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Detection and Characterization of Defective Mycoplasmacidal Antibody Produced by Rodents Against *Mycoplasma arthritidis*

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A procedure has been developed for the detection of mycoplasmacidal antibody in the serum of rats and mice infected with *Mycoplasma arthritidis*. Antibody activity can apparently be measured only by using resting mycoplasma cells. The reaction is complement dependent and is usually complete within 3 h. Early antibody (5 days) could be detected only within the immunoglobulin (Ig)M fraction, whereas late antibody (42 days) is found within the IgG fraction. The mycoplasmacidal antibody was highly specific and cross reactions were not observed with other mycoplasma species. Antibody activity could be removed from serum by absorption with either viable or nonviable *M. arthritidis*. Cidal antibody produced by rats against other mycoplasma species was active against both resting and multiplying cells. Mycoplasmacidal antibody against *M. arthritidis* was detected in rats 2 days after injection of the organisms and persisted at least through 300 days. Mice usually developed antibodies by 3 days after injection of the organisms and moderate titers have been observed for as long as 487 days. Since viable mycoplasmas can persist in the peripheral circulation in the presence of antibody, it is likely that viable mycoplasma-antibody complexes are also present.

In 1953 Edward and Fitzgerald reported that rabbit antisera against mycoplasma species were inhibitory to the growth of the organisms when incorporated into the base agar (14). Variations on their procedure resulted in a simple test in which paper disks impregnated with specific antisera produced zones of growth inhibition on agar plates previously seeded with the homologous organism (7, 24). More sensitive techniques were devised in which inhibition was measured by the ability of the antiserum to inhibit a color change produced in broth media as a result of the metabolism of a particular substrate by the mycoplasma. Metabolic inhibition tests have now been developed for glucose (25), arginine (20), and urea (21) metabolizing mycoplasma species. Growth inhibition by antiserum has also been measured in broth in which reduction of the numbers of colony-forming units (CFU) is observed (23). Variations on this procedure have resulted in increased sensitivity of the test system (2). Antibody tests based upon immune inhibition have been found to be highly species specific (7) and have been widely used as indicators of active or past infection.

Previous studies in our laboratories failed to demonstrate the presence of metabolic-inhibiting (MI) or growth-inhibiting (GI) antibody in the sera of rats injected intravenously (i.v.) with *Mycoplasma arthritidis* (8). On the other hand, MI antibody was produced against other mycoplasma species similarly injected into rats. Rats did respond weakly to the injection of a mixture of *M. arthritidis* and Freund adjuvant (9). Mice exhibited only a weak MI antibody response to *M. arthritidis* (12). Recently, evidence was obtained that rats are capable of producing GI antibodies in response to infection by *M. arthritidis* (11). Thus, during attempts to demonstrate the phagocytosis of *M. arthritidis* by murine peritoneal macrophages, it was unexpectedly observed that rat and mouse convalescent sera inhibited the growth of the organisms in the absence of macrophages and yet the same sera had no effect upon the organisms in the presence of macrophages. The base medium alone, consisting of Eagle minimal essential medium (MEM) with 10% (vol/vol) fetal calf serum (FCS), allowed survival of the organisms but did not promote multiplication. When macrophages were present, multiplication of the

mycoplasmas occurred. As a result of these studies we suggested that rat and mouse convalescent sera might contain an antibody which was inhibitory to resting mycoplasmas but which had no effect on growing mycoplasmas. The present study was undertaken to confirm the existence of this antibody, to develop procedures for its detection and characterization, and to determine at which stage of the disease it was present.

MATERIALS AND METHODS

Strains used. *M. arthritidis* strains 158 P10 (8) and 158 P10 P9 (13) were used throughout this study. *M. hominis* strain 14027 and *M. arginini* 19388 were obtained from the American Type Culture Collection (Rockville, Md.) Cultures and antisera of *M. felis*, *M. leonis*, *M. hyorhinis*, *M. maculosum*, and *M. meleagridis* were as previously described (8, 12).

Cultivation of mycoplasmas. The base medium used consisted of PPLO agar or broth (Difco) supplemented to a final concentration of 15% (vol/vol) sterile horse serum, 5% (vol/vol) sterile yeast extract, and 1,000 U of penicillin G per ml (6, 19). Mycoplasma suspensions were prepared and counted as described previously (18).

Injection of animals. Male Holtzman rats (120–150 g) were injected i.v. with 10^{10} CFU of *M. arthritidis* strain 158 P10 or strain 158 P10 P9. Serum samples were collected for antibody studies 3, 5, 7, 10, 14, 21, and 28 days after injection. Arthritis scores were made at similar intervals of time, as described previously (8). All of the above determinations were made on the same animals. Additional rats were injected with 2×10^9 CFU of *M. arthritidis* 158 P10 for antibody studies at 1 and 2 days. All other studies were performed on rat sera prepared by injection of *M. arthritidis* strain 158 P10 P9.

Swiss-Webster mice (male, 35 g) were injected i.v. with 2.1×10^9 CFU of *M. arthritidis* strain 158 P10 P9. After 3, 6, 8, 10, 14, 21, 28, and 35 days, blood samples were taken from each of three mice and were tested (10) for circulating mycoplasmas. The mice were then exsanguinated and the resulting sera were tested for circulating antibody. Arthritis scores were read as before.

CF and MI antibody tests. Complement-fixing (CF) antibodies were detected by using the micro technique described by Casey (5). Details of antigen preparation were as reported previously (8). MI antibodies were tested using the micro technique of Purcell et al. (20) with 1% (wt/vol) arginine monohydrochloride as the test substrate.

Mycoplasmacidal antibody test. Mycoplasma suspensions were prepared from cultures frozen at −20 C and previously counted. In initial experiments the tests were performed in screw-capped test tubes (13 by 100 mm). After standardization of the procedure, a microtest adaptation was used to conserve animal serum samples. The final procedure adopted was as follows. Disposable microtitration plates were sterilized by exposure to ultraviolet light for 2 h. Serum samples were heated at 56 C for 30 min. All dilutions of serum, guinea pig complement, and mycoplasma suspensions were prepared in MEM supplemented to a final concentration of 10% (vol/vol) FCS and 1% (vol/vol) of a 200 mM solution of L-glutamine. The pH was adjusted to 7.8 by using a sterile solution of sodium bicarbonate. Microdiluters and micropipette droppers (Flow Laboratories, Inc.) were used.

Base medium (0.025 ml) was added to each well and 0.025 ml of a 1:5 dilution of serum was added to the first well. Serial twofold dilutions (usually to 1:40,960) were made. The mycoplasma suspension, containing approximately 5×10^3 CFU, was added (0.15 ml) to each well. Guinea pig complement (BBL, Cockeysville, Md.) diluted 1:10 in base medium was also added (0.025 ml) to each well to give a final concentration of 1:100. Control wells were set up without serum. The plates were sealed with tape and incubated at 37 C for 24 h. By using a microdiluter the contents of each well were then diluted 1:10 in MEM. Samples of 0.05 or 0.1 ml of both the diluted or undiluted reaction mixtures were then transferred to one-half of a mycoplasma agar plate. Mycoplasma colonies were counted after 3 to 4 days of incubation at 37 C. In later tests shorter incubation times were used. Appropriate controls were set up with each test, substituting normal serum or MEM for immune serum. The mycoplasmacidal antibody titer of the serum was recorded as the highest dilution which exhibited a reduction of 75% or more in the number of viable organisms. Due to the dilution factor in the test, the actual antibody levels of the sera would be eightfold higher than those recorded.

Effect of complement on mycoplasmacidal activity. Sera were collected from rats 5 and 28 days after infection with *M. arthritidis*. By using the microtiter procedure, the following systems were compared for mycoplasmacidal antibody titers: (i) unheated serum; (ii) serum heated at 56 C for 30 min; (iii) heated serum plus 1:25, 1:50, 1:100, 1:200, or 1:400 final dilutions of guinea pig complement. Controls to test the inhibitory effects of all dilutions of guinea pig complement in the absence of antiserum were also set up.

Effect of the growth phase of mycoplasma on susceptibility to antibody. Since *M. arthritidis* does not actively multiply in complete MEM, mycoplasma broth supplemented with 10% (vol/vol) horse serum was used in these studies in addition to MEM supplemented with calf serum. Microtiter plates were set up containing samples of mycoplasma broth and 5×10^3 CFU of *M. arthritidis* per well. The dilution of organisms used was prepared from a frozen culture. Serum dilutions containing complement were prepared in advance. In the first experiment the mycoplasma suspensions were counted just before addition of serum and also after 1 and 3 h of incubation after the addition of serum. In the second experiment, the wells containing the mycoplasma suspensions were preincubated for 1 h at 37 C before addition of serum. As before, the reaction mixtures were counted for mycoplasma after a further 1 and 3 h of incubation. In the third experiment, the mycoplasma suspensions were preincubated 3 h before addition of the serum

dilutions. As controls, all experiments were repeated by using normal rat serum.

Absorption of serum. Formalin-killed antigen was prepared from a frozen concentrated suspension of *M. arthritidis*, previously adjusted to contain 4×10^{10} CFU per ml. Formalin was added to give a final concentration of 0.5% (vol/vol) and the mixture was incubated overnight at 37 C. The suspension was then washed three times by centrifugation and was suspended in complete MEM.

Viable antigen was prepared by centrifuging a fresh culture of *M. arthritidis* and adjusting the optical density to that of the Formalin-killed antigen suspension. Additional dilutions containing 4×10^8 and 4×10^6 CFU per ml were prepared for both antigen suspensions. Absorption was carried out by mixing 0.5-ml amounts of 5-day rat serum (diluted 1:5 in MEM) with 0.5-ml amounts of the three concentrations of either Formalin-killed antigen or viable antigen. The mixtures were incubated for 5 h at 37 C on a shaker. Antigen was removed by centrifugation, followed by filtration through a 0.1-μm membrane filter.

Kinetics of mycoplasmacidal reaction. The test used was a modification of the procedure described by Brunner et al. (2) for the determination of neutralization of *M. pneumoniae*. Complete MEM at 37 C containing 2×10^5 CFU per ml of *M. arthritidis* strain 158 P10 P9 and 1% (vol/vol) guinea pig complement was distributed into sterile screw-capped tubes (13 by 100 mm). Sera taken 5 and 28 days after injection of rats with *M. arthritidis* were heated at 56 C for 30 min and were added to the reaction tubes. The mixtures were incubated at 37 C and 0.2-ml samples were removed after 5, 15, and 30 min and after 1, 2, 4, 6, and 24 h. The samples were immediately added to 1.8 ml of ice-cold MEM, thus giving a 1:10 dilution of the reaction mixture. Further dilutions of 1:100 and 1:1,000 were made, also in ice-cold MEM. The dilutions were plated to mycoplasma agar and were counted by using the standard procedure.

Fractionation of serum. Rat serum was taken from animals 4, 14, and 32 days after i.v. injection of 2×10^9 CFU of *M. arthritidis* strain 158 P10 P9. Mouse serum was collected 5 days after the i.v. injection of 1.5×10^9 CFU of *M. arthritidis* strain 158 P10 P9. Gamma globulin fractions were prepared by ammonium sulfate precipitation as described by Goldman (17) and redissolved to give a twofold concentration over the original serum. The gamma globulin solutions were fractionated on a Sepharose 4B column, as described previously (12). Protein peaks were measured spectrophotometrically at 280 nm. Selected fractions were dialyzed overnight against distilled water at 6 C and were concentrated 10-fold by lyophilization prior to testing for mycoplasmacidal activity.

RESULTS

Standardization of procedure. Mycoplasmacidal antibody could not be detected employing the usual mycoplasma broth containing horse serum. Thus, all preliminary work was performed by using MEM supplemented to 10% FCS. Mycoplasmacidal antibody titers were variable unless a high titered culture of *M. arthritidis* was used as antigen. The likely presence of large numbers of nonviable cells in older cultures could explain these results. The test might also be influenced by clumps of organisms in the mycoplasma suspension as reported by Brunner et al. for *M. pneumoniae* (2). Thus, several antisera were tested for antibody titers before and after filtration through a 0.45-μm membrane filter. No difference was observed in the titers, indicating that *M. arthritidis* does not readily agglutinate or clumps.

In order to determine the most suitable number of organisms, reaction mixtures consisting of 5×10^2 to 5×10^5 CFU per ml were tested against both 5- and 28-day rat serum samples. The 5×10^2 inoculum resulted in a barely adequate number of countable colonies. Complete inhibition was rarely achieved by using the 5×10^4 and 5×10^5 inocula, thus requiring extra dilutions to be made and requiring all dilutions of serum to be counted. Thus, the most suitable mycoplasma inoculum was considered to be 5×10^3 CFU per ml of reaction mixture. The survival of some organisms irrespective of serum concentration suggested that a certain proportion were resistant or more resistant to the action of antibody.

Effect of complement. The results are summarized in Fig. 1 and 2, and in Table 1. Guinea pig complement was not inhibitory to *M. arthritidis* at any dilution when incubated in the absence of rat antiserum. Both early (5-day) and late (28-day) mycoplasmacidal antibodies against *M. arthritidis* were complement dependent. Thus, a 1:25 dilution of complement increased the titer of heated 5-day convalescent

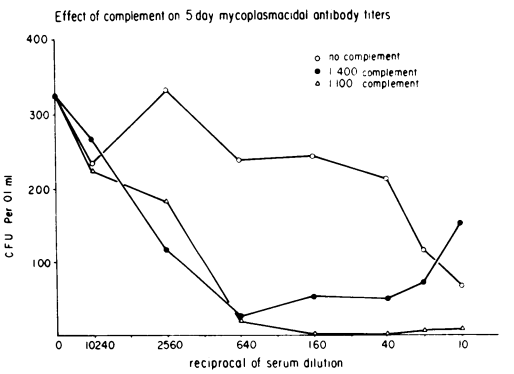

FIG. 1. *The base medium (MEM plus 10% FCS) was supplemented with final concentrations of 1:100 and 1:400 guinea pig complement. Serum antibody taken at 5 days was found to be dependent upon complement.*

serum as much as 256-fold. A similar enhancement of antibody titers was observed with 28-day convalescent serum. In the absence of sufficient complement, an increase in the numbers of surviving mycoplasmas occurred with higher concentrations of serum (Fig. 1). This was presumably due to the nutritive properties of the serum enhancing mycoplasmal multiplication, thereby counteracting the inhibitory effects of the antiserum. This phenomenon was observed on numerous occasions and occurred only at serum dilutions of 1:20 and lower.

Effect of medium composition. The base media consisted of (i) MEM with 10% FCS or 10% horse serum (HS); (ii) mycoplasma broth (Difco) with 10% FCS, 10% HS, or 2% HS. Rat serum collected 7 days after infection was used as the source of antibody. By using the microtiter system, the reaction mixtures were plated to agar after 3 h of incubation at 37 C. All media containing horse serum exhibited antibody titers of <1:10. MEM with 10% FCS exhibited a titer of 1:1280 and mycoplasma broth with 10% FCS exhibited a titer of 1:160. Thus, the choice of serum appeared to be the critical factor.

In order to check for possible Ca^{2+} and Mg^{2+} deficiency of the medium (2), another experiment was set up using MEM supplemented with 0.02% (wt/vol) of $CaCl_2$ and $MgSO_4$ and 10% (vol/vol) HS. Antibody activity was still not detected in the HS-containing medium.

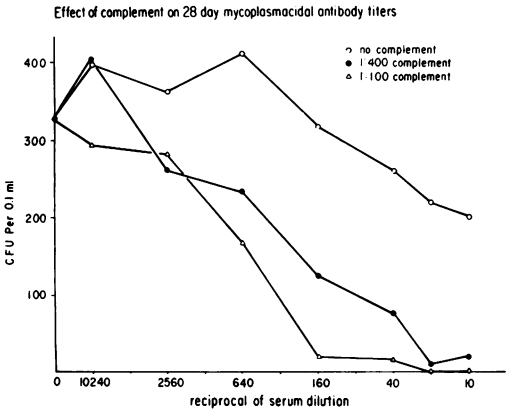

FIG. 2. *The base medium (MEM plus 10% FCS) was supplemented with final concentrations of 1:100 and 1:400 guinea pig complement. Serum antibody taken at 28 days was found to be dependent upon complement.*

Effect of growth phase of organism. The next experiment was undertaken in an attempt to explain the failure to detect inhibiting antibodies when complete mycoplasma broth was used as the base medium.

Either 5-day convalescent rat serum or normal rat serum mixed with complement were added to the mycoplasma suspension at zero time and after 1 and 3 h of incubation at 37 C. The reaction mixtures were plated out just before addition of serum and after 1 and 3 h of further incubation at 37 C.

By using complete mycoplasma medium, cidal activity was demonstrable only with 1:10 convalescent rat serum. The greatest inhibiting activity occurred when the serum and complement were added without prior incubation of the organisms (Table 2). A preincubation period of 1 h resulted in less inhibition of organisms and a 3-h preincubation period resulted in no inhibition when serum and complement were added to the system. The organisms did not multiply during the preincubation periods.

Using the MEM base medium preincubation of the organisms before addition of serum had no effect on the mycoplasmacidal titers of the serum which exceeded 1:160.

Effect of absorption of antiserum with M. arthritidis antigens. The absorption of rat sera with either 10^{10} CFU of viable *M. arthritidis* or an identical concentration of Formalin-killed mycoplasma completely eliminated antibody activity. Absorption with 10^{8}- or 10^{6}-CFU quantities of antigen reduced the titers two- to fourfold with no difference being apparent between the effectiveness of viable or Formalin-killed antigen preparations in absorbing ability.

Kinetics of mycoplasmacidal reaction. The results, using 5-day convalescent rat serum and 2×10^5 CFU of *M. arthritidis* per ml, are summarized in Fig. 3. Cidal activity was rapid and within 30 min the 1:40 dilution of serum resulted in a 99.7% inactivation of organisms and the 1:160 serum dilution exhibited a 92.5% death of organisms. No further killing occurred

TABLE 1. *Complement dependency of mycoplasmacidal antibody*

Rat serum	Reciprocal of mycoplasmacidal antibody titers using:						
	Unheated serum	Heated serum	Heated plus 1:25 C′	Heated plus 1:50 C′	Heated plus 1:100 C′	Heated plus 1:200 C′	Heated plus 1:400 C′
5-day	20	10	2560	1280	1280	1280	640
28-day	20	10	640	160	160	160	40

TABLE 2. *Effect of growth phase on susceptibility to antibody*

Serum added (1:10 serum dilution)	CFU/0.1 ml after the following treatments:					
	5-Day convalescent serum			Normal rat serum		
	Before addition of serum	After addition of serum, 1 h	After addition of serum, 3 h	Before addition of serum	After addition of serum, 1 h	After addition of serum, 3 h
No preincubation: mycoplasma added with serum	1,200	7	7	1,300	1,200	2,300
Mycoplasma preincubated 1 h before addition of serum	1,140	12	114	1,160	1,000	630
Mycoplasma preincubated 3 h before addition of serum	600	970	730	730	700	800

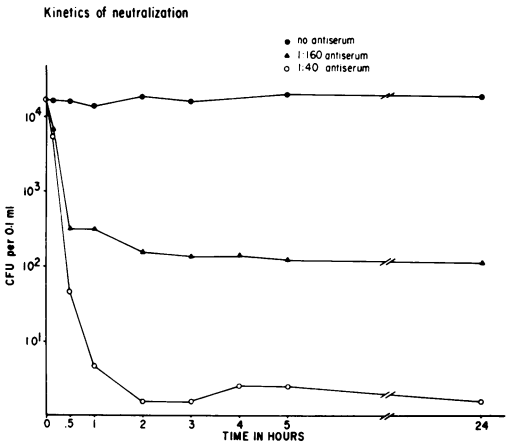

FIG. 3. *Kinetics of mycoplasmacidal action using 5-day convalescent rat serum. Counts for mycoplasma were performed at various times after the addition of serum.*

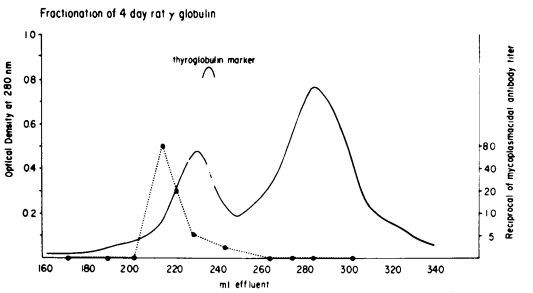

FIG. 4. *Fractionation of gamma globulin prepared from sera taken from rats 4 days after injection of M. arthritidis. Continuous lines represent protein levels and dotted lines represent mycoplasmacidal antibody titers. The 4-day gamma globulin exhibited mycoplasmacidal activity only within the IgM fraction.*

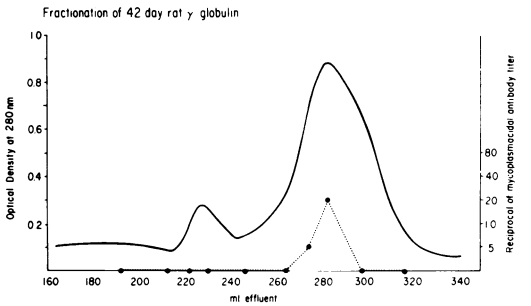

FIG. 5. *Same as in Fig. 4 except that sera were taken from rats 42 days after injection of M. arthritidis. The 42-day gamma globulin exhibited only IgG mycoplasmacidal antibody.*

after 2 h and a small proportion of viable organisms persisted through 24 h.

The rapidity of the reaction was confirmed in another experiment in which 5-day rat serum was tested in a reaction mixture containing 6×10^5 CFU of *M. arthritidis* per ml. A final antiserum concentration of 1:50 resulted in a 95% kill by 30 min and a 99% kill by 1 h. By using 28-day serum, a 90% kill occurred within 30 min and a 99% kill was observed after 2 h.

Fractionation of sera. The 4-day rat serum exhibited antibody exclusively within the immunoglobulin (Ig)M fraction (Fig. 4). The identity of these antibodies with IgM was confirmed by using a thyroglobulin marker and also by reacting those fractions exhibiting the highest antibody titers with specific goat anti-rat IgM antiserum in a gel diffusion test. Serum taken at 6 weeks was found to exhibit exclusively IgG activity in the mycoplasmacidal antibody test (Fig. 5). Again, the identity of this antibody with IgG was confirmed by using goat anti-rat IgG antiserum.

The antibody titers of whole 14-day rat sera were low and fractionation of this serum resulted only in the detection of low titers of IgG mycoplasmacidal antibody.

Mouse serum collected 5 days after infection by *M. arthritidis* exhibited weak mycoplasmacidal activity only within the IgM protein peak.

Specificity of mycoplasmacidal antibody. Rat serum was tested for activity against *M. arthritidis* strain 158 P10 P9, *M. hominis*, and *M. arginini*. Serum taken at 28 days was used in these experiments since it should be less specific than serum taken earlier in the course of infection. The serum exhibited an antibody titer of 1:1,280 with *M. arthritidis* antigen but <1:5 with the *M. hominis* and *M. arginini* antigens.

Mycoplasmacidal and MI activity of hyperimmune rabbit antiserum against M. arthritidis. Rabbit antiserum against *M. arthritidis* strain 14152 P13 (18) exhibited high titers of complement-dependent mycoplasmacidal antibody against *M. arthritidis* (Table 3). The low titers observed with unheated serum alone reflect the loss of complement during storage of the serum. The cidal antibody titers were 16- to 32-fold higher than the corresponding MI antibody titers.

Relationship of antibody production to the course of experimental disease. Mycoplasmacidal antibody was detected in rats as early as 2 days after infection and the highest mean antibody titers occurred 7 days after infection (Fig. 6). A decrease in antibody levels was observed at 10 to 14 days. CF antibody was not detected until 5 days after infection. Mycoplasmas were present in the peripheral circulation at least through 5 days, a time at which mycoplasmacidal antibody levels were high. Furthermore, mycoplasmacidal antibody was present at the onset of arthritis and, in one animal, occurred before the onset of arthritis. Comparisons of the data obtained from individual rats failed to demonstrate a relationship between severity of arthritis and mycoplasmacidal antibody levels. As observed before (8), MI antibody was not detected.

Similar results were obtained with mice (Fig. 7) although the titers of both mycoplasmacidal and CF antibody were lower than in rats. No MI antibody was detected in these experiments. There was a decrease in mycoplasmacidal antibody at 8 to 10 days, after which the levels increased. As was observed in rats, mycoplasmacidal antibody was present together with circulating viable mycoplasmas.

Rats and mice infected many months previously with *M. arthritidis* were also tested for antibody. Serum samples, taken from three rats approximately 300 days after infection with 5×10^8 CFU of strain 158 P10 P9, exhibited mycoplasmacidal antibody titers of 1:40, 1:40, and 1:640, respectively. Sera taken from mice after

TABLE 3. *Mycoplasmacidal and metabolic-inhibiting antibody activity of hyperimmune rabbit anti-M. arthritidis serum*

Serologic test	Reciprocal of antibody titer using:			
	Unheated serum	Unheated serum plus complement	Heated serum	Heated serum plus complement
Mycoplasmacidal antibody	20	20,480	20	40,960
Metabolic-inhibiting antibody	40	1,280	10	1,280

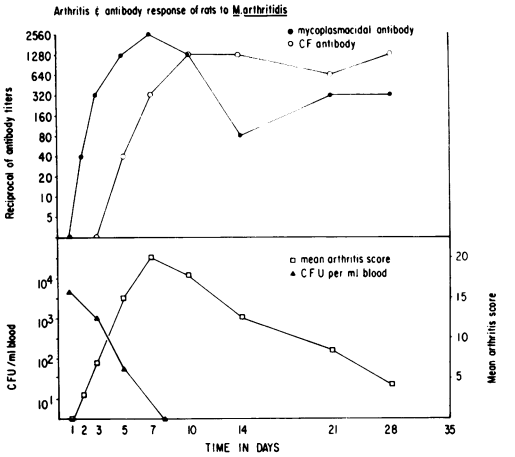

FIG. 6. *Arthritis development and antibody response of rats produced by the i.v. injection of 10^{10} CFU of M. arthritidis.*

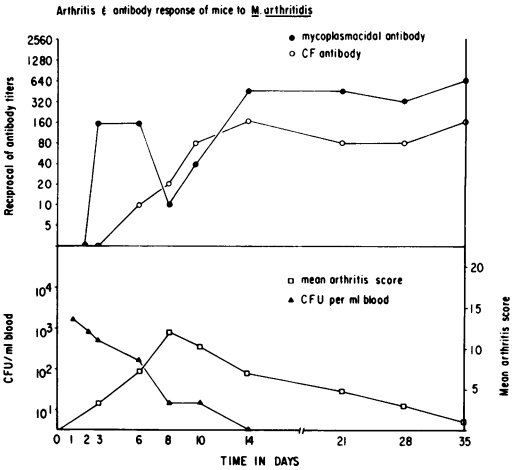

FIG. 7. *Arthritis development and antibody response of mice injected i.v. with 2.1×10^9 CFU of M. arthritidis.*

196 days of infection had antibody titers of 1:320 and 1:640, respectively. Mouse sera collected 487 days after infection with *M. arthritidis* exhibited antibody titers of 1:40 and 1:160, respectively. The mice had low arthritis scores ranging from 2 to 4. The rats had all resolved their arthritis.

Production of mycoplasmacidal antibodies in rodents against other mycoplasma species. The following experiments were undertaken to determine whether the defective antibody response of rats toward *M. arthritidis* was specific for the latter organism or whether the response of rats to all mycoplasmas was defective.

Rat sera previously prepared (8) against *M. felis*, *M. leonis*, *M. hyorhinis*, *M. maculosum*, and mouse sera prepared against *M. meleagridis* (11) were tested for the presence of antibodies cidal to mycoplasma cells suspended in MEM with 10% FCS or mycoplasma broth with 10% HS. For comparison, MI antibody determinations were also carried out. The results are summarized in Table 4.

In contrast to *M. arthritidis* antibody titers against nonmurine mycoplasmas were similar irrespective of the test system used. Thus, cidal antibody titers were virtually identical using either the MEM with fetal calf serum or mycoplasma broth with horse serum. In addition, MI antibody titers correlated well with mycoplasmacidal titers.

TABLE 4. *Cidal antibody response of rodents to various Mycoplasma species*

Antisera	Reciprocal of homologous antibody titers		
	Mycoplasmacidal using:		
	MI	MEM + 10% FCS	Mycoplasma broth plus 10% HS
Rat anti-*M. felis*	64	40	80
Rat anti-*M. leonis*	8	20	40
Rat anti-*M. hyorhinis*	128	80	160
Rat anti-*M. maculosum*	128	160	320
Mouse anti-*M. meleagridis*			
6 day serum	128	320	320
12 day serum	256	320	320
Rat anti-*M. arthritidis*[a]	<10	40–5120	<10–10

[a] Titers of individual sera were within the ranges indicated.

DISCUSSION

These investigations have demonstrated that rats and mice are capable of producing inhibiting antibodies in response to infection by *M. arthritidis*. The mycoplasmacidal test described is thus more sensitive than the MI test in detecting antibodies in rats and mice against *M. arthritidis*. In addition, mycoplasmacidal antibody titers in rabbits were greater than the corresponding MI antibody titers.

Rat mycoplasmacidal antibody has been detected as early as 2 days after injection of the organisms and can persist for 300 days. The antibody is specific and can be absorbed out with appropriate antigen. Both IgM and IgG globulins possess cidal properties and both are dependent upon the presence of a heat-labile factor, presumably complement. Both *M. pneumoniae* (2, 3, 15, 16) and *M. gallisepticum* (1) require complement for immune inhibition and complement clearly enhances the inhibition of other mycoplasmas (22). The activity of rat antiserum against *M. arthritidis* is rapid and the reaction is at least 95% complete by 1 h. *M. pneumoniae* is also rapidly killed by antibody (16).

Present evidence suggests that mycoplasmacidal antibody produced in rats against *M. arthritidis* is effective only against "resting" mycoplasmal cells. Thus, rat and mouse cidal antibody was first detected during experiments designed to demonstrate phagocytic action against *M. arthritidis* (11). In the absence of macrophages, the mycoplasmas were inhibited by convalescent sera. The addition of macrophages resulted in increased mycoplasmal growth and resistance to the cidal properties of serum.

The present study also suggests that antibody is not active in media which permit growth of the organisms, i.e., complete mycoplasma broth. In addition, when high concentrations of convalescent rat serum are used a decreased cidal effect is observed due to the nutritive properties of the serum which render the mycoplasmas more resistant to killing. These observations explain our previous failure to detect metabolic-inhibiting antibody in rats infected with *M. arthritidis* (8) since the test system requires active growth of the organisms to effect an indicator change.

Preliminary evidence was obtained that increased metabolism rather than cell division was responsible for resistance to antibody. Thus, in one experiment, preincubation of resting mycoplasmas in complete medium for 1 and 3 h prior to the addition of serum resulted in a gradual increase in resistance to the cidal activity of the serum. A control experiment showed

that the mycoplasmas had not yet begun to multiply during this time.

There are several reasons why actively metabolizing mycoplasmas might be more resistant to lethal antibody. It was considered that the antibody might be directed against some vital enzyme system rather than against a purely structural component of the cell membrane. Thus, inhibition of this enzyme in the resting cell would be fatal but, in the actively growing cell, an excess of excreted enzyme molecules would effectively bind the antibody before it could inhibit cell functions. This explanation is unlikely since both Formalin-killed antigen and freshly prepared viable antigen were equally effective in absorbing out the antibody activity. Secondly, metabolizing cells may be able to repair minor damage to the cell membrane, whereas resting cells cannot. Membrane repair may require adequate substrate in the medium which is not supplied by calf serum.

Irrespective of the number of organisms and the concentration of antiserum in the reaction mixtures, a certain proportion of cells appeared to be resistant or more resistant to the action of antibody. Previous investigations on the clearance of *M. arthritidis* from the blood stream of rats indicated that irrespective of the dose of organisms injected, a constant proportion of the organisms survived the clearance mechanism (10). This effect was not due to reticuloendothelial blockade. The presence of antibody-resistant cells might also be explained by agglutination resulting in the protection of some organisms. The latter possibility has been found to explain similar observations obtained during virus-neutralization studies (26).

Mice are also capable of producing a mycoplasmacidal antibody against *M. arthritidis*. The antibody has been detected as early as 3 days after injection of the organisms and persists for at least 487 days. Whilst the antibody has not yet been characterized, its requirements for activity appear to be similar to those of rat mycoplasmacidal antibody.

In previous studies the failure of rats to produce MI antibody led us to postulate the existence of a heterogenetic antigen common to parasite and host (4, 8). Although this study indicates that convalescent rat serum can inhibit resting mycoplasmas, it appears that the immunological response of rats to *M. arthritidis* is defective in comparison with the response exhibited towards other mycoplasma species. Thus, rats are able to produce MI antibody against *M. hominis*, *M. gallinarum*, *M. bovigenitalium*, *M. felis*, *M. maculosum*, *M. leonis*, and *M. hyorhinis*. In addition, cidal antibody produced by rats against those species tested was equally active on both resting and multiplying organisms. Mouse anti-*M. meleagridis* serum exhibited similar cidal properties. It is possible that the mycoplasmacidal antibody produced by rats against *M. arthritidis* is directed toward only minor components of the mycoplasma membrane, thus accounting for the lack of activity toward growing cultures. As discussed previously (8), the major membrane antigen may be nonantigenic by virtue of its similarity to host antigens.

What is the significance of these antibodies in the control of *M. arthritidis* infections of rodents? The rapid production of mycoplasmacidal antibody by rats may contribute to the control of infection. However, since the antibody appears to be noneffective (at least at the dilutions used in vitro) on actively multiplying organisms, it would be expected that the mycoplasmas would also be resistant in vivo unless other host factors are operative. The coexistence of viable circulating *M. arthritidis* with circulating mycoplasmacidal antibody supports this view.

Whilst these antibodies may play no role in immunity to *M. arthritidis*, they may have other consequences in the disease process. Thus, since the antibody can be absorbed from the serum by use of washed antigen, it is likely that binding to the mycoplasma membranes occurs. Since the mycoplasmas survive and presumably multiply in vivo, it might be expected that viable antigen-antibody complexes are formed. An investigation of the role of these complexes in the initiation of the acute and chronic forms of murine arthritis would provide a fascinating new area of study.

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