B abortus O polysaccharide and stearoyl chloride that was hydrolyzed with 0.1 M NaOH to remove O stearoyl groups. Endpoint titer were determined to be the highest dilution at which absorbance readings were 0.2 U greater than those of the control.

Nonspecific esterase (NSE) stain—Cell suspensions were stained by the method of Miller and Morahan. Percentage of nonspecific esterase-staining cells was determined by counting 100 leukocytes in a hemacytometer. Cells stained deep red to light pink were counted as positive.

Estimation of bacterial numbers—Mice were killed by cervical dislocation. The spleen and, in some experiments, the left lateral lobe of the liver, marrow from the femur, blood, axillary lymph nodes, right kidney, right lung, uterus, and small and large intestine (including contents) were removed aseptically and were homogenized individually in 10 ml of PBSS. Homogenates were diluted serially and plated in triplicate on Schaedler blood agar plates. Intestines and contents were plated on blood agar containing, per milliliter of medium, 25,000 U of bacitracin, 6,000 U of polymyxin B sulfate, and 0.25 μg of neomycin sulfate. Colonies of B abortus were counted after 3 days of incubation at 37 C in an atmosphere of 10% CO₂. Colonies of L monocytogenes were counted after 24 hours of aerobic incubation at 37 C.

Tissue processing, staining, and examination—Tissues were processed and were stained with H&E and for nonspecific esterase activity as described by Enright et al. Tissue reaction scores for granuloma formation, infiltration of polymorphonuclear (PMN) or mononuclear cells, extramedullary hematopoiesis, and NSE staining were assigned according to previous criteria, in which scores ranged from 0 (no reaction) to 3 (extensive reaction). Splenic periarteriol lymphoid sheaths were scored from 4 (normal pattern) to 0 (complete loss of lymphoid architecture).

Experimental design—Two principal types of experiments were performed. Experimental groups consisted of 5 mice, unless otherwise stated.

Growth curves—Mice were injected iv with approximately 5 x 10⁴ brucellae in 0.1 ml of PBSS and were killed in groups of 5 at established intervals thereafter. In some experiments, blood samples (100 μl) were taken and foot pads were injected 2 days before mice were scheduled to be killed. Foot pad thickness was measured just before mice were killed. Two thin (1.0 to 1.5 mm) transverse slices of spleen and one of liver were taken at the time the mice were killed. One piece of spleen was used to prepare a single-cell suspension for the NSE assay. Other pieces of spleen and liver were fixed in a cold solution of

Figure 1—Effect of irradiation on implantation and growth of Brucella abortus strain 2308 in spleen (a) and liver (b) of BALB/c mice. Mice were inoculated iv with approximately 5 x 10⁴ organisms. Irradiated mice were treated with 500 rads of gamma radiation 4 hours before infection. There were 5 mice/group. Bars = se.
Figure 2—Growth curve of *B abortus* strain 2308 in the spleen of BALB/c mice inoculated iv with approximately $5 \times 10^4$ organisms. At 4 weeks after inoculation, half of the remaining animals were irradiated (500 rads gamma radiation). There were 5 mice/group. Bars = SE.

2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.5, and were stored at 4 C. The remainder of each spleen and liver was homogenized in 10 ml PBS and plated for colony counts.

Nonspecific resistance to infection—Mice that had been infected with *B abortus* for designated lengths of time, as well as uninfected age-matched controls, were injected iv with $1 \times 10^5$ live *L monocytogenes* bacteria. In a previous study, we had determined this dose to be lethal in 100% of normal BALB/c mice by 4 days after inoculation. Animals in groups of 5 were killed 1 day after *Listeria* injections, and numbers of *Listeria* in the spleen were determined by quantitative counts.

Statistical methods—Mean value for each bacterial count was obtained by averaging the triplicate values after log conversion. Hepatic bacteria counts were multiplied by 3 prior to log conversion to compensate for using only the left lateral lobe, which constituted one-third of the liver, in bacteriologic cultures. Statistical analyses were performed with paired one-tailed Student *t* test or, for multiple comparisons, the Studentized range test. Tissue reaction scores were subjected to analysis of variance, using a general linear-model program.

Results

Effect of irradiation on the course of infection with *B abortus*—Irradiation 4 hours prior to infection did not induce a significant effect on the implantation of *B abortus* in the spleen or liver (Fig 1). *Brucella abortus* replicated in the spleen of both irradiated and unirradiated mice between postinoculation days 1 and 5, but the rate of replication was lower in irradiated mice and, by day 5, numbers of brucellae in irradiated mice were significantly (*P* < 0.01) less than those in unirradiated mice. Kinetics of bacterial growth in the liver did not differ between the 2 treatment groups during the first week after inoculation. Between 1 and 2 weeks after inoculation, bacterial numbers increased in the unirradiated mice, but decreased in those that had been irradiated. At 2 weeks after inoculation, bacterial counts were significantly lower in the spleen (*P* < 0.001) and liver (*P* < 0.01) of irradiated mice, compared with unirradiated mice. In 3 other experiments, we demonstrated that peak reductions in bacterial numbers occurred in the spleen and liver of irradiated mice at 3 and 4 weeks after inoculation.
Table 1—Tissue reaction scores (mean ± so) in spleens of irradiated and unirradiated BALB/c mice inoculated with Brucella abortus strain 2308*

<table>
<thead>
<tr>
<th>Weeks after inoculation</th>
<th>Treatment</th>
<th>GRAN</th>
<th>PALS</th>
<th>EMH</th>
<th>NSE</th>
<th>PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>0.0</td>
<td>2.6±0.32</td>
<td>1.4±0.12</td>
<td>1.9±0.14</td>
<td>1.2±0.17</td>
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<td></td>
<td>Irradiation</td>
<td>0.0</td>
<td>2.0±0.0</td>
<td>1.1±0.21</td>
<td>1.0±0.3</td>
<td>0.8±0.34</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>1.2±0.76</td>
<td>1.7±0.67</td>
<td>1.2±0.10</td>
<td>1.3±0.21</td>
<td>1.6±0.23</td>
</tr>
<tr>
<td></td>
<td>Irradiation</td>
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<td>1.5±0.00</td>
<td>0.6±0.08</td>
<td>0.7±0.27</td>
<td>0.5±0.05</td>
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<tr>
<td>3</td>
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<td>1.5±0.35</td>
<td>1.1±0.13</td>
<td>1.2±0.04</td>
<td>1.6±0.31</td>
<td>1.4±0.27</td>
</tr>
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<td></td>
<td>Irradiation</td>
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<td>1.2±0.09</td>
<td>1.7±0.27</td>
<td>0.7±0.27</td>
<td>2.1±0.65</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>1.7±0.24</td>
<td>1.1±0.15</td>
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<td>1.1±0.34</td>
<td>1.6±0.30</td>
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<td>1.4±0.42</td>
<td>0.9±0.36</td>
<td>2.5±0.57</td>
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<td>5</td>
<td>None</td>
<td>1.6±0.14</td>
<td>1.2±0.27</td>
<td>1.5±0.33</td>
<td>1.2±0.27</td>
<td>1.8±0.21</td>
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<tr>
<td></td>
<td>Irradiation</td>
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<td>1.8±0.27</td>
<td>1.3±0.27</td>
<td>0.5±0.01</td>
<td>2.1±0.06</td>
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<tr>
<td>6</td>
<td>None</td>
<td>1.8±0.25</td>
<td>1.4±0.55</td>
<td>1.6±0.14</td>
<td>1.4±0.10</td>
<td>1.9±0.22</td>
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<tr>
<td></td>
<td>Irradiation</td>
<td>2.2±0.37</td>
<td>1.6±0.22</td>
<td>1.5±0.00</td>
<td>0.7±0.01</td>
<td>2.2±0.22</td>
</tr>
</tbody>
</table>

* Mice (groups of 5/time point) were inoculated iv with approximately 5 x 10⁶ organisms. †, ‡ Significantly different from comparable value in unirradiated mice († P < 0.05, ‡ P < 0.01, § P < 0.001).

Effects of irradiation on the inflammatory and immune response against Brucella abortus—Numbers of organisms in the spleen of irradiated mice were, as before, significantly lower than numbers in unirradiated mice at all time periods (P < 0.01), with peak reduction (2.9 logs) at 3 weeks after inoculation (Fig 3). Antibody titers were reduced in irradiated mice, most markedly at 3 and 4 weeks after inoculation. Foot pad thickness increases in irradiated mice were significantly (P < 0.05) less at 1 and 2 weeks after inoculation than in unirradiated mice. Whereas the histologic changes in foot pads of irradiated mice were typical of DTH, the thickened foot pads of irradiated mice were markedly depleted of cells, a finding that also has been reported by Volkman and Collins. Suspensions of splenic tissue from irradiated mice were extremely deficient in NSE-staining cells between weeks 2 and 6 after inoculation, with differences from control groups most marked at 4 and 5 weeks after inoculation.

Histologic examinations were performed in 3 experiments (Table 1). In unirradiated mice, splenic infection with Brucella abortus strain 2308 over a 6-week period was characterized by a moderate degree of granuloma formation, low-to-moderate increases in PMN, NSE-staining cells, and extramedullary hematopoiesis, and depletion of lymphoid tissue peaking at 3 to 5 weeks after inoculation. These findings were comparable to those reported by Enright et al. Principal differences observed in the spleen of irradiated mice were a delay in the formation of granulomas, significant (P < 0.05) decrease in extramedullary hematopoiesis at weeks 1 and 2, significant (P < 0.01) decrease in NSE-staining cells at all periods except week 4, and a significant (P < 0.001) decrease in PMN at week 2 (Table 1). Mean number of PMN in irradiated mice exceeded that in controls at weeks 3 to 6 and was significantly (P < 0.05) higher at weeks 4 and 6.

Lesions in the liver of infected control mice were mild, as observed previously, and were characterized by small numbers of granulomas and a low-to-moderate influx of PMN and monocytes. Irradiation induced a change only in the numbers of PMN, which were lower from weeks 1 to 4, with significant differences at weeks 2 (P < 0.05) and 4 (P < 0.01; data not shown).

Irradiation alone caused significant increases in Listeria killing at 1 and 4 weeks after inoculation, (P < 0.01; Fig 4). Significant (P < 0.001) increases in Listeria killing occurred at all time intervals in spleens of irradiated and unirradiated B abortus-infected mice. At 1 week after inoculation, however, the logs of protection against Listeria were significantly (P < 0.01) greater in the Brucella-infected and irradiated group than in the Brucella-infected, unirradiated group, whereas the opposite situation prevailed at weeks 2, 3, and 4. From weeks 3 to 6, the number of NSE-staining cells in specimens from the spleen of unirradiated, Brucella-infected mice greatly exceeded that in the other treatment groups.

Effect of AZA treatment on infection with B abortus—Subcutaneous injections of AZA at 100 mg/kg daily for 11 days caused no enhanced killing of L monocytogenes (data not shown). Mice were inoculated with B abortus on the eleventh day after initiation of AZA treatment, when circulating monocytes were undetectable and neutrophil counts had been decreased by two-thirds (Fig 5) in accord with prior reports. Initially, significantly (P < 0.01) fewer organisms were implanted in the spleen of AZA-treated mice; however, on subsequent days there were no signif-
Discussion

Effects of sublethal irradiation have been assessed in several studies involving facultative intracellular parasites in mice. Infection of irradiated CBA mice with *Brucella abortus* by iv inoculation and challenge 4 hours later with *Listeria monocytogenes* resulted in significant differences in counts of organisms in the spleen. Significant ($P < 0.05$) differences in number of organisms in the liver occurred only at 2 and 4 days after inoculation, when numbers of organisms in AZA-treated mice were below those in control groups. Cells staining with NSE were virtually absent from suspensions of spleen cells from AZA-treated mice and were significantly ($P < 0.05$) lower in spleen sections at all time periods (Table 2). Extra-medullary hematopoietic activity and PMN count in AZA-treated mice were significantly ($P < 0.05$) lower on post-inoculation days 2, 4, 7, and 11. Monocyte and PMN infiltrations in the liver of AZA-treated mice were uniformly diminished, with significant ($P < 0.05$) differences at 4, 7, and 11 days after inoculation (data not shown).

**Table 2—Tissue reaction scores in spleens of azathioprine (AZA)-treated and untreated BALB/c mice inoculated with *Brucella abortus* strain 2308***

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Treatment</th>
<th>PMN</th>
<th>NSE</th>
<th>ESH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (2 h)</td>
<td>None</td>
<td>1.5 ± 0.31</td>
<td>2.9 ± 0.35</td>
<td>1.2 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>AZA</td>
<td>1.6 ± 0.11</td>
<td>0.7 ± 0.48</td>
<td>1.0 ± 0.11</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>1.8 ± 0.38</td>
<td>2.1 ± 0.55</td>
<td>1.2 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>AZA</td>
<td>0.9 ± 0.21</td>
<td>0.5 ± 0.22†</td>
<td>0.7 ± 0.21†</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>1.6 ± 0.33</td>
<td>2.4 ± 0.22</td>
<td>1.4 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>AZA</td>
<td>0.6 ± 0.52†</td>
<td>0.8 ± 0.13†</td>
<td>0.4 ± 0.11§</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>1.4 ± 0.42</td>
<td>2.5 ± 0.31</td>
<td>1.4 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>AZA</td>
<td>0.3 ± 0.11‡</td>
<td>0.5 ± 0.09</td>
<td>0.4 ± 0.14§</td>
</tr>
<tr>
<td>11</td>
<td>None</td>
<td>1.6 ± 0.27</td>
<td>1.7 ± 0.45</td>
<td>1.4 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>AZA</td>
<td>0.3 ± 0.11‡</td>
<td>0.5 ± 0.24‡</td>
<td>0.4 ± 0.24§</td>
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</table>

*Mice (groups of 5/time point) were inoculated iv with approximately $5 \times 10^6$ organisms at time 0. †, ‡, § Significantly different from comparable value in untreated mice ($P < 0.05$, †$P < 0.01$, § $P < 0.001$).

Azathioprine treatment was begun 11 days prior to infection. The experiment is depicted in Figure 5.

See Table 1 for key.
L monocytogenes or of mice of the resistant A/J strain with Salmonella typhimurium resulted in fatal infections from otherwise sublethal challenge doses. This finding was attributed to the destruction of monocyte precursors essential for control of these infections. Whereas sublethal irradiation had no apparent effect on accelerating or suppressing the course of Mycobacterium tuberculosis infection, fatal infection of the hypersusceptible BALB/c mouse with Leishmania major was prevented by prior irradiation, and the number of B abortus strain 19 in the spleen of irradiated CBA mice was substantially decreased during the first week of infection. The ameliorating effect of irradiation on mice with leishmaniasis is now attributed to a favored expansion of CD4 T-cell subsets with protective properties over T cells that exert a suppressive effect. The reduced number of brucellae in the spleen of irradiated mice was proposed to have resulted from an early activation of resident macrophages by irradiation, a phenomenon that has been subsequently verified and was confirmed in the study reported here (Fig 1). The experiments reported here extend the findings of Cheers and Waller in demonstrating that, in irradiated mice, maximum reduction in the number of organisms in the spleen occurred at 3 weeks after inoculation, and was followed by a gradual diminution of the effect (Fig 3); the course of events in the liver was similar, but was delayed for about 1 week in relation to that in the spleen; and these phenomena were not attributable to differences in implantation of brucellae in the liver or spleen (Fig 1), nor to an abnormal distribution of organisms in other organs of irradiated mice. We obtained similar results in irradiation studies involving C57BL/10 and CBA mice.

The significant reduction of B abortus in the spleen of irradiated mice at 1 week after inoculation occurred at a time when macrophage activation, as determined by lysis of infected erythrocytes, was significantly heightened in irradiated, B abortus-infected mice in comparison with B abortus-infected controls (Fig 4). Numbers of brucellae in the spleen of control infected mice had increased by 2 weeks after inoculation to plateau values; whereas in irradiated mice, a decrease in splenic counts was observed consistently between 1 and 2 weeks after inoculation (Fig 1 and 3). This decrease occurred over a period of time when irradiation-induced activation of macrophages had waned, immune responses remained suppressed, and the influx of monocytes and neutrophils into the spleen was significantly diminished (Table 1). At 3 weeks after inoculation, when splenic counts remained low, the same relationships generally held, except that by this time, DTH reactions in irradiated mice were equivalent to those in controls, and mean numbers of PMN in the spleen of irradiated mice exceeded those in control mice. At later time periods, when numbers of brucellae in spleens of irradiated mice approached those of control mice, there was a recovery of the immune response to B abortus and a gradual increase in numbers of NSE-staining cells in suspensions of spleen cells from irradiated mice.

A working hypothesis to account for our observations encompasses some proposals put forward earlier. Sublethal irradiation may have, within a period of a few hours, activated the radioresistant population of macrophages of the spleen to a brucellacidal state, thus limiting replication of the organism during the first week after inoculation. The advantage thereby gained by the host in its containment of the parasite may have been sustained during the next 2 weeks by maintaining macrophage activation through nonspecific stimuli derived from the infecting organism, in particular lipopolysaccharide and muramyl dipeptide. Equally important, during much of this time there would have been a markedly reduced emigration of neutrophils and monocytes into the infected spleen, because of suppression of hematopoiesis caused by ionizing radiation. Newly formed monocytes may serve as "safe targets" in which B abortus organisms survive during the plateau phase. Proliferation of B abortus in nonactivated monocytes might result in development of macrophages that could not be activated sufficiently to eliminate the bacteria. Such macrophages, which might exhibit the properties of suppressor macrophages in vitro, may still have been capable of killing L monocytogenes (Fig 4). An explanation for the reduction in the number of B abortus in the spleen of mice irradiated during the plateau phase (Fig 2) also remains speculative, but it could have involved the activation of a brucellacidal state of newly emigrated monocytes, of tissue macrophages already harboring B abortus, or of both of these populations. According to this hypothesis, the enhanced resistance to brucellosis conferred by irradiation would gradually wane as the influx of susceptible leukocytes into the spleen increased, thus allowing establishment of chronic infection. Events in the liver could be explained by the same hypothesis, with the temporal delay in reduction of bacterial numbers perhaps linked to the increased time required for effective activation of Kupffer cells.

Treatment with azathioprine was studied in an attempt to dissect the elements of the hypothesis by testing whether elimination of the putative cell type responsible for perpetuation of chronic infection would suffice to control infection in the absence of early macrophage activation. The failure to demonstrate reduced infections in AZA-treated mice (Fig 5) is consistent with the dual hypothesis that has been advanced. In contrast to murine listeriosis, however, in which 1 injection of azathioprine prior to infection with a sublethal dose resulted in uncontrolled infection, there was no increase in bacteria counts in mice challenged-inoculated with B abortus at the height of AZA-induced monocytopenia and neutropenia. This finding suggests that during the first 11 days after inoculation, newly formed monocytes and neutrophils have no essential role in containing B abortus infection in mice.

References


