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"*Haemophilus somnus*," a Facultative Intracellular Pathogen of Bovine Mononuclear Phagocytes

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We have reported previously that bovine neutrophils are unable to kill the bovine respiratory pathogen "*Haemophilus somnus*." In the present study we expanded our efforts and examined the interaction of bovine mononuclear phagocytes with this important veterinary pathogen. Bovine alveolar macrophages and blood monocytes ingested but did not kill opsonized "*Haemophilus somnus*" in vitro, whereas these same cells ingested and killed opsonized *Escherichia coli*. Because this suggested that "*H. somnus*" was a facultative intracellular pathogen, we developed an assay to monitor the intracellular fate of ingested "*H. somnus*" within bovine monocytes. Our results indicated that ingested "*H. somnus*" multiplied within bovine monocytes (1- to 2-$\log_{10}$ increase in 4 h); equivalent intracellular growth was noted for both a laboratory strain and a recent field isolate of "*H. somnus*." Bovine monocytes killed ingested *E. coli* (1- to 2-$\log_{10}$ decrease in 4 h) under the same assay conditions that were used to follow intracellular growth of "*H. somnus*," thus indicating that the assay conditions did not induce a generalized defect in monocyte antibacterial activity. Light and electron microscopic examination of "*H. somnus*"-infected monocytes confirmed that intracellular growth had occurred. We did not observe an obvious correlation between the release of superoxide anion from bovine mononuclear phagocytes that had ingested opsonized "*H. somnus*" and *E. coli* and the subsequent intracellular survival of the bacteria. The results of this study suggest that infected mononuclear phagocytes sustain "*H. somnus*" infections in cattle and thus contribute to the subacute to chronic clinical course that has been reported.

Pathogenic bacteria have developed various tactics for evading destruction by host defense mechanisms (7). The ability to survive and multiply within cells of the mononuclear phagocyte system is a strategy that has been utilized by a number of important pathogens of humans and domestic animals, including *Mycobacteria* (16), *Brucella* (21), *Salmonella* (8), *Yersinia* (2), and *Listeria* (17) species. By so doing these microbes are able to establish a protected niche within host tissue that sustains local infection and serves as a nidus for dissemination of microorganisms to other sites via migration of infected mononuclear phagocytes through vascular and lymphatic channels.

"*Haemophilus somnus*" is a ruminant pathogen that is of significant economic importance for the beef and dairy cattle industries (4, 14, 22). "*H. somnus*" can infect various organ systems resulting in clinical signs of respiratory disease, arthritis, septicemia, abortion, or fulminant thromboembolic meningoencephalitis (22). Spontaneously occurring and experimentally induced "*H. somnus*" respiratory infections are typically subacute to chronic, thus suggesting that innate host defense mechanisms are unable to eliminate the organism during the early stages of infection (1; L. N. D. Potgieter, R. G. Helman, and W. H. Green, Abstr. 66th Annu. Meet. Conf. Res. Workers Anim. Dis. 1985, Abstr. no. 238, p. 44; S. C. Groom and P. B. Little, Abstr. 66th Meet. Conf. Res. Workers Anim. Dis. 1985, abstr. no. 240, p. 44). We recently reported that bovine neutrophils, which are a prominent cell type in "*H. somnus*"-infected lesions, are unable to kill ingested "*H. somnus*" (5). As a result of this study we became interested in examining the interaction of bovine mononuclear phagocytes with "*H. somnus*" in hopes of identifying how this encounter contributes to the pathogenesis of "*H. somnus*" infections in cattle. Our initial experiments indicated that bovine alveolar macrophages and blood monocytes, like blood neutrophils, were able to ingest but not kill opsonized "*H. somnus*." We then devised an assay that allowed us to follow the fate of ingested "*H. somnus*" under conditions in which extracellular growth of uningested bacteria was inhibited. Our results indicated that "*H. somnus*" can multiply within bovine mononuclear phagocytes under experimental conditions in which monocytes are not compromised in their ability to kill other ingested bacteria such as *Escherichia coli*. These data indicate that "*H. somnus*" should be considered a facultative intracellular pathogen and that intracellular survival and growth within bovine mononuclear phagocytes may be a virulence mechanism for "*H. somnus*" in cattle.

MATERIALS AND METHODS

Bacteria. "*H. somnus*" was obtained from the Wisconsin Central Animal Health Laboratory (Madison). *E. coli* ATCC 25922 was obtained from the clinical pathology laboratory of the University of Wisconsin School of Veterinary Medicine. Stationary-phase cultures of "*H. somnus*" and *E. coli* were maintained at −70°C. Before each experiment a sample was thawed, inoculated into brain heart infusion broth that contained 0.5% yeast extract, and incubated at 37°C in the presence of 5% CO_2 for 16 to 22 h. Bacteria were recovered and washed twice by centrifugation in cold Hanks balanced salt solution that contained 0.25% bovine serum albumin (HBSA) before being used in that day's experiment.

Source of bovine mononuclear phagocytes. Lungs and blood were obtained from healthy cattle at a local slaughterhouse. With sodium citrate (0.38% final concentration) as an anticoagulant, heart blood was collected within 2 min after

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stunning of the animal. Lungs were dissected out of the same animal within 20 min after death and immediately lavaged to obtain alveolar macrophages.

Preparation of bovine alveolar macrophages. Lung cells were recovered by lavaging the excised lungs twice with sterile pH-balanced (7.2) saline that contained 0.2% EDTA. The lavage fluid was poured into a sterile siliconized Erlenmeyer flask and then aliquoted into 50-ml plastic centrifuge tubes that were kept on ice while being transported to the laboratory. The lung cells were washed three times (300 × *g* for 10 min at 4°C) and suspended at 4.1×10^6 viable cells per ml in HBSA. Cell viability was measured by trypan blue exclusion (mean viability of 70% for the five cell preparations reported in this study). Lung cell suspensions contained an average of 88% alveolar macrophages as determined by microscopic examination of Diff-Quik-stained cytospin smears.

Isolation of bovine blood mononuclear cells. Citrated blood was centrifuged (300 × *g* for 20 min at 22°C) to separate blood cells from platelet-rich plasma. The buffy coat cells were removed, diluted 1:4 in sterile phosphate-buffered saline, and separated on Ficoll-Hypaque gradients to obtain mononuclear cell-rich populations. These were removed, washed three times, and suspended in cold HBSA at 4.2×10^6 cells per ml (20 to 25% monocytes). Mononuclear cell viability as determined by trypan blue exclusion averaged 95% or better.

Immune serum. Serum was obtained from a calf that was immunized repeatedly with Formalin-killed "*H. somnus*" as described previously (5).

Phagocytosis assay. Phagocytosis of "*H. somnus*" and *E. coli* by bovine alveolar macrophages and blood monocytes was determined as described previously (5) by mixing 2.5×10^6 phagocytes, 2.5×10^7 bacteria, and 10% immune serum in a total volume of 1.0 ml of HBSA in duplicate plastic tubes (Falcon; 12 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.). The tubes were rotated (Labquake; Labindustries, Berkeley, Calif.) at 8 rpm for 10 to 60 min at 37°C. Extracellular bacteria were removed by three slow-speed centrifugations (100 × *g* for 5 min at 4°C) in 1.0 ml of HBSA. The washed phagocytes were suspended in 1.0 ml of cold HBSA and used to prepare cytospin smears that were stained with Diff-Quik and examined by light microscopy with an oil immersion objective. At least 100 phagocytes per slide were scored for the percentage of phagocytes that ingested bacteria and for the number of bacteria associated with each ingesting phagocyte. The results were expressed as a phagocytic index (PI) where PI = (the percentage of ingesting phagocytes × the average number of bacteria per ingesting phagocyte) × 100. The validity of this assay for assessing phagocytic activity has been confirmed previously by electron microscopy (5).

Bactericidal assay. The bactericidal activity of bovine alveolar macrophages and blood mononuclear cells was determined by incubating "*H. somnus*" or *E. coli* (2.5×10^6 cells) with an equal number of phagocytes and 10% immune serum in triplicate ("*H. somnus*") or duplicate (*E. coli*) plastic tubes (12 by 75 mm) in a total volume of 1.0 ml of HBSA. The capped tubes were rotated at 8 rpm for 2 h at 37°C. At the beginning of the assay, and after the 2-h incubation period, 0.1-ml samples were removed, serially diluted in sterile distilled water, and plated in duplicate on chocolate agar. The plates were incubated overnight at 37°C in the presence of 5% CO_2, and then the number of CFU was determined. Results were expressed as the mean ± standard error of the mean (five separate experiments) $\log_{10}$ change (as compared with the inoculum) in the number of viable bacteria.

Intracellular growth of "*H. somnus*." We modified the phagocyte bactericidal assay of Leijh et al. (15) to follow the fate of "*H. somnus*" within bovine monocytes. Blood mononuclear cells were obtained from living healthy cattle that were maintained in the School of Veterinary Medicine teaching and research herd. Suspensions that contained 1×10^7 mononuclear cells (20 to 25% monocytes), 2.5×10^6 "*H. somnus*" (approximately one bacterium per monocyte), and 10% immune serum in a total volume of 1.0 ml of HBSA were rotated for 30 min at 37°C. After this the uningested bacteria were removed by three slow-speed washes with cold HBSA (100 × *g* for 5 min at 4°C), and the bacteria-containing mononuclear cell pellets were suspended in 1.0 ml of RPMI 1640 medium that contained 10% fetal bovine serum (which does not facilitate ingestion of additional bacteria), and 1 μg of gentamicin per ml. Because gentamicin does not readily cross the bovine monocyte cell membrane (6), this system provided a means to follow the intracellular fate of "*H. somnus*" in the absence of significant extracellular growth by any uningested bacteria. The suspended pellets were incubated at 37°C while being rotated continuously. At 1, 2, 4, and 18 h triplicate tubes were removed and again subjected to the same slow-speed washing procedure described above. This allowed us to remove the inhibitory effects of gentamicin before the cell pellets were diluted in sterile water and plated on chocolate agar. Colonies were counted after 48 h of incubation at 37°C in 5% CO_2, and the results are expressed as the $\log_{10}$ increase in "*H. somnus*" at that time point as compared with the number of "*H. somnus*" recovered from the infected monocytes at the beginning of the incubation period. Microscopic examination of Diff-Quik-stained cytospin smears revealed large numbers of intracellular bacteria within monocytes after 4 to 18 h of incubation. Cell-free control tubes, which were included in all experiments, indicated that in the absence of gentamicin there was a 1.5-$\log_{10}$ increase in viable "*H. somnus*" cells at 4 h and at least a 3-$\log_{10}$ increase at 18 h, whereas in the presence of 1 μg of gentamicin per ml the number of viable "*H. somnus*" remained static throughout the incubation period. We also determined that bovine mononuclear cells did not absorb the inhibitory activity of gentamicin for "*H. somnus*" out of the tissue culture medium. Using this same assay, bovine monocytes were able to kill intracellular *E. coli*, thus indicating that the observed intracellular growth of "*H. somnus*" was not caused by a generalized defect in monocyte antibacterial activity that was induced by the experimental conditions. Infected mononuclear cells were fixed and prepared for electron microscopy as we have described previously (5).

Superoxide assay. The extracellular release of superoxide anion (O_2^-) was determined by measuring the reduction of cytochrome *c* as described originally by Johnston (13) and adapted for use with bovine mononuclear phagocytes in our laboratory (26). Briefly, phagocytes were harvested as described above and washed three times in Hanks balanced salt solution without phenol red. The bacteria were grown overnight and washed as described above, suspended in sterile Dulbecco modified phosphate-buffered saline (GIBCO Laboratories, Grand Island, N.Y.), and preopsonized by incubation with an equal volume of immune serum for 30 min in a 37°C water bath. The reaction mixtures contained 2.5×10^6 mononuclear phagocytes and 2.5×10^8 preopsonized bacteria (100 bacteria per phagocyte) or phorbol myristic acetate (PMA, 0.5 μg) in a total volume of

1.0 ml of Hanks balanced salt solution that contained 80 μM cytochrome *c*. Some reaction mixtures contained superoxide dismutase (40 μg) to confirm that reduction of cytochrome *c* was O_2^- dependent (PMA, superoxide dismutase, and cytochrome *c* were obtained from Sigma Chemical Co., St. Louis, Mo.). These reaction mixtures were rotated in capped plastic tubes (12 by 75 mm) for 90 min at 37°C and then pelleted (8,000 × *g* for 3 min) in a microcentrifuge (Eppendorf). The absorbance of the supernatants was determined at 550 nm with a Beckman DU-6 spectrophotometer (1-nm slit width), and the readings were converted to nanomoles of cytochrome *c* reduced by using the molar extinction coefficient $\Delta E_{550} = 21.0 \times 10^3\ M^{-1}\ cm^{-1}$ as described previously (13). Results were expressed as the mean ± standard error of the mean nanomoles of cytochrome *c* reduced per 10^6 phagocytes (at least four separate experiments).

Statistical analysis. Data were analyzed for statistical significance by a one-way analysis of variance with the BMDP statistical software package (University of California Press, 1981). If a significant *F* value ($P < 0.05$) was obtained, relevant comparisons were made by the paired *t* test with the same statistical package. The level of significance was set at $P < 0.05$.

RESULTS

Can suspensions of bovine alveolar macrophages and blood monocytes ingest and kill "*H. somnus*" in vitro? Monocytes and alveolar macrophages rapidly ingested comparable numbers of "*H. somnus*" in the presence of 10% immune serum (Fig. 1), an observation that is similar to what we reported previously for bovine neutrophils (5). Ingestion plateaued by 30 min of incubation; increasing the incubation period to 60 min did not result in continued uptake of "*H. somnus*" (data not shown). Neither alveolar macrophages nor blood

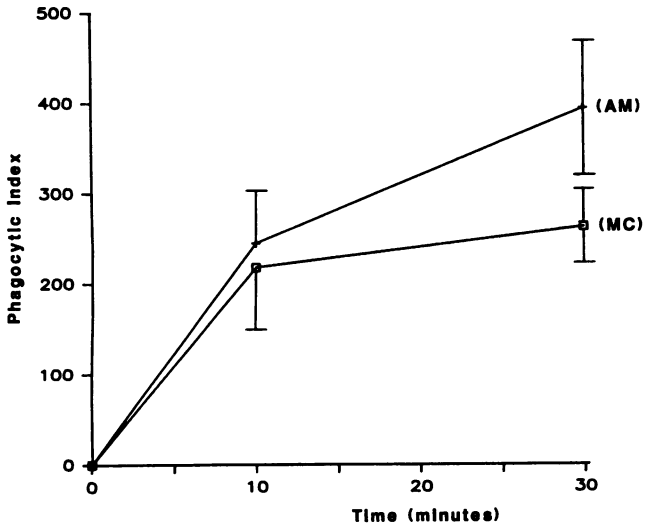

FIG. 1. Ingestion of "*H. somnus*" by bovine alveolar macrophages (AM) and blood monocytes (MC) obtained from the same donors. Suspensions containing 2.5×10^6 cells and 2.5×10^7 "*H. somnus*" were incubated in the presence of 10% immune serum for 10 to 30 min. The uningested bacteria were removed by three washes in cold HBSA (100 × *g* for 5 min at 4°C), and the cell pellets were used to prepare cytospin smears that were Diff-Quik stained and examined microscopically. Results are expressed as the mean ± standard error of the mean phagocytic index (as described in Materials and Methods) for cells from five separate donors.

TABLE 1. Bovine alveolar macrophages and blood mononuclear cells failed to kill "*H. somnus*" under conditions in which they killed *E. coli*

Treatment group	Log_{10} change in viable bacteria[a]	
	"*H. somnus*"	*E. coli*
Alveolar macrophages	+0.45 ± 0.20	−0.51 ± 0.12
Blood macrophages	+0.47 ± 0.23	−0.42 ± 0.08
Cell-free control	+0.47 ± 0.27	+0.46 ± 0.22

[a] Mean ± standard error of the mean (five separate experiments) $\log_{10}$ change in CFU, as compared with the inoculum after a 2-h incubation at 37°C.

monocytes killed "*H. somnus*" during a 2-h incubation period in vitro, whereas these same populations of mononuclear phagocytes killed *E. coli* (Table 1).

Can "*H. somnus*" grow within bovine mononuclear phagocytes? The above experiments indicated that bovine mononuclear phagocytes had little ability to kill "*H. somnus*" cells in vitro. We therefore became interested in determining whether "*H. somnus*" is actually a facultative intracellular pathogen that can multiply within bovine mononuclear phagocytes. To answer this question, we modified the procedure of Leijh et al. (15) and devised an in vitro assay that largely eliminated uningested bacteria by repeated slow-speed centrifugation (100 × *g* for 5 min at 4°C) of the infected mononuclear cells. Extracellular growth of any remaining bacteria was inhibited by the addition of gentamicin (1 μg/ml). Because gentamicin does not readily cross the bovine monocyte cell membrane (6) and subsequent washing removed the gentamicin before the infected monocytes were lysed, this system did not interfere with our ability to quantitate accurately the number of monocyte-associated "*H. somnus*." Using this assay we observed a significant increase ($P < 0.05$) in the number of intracellular "*H. somnus*" within 4 h of incubation (Fig. 2). The number of "*H. somnus*" continued to increase with time; at 18 h the number of viable "*H. somnus*" recovered from infected monocytes had increased by approximately 2.2 $\log_{10}$ (Fig. 2). In each experiment we included cell-free control tubes, both with and without gentamicin, to confirm that the antibiotic had indeed restricted extracellular bacterial growth. We noted a similar increase ($P < 0.05$ at 2 h of incubation) in the number of monocyte-associated "*H. somnus*" when we used a recent field isolate of "*H. somnus*" (data not shown). The experimental conditions used in these experiments did not compromise the general bactericidal activity of bovine monocytes as documented by their ability to kill ingested *E. coli* (Fig. 3; $P < 0.05$ at 2 h of incubation). Light and electron microscopy confirmed our microbiological determination of the intracellular growth of "*H. somnus*." Initially we observed few bacteria within "*H. somnus*"-infected monocytes (Fig. 4A). By 4 h of incubation additional bacteria were observed within monocytes, and by 18 h large numbers of bacteria could be seen enmeshed within the cytoplasmic debris of degenerating monocytes (Fig. 4B). Extracellular bacteria were very rare initially, thus confirming that our washing procedure had effectively removed most of the uningested bacteria (5, 14). Although at 18 h of incubation we observed clumps of extracellular bacteria, these typically were adherent to what appeared to be monocyte debris. On the basis of these visual observations and on our recovery of low numbers of bacteria from the supernatant fluid and from unwashed monocyte pellets, we conclude that as "*H. somnus*" multiplied intracellularly they eventually damaged the host monocytes, which then released "*H.*

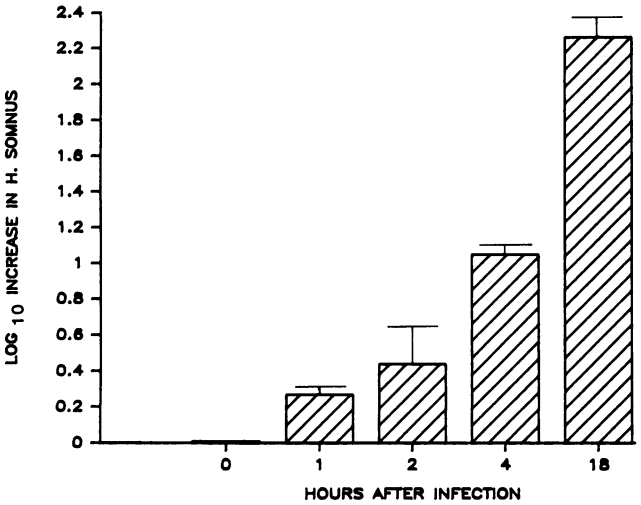

FIG. 2. Intracellular growth of "*H. somnus*" in bovine monocytes. Suspensions of blood mononuclear cells (10^7) were incubated with "*H. somnus*" in the presence of 10% immune serum for 30 min at 37°C. The uningested bacteria were removed by three washes with cold HBSA (100 × *g* for 5 min at 4°C) before samples were removed, diluted in sterile water, and plated on chocolate agar to determine the number of viable "*H. somnus*" initially present in the monocytes. The infected mononuclear cell pellets were suspended in RPMI 1640 medium that contained 5% fetal bovine serum and an inhibitory concentration of gentamicin (1 μg/ml) and rotated at 37°C for 1 to 18 h, and at the times indicated the mononuclear cells were washed free of gentamicin and samples were removed for dilution and plating on chocolate agar. Results are expressed as the mean ± standard error of the mean $\log_{10}$ increase in the number of "*H. somnus*" for four separate experiments (time points determined in triplicate for each experiment).

somnus" into the extracellular milieu where further bacterial growth was inhibited by gentamicin.

Is intracellular growth of "*H. somnus*" reflected in a decreased release of superoxide anion by infected mononuclear phagocytes? We reported previously that the inability of bovine neutrophils to kill "*H. somnus*" was associated with a relatively weak oxidative response after ingestion of opsonized "*H. somnus*" (5). We therefore decided to determine whether the ability of "*H. somnus*" to parasitize bovine monocytes was associated with a similar depression of phagocyte oxidative activity. Although bovine alveolar macrophages and monocytes released sizeable amounts of superoxide anion when stimulated with PMA ($P < 0.05$), they produced relatively little superoxide anion ($P > 0.05$) when stimulated with either opsonized "*H. somnus*" or *E. coli* (Table 2). Thus, we did not demonstrate an obvious association between bacterial killing and release of superoxide anion by bovine mononuclear phagocytes ($P > 0.05$) for comparisons of "*H. somnus*"- and *E. coli*-stimulated mononuclear phagocytes with each other in Table 2).

DISCUSSION

Spontaneously occurring and experimentally induced "*H. somnus*" respiratory infections are characteristically subacute to chronic in nature and typically result in necrotic bronchopneumonia (1; Potgieter et al., abstr. no. 238; Groom and Little, abstr. no. 240). Infiltration of inflammatory neutrophils was an especially prominent histological feature; however, inflammatory macrophages and occasional giant cells were also observed (1). We previously reported that bovine neutrophils did not kill ingested "*H. somnus*" in vitro, thus suggesting that accumulation of large numbers of neutrophils in the lung does not eliminate "*H. somnus*" but instead might actually contribute to the pulmonary damage that occurs (5). Because alveolar macrophages have been shown to play a prominent role in protection of the lung against bacterial pathogens (10), and recently emigrated blood monocytes also contribute to pulmonary antibacterial defense (24), we decided to examine the interaction of bovine mononuclear phagocytes with "*H. somnus*" in vitro in hopes of delineating their role in the pathogenesis of "*H. somnus*" infections. As noted previously for bovine neutrophils (5), bovine alveolar macrophages and blood monocytes ingested opsonized "*H. somnus*" but failed to kill the internalized bacteria (Table 1). By documenting that these same cells were capable of killing *E. coli* we were able to exclude the possibility that our procedures for recovery of these cell populations had adversely affected their antibacterial activity.

The above-described experiments suggested to us that by its ability to survive within bovine mononuclear phagocytes, "*H. somnus*" might provide itself with a niche, protected against the adverse effects of bactericidal serum components (20), in which to multiply and sustain the infection. We therefore decided to determine the fate of ingested "*H. somnus*" within bovine monocytes. Because our assay used several slow-speed centrifugations to gently pellet the infected mononuclear cells, it allowed us largely to remove uningested bacteria from the reaction mixtures (verified by electron microscopy). The addition of an inhibitory dose (1 μg/ml) of gentamicin to the reincubated infected monocytes suppressed the extracellular growth of any residual bacteria that might have confounded our ability to determine the intracellular growth of "*H. somnus*." Using this assay we obtained convincing microbiological evidence that both a laboratory strain and a recent field isolate of "*H. somnus*" multiplied within bovine monocytes (Fig. 2) under conditions in which intracellular *E. coli* (Fig. 3) were killed, thus confirming and extending the above-described results that

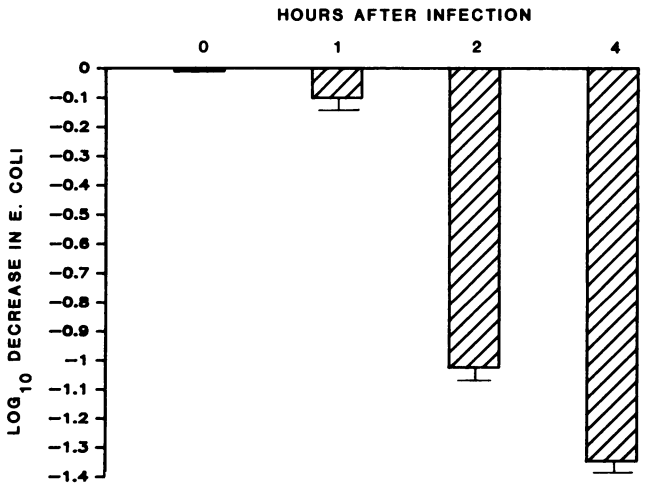

FIG. 3. Intracellular killing of *E. coli* within bovine blood monocytes. Experimental conditions are identical to those described in the legend to Fig. 2 except that the bacteria were plated on blood agar rather than on chocolate agar. Results are expressed as the mean ± standard error of the mean $\log_{10}$ decrease in the number of viable *E. coli* for two separate experiments.

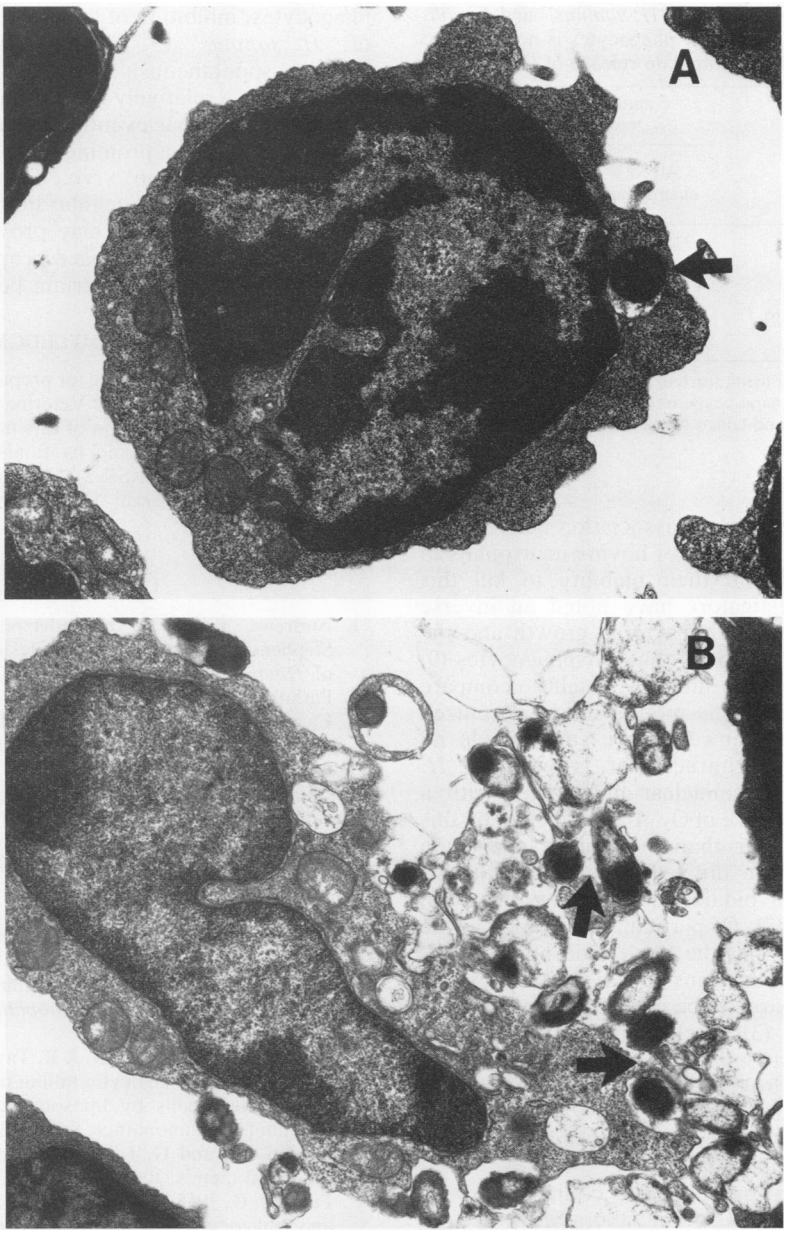

FIG. 4. Electron micrographs of "*H. somnus*"-infected bovine monocytes. The monocyte in panel A was fixed for electron microscopy after 2 h of incubation and contains a single intracellular "*H. somnus*" (arrow). After 18 h of incubation, the monocyte in panel B appears to be undergoing dissolution and is releasing a number of "*H. somnus*" (arrows) into the extracellular milieu where their continued growth was inhibited by gentamicin.

were obtained with suspensions of mononuclear phagocytes (Table 1).

Electron microscopic examination of infected monocytes indicated that initially most infected monocytes contained a single or in some instances two to three bacteria and that extracellular bacteria were not readily apparent (Fig. 4A). By 4 h of incubation we observed monocytes that contained increased numbers of bacteria, and by 18 h of incubation large numbers of "*H. somnus*" could be seen enmeshed in the cytoplasmic debris of degenerating monocytes (Fig. 4B). Light microscopic examination of Diff-Quik-stained cytospin smears and adherent monolayers of "*H. somnus*"-infected monocytes also suggested that intracellular multiplication of "*H. somnus*" had occurred. Although the physical stress associated with centrifuging the bacteria-laden monocytes and preparing them for electron microscopic examination may have contributed to the monocyte damage that we observed after 18 h of incubation, these observations are also consistent with a detrimental effect of "*H. somnus*" components on bovine monocytes. "*H. somnus*" has previously been reported to be cytotoxic to bovine endothelial cells (23) and alveolar macrophages (D. Liggitt, L. Huston, and L. Corbeil, Abstr. 65th Annu. Meet. Conf. Res. Workers Anim. Dis. 1984, abstr. no. 174, p. 31) and to contain both high- and low-molecular-weight components that are inhibitory for various functions of bovine neutrophils (3, 12); these components might adversely affect bovine monocytes as well.

TABLE 2. Difference in the ability of "*H. somnus*" and *E. coli* to resist killing by bovine mononuclear phagocytes is not reflected in their relative ability to stimulate release of O_2^-

Stimulant	nmol of cytochrome *c* reduced/10^6 cells[a]	
	Alveolar macrophages	Blood mononuclear cells
"*H. somnus*"[b]	2.46 ± 0.46	0.27 ± 0.08
E. coli[b]	3.80 ± 1.32	0.23 ± 0.07
PMA (0.5 μg)	11.18 ± 2.42	3.62 ± 0.22
PMA (0.5 μg) + SOD[c] (40 μg)	2.46 ± 0.63	0.22 ± 0.05
Unstimulated	0.48 ± 0.19	0.42 ± 0.10

[a] Mean ± standard error of the mean for four (alveolar macrophages) and five (blood mononuclear cells) separate experiments.
[b] Bacteria were preopsonized and added at a 100:1 bacteria-to-phagocyte ratio.
[c] SOD, Superoxide dismutase.

We have previously reported an association between the relatively weak oxidative response of bovine neutrophils to opsonized "*H. somnus*" and their inability to kill this organism (5). Other investigators have noted an inverse association between intracellular bacterial growth and the oxidative response of infected mononuclear phagocytes (9, 19, 25). In the present study we similarly sought to compare the oxidative response of bovine monocytes to opsonized "*H. somnus*" and *E. coli*; however, we can provide no direct evidence linking the intracellular growth of "*H. somnus*" within bovine mononuclear phagocytes with a relative deficiency in the release of O_2^- that is elicited by this organism. Mechanisms other than evasion of phagocyte oxidative activity have been utilized by other facultative intracellular pathogens to avoid destruction and grow within mononuclear phagocytes (7). Of particular relevance to the present study are reports that the nucleotide pool of "*H. somnus*" inhibits various functions of bovine neutrophils. This inhibitory effect appeared to be mediated principally by adenosine and guanosine (3), nucleosides that have been reported to influence human monocyte function as well (18). The contributions of "*H. somnus*"-released nucleosides to the intracellular survival and growth of "*H. somnus*" within bovine monocytes remains an intriguing possibility that will be considered in future studies.

The results obtained in this study, in conjunction with previous reports from this and other laboratories, greatly increase our understanding of the role that bovine phagocytic cells play in the pathogenesis of "*H. somnus*" infections. If we extrapolate from these principally in vitro studies to the events that occur during in vivo infection, it would appear that inflammatory neutrophils that accumulate at sites of infection are unable to kill substantial numbers of "*H. somnus*." As a result of this encounter, however, the neutrophils may release various inflammatory mediators that cause local tissue damage and result in formation of the thrombotic lesions that are characteristic of "*H. somnus*" infections. Some "*H. somnus*" will be engulfed by tissue macrophages or recently emigrated blood monocytes. Instead of killing the ingested "*H. somnus*," the mononuclear phagocytes may protect the bacteria against bactericidal serum components (20) and provide them with a privileged site in which to multiply. Infected macrophages thus may prolong the infection, a situation that could explain in part the subacute to chronic course of "*H. somnus*" infections (1, 11). Although at this time we do not know the mechanism that allows "*H. somnus*" to avoid destruction by bovine phagocytes, inhibition of leukocyte function by components of "*H. somnus*" (3, 12) may contribute to this process. Because spontaneously occurring "*H. somnus*" infections usually have a relatively low mortality rate, it seems reasonable to assume that eventual activation of cellular defense mechanisms plays a prominent role in restricting the growth of "*H. somnus*" in vivo. Future studies of the immunoregulation of the cellular immune response of cattle to "*H. somnus*" therefore may provide valuable information that ultimately could be used prophylactically or therapeutically to combat this important bovine pathogen.

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