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Lack of a Role for Natural Killer Cells in Early Control of *Brucella abortus* 2308 Infections in Mice

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Studies were conducted to determine if natural killer (NK) cells are important for early control of the virulent strain *Brucella abortus* 2308 following infection of mice with high or low challenge doses. Splenocytes from C57BL/10 and BALB/c mice that had been infected with the lower dose of *B. abortus* displayed increased cytotoxicity against YAC-1 cells during the first week after infection, while infection of C57BL/10 mice with the higher challenge dose either did not alter the level of NK cytotoxic activity or decreased it, depending upon the time postinfection. In vivo depletion of NK cells by monoclonal antibody anti-NK1.1 or polyclonal anti-asialo-GM_1 antiserum did not result in an increase in the number of brucellae recovered from the spleens or livers of the brucella-resistant C57BL/10 mice or from the spleens of the susceptible BALB/c mice during the first week after infection. Treatment of control mice with the NK-reactive antibodies, however, decreased killing of the NK-sensitive target YAC-1, indicating that the NK cell depletion regimes were effective. Our results suggest that NK cells are not crucial for early control of *B. abortus* 2308 even though they may be activated following infection. Further experiments indicated that treatment of C57BL/10 mice with poly(A:U) did not decrease the number of brucellae recovered from their spleens although it did decrease the CFU in livers of mice infected with the high challenge dose.

Brucella abortus is a gram-negative facultative intracellular bacterium and a causative agent of brucellosis, a chronic disease of cattle, rodents, and humans. Infections in mice with the virulent strain *B. abortus* 2308 show a plateau in bacterial numbers in the spleen, the main site of infection, around 1 week after infection. However, a diminution in CFU does not occur until many weeks later (18). The results of in vivo studies indicate that cell-mediated immune responses involving CD4 and CD8 T cells are principally important for control of the infection both during the initial stages of infection and at clearance (2, 21). Gamma interferon (IFN-γ) is also instrumental in mediating resistance. For instance, in vivo studies have shown that administration of recombinant IFN-γ to infected mice reduces the number of organisms in their spleens (26), while depletion of endogenous IFN-γ by administration of neutralizing antibodies increases it (7, 13). Activation of macrophages by IFN-γ has been shown in vitro to increase the ability of macrophages to kill or inhibit replication of intracellular brucellae (10, 11). While both CD4 and CD8 T cells produce IFN-γ, and therefore their role in mediating resistance to brucellosis is likely to involve production of IFN-γ, natural killer (NK) cells also produce IFN-γ. Moreover, NK cells have been shown to be important for control of infections of intracellular bacterial and protozoan parasites (6, 15), and this role has been shown specifically to relate to their ability to produce IFN-γ (6, 23).

NK cells are also capable of killing microbe-infected cells (14) and directly lysing gram-negative bacteria (8). Such antimicrobial activities potentially could contribute to control of infections with *B. abortus*. While the involvement of NK cells in resistance to *B. abortus* has not been evaluated, an aqueous ether extract of *B. abortus* strain 456, known as Bru-Pel, has been shown to stimulate NK cytolytic activity against YAC-1 cells in vitro (27). The observation lends support to the conjecture that NK cells may be involved in controlling brucellosis. Conversely, however, human patients with brucellosis have been noted to have impaired NK cytolytic activity against YAC-1 cells (22), which, if NK cells are important for control, may be a factor in promoting the chronicity of the infection. Here, we evaluated the role of NK cells in mice during the early phase of infection with the virulent strain *B. abortus* 2308. In contrast to their role in controlling other intracellular microbes, our results suggest that NK cells do not contribute to the early control of murine brucellosis.

MATERIALS AND METHODS

Infection of mice. Female C57BL/10 and BALB/c mice were purchased at 7 to 8 weeks of age from Harlan Sprague Dawley (Indianapolis, Ind.) or Jackson Laboratories (Bar Harbor, Maine) and used at 8 to 12 weeks of age. Mice were infected by intraperitoneal injection of approximately 5×10^3 or 5×10^6 CFU of the virulent strain *B. abortus* 2308 suspended in 0.1 ml of phosphate-buffered saline (PBS). At 2, 4, or 7 days after infection, the mice were sacrificed by cervical dislocation, and their spleens and livers were removed and homogenized in PBS. Serial dilutions of the homogenate were plated on Schaedler blood agar, and the CFU of *B. abortus* were enumerated after incubation at 37°C in 5% CO_2 in air for 3 days.

In vivo depletion or activation of NK cells. To deplete NK cells, mice were injected intraperitoneally with either 30 or 50 μl of the polyclonal rabbit antiserum anti-asialo-GM_1 (Wako Bioproducts, Richmond, Va.) or 1 mg of the murine anti-NK1.1 monoclonal antibody (MAb) produced by the hybridoma PK 136 (cell line HB191 purchased from the American Type Culture Collection, Rockville, Md.) diluted to 0.2 ml with PBS. Anti-NK1.1 MAb was prepared as ascites in pristane-primed nude mice and enriched for immunoglobulin by 45% ammonium sulfate precipitation as described previously (1). Equal amounts and volumes of control rabbit serum (Sigma, St. Louis, Mo.), control rabbit gamma globulin (Accurate Chemical Co., Westbury, N.Y.), or mouse immunoglobulin (IgG; Sigma) were used as controls. To activate NK cells, mice were given 300 μg of poly(A:U) (Sigma) as described previously (4). Concentrations of reagents and schedules for administration relative to infection were similar to those described in previous publications (6, 15) and are detailed for each experiment.

Assessment of NK cytolytic activity. Mononuclear cells isolated from suspensions of mouse splenocytes by density gradient centrifugation over Ficoll-Paque (Pharmacia, Piscataway, N.J.) were assessed for NK cytolytic activity against target cells with a standard ^{51}Cr-release cytotoxicity assay and murine lymphoma

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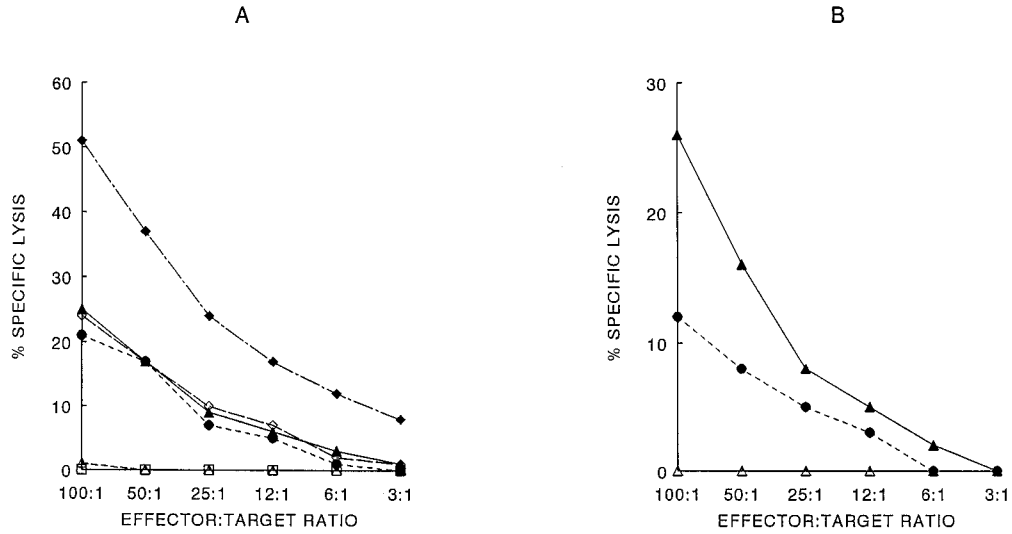

FIG. 1. Determination of the ability of anti-asialo-GM_1, anti-NK1.1, and poly(A:U) to affect NK activity in the spleens of C57BL/10 mice. Cytotoxicity was measured in a standard 4-h ^{51}Cr-release cytotoxicity assay against YAC-1 cells 24 h after injection of the various reagents. Percentage specific lysis was calculated as described in the text. Each value is calculated from quadruplicate test wells for each of two mice. Variability was less than 10% among replicates. (A) Mice were treated with PBS (closed triangles), 30 μl (open triangles) or 50 μl (open squares) of anti-asialo-GM_1 antiserum, 30 μl (closed circles) or 50 μl (open diamonds) of control rabbit serum, or 300 μg of poly(A:U) (closed diamonds). (B) Mice were treated with PBS (closed triangles), 0.5 mg of anti-NK1.1 MAb (open triangles), or mouse IgG (closed circles). The various reagents were diluted with PBS so that a total volume of 0.2 ml was given for each treatment.

YAC-1 target cells (cell line purchased from the American Type Culture Collection). Briefly, 2×10^6 YAC-1 target cells were labeled with 0.1 mCi of $Na_2Cr_2O_4$ (ICN, Irvine, Calif.) for 2 h at 37°C in an atmosphere of 5% CO_2 in air. After washing, 10^4 target cells were mixed with effector cells at effector-to-target ratios ranging from 100:1 to 3:1, in a total volume of 0.2 ml in 96-well round-bottom plates. Plates were centrifuged briefly to promote contact and incubated at 37°C, in 5% CO_2, for 4 h. After incubation, the cells were resuspended and then centrifuged at $400 \times g$ for 5 min, after which 100 μl of supernatant was removed and counted in a gamma counter. Maximum-release controls were obtained by thrice freeze-thawing labeled cells suspended in distilled water. Results were expressed as follows: % specific lysis = $[(cpm_{experimental} - cpm_{spontaneous})/(cpm_{maximum} - cpm_{spontaneous})] \times 100$, where cpm is counts per minute. To assess the effectiveness of the NK cell depletion or activation regimes described above, mononuclear splenocytes from uninfected control mice were used. In addition, mononuclear splenocytes from mice infected with 5×10^3 or 5×10^6 CFU of *B. abortus* 2308 were assessed 4 or 7 days after infection for cytotoxicity against YAC-1 cells.

RESULTS

Efficacy of NK-depleting or -activating regimes. The efficacy of the reagents used in studies presented here was established in preliminary experiments by assessing the NK cytolytic activity of splenocytes from treated mice. The epitope NK1.1 is polymorphic among mouse strains, being expressed by NK cells from C57BL/10 mice but not from BALB/c mice. Initial experiments involving evaluation of C57BL/10 mice employed a MAb reactive with NK1.1 to deplete NK cells. Subsequent experiments used anti-asialo-GM_1 antiserum so that the same reagent could be used in experiments involving BALB/c mice. Both reagents were effective: either 30 or 50 μl of anti-asialo-GM_1 antiserum or 0.5 mg of anti-NK1.1 MAb abrogated NK cytolytic activity against YAC-1 cells in C57BL/10 (Fig. 1) and BALB/c mice (data not shown). In contrast, administration of 300 μg of poly(A:U) to mice significantly increased NK cell activity by their splenocytes.

Role of NK cells in early control following infection of resistant C57BL/10 mice with a high challenge dose of *B. abortus*. Since C57BL/10 mice are resistant to infections with the virulent strain *B. abortus* 2308, initial experiments evaluated the role of NK cells in controlling infections in these mice. The effect of NK cell depletion on control of infection employing a challenge dose of 5×10^6 CFU of *B. abortus* 2308 was determined during the first week of infection (Tables 1 and 2). As shown, depletion of NK cells did not result in a significant increase in the number of CFU recovered from spleens of infected C57BL/10 mice at either 2 days (Table 1) or 1 week (Table 2) after infection when compared with controls given control serum or immunoglobulin. In only one of three experiments was the CFU in NK-depleted mice greater than that in the control group given PBS (Table 2, experiment 3). However, the difference between the two control groups (PBS and rabbit serum) in that experiment was also very pronounced for reasons which are unclear, i.e., there were no obvious mitigating circumstances. Similar results were found for livers of infected mice evaluated after 7 days of infection (Table 2). The CFU in livers of NK-depleted mice were never significantly higher than those in the control groups receiving rabbit serum or immunoglobulin (Table 2). As for the CFU in the spleen, only in experiment 3 was the number of CFU in the livers of mice

TABLE 1. Effect of in vivo depletion of NK cells by use of anti-NK1.1 MAb on infection of C57BL/10 mice with *B. abortus* 2308, evaluated 2 days after infection[a]

Infection dose	Treatment[b]	Log_{10} CFU/spleen[c]
5×10^6	PBS	6.07 ± 0.11
	Normal mouse IgG	6.09 ± 0.08
	Anti-NK1.1	6.10 ± 0.23
5×10^3	PBS	4.57 ± 0.12
	Normal mouse IgG	4.77 ± 0.21
	Anti-NK1.1	4.48 ± 0.21

[a] C57BL/10 mice were infected intraperitoneally with either 5×10^6 or 5×10^3 CFU of *B. abortus* 2308.

[b] Mice were given either PBS, normal mouse IgG, or anti-NK1.1 MAb 24 h before infection.

[c] Each value represents the mean ± standard error of the mean of CFU of *B. abortus* 2308 in the spleens of five mice. No significant differences were detected between test and control groups by the Wilcoxon or Student's *t* test ($P \geq 0.05$).

TABLE 2. Effect of NK cell depletion or poly(A:U) treatment on infection of C57BL/10 mice with high challenge doses of *B. abortus* 2308, evaluated 1 week after infection[a]

Expt no.	Treatment[b]	Log_{10} CFU/spleen[c]	Log_{10} CFU/liver[c]
1	PBS	6.42 ± 0.06	7.58 ± 0.09[d]
	Rabbit serum	6.41 ± 0.10	7.57 ± 0.03
	Anti-asialo-GM_1	6.48 ± 0.03	6.70 ± 0.33
	Poly(A:U)	6.59 ± 0.05	6.86 ± 0.25[e]
2	Rabbit gamma globulin	6.44 ± 0.06	6.52 ± 0.23
	Anti-asialo-GM_1	6.32 ± 0.21	6.12 ± 0.35
3	PBS	5.36 ± 0.22[f]	4.90 ± 0.13[f]
	Rabbit serum	6.54 ± 0.06	7.11 ± 0.09[g]
	Anti-asialo-GM_1	6.47 ± 0.11[h]	5.84 ± 0.35[h]
	Rabbit serum + poly(A:U)	6.26 ± 0.20	5.28 ± 0.13[i]
4	Rabbit serum	6.91 ± 0.05	6.35 ± 0.06
	Anti-asialo-GM_1	6.16 ± 0.13	5.97 ± 0.26

[a] C57BL/10 mice were infected intraperitoneally with 5×10^6 CFU of *B. abortus* 2308.

[b] In experiments 1 and 2, mice received PBS, anti-asialo-GM_1 antiserum, or an equivalent amount of control rabbit gamma globulin 24 h before and 3 days after infection, while poly(A:U) was given 2 h before as well as 2, 4, and 6 days after infection. In experiment 3, mice were given PBS, control rabbit serum, or anti-asialo-GM_1 antiserum 24 h before infection and 4 days after infection. Poly(A:U) was given 2 h after infection.

[c] Each value represents the mean ± standard error of the mean of CFU recovered from livers and spleens of three to five mice 1 week after infection. In no case did depletion of NK cells result in a significant increase in CFU recovered when experimental groups receiving anti-asialo-GM_1 antisera were compared with control groups receiving rabbit serum or rabbit immunoglobulin. There was a significant increase in the CFU from mice receiving anti-asialo-GM_1 compared with those which received PBS in experiment 3 (*f* versus *h* in same column, groups significantly different from one another). Treatment with poly(A:U) significantly decreased the CFU relative to that of the group receiving PBS in experiment 1 (*d* versus *e* in same column, groups significantly different from one another), while treatment with poly(A:U) plus rabbit serum significantly decreased the CFU relative to that in mice receiving rabbit serum alone in experiment 3 (*g* versus *i* in same column, groups significantly different from one another). Significance means P of < 0.05 by the Wilcoxon test.

depleted of NK cells significantly greater than that in mice which received PBS. These results suggest that control of high-dose infection of *B. abortus* 2308 in the spleens and livers of C57BL/10 mice does not rely on NK cells.

In converse experiments, injection of poly(A:U) to activate NK cells did not significantly decrease the CFU recovered from spleens of C57BL/10 mice (Table 2, see experiments 1 and 3). Thus, the potential exogenous stimulation of NK cells by administration of poly(A:U) did not increase control in the spleen. However, in the liver, poly(A:U) decreased the CFU relative to that in control mice given PBS in experiment 1, the appropriate control in this experiment; in a second experiment (experiment 3), poly(A:U) mixed with rabbit serum decreased the CFU relative to that in mice given rabbit serum alone (Table 2). Since poly(A:U) has pleiotropic effects, including direct effects on macrophages and T cells (3, 19, 20), it was not clear from these experiments if the observed effect is related to NK activation.

Activation of NK cell cytolytic activity following infection of mice with *B. abortus*. Further experiments were performed to evaluate the status of NK cell activation in mice infected with either a high or low challenge dose of *B. abortus* 2308. Results of other studies performed by us suggest that the relative importance of some control mechanisms differs with challenge dose of brucellae. That is, the beneficial effect of anti-interleukin-10 MAb was evident in BALB/c mice at an infection dose of 5×10^3 CFU (7) but not at an infection dose of 5×10^6 CFU (6a). Results presented in Fig. 2 indicate that in C57BL/10 mice infected with the high challenge dose, NK cytolytic activity against YAC-1 cells was nonexistent. This may explain the lack of effect of NK cell depletion in these mice. However, NK cells were activated following infection of C57BL/10 mice with a low challenge dose of *B. abortus*, and the level of NK activity was equivalent to that measured following treatment of mice with poly(A:U).

Effect of NK cell activation and depletion following low-dose infection of C57BL/10 mice. Because we observed a significant activation of NK cells in the spleens of mice infected with the low challenge dose, we evaluated the effect which NK cell depletion had on control of brucellosis in these mice. Results of these experiments indicated that depletion of NK cells did not result in a significant increase in the number of brucellae recovered from spleens of C57BL/10 mice 2 days (Table 1), 4 days, or 1 week (Table 3) after infection. Similarly, when the converse experiment was performed, i.e., mice were given poly(A:U) to potentially increase activation of NK cells in the spleen, the number of CFU recovered from C57BL/10 mice 1 week after infection was not significantly decreased (Table 3). This latter observation might have been predicted since the results in the previous section suggest that activation of NK cells occurs as a result of the infection alone. The observation that depletion of NK cells in mice infected with the low challenge dose did not have a deleterious effect on control of the bacteria despite the clear NK cell activation further supports the conclusion that NK cells are not important for control of brucellae in the spleen. Because of the low number of CFU recovered from livers of mice infected with low doses of *B. abortus* (see reference 9), the CFU in this organ were not evaluated.

Role of NK cells in control of infections in the more-susceptible BALB/c mice. NK cells might be expected to play a role in the susceptible BALB/c mice that was absent or inapparent in the resistant C57BL/10 mice. When these mice were evaluated for NK cytolytic activity following infection with a low challenge dose ($\leq 5 \times 10^3$ CFU), we found that it was increased two- to threefold above that in uninfected control mice (data not shown), similar to results reported for C57BL/10 mice infected with low doses (Fig. 2). When infected BALB/c mice were depleted of NK cells, however, there was not a significant increase in the number of CFU recovered from their spleens 1 week after infection (Table 4). Although livers were also evaluated, the results were generally not informative since the number of CFU was at least 10- to 100-fold lower than that in the spleens, and in some mice, no CFU were recovered (data not shown). This observation is consistent with previous reports (9).

DISCUSSION

In infections with other intracellular bacteria, such as murine listeriosis, NK cells are known to be crucial for early control of infection. The mechanism by which they mediate protection is linked to IFN-γ production (6). Similar results have been reported for experiments with the intracellular protozoan parasites *Toxoplasma gondii* (5, 24, 25) and *Leishmania major* (15, 23). In infections with *L. major*, NK cell production of IFN-γ during the early phase of the infection is important for biasing the CD4 T-cell response towards the Th1 axis and thereby control of leishmaniasis (15, 23). Those results contrast with the data reported here which indicate that NK cells are not important for control of murine brucellosis. This is in the face of extensive evidence that, as for the other intracellular microbes discussed above, IFN-γ plays an important role in host control of *B. abortus*, including during the first week of infection (10, 11, 13, 26).

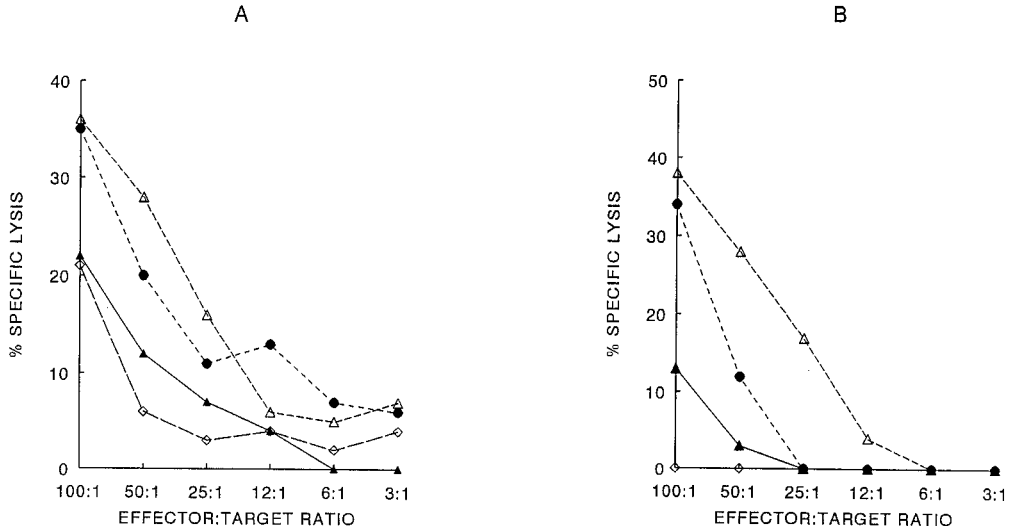

FIG. 2. NK cytolytic activity against YAC-1 cells by splenocytes from C57BL/10 mice. Mice were infected with either 5×10^3 (open triangles) or 5×10^6 (open diamonds) CFU of *B. abortus* 2308, and their splenocytes were evaluated for cytolytic activity either 4 (A) or 7 (B) days after infection. Additional mice were treated with 0.2 ml of PBS (closed triangles) or with 300 μg of poly(A:U) in 0.2 ml of PBS (closed circles) at times corresponding to 0 and 3 days after infection for mice evaluated at 4 days (A) or at times corresponding to 0, 3, and 6 days for mice evaluated at 7 days (B). The average of results from four replicates for each of two mice per treatment and time point is indicated. The variability among replicates was less than 10%.

While not all intracellular microbial parasites have the ability to stimulate NK cells, e.g., *Leishmania donovani* (12, 13), our results indicate that lack of a beneficial role for NK cells in brucellosis was not attributable to a generalized lack of ability of *B. abortus* to stimulate NK cells in infected mice. This was evident from data presented here for both C57BL/10 and BALB/c mice which received the lower infection doses ($\leq 5 \times 10^3$ CFU). Extracts of *B. abortus* have also been shown by others to stimulate NK activity of peritoneal exudate cells and splenocytes in vitro (27). Moreover, we have also shown by biological assays that supernatants from peritoneal exudate cells from both BALB/c and C57BL/10 mice stimulated with *B. abortus* 2308 have interleukin-12 (NK stimulatory factor)-like activity (6b).

TABLE 3. Effect of NK cell depletion or poly(A:U) treatment in C57BL/10 mice infected with the low challenge dose of *B. abortus* 2308, evaluated during the first week of infection

Expt no.	Treatment[a]	Log_{10} CFU/spleen[b]	
		Day 4 p.i.[c]	Day 7 p.i.
1	PBS	3.74 ± 0.35	4.57 ± 0.25
	Rabbit serum	4.65 ± 0.06	5.09 ± 0.05
	Anti-asialo-GM_1	3.47 ± 0.04	4.18 ± 0.24
	Poly(A:U)	ND[d]	5.07 ± 0.10
2	PBS	5.28 ± 0.09	5.24 ± 0.19
	Rabbit serum	5.18 ± 0.10	5.44 ± 0.15
	Anti-asialo-GM_1	4.16 ± 0.04	4.14 ± 0.37
	Poly(A:U)	ND	4.90 ± 0.47

[a] Mice received PBS, anti-asialo-GM_1 antiserum, or control rabbit serum 24 h before and 4 days after infection. Poly(A:U) was given 2 h as well as 2, 4, and 6 days after infection to mice euthanized 1 week after infection, whereas mice euthanized at 4 days after infection were given poly(A:U) at 2 h and 2 days after infection.

[b] Each value represents the mean ± standard error of the mean of CFU recovered from the spleens and livers of five mice. Depletion of NK cells did not result in a significant increase in CFU recovered nor did treatment with poly(A:U) result in a significant decrease.

[c] p.i., postinfection.

[d] ND, not done.

The reasons for lack of importance of NK cells in control of murine brucellosis may be as follows. *B. abortus* replicates relatively slowly and causes chronic rather than acute infections. In contrast, more rapidly replicating organisms such as *Listeria monocytogenes* may result in death of the host within 1 week after infection. Given the slower onset of brucellosis, early production of IFN-γ by NK cells may not be as crucial to control. Furthermore, CD4 T cells which produce IFN-γ in vitro in response to stimulation with brucella antigens are evident in splenocyte populations as early as 4 days after infection of mice with *B. abortus* 2308 (6c). It is possible that the amount of IFN-γ produced by NK cells is minor compared with that produced by $CD4^+$ T cells during this time and therefore not critical to control of brucellosis. Moreover, while Scharton and Scott (23) have shown that production of IFN-γ by NK cells is crucial for biasing $CD4^+$ T-cell responses in

TABLE 4. Effects of NK cell depletion in BALB/c mice infected with the low challenge dose of *B. abortus* 2308 at 1 week postinfection[a]

Expt no.	Treatment[b]	Log_{10} CFU/spleen[c]
1	PBS	5.53 ± 0.09
	Rabbit serum	4.97 ± 0.32
	Anti-asialo-GM_1	5.08 ± 0.21
2	PBS	4.32 ± 0.30
	Rabbit serum	4.44 ± 0.33
	Anti-asialo-GM_1	3.87 ± 0.32
3	Rabbit serum	4.38 ± 0.22
	Anti-asialo-GM_1	3.64 ± 0.98

[a] Mice in experiment 1 received 5×10^3 CFU, while those in experiments 2 and 3 received 3×10^3 as a result of a technical error.

[b] Mice were injected with 0.2 ml of PBS, 50 μl of rabbit serum diluted to a volume of 0.2 ml with PBS, or 50 μl of anti-asialo-GM_1 antiserum diluted to 0.2 ml with PBS 24 h before and 4 days after infection.

[c] Each value represents the mean ± standard error of the mean of three to five mice. There was no significant difference ($P \geq 0.05$ by the Wilcoxon and Student's *t* test) in CFU recovered from mice treated with anti-asialo-GM_1 antiserum and those treated with control rabbit serum.

C3H/HeN mice towards the Th1 axis for control of *L. major*, they also indicate that this is not the case in C57BL/6 mice. The absence of detectable NK cytolytic activity in splenocytes from C57BL/10 mice infected with the higher challenge dose of *B. abortus* (i.e., 5×10^6 CFU) suggests that NK cells also play no role in the development of the Th1 response to *B. abortus* in C57BL/10 mice (6c). However, it is also possible that the lack of detectable NK cytolytic activity in mice as well as in patients with brucellosis (22) reflects only suppression of NK cytolytic activity rather than cytokine production as well. Others have shown that in mice infected with toxoplasma, NK cells are present and produce IFN-γ in the absence of detectable cytolytic activity against YAC-1 cells (10, 25). However, this is purely speculative with regard to brucellosis at this time.

While we found no evidence for a positive role for NK cells in control of brucellosis in mice that did not receive immunopotentiators, regardless of the infection dose, our results indicated increased control of brucellosis in the livers (but not the spleens) of C57BL/10 mice infected with the high challenge dose and treated with poly(A:U). Poly(A:U) is known to affect cells other than NK cells (3, 19, 20), and it has been reported previously by others that the effectiveness of poly(A:U) at reducing the number of brucellae in CBA and athymic congenic mice infected with the attenuated *B. abortus* strain 19 (4, 16) was by its macrophage-activating capacity (17). The role of poly(A:U) for facilitating increased clearance in the studies conducted here may be similar. The generalized lack of a role for NK cells in all other situations evaluated here would support this.

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