INFECTION AND IMMUNITY, May 1973, p. 691-699

Vol. 7, No. 5
Printed in U.S.A.

Interaction of *Mycoplasma arthritidis* and Other Mycoplasmas with Murine Peritoneal Macrophages

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Received for publication 11 October 1972

Neither mouse nor rat peritoneal macrophages were able to kill *Mycoplasma arthritidis* to any observable degree in the absence of specific hyperimmune rabbit antiserum. Although convalescent mouse and rat serum were somewhat inhibitory to *M. arthritidis* in the absence of macrophages, these sera did not promote active phagocytosis by peritoneal macrophages. In fact, the macrophages appeared to protect the mycoplasmas against the inhibitory effects of the antisera by stimulating their growth. Hyperimmune rabbit antiserum against *M. arthritidis* initiated phagocytic action and resulted in a 50-fold decrease in numbers of viable mycoplasmas by 6 h. In contrast with *M. arthritidis*, *M. pulmonis* rapidly adsorbed to the surface of peritoneal macrophages. Upon addition of specific rabbit antiserum, a rapid decrease in viable organisms occurred, and a more complete destruction of organisms ensued in comparison with *M. arthritidis*. *M. gallinarum*, as with *M. arthritidis*, did not adsorb to the macrophages to any great extent. Phagocytic action was observed only in the presence of homologous rabbit antiserum and was not marked until after 6 h of incubation.

The significance of the interactions between mycoplasmas and mammalian cells has only recently begun to be realized. Current knowledge on this subject was recently reviewed by Stanbridge (23). A number of investigations have shown that certain mycoplasma species are capable of adsorbing to the surfaces of animal cells (14, 17, 18, 19, 24, 27). Several different receptor sites appear to be involved in this process (18, 19). Electron microscopic studies by Zucker-Franklin et al. (26) indicated that *Mycoplasma pneumoniae*, *M. neurolyticum*, and *M. gallisepticum* rapidly adsorbed to human blood leukocytes and were promptly phagocytized in the absence of specific antiserum. Jones and Hirsch (15) used *M. pulmonis* and observed the adsorption of this species to mouse peritoneal macrophages but reported that homologous rabbit antiserum was required for ingestion and killing. Simberkoff and Elsbach (22) reported that *M. hominis* and *M. arthritidis* did not adsorb to human and rabbit polymorphonuclear leukocytes and that phagocytic action could not be induced even in the presence of rabbit anti-mycoplasmal sera. Evidence that these mycoplasma species impaired macrophage function was also obtained.

The significance of mycoplasma-cell associations in vivo has recently received attention, particularly in regard to experimental *M. pneumoniae* disease. Thus, the ability of this species to adsorb to and multiply at the surface of the bronchial epithelium is believed to contribute to the cytotoxicity induced in cells as a result of hydrogen peroxide production by the organisms (5, 10). There is also evidence that a delayed hypersensitivity reaction of the host cells to the invading organisms occurs at the site of the lesion (11). The interaction of *M. arthritidis* with murine macrophages has not been studied. Previous investigations in our laboratories have failed to demonstrate a relationship between circulating antibody and resistance of rodents to *M. arthritidis* (6, 8). Other studies have shown that both mice and rats are deficient in their ability to eliminate virulent *M. arthritidis* from the peripheral circulation (in press), and persistence of the organisms is observed well after the appearance of circulating complement-fixing (CF) antibody. Whereas

rats develop an acute, suppurative, short-term arthritis after injection of *M. arthritidis* (25), mice develop a chronic, proliferative disease with periods of remission and exacerbation (9). The reasons for the differing pathological responses of rats and mice to *M. arthritidis* remain unknown.

The present study was undertaken to study the response of mouse and rat macrophages toward *M. arthritidis* and to attempt to relate this to virulence of the organisms and mechanisms of immunity.

MATERIALS AND METHODS

Mycoplasma strains used. Unless otherwise stated, all experiments were performed with *M. arthritidis* strain 158 P10 P9. This culture is highly virulent for both mice (9) and rats (unpublished observations). The virulent DL strain was used in some experiments as were the less virulent strains H606 and PG6 (12). *M. pulmonis* strain 19612 was obtained from the American Type Culture Collection (Rockville, Md.) and *M. gallinarum* strain ST114 was received from D. L. Madden (Bethesda, Md.).

Cultivation and enumeration of mycoplasmas. The basic medium used consisted of PPLO agar or broth (Difco) supplemented to a final concentration of 15% (vol/vol) horse serum, 5% (vol/vol) fresh yeast extract, and 1,000 U of penicillin per ml (4, 13). The preparation and counting of mycoplasma suspensions were as described previously (12).

Antibody studies. CF and metabolic inhibition (MI) antibody tests were performed as described previously (2, 12, 20).

Growth of mycoplasmas in cell culture media. Eagle minimal essential medium (MEM) was supplemented to a final concentration of 1% (vol/vol) of a 200 mM sterile solution of L-glutamine and 0%, 5%, 10%, or 20% (vol/vol) sterile fetal calf serum (FCS). Two-milliliter samples of the media were added to sterile disposable petri dishes, 35 × 10 mm which then were inoculated with 0.2 ml of MEM containing 3×10^4 colony-forming units (CFU) of *M. arthritidis*. Samples were counted at 6 h and at 1, 2, 3, 4, and 6 days after incubation at 37 C in a CO_2 incubator.

Collection of macrophages. "Unstimulated" macrophages were collected by injecting mice intraperitoneally (i.p.) with 2 ml of cold phosphate-buffered saline at pH 7.2 containing 5 U of heparin per ml. Rats received 10 to 40 ml of the heparinized saline. After 1 h, the animals were killed with ether and, after gentle kneading of the abdomen, the peritoneal contents were removed with a pipette. Macrophage suspensions were washed twice by a centrifugation at 600 × *g* and finally resuspended in MEM supplemented to a final concentration of 10% (vol/vol) FCS and 1% (vol/vol) of a 200 mM sterile solution of glutamine (Microbiological Associates Inc.).

"Stimulated" macrophages were prepared by injecting mice i.p. with 2 ml of thioglycollate broth (1). Rats were given 10 ml of thioglycollate broth. After 3 to 4 days the animals were treated with heparinized phosphate-buffered saline, and the macrophages were collected as before.

Effect of trypsin and macrophage extracts on mycoplasma growth. Since the assay procedure which was used employed trypsin to aid in the removal of adhered macrophages, a preliminary experiment was set up to determine whether trypsin was inhibitory to *M. arthritidis*. Mycoplasma suspensions containing approximately 10^5 and 10^6 CFU of *M. arthritidis* per ml were exposed to 0.25% (wt/vol) trypsin. Samples were counted for mycoplasmas at 30 and 90 min.

Cell extracts have been reported to be inhibitory to mycoplasmas (16). Since macrophages were to be homogenized during the assay procedure, the effect of macrophage extracts on the viability of *M. arthritidis* was investigated. A suspension of unstimulated rat macrophages, 2.8×10^7 cells per ml of mycoplasma broth, was homogenized in a teflon grinder until the cells were disrupted. The homogenate was passed through a 0.45-μm membrane filter and then through a 0.1-μm Seitz filter. This filtrate was added to a suspension of 5×10^6 CFU of *M. arthritidis* strain 158 P10 P9 per ml with final concentrations of extract of 1:2, 1:10, 1:50, and 1:250. Samples of 0.2 ml were counted for mycoplasmas after 3, 24, 48, and 72 h.

Interaction of macrophages with mycoplasmas. The phagocytic potential of the peritoneal cells was judged to be greater than 95% as evidenced by their ability to ingest carbon particles. A variety of procedures were used to initiate phagocytic action by unstimulated and stimulated peritoneal macrophages. The procedure most commonly used was as follows. Mouse or rat macrophages were collected as described above, and 2×10^6 cells in 2 ml of MEM were distributed in petri dishes, 35 × 10 mm in diameter. After 2 h of overnight incubation at 37 C in a CO_2 incubator, the plates were washed in warm MEM to remove lymphocytes, and MEM containing approximately 2×10^5 CFU of the mycoplasma culture being tested was added. Mycoplasma counts were usually performed at zero time, 2 h, 5 to 6 h, 24, and 48 h after infection. The supernatants were removed and 1 ml of fresh MEM was added to each plate to wash the attached macrophages. The washings and the original supernatants were pooled, centrifuged at 600 × *g*, and separated from any macrophages which sedimented. The macrophage monolayers were incubated at 37 C with 0.25% (wt/vol) trypsin and, if necessary, were removed with a rubber policeman. The resulting macrophage suspension was added to the sediment from the supernatants and was homogenized in a sterile teflon homogenizer. Microscopic examination confirmed that cell disruption had occurred. Supernatant and macrophage samples were counted for mycoplasmas. Variations on the technique will be alluded to below.

Control experiments to determine the growth characteristics of the mycoplasmas in the absence of macrophages were always performed in petri dishes incubated at 37 C in a CO_2 incubator.

Effect of immune serum. The convalescent mouse serum employed was that described previously (8). It exhibited a CF titer of 1:320 and an MI titer of 1:2 at the time of the experiment. After overnight incubation of mouse macrophage monolayers (2×10^6 cells per dish), the medium was removed and replaced with complete base medium containing *M. arthritidis* strain 158 P10 P9 (5×10^5 CFU per dish). Two hours later, macrophage and supernatant counts for mycoplasma were carried out on two dishes. The remaining dishes were immediately treated with either: (i) 0.2 ml of 1:10 pooled normal serum, (ii) 0.2 ml of 1:10 pooled 41-day convalescent mouse serum, (iii) 0.2 ml of 1:50 41-day pooled convalescent mouse serum. All serum dilutions were made in complete base medium. Macrophage and supernatant counts for mycoplasmas were made on two dishes of each of the above after 2 h, 6 h, 24 h, and 48 h of incubation at 37 C in a CO_2 incubator. A control experiment was also set up as above but in the absence of macrophages. Final serum dilutions of 1:10, 1:100, and 1:500 were tested for their inhibiting effects on *M. arthritidis*.

A similar experiment was set up with stimulated rat macrophages in the presence of 1:100 and 1:500 convalescent rat serum and 1:100 normal rat serum. The serum used was taken from rats 8 weeks after infection by 2×10^9 CFU of *M. arthritidis* strain 158 P10 P9, and it exhibited a CF titer of 1:640 and an MI titer of less than 1:2. Control runs were undertaken as before in the absence of macrophages.

The above procedure was used to determine the effect of hyperimmune rabbit antiserum against *M. arthritidis* on the phagocytic activity of peritoneal rat macrophages. The hyperimmune rabbit antiserum was prepared against *M. arthritidis* strain 14152 P13 sc as described previously (12) and exhibited a CF titer of 1:640 and an MI titer of 1:1,280 at the time of the experiment.

RESULTS

Growth of M. arthritidis in various media and effects of trypsin and macrophage extracts. The most suitable medium was considered to be MEM supplemented to a final concentration of 10% (vol/vol) FCS, since it permitted survival but not multiplication of the mycoplasmas.

Trypsin was not inhibitory toward *M. arthritidis*. Only minimal inhibitory effects were observed with the macrophage extract. The 1:2 dilution of the extract reduced the mycoplasma count 10-fold after 24 h at 37 C. Inhibition did not occur at any of the other dilutions. Since the assay procedure for phagocytosis employs fewer macrophages and a shorter time of exposure to the macrophage extract, the weak inhibition observed would be negligible under the assay conditions.

Interaction of M. arthritidis with peritoneal macrophages. Since detailed morphological observations using electron microscopy were not performed in these studies, the term "phagocytosis", when used, denotes killing of mycoplasmas by viable peritoneal macrophages.

Although mouse and rat peritoneal macrophages survived for the duration of the experiment, no mycoplasmacidal activity was detected. In the experiment illustrated (Fig. 1), monolayers of normal mouse macrophages were incubated overnight and then infected with 7.5×10^5 CFU of *M. arthritidis* strain 158 P10 P9 per dish. The mean values of three determinations are recorded. When supernatant and macrophage-associated mycoplasmal counts were totaled there was evidence of some enhancement of mycoplasmal growth as compared to cultures containing no macrophages. A similar enhancement of growth was observed in subsequent studies in which both rat and mouse macrophages were used.

A variety of procedures were investigated to stimulate "phagocytic" activity. Freshly prepared, unwashed macrophage-mononuclear cell cultures were exposed to *M. arthritidis* within 2 h of collection; no change in CFU per dish was obtained. Similarly, the addition of 1:100 final concentration of guinea pig complement was without effect. To counteract possible arginine deficiency due to the multiplication of the mycoplasmas, the complete MEM medium was supplemented with 0.05% (wt/vol) and 0.25% (wt/vol) arginine monohydrochloride. This procedure resulted in slightly enhanced mycoplasmal growth with no evidence of increased uptake or killing by peritoneal macrophages. Decreasing the inoculum to 1×10^5 CFU of *M. arthritidis* per dish, i.e., a macrophage to mycoplasma ratio of 20:1, did not induce killing. Stimulated macrophages from animals treated i.p. with thioglycollate broth did not differ in their activity toward *M. arthritidis*, although

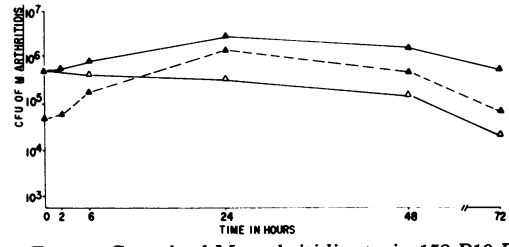

FIG. 1. *Growth of M. arthritidis strain 158 P10 P9 in the presence of unstimulated mouse macrophage monolayer cultures. Supernatant count of mycoplasmas (—▲—); macrophage-associated mycoplasmas (--▲--); control of M. arthritidis growth in the absence of macrophages (—△—). Mean values of three determinations.*

these cells were uniformly larger than those recovered from unstimulated animals.

Since the failure of macrophages to kill *M. arthritidis* strain 158 P10 P9 might be related to the high virulence of this organism for mice and rats, the less virulent strains, H606 and PG6, were also tested. Rat macrophages were used in these experiments since the latter strains are non-arthritogenic for rats (12). The results were almost identical with those obtained for strain 158 P10 P9 during the first 48 h of incubation, and again the presence of macrophages appeared to stimulate the growth of the organisms. However, after 48 h, both H606 and PG6 exhibited a sharper decline in numbers of viable organisms than did the virulent strain. An identical experiment was set up with another virulent strain (DL) of *M. arthritidis*. In the absence of macrophages, the growth of the DL strain gradually declined until, by 96 h, 2 of 3 of the dishes were devoid of mycoplasmas. In the presence of rat macrophages, the numbers of viable organisms remained constant for the 96-h duration of the experiment.

Thus, although "phagocytosis" appears not to be dependent upon strain virulence in the absence of specific antiserum, differences in long-term survival rates in the presence of macrophages are apparent between virulent and avirulent strains of *M. arthritidis*.

Effect of immune sera. In preliminary experiments with unstimulated mouse macrophages, hyperimmune rabbit antiserum appeared to promote macrophage killing of the mycoplasmas. Stimulated macrophages were used in subsequent experiments since greater killing was observed.

The results with convalescent mouse sera are summarized in Fig. 2 and 3. Convalescent mouse serum at a concentration of 1:10, and to a lesser extent at 1:100, inhibited the growth of *M. arthritidis* in the absence of macrophages (Fig. 2). Normal serum was without effect. In the presence of macrophages, no inhibition of the mycoplasmas occurred (Fig. 3). Macrophages stimulated the growth of *M. arthritidis* with 1:100 normal serum and 1:500 convalescent serum. When the macrophage and supernatant counts were totaled, increased growth was also apparent in cultures exposed to 1:100 convalescent serum. Thus, the macrophages protected against the inhibitory effects of the convalescent sera and promoted multiplication of the organisms. Similar results were obtained with *M. arthritidis* exposed to rat peritoneal macrophages and rat convalescent serum taken from arthritic animals. Thus, in the absence of macrophages the 1:100 convalescent rat serum

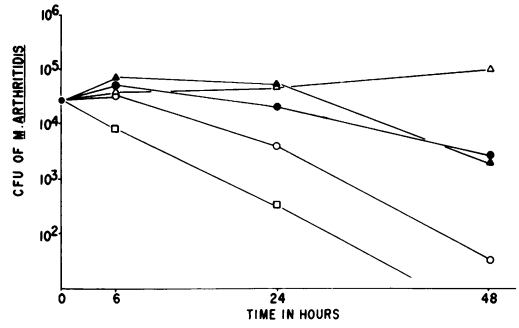

FIG. 2. *Inhibitory effects of convalescent mouse serum on the growth of M. arthritidis strain 158 P10 P9. Control experiment for Fig. 3. Normal mouse serum 1:10 (△); normal mouse serum 1:100 (▲); convalescent mouse serum 1:10 (□); convalescent mouse serum 1:100 (○); convalescent mouse serum 1:500 (●). Mean values of two determinations.*

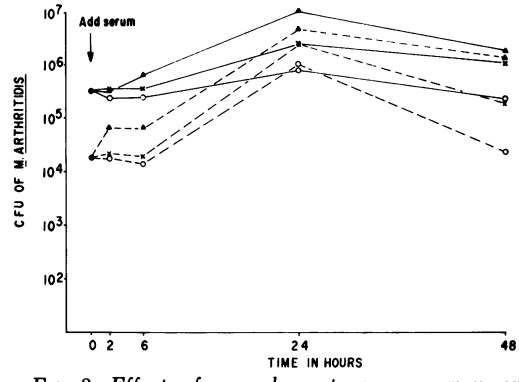

FIG. 3. *Effect of convalescent mouse serum on phagocytosis of M. arthritidis by stimulated mouse macrophages. Continuous lines represent supernatant counts of mycoplasma, dashed lines represent macrophage-associated mycoplasma counts. The sera were added 2 h after infection by the mycoplasmas. Final serum concentrations were: normal mouse serum 1:100 (▲); convalescent mouse serum 1:100 (○); and convalescent mouse serum 1:500 (×). Mean values of two determinations are recorded.*

resulted in a 1,000-fold decrease of viable organisms after 48 h. In the presence of macrophages and 1:100 normal convalescent rat serum, the mycoplasma counts decreased by only a factor of 3 after 48 h. Macrophage cultures treated with 1:500 convalescent serum exhibited a slight increase in numbers of mycoplasma.

The effect of hyperimmune rabbit anti-*M. arthritidis* serum was investigated by using stimulated mouse macrophages infected with *M. arthritidis* strain 158 P10 P9, 1×10^5 CFU per plate. After 2 h, either MEM, 1:100 normal rabbit serum, or 1:100 anti-*M. arthritidis* serum was added. The results are summarized

in Fig. 4. Both macrophage and supernatant counts decreased 2 h after administration of the rabbit antiserum and continued to fall rapidly through 6 h. Normal rabbit serum had no inhibiting effects as compared with those cultures receiving MEM. By 48 h, the inhibitory effects of rabbit antiserum were the most pronounced. When *M. arthritidis* was grown under identical conditions, but in the absence of macrophages, no decrease in counts occurred through 24 h (Fig. 5). By 48 h, both control and antiserum-treated cultures showed a slight decrease in viable mycoplasmas.

Interaction of other mycoplasma species with mouse macrophages. The failure to obtain significant killing of *M. arthritidis* in the absence of hyperimmune sera when incubated with peritoneal macrophages led us to determine whether this was characteristic for *M. arthritidis*.

The "phagocytosis" of *M. pulmonis* was investigated by using both unstimulated and stimulated macrophages. MEM was supplemented with 10% (vol/vol) PPLO broth (Difco) in addition to 10% FCS. The experiments were run in duplicate. In the absence of macrophages, rabbit anti-*M. pulmonis* serum (1:100 and 1:1,000) had no marked effects on the survival of *M. pulmonis* (Fig. 6).

The behavior of *M. pulmonis* on normal mouse macrophages is summarized in Fig. 7. Macrophages were infected with *M. pulmonis* 20 h before the addition of the serum. Enhanced multiplication of *M. pulmonis* in the presence of mouse macrophages was apparent, since combined macrophage-associated and supernatant counts of mycoplasma were 50-fold higher in the presence of normal serum after 20 h as compared with the 24-h counts in the absence of macrophages. In contrast with *M. arthritidis*, the macrophage counts were 10-fold higher than the supernatant counts, thus confirming Jones and Hirsch's observation that *M. pulmonis* multiplies on the surface of the macrophage (15). The addition of anti-*M. pulmonis* serum resulted in a prompt killing of macrophage-associated mycoplasmas. Thus, 24 h after the addition of 1:100 rabbit antiserum, the macrophage counts had decreased by a factor of 2.5×10^4. Normal rabbit serum (1:100) and anti-*M. pulmonis* serum (1:1,000) both exhibited some activity and resulted in decreased macrophage counts of 100-fold. Up to this time supernatant

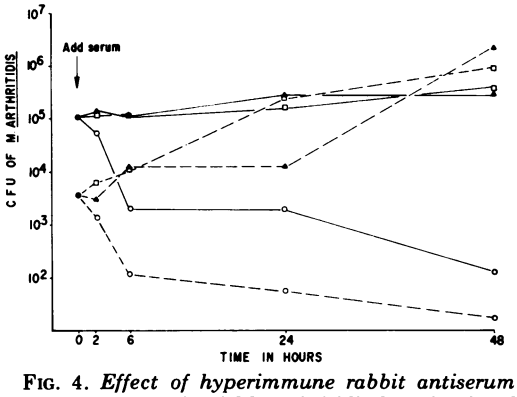

FIG. 4. *Effect of hyperimmune rabbit antiserum on the phagocytosis of M. arthritidis by stimulated mouse macrophages. The sera were added 2 h after infection by M. arthritidis. Continuous lines represent supernatant counts, dashed lines represent macrophage-associated mycoplasma counts. Sera used were: normal rabbit serum 1:100 (▲); rabbit anti-M. arthritidis serum 1:100 (○); and controls using no serum (□). Mean values of two determinations are recorded.*

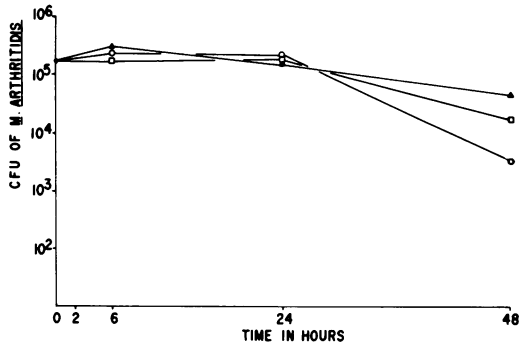

FIG. 5. *Effect of rabbit antiserum on viability of M. arthritidis. Control experiments for Fig. 4. Growth of M. arthritidis in the absence of macrophages. Sera used were: normal rabbit serum 1:100 (▲); rabbit anti-M. arthritidis serum 1:100 (○); control with no serum (□). Mean values of two determinations.*

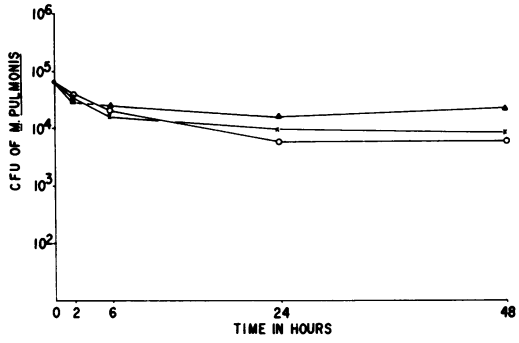

FIG. 6. *Effect of rabbit antiserum on viability of M. pulmonis. Control experiment for Fig. 7 and 8. Sera used were: normal rabbit serum 1:100 (▲); rabbit anti-M. pulmonis serum 1:100 (○); rabbit anti-M. pulmonis serum 1:1,000 (×). Mean values of two determinations are recorded.*

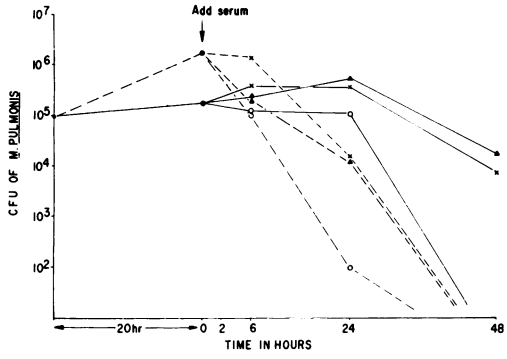

FIG. 7. *Effect of rabbit antiserum on phagocytosis of M. pulmonis by unstimulated mouse macrophages. Unstimulated mouse macrophages were infected for 20 h with M. pulmonis, and then the following sera were added: normal rabbit serum 1:100 (▲); rabbit anti-M. pulmonis serum 1:100 (○); rabbit anti-M. pulmonis serum 1:1,000 (×). Continuous lines represent supernatant counts, and dashed lines represent macrophage-associated counts of mycoplasmas. Mean values of two determinations are recorded.*

counts of *M. pulmonis* remained unchanged. By 48 h, mycoplasmas were completely eliminated from both macrophages and supernatants in cultures exposed to 1:100 anti-*M. pulmonis* serum. Cultures treated with 1:1,000 anti-*M. pulmonis* serum and normal rabbit serum showed a slight decrease in supernatant counts, but a complete elimination of mycoplasmas from the macrophages.

Similar results were obtained by employing stimulated mouse macrophages (Fig. 8). In this experiment, the medium was removed from the macrophages 20 h after infection by *M. pulmonis* and was replaced with fresh medium containing the antiserum dilutions. Within 2 h a dramatic decrease in numbers of macrophage-associated mycoplasmas had occurred. By 6 h, cells treated with 1:100 antiserum exhibited a 400-fold decrease in organisms, whereas cells exposed to 1:100 normal rabbit serum exhibited a decrease of only sevenfold. Both supernatants and macrophages were negative for mycoplasmas after 6 h in the presence of antiserum, whereas mycoplasmas could still be detected at 48 h in cultures exposed to 1:100 normal serum.

The marked affinity of *M. pulmonis* for peritoneal macrophages was confirmed in another experiment in which the mycoplasmas were added to stimulated macrophages only 2 h before the addition of serum. The macrophages had adsorbed 90% of the *M. pulmonis* inoculum by the time of the addition of serum.

The interaction of *M. gallinarum* with stimulated mouse macrophages was also investigated. Two hours after the addition of *M. gallinarum*, the cultures were treated with MEM, 1:160 normal rabbit antiserum, or 1:160 rabbit anti-*M. gallinarum* serum. Control dishes without macrophages were also set up. The results are summarized in Fig. 9 and 10.

Normal rabbit serum appeared to promote the survival of *M. gallinarum* in the absence of macrophages (Fig. 9). No differences in growth were apparent between media with and without rabbit anti-*M. gallinarum* serum, and both cultures were sterile at 48 h. When *M. gallinarum* was cultured in the presence of macrophages, no decrease in titer occurred

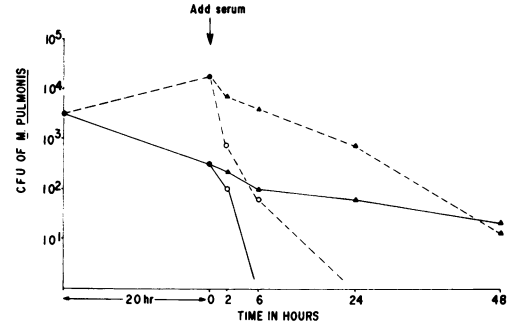

FIG. 8. *Effect of rabbit antiserum on phagocytosis of M. pulmonis by stimulated mouse macrophages. Stimulated mouse macrophages were infected for 20 h with M. pulmonis. The supernatants were then removed and replaced with medium containing: normal rabbit serum 1:100 (▲); rabbit anti-M. pulmonis serum 1:100 (○). Continuous lines represent supernatant counts, and dashed lines represent macrophage-associated counts for mycoplasmas. Mean values of three determinations are recorded.*

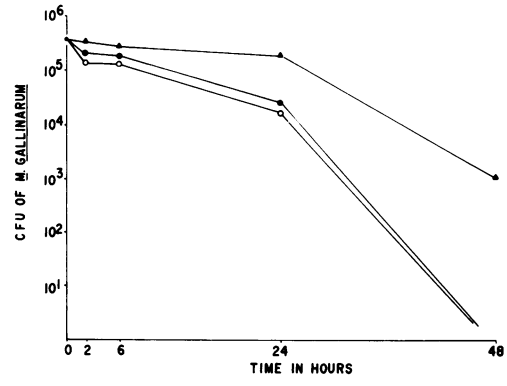

FIG. 9. *Effect of rabbit antiserum on the viability of M. gallinarum. Control experiment for Fig. 10. Sera used were: normal rabbit serum 1:160 (▲); rabbit anti-M. gallinarum serum 1:160 (○); control with no serum (●). Mean values of two determinations are recorded.*

through 48 h in media containing normal rabbit serum or no serum (Fig. 10). Thus, the macrophages clearly promoted the survival of the mycoplasmas. When 1:160 rabbit anti-*M. gallinarum* serum was added to the cultures, a rapid decline of viable organisms was apparent in both macrophage and supernatant-associated macrophages. This decline was pronounced 6 h after the addition of the serum. In the control experiment without macrophages, antiserum exhibited no effect by 6 h. Thus, the results with *M. gallinarum* closely paralleled those obtained in which *M. arthritidis* was used in the presence of homologous hyperimmune rabbit antiserum (Fig. 4).

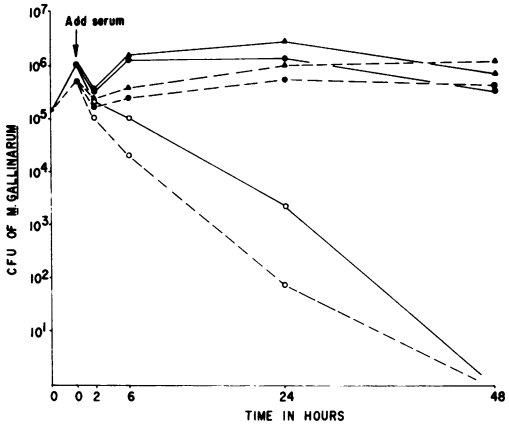

FIG. 10. *Effects of rabbit antiserum on the phagocytosis of M. gallinarum by stimulated mouse macrophages. Stimulated mouse macrophages were infected with M. gallinarum, and 2 h later the following final concentrations of serum were added; normal rabbit serum 1:160 (▲); rabbit anti-M. gallinarum serum 1:160 (○); control with no serum (●). Continuous lines represent supernatant counts, and dashed lines represent macrophage-associated counts of mycoplasmas. Mean values of two determinations are recorded.*

DISCUSSION

In the absence of homologous hyperimmune rabbit antiserum neither mouse nor rat peritoneal macrophages were capable of killing *M. arthritidis*. A wide range of procedures using both normal and stimulated macrophages failed to induce "phagocytic" activity. In the majority of experiments, the presence of the macrophages promoted the survival and multiplication of the organisms.

By 2 h after the addition of rabbit anti-*M. arthritidis* serum to infected macrophages, only minimal killing of the mycoplasmas was observed. However, by 6 h the numbers of mycoplasmas in the supernatant extract had decreased by 30-fold, and the macrophage-associated mycoplasma counts had decreased by 50-fold. Simberkoff and Elsbach (22) used rabbit polymorphonuclear leukocytes and reported the inability of these cells to kill *M. arthritidis* and *M. hominis* even in the presence of homologous rabbit antiserum. However, these investigators examined the "phagocytic" response for only 2 h after exposure to the antiserum. Our results indicated that killing is not maximal at this time. The inability of peritoneal macrophages to ingest and kill *M. arthritidis* in the absence of homologous rabbit antiserum does not appear to be related to virulence of the organism, since similar results were obtained with less virulent *M. arthritidis* strains and also with the non-murine mycoplasma, *M. gallinarum*.

When macrophages infected with *M. pulmonis* were treated with immune rabbit antiserum, a rapid and more complete killing of macrophage-associated mycoplasmas was observed than with *M. arthritidis*. In addition, normal rabbit serum exhibited some opsonizing capabilities, although the reduction in the numbers of mycoplasmas was much less than for the hyperimmune serum. In the absence of antiserum, the growth of *M. pulmonis* was stimulated by the presence of macrophages. The striking difference between *M. pulmonis* and the other mycoplasmas examined was the ability of the *M. pulmonis* to rapidly adsorb to the surface of the macrophages. Other investigators have reported the ability of this species to adsorb to mammalian cells (15, 17, 24). In contrast, Simberkoff and Elsbach (22) reported that neither *M. hominis* nor *M. arthritidis* adsorbed to rabbit or human granulocytes. Our studies largely confirm their observations for the first few hours after infection. However, evidence was obtained that, on continued incubation, the macrophage-associated mycoplasma counts increased relative to the supernatant counts. A similar phenomenon was observed with *M. gallinarum*. This effect may be due to increased multiplication around the macrophages after exhaustion of medium nutrients rather than to a specific attachment to the macrophages. Comparatively little work has been conducted on the ability of *M. arthritidis* to adsorb to cell surfaces. Preliminary work in these laboratories indicates that neither virulent nor avirulent *M. arthritidis* adsorbs to mammalian erythrocytes or to mouse fibroblasts as tested by adsorption of cells to mycoplasma colonies (unpublished observations). Manchee and Taylor-Robinson also failed to obtain ad-

sorption of erythrocytes to colonies of *M. arthritidis* (17). However, low levels of adsorption of *M. arthritidis* to human diploid cells have been observed by Stanbridge as indicated by the failure to remove a small percentage of cell-associated mycoplasmas after repeated washing procedures (personal communication). Zucker-Franklin et al. reported that *M. pneumoniae*, *M. gallisepticum*, and *M. neurolyticum*, which all adsorb to cells or hemagglutinate (17, 24), can be phagocytized by polymorphonuclear leukocytes apparently in the absence of specific antiserum (26). Thus, there may be a relationship between avidity of adsorption to cell surfaces and susceptibility to phagocytosis.

In the absence of macrophages, convalescent mouse and rat sera exhibited inhibitory effects on the growth of *M. arthritidis* as compared with normal sera. Macrophages counteracted this effect, and no inhibition was observed. It would thus appear that, in contrast to rabbit antiserum, rat and mouse convalescent sera are devoid of effective opsonizing antibodies against *M. arthritidis*. The observations on the inhibitory properties of convalescent sera are paradoxical in view of previous findings that rats do not produce metabolic inhibiting or growth inhibiting antibodies against *M. arthritidis* (6). However, in the latter studies, tests for inhibiting antibodies were performed using a medium which permitted multiplication of the mycoplasmas. It would appear from the present study that neutralization of *M. arthritidis* can be demonstrated provided that the organisms are not in an active growth phase. Since macrophages apparently stimulate mycoplasmal growth, they are thus able to counteract the inhibitory properties of the serum. Whether this neutralizing antibody has any role in controlling *M. arthritidis* infections remains to be determined. The failure of convalescent murine sera to exhibit effective opsonizing capabilities correlates with previous findings that these sera exhibit negative or only partially protective properties when used to passively immunize rodents against *M. arthritidis* (6, 8). Furthermore, they exhibit similar low activity in eliminating mycoplasmas from the peripheral circulation (Cole and Ward, in press). In comparison, hyperimmune rabbit antiserum against *M. arthritidis* is highly efficient in all of these regards.

What is the explanation of the failure of murine macrophages to efficiently "phagocytize" *M. arthritidis*? Firstly, the small size of the mycoplasma cells may render the organisms less susceptible to ingestion. The greater tendency of *M. pulmonis* to grow in clumps and to autoagglutinate as compared with *M. arthritidis* might explain the more complete "phagocytosis" of the former species when exposed to specific rabbit antiserum. The reason for the complete lack of "phagocytic" activity in the absence of specific rabbit antiserum could be explained by the failure of the macrophage to recognize the mycoplasmas as foreign. Similarly, the murine host may have an impaired immunological response to the invading organisms, thus resulting in a lack of effective opsonizing antibodies. Evidence for an immunological similarity between *M. arthritidis* and rat tissues was reported earlier (2, 6).

In similar studies with *Salmonella typhimurium* infections of mice, Rowley and Jenkin (21) obtained evidence to indicate that a heterogenetic antigen(s) common to parasite and host enabled the organism to resist the defenses of the host. Thus, opsonizing antibodies were not produced by mice and phagocytosis of the organisms did not occur. Heterologous serum resulted in prompt phagocytosis, indicating that the macrophages were capable of phagocytic activity.

Whereas mice developed a chronic disease after exposure to *M. arthritidis*, rats rapidly resolve the arthritis and exhibit a high degree of immunity to subsequent infections. The present study suggests that macrophages do not play a major role in rat immunity. Preliminary experiments employing macrophages collected from convalescent rats have likewise failed to demonstrate "phagocytic" activity even in the presence of convalescent sera (unpublished observations). Thus, it is apparent that additional studies must be undertaken to determine the mechanism of immunity to *M. arthritidis*. To date, no work has been conducted on the production of local antibody at the site of infection. In addition, the role of the polymorphonuclear leukocyte in controlling *M. arthritidis* infections remains to be explored. Histological studies have shown that this cell type predominates in the early infiltrates of infected tissues (9, 25). Likewise, the role of the cellular responses of the host in the development of the chronic mouse lesion is another vital area which must be studied in order to gain a thorough understanding of the mechanism of pathogenesis of this disease.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AM-02255 from the National Institute of Arthritis and Metabolic Diseases.

We thank Laura Golightly-Rowland and Benjamin Santistevan for excellent technical assistance.

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