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Immunological Responses of the Rat to *Mycoplasma arthritidis*[1]

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Arthritis was produced in rats by the intravenous injection of *Mycoplasma arthritidis*. Metabolic inhibiting antibody and indirect hemagglutinating antibody could not be detected in the sera of arthritic or convalescent animals. Nonmurine species of mycoplasma were capable of inducing metabolic inhibiting antibody in the rat. A hypothesis based upon the possible occurrence of heterogenetic antigens common to *M. arthritidis* and rat tissue was brought forward to explain these findings. Complement-fixing antibody to *M. arthritidis* was detected 3 to 4 days after injection and subsequently rose to high levels, depending upon the severity of arthritis and number of organisms injected. Animals that had recovered from intravenous or subcutaneous inoculation with *M. arthritidis* were resistant to subsequent infections by the organism. Immunity could be passively transferred by the intravenous injection of convalescent serum. Adsorption of the convalescent serum with antigen greatly reduced the complement fixation titer but did not significantly alter the protective properties of the serum. The presence of complement-fixing antibody could not be related to the development of immunity. An avirulent strain of *M. arthritidis* and a strain previously classified as *M. hominis* type 2 were capable of inducing resistance to subsequent injection by virulent *M. arthritidis*.

Histological studies of rat arthritis induced by *Mycoplasma arthritidis* have been well described in the literature (8, 15, 16, 21), but little detailed work has been reported on antibody response or the mechanisms involved in the development of immunity.

Early studies by Collier (5) and Woglom and Warren (22) indicated that the subcutaneous (sc) or intravenous (iv) injection of rats with *M. arthritidis* resulted in the development of resistance to reinfection. Both of these studies failed to demonstrate the presence of neutralizing antibodies in the sera of convalescent animals. The incubation of viable organisms with immune serum prior to injection had no effect on the severity of the disease.

More recently, other studies have been conducted by use of the complement fixation (CF) technique (12, 14). Rats were injected sc with *M. arthritidis*, and antibody production was measured throughout the development and resolution of the ensuing abscesses. Klieneberger-Nobel (12) showed that the maximal (CF) titers of 1:2,560 were produced 4 to 5 weeks after injection. Lemcke (14), using a less virulent strain of *M. arthritidis*, demonstrated maximal CF titers of 1:320. The titer of the serum was found to be directly related to the severity of the infection.

This study was undertaken to investigate in more detail the antibody responses of the rat to experimentally induced mycoplasmal arthritis and to determine the role of antibody in resistance to reinfection.

MATERIALS AND METHODS

Strains used. The sources of the mycoplasmas used in this study are listed in Table 1. The pathogenicity for rats of strains previously called *M. hominis* type 2, Campo, was established previously (4). To increase virulence, *M. arthritidis* strains 158 and 14124 were passaged in rats 10 times by sc injection. Mycoplasmas cultured from the resulting abscesses were injected sc into normal rats. The passaged strains were designated 158 P10 and 14124 P10, respectively. The virulence of these organisms was similar to that of freshly isolated strains (B. C. Dole and L. Rowland, *unpublished data*). Unless otherwise stated, all experiments reported in this study were conducted with *M. arthritidis* strain 158 P10.

Media. The medium used for maintenance of mycoplasma strains consisted of mycoplasma agar

[1] Portions of this study were submitted to the University of Utah by J. F. Cahill in partial fulfillment of the requirements of the M. S. degree.

TABLE 1. *Source of Mycoplasma strains*

Species and strains	Obtained from
M. arthritidis 158[a]	M. F. Barile, National Institutes of Health, Bethesda, Md.
M. arthritidis 14124	American Type Culture Collection, Rockville, Md.
M. arthritidis H606	M. Davidson, New York University Medical Center, New York, N.Y.
M. hominis (type 1) 14027	American Type Culture Collection, Rockville, Md.
M. bovigenitalium 14173	American Type Culture Collection, Rockville, Md.
M. maculosum PG15	American Type Culture Collection, Rockville, Md.
M. hyorhinis GDL	W. P. Switzer, Iowa State University, Ames, Iowa.
M. gallinarum ST 114	D. L. Madden, National Institutes of Health, Bethesda, Md.
M. felis CO	Authors (3)

[a] Cloned three times from strain PG27, Campo, of D. G. Edward.

(Difco) or broth supplemented with 10% (v/v) horse serum, 5% (v/v) fresh yeast extract, and 1,000 units of penicillin G per ml (2, 9). The medium used for the metabolic inhibition (MI) tests was further supplemented with 1% (w/v) L-arginine hydrochloride or 1% (w/v) D-glucose and 0.002% (w/v) phenol red. The arginine broth medium was adjusted to *p*H 7.2 and the glucose medium to *p*H 7.6.

Inoculation and bleeding of rats and recording of polyarthritis. Holtzman white male rats weighing 100 to 120 g or 350 to 400 g were used. Mycoplasma suspensions or sterile broth were injected into rats via the caudal vein in 0.5-ml amounts. The severity of arthritis was recorded by scoring the inflammation of each involved joint from 1 to 4. Paralysis and partial paralysis were scored 1 and 0.5, respectively, for each limb involved. Scores for each rat were totaled and recorded as the degree of polyarthritis. The animals were bled initially by cardiac puncture, but because this procedure resulted in a high fatality subsequent bleedings were done via the orbital sinus. The sera were harvested after overnight storage at 4 C and were stored at −20 C until used.

CF test. Complement-fixing antibody titers were determined by the 20% volume technique of Kolmer and Boerner (13). Antigens were prepared by growing the mycoplasmas for 3 to 4 days in mycoplasma broth, concentrating the organisms in a model RC-2 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) by centrifuging at 27,000 × *g* for 20 min in a SS-34 rotor, washing three times in physiological saline, and resuspending in saline containing 0.2% (v/v) Formalin. The suspension of organisms was subjected to ultrasonic vibration for 2 to 3 min using a model S 75 sonifier fitted with a 0.32-cm microtip (Branson Instruments Inc., Danbury, Conn.) and set at 2.5 to 3 ma.

Metabolic inhibition (MI) test. The method used was a modification of the technique described by Purcell et al. (17). The tests were performed in screw-cap test tubes (11 by 100 mm). Serum samples (0.25 ml), diluted 1:10 and heated at 56 C for 15 min, were added to the first tube of each series and diluted serially in twofold steps. A broth suspension containing 1,000 colony-forming units (CFU) of the organisms in a 0.2-ml volume was added to all tubes except the medium control series. Guinea pig complement (BBL), used as a source of heat-labile accessory factor (1), was diluted 1:10 and delivered in 0.1-ml amounts to each tube. The volume was brought to 0.9 ml by addition of broth medium. The tubes were incubated aerobically at 37 C until the indicator in the tubes which contained organisms but no antiserum had changed from orange to red, in the case of the arginine broth, or from red to yellow in the case of the glucose broth. The highest serum dilution which prevented a color change was recorded as the end point. In a positive test, the addition of specific antiserum to the medium inhibits mycoplasma growth and subsequent metabolism of arginine or glucose. This is manifested by failure of the indicator to change color.

Indirect hemagglutination (IHA) test. The method used was that described by Taylor-Robinson et al. (20).

Passive immunization. Rats weighing 100 to 120 g were grouped and injected iv as follows: group 1, 0.7 ml of pooled normal rat serum; group 2, 0.7 ml of rat antiserum taken from animals exhibiting severe mycoplasmal polyarthritis; and group 3, 0.7 ml of rat antiserum absorbed with *M. arthritidis* antigen. The antiserum was absorbed by mixing 11 ml of serum and 1 ml of concentrated antigen on a shaker at 37 C. After 2 hr, the mixture was stored for 18 hr at 4 C and then centrifuged to remove the precipitate (6). The procedure was repeated once more. Control, untreated rats were designated group 4.

At 3 hr after iv injection of the sera, all groups of rats were injected intravenously with 1.4×10^{10} CFU of viable *M. arthritidis*.

RESULTS

Iv inoculation of rats with M. arthritidis strain 158 P10. Groups of rats weighing 100 to 120 g were injected iv with 6×10^6, 6×10^7, 6×10^8, and 6×10^9 CFU of *M. arthritidis*. After injection, serum samples were taken from the animals at 4, 8, and 14 days, and thereafter once each week until 21 weeks. Tests for CF antibody titers were conducted on the individual sera from those animals which survived for the duration of the experiment. The deaths which occurred during

the early part of the experiment were due to the trauma of cardiac puncture. Arthritis scores and CF titers observed were averaged and 95% confidence limits were calculated by t distribution (23). The severity of arthritis and the levels of complement-fixing antibodies were proportional to the number of organisms injected. No arthritis occurred when an inoculum of 6×10^6 CFU of *M. arthritidis* was injected, although one animal developed paralysis. However, complement-fixing antibody titers averaging 1:160 were detected 4 days after injection. The results obtained with the larger inocula are recorded in Fig. 1–3. Rats injected with these doses produced mean CF titers of 1:1,280 to 1:2,560 after 4 days. To determine more exactly the time at which complement-fixing antibody was first produced, 10 animals were given iv injections with *M. arthritidis* and bled after 12, 24, 48, 72, and 144 hr. Complement-fixing antibody was not detected until 72 hr after injection of the organisms.

After the initial rise in CF antibody, considerable variation in the titers was observed, presumably due to periodic septicemias. This variation was also apparent upon examination of the antibody responses of individual animals.

Of considerable importance was the finding that complement-fixing antibody titers were usually high at the onset of arthritis and were sometimes present before the clinical symptoms of the disease were evident. Rats inoculated with 6×10^7 CFU of *M. arthritidis* did not develop arthritis until 5 to 8 days. The mean CF titers of these animals after 4 days was 1:1280.

With doses of 6×10^8 and 6×10^9 CFU, complement-fixing antibody titers of 1:40,960 were commonly produced between 4 and 8 weeks after injection. The antibody titers gradually declined after 8 weeks, although they remained at fairly high levels (1:320 to 1:1,280) until completion of the experiment at 21 weeks. The data

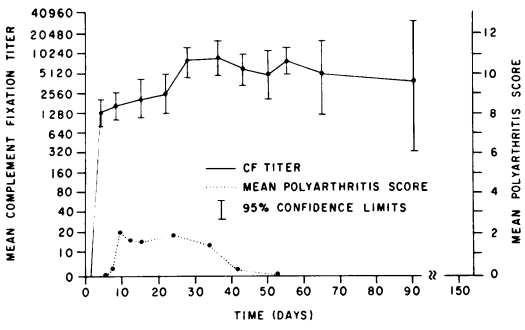

FIG. 1. *Complement-fixing antibody response and arthritis produced by iv injection of 6×10^7 CFU of viable M. arthritidis strain 158 P10.*

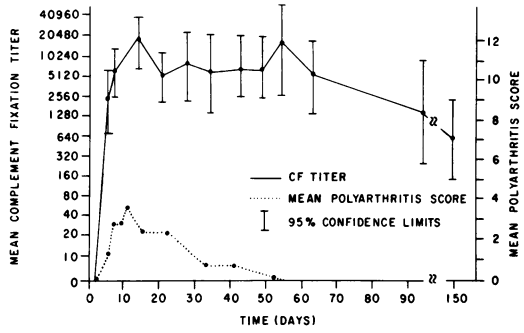

FIG. 2. *Complement-fixing antibody response and arthritis produced by the iv injection of 6×10^8 CFU of viable M. arthritidis.*

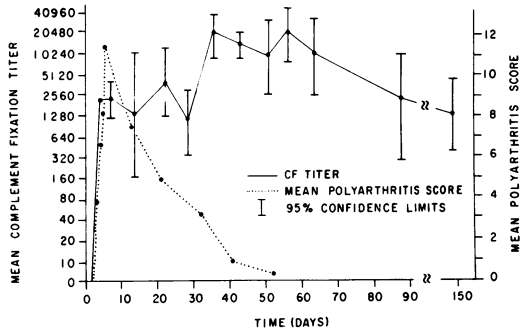

FIG. 3. *Complement-fixing antibody response and arthritis produced by iv injection of 6×10^9 CFU of viable M. arthritidis.*

obtained using the various inocula are summarized in Table 2.

For the purposes of comparison, groups of rats were injected with the same doses of suspensions of Formalin-killed *M. arthritidis*. The animals were bled at intervals up to 5 weeks (Table 3). Complement-fixing titers were much lower with the Formalin-killed organisms, and they reached significant levels only after 15 to 33 days.

Sc inoculation of rats with M. arthritidis strain 158 P10. Ten rats weighing 100 to 120 g were inoculated sc with 6×10^9 CFU of viable *M. arthritidis*. Serum samples were taken up to 33 days after injection and complement-fixing antibody titers were determined. Circulating antibodies were much slower to appear than with animals injected iv. Most rats did not produce detectable CF antibody titers until 8 days after injection. The maximal titer observed was 1:2,560 at 33 days. The mean titer at 33 days was 1:1,280. The results obtained closely correspond with those of Klieneberger-Nobel (12).

Resistance of rats to reinfection. Groups of rats

TABLE 2. *Arthritis and complement-fixing antibody response induced by iv injection of rats with various doses of M. arthritidis strain 158 P10*

Dose	Arthritis				Surviving animals			
	Incidence	Time of onset (days)	Mean time of maximal arthritis (days)	Mean maximal score	No.	Mean CF titer at 4 days	Mean of maximal CF titers	Mean titer after 21 weeks
CFU								
6×10^6	1/9			<1	4	1:160	1:2,560	1:320
6×10^7	10/14	5–8	12	3	11	1:1,280	1:20,480	—[a]
6×10^8	12/12	3–4	9	7	5	1:2,560	1:20,480	1:640
6×10^9	13/13	3	8	17	5	1:2,560	1:40,960	1:1,280

[a] Animals in this group were used in other experiments.

TABLE 3. *Complement-fixing antibody titers produced in rats by iv injection of Formalin-killed M. arthritidis strain 158 P10*

Dose	No. of rats	Reciprocal of mean CF antibody titers: days after injection				
		0	4	8	15	33
CFU						
6×10^6	4	<10	40	<10	20	40
6×10^8	5	<10	40	20	40	640
6×10^9	10	<10	80	20	320	640

previously injected iv with 6×10^6 to 6×10^9 CFU of *M. arthritidis* were challenged by iv injection 9 weeks later with 1.8×10^{10} CFU of the organism. As controls, 10 uninoculated rats of the same weight (350 to 400 g) were injected with an identical dose. As additional controls, two groups of rats previously injected with sterile broth were reinjected with either *M. arthritidis* or broth. All animals were tested for complement-fixing antibody immediately prior to reinjection (Table 4).

Rats exhibiting CF titers of 1:160 or greater were completely resistant to reinfection. The only rat which developed arthritis had a titer of 1:10 prior to reinjection. Some of the animals (3 of 9) receiving sterile broth as the first injection developed complement-fixing antibody titers of 1:10 to 1:20 against *M. arthritidis* antigen. Animals which received no previous injection and animals receiving a primary injection of sterile broth developed identical, high scores of arthritis after injection with *M. arthritidis*. These results demonstrate the inability of antibodies against broth constituents to stimulate resistance to infection by *M. arthritidis*. Animals previously injected with broth and then reinjected with broth exhibited no inflammatory response.

In another experiment, untreated rats and rats

TABLE 4. *Immunity induced by iv injection of rats with viable M. arthritidis strain 158 P10*

Previous treatment[a]	Reciprocal of CF titers prior to reinjection	Effect of iv reinjection with 1.8×10^{10} CFU of viable 158 P10	
		Incidence of arthritis	Mean score of maximal arthritis
None	<10	10/10	61
Sterile serum broth	<10	10/10	58
M. arthritidis strain 158 P10	160–40,960	0/12	0

[a] Injections were given iv. Injection of *M. arthritidis* was between 6×10^7 and 6×10^9 CFU.

injected iv with Formalin-killed suspensions of *M. arthritidis* were challenged by iv injection 5 weeks later with 4×10^{10} CFU of viable organisms (Table 5). Animals exhibiting CF titers of 1:80 or greater were completely resistant to reinfection. Animals with lower CF titers developed arthritis which was less severe than that of the controls.

An experiment was set up to measure the immunity induced by the sc injection of rats with *M. arthritidis* strain 158 P10 (Table 6). Five weeks after the primary injection, all rats were completely resistant to subsequent sc or iv injections of 4×10^{10} CFU of *M. arthritidis*. Control groups exhibited a 100% incidence of abscesses or arthritis.

Experiments were set up to determine whether the injection of rats with other strains of *M. arthritidis* induced protection against strain 158 P10. Groups of rats were inoculated intravenously with *M. arthritidis* strains H606 and 14124 P10. The former strain had previously been found to be nonarthritogenic (B. C. Cole and L. Rowland, *unpublished data*). After primary in-

TABLE 5. *Immunity induced in rats by Formalin-killed suspensions of M. arthritidis 158 P10 injected iv*

Primary injection using Formalin-killed organisms	Reciprocal of CF titers prior to re-injection (33 days)	Effect of iv reinjection with 4×10^{10} CFU of viable organisms	
		Incidence of arthritis	Mean maximal score or arthritis
CFU			
6×10^6	10–40	3/3	12.5
6×10^8	80–1,280	0/4	0
6×10^9	160–2,560	0/10	0
None	<10	10/10	31

TABLE 6. *Immunity induced in rats by viable M. arthritidis strain 158 P10*

Treatment given	Reciprocal of mean CF titers prior to second injection	Effect of second injection on incidence of	
		Abscesses	Arthritis
(A) First injection, 6×10^9 CFU sc; second injection 4×10^{10} CFU sc..	1,280	0/10	
(B) First injection, none; second injection 4×10^{10} CFU sc..	<10	10/10[a]	
(C) First injection, 6×10^9 CFU sc; second injection 4×10^{10} CFU iv.....	1,280		0/10
(D) First injection, none; second injection 4×10^{10} CFU iv.....	<10		10/10[b]

[a] Average size, 2 cm in diameter.
[b] Average score, 31.

jection, the animals were bled at 5 and 9 weeks, respectively. The sera were tested for complement-fixing antibodies by the use of antigens of the strain injected. The serum samples taken immediately prior to reinjection with strain 158 P10 were tested by use of the homologous antigen and 158 P10 antigen. The results obtained with strain H606 are summarized in Table 7. With the exception of two animals previously injected with strain H606 and which had titers of only 1:10 with strain 158 P10 antigen, all rats were completely resistant to reinfection. Thus, it is apparent that avirulent strains can induce immunity against subsequent infection by virulent organisms. All 19 animals that had received a primary injection of *M. arthritidis* strain 14124 P10 developed complete resistance to subsequent infection by *M. arthritidis* strain 158 P10. Furthermore, rat antisera against the different strains cross-reacted almost identically when tested in the CF test against 158 P10 antigen.

Passive immunity. The rat antiserum used in these experiments had a CF titer of 1:81,920. Absorption with *M. arthritidis* antigen reduced this titer to less than 1:1,280. The normal rat serum had a titer of less than 1:10. The protective properties of these sera are illustrated in Fig. 4. Untreated rats and rats injected with normal serum developed severe arthritis, whereas those inoculated with anti-158 P10 rat serum exhibited significantly less arthritis. Absorption of the antiserum with *M. arthritidis* antigen did not significantly alter its protective properties.

The experiment was repeated using a different batch of convalescent serum. In this case, the original CF titer of 1:5,120 was reduced to 1:10 by the absorption procedure. As before, absorbed serum still possessed protective properties.

For the purposes of comparison, the protective properties of rabbit antiserum against *M. arthritidis* were investigated. The rabbit antiserum used had a homologous CF titer of 1:10,240. Groups of rats were treated with 0.5-ml amounts of undiluted serum and of 1:5 and 1:25 dilutions of the serum. All rats, with the exception of those receiving normal rabbit serum, were completely protected against the subsequent injection of 3.3×10^8 CFU of *M. arthritidis*.

Indirect hemagglutinating and metabolic inhibiting antibody. At no time could indirect hemagglutinating or metabolic inhibiting antibody be detected in the sera of rats inoculated with *M. arthritidis*. Serum samples were taken at various stages of the disease and from animals injected with various doses of organisms. All sera were negative in these tests. On the other hand, rabbits immunized with *M. arthritidis* produced antibody titers of 1:10,240 in the MI test and 1:5,120 in the IHA test.

The ability of the rat to produce metabolic inhibiting antibody was further explored. *M. arthritidis* and six nonmurine species of mycoplasma were injected into rats using either of the following immunization procedures. (i) The hind foot pads were injected with 0.15-ml amounts of an antigen/adjuvant (Freund's complete) mixture; the rats were challenged 4 weeks later in the shoulder muscles and serum was collected after

TABLE 7. *Cross reactivity between virulent (strain 158 P10) and avirulent (strain H606) M. arthritidis by complement fixation and immunity*

Primary iv injection	Reciprocal of CF titers prior to reinjection		Effect of iv injection of 4×10^{10} CFU of strain 158 P10	
	Homologous antigen	Strain 158 P10 antigen	Incidence of arthritis	Mean maximal score of arthritis
Strain H606 (6×10^9)[a]	20–40	10	2/2	6
Strain H606 (6×10^9 to 6×10^{10})	160–1,280 (mean 640)	80–2,560 (mean 640)	0/7	0
None	<10	<10	10/10	31

[a] Serum taken 5 weeks after injection.

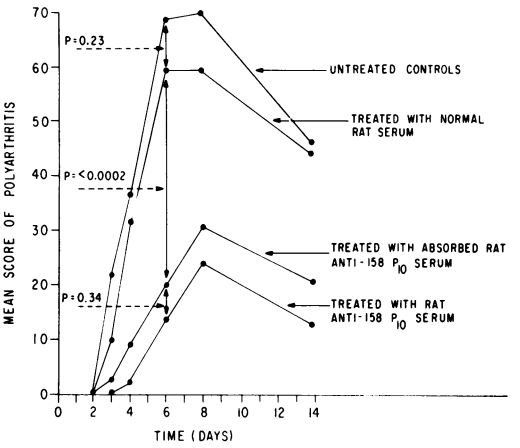

FIG. 4. *Passive immunization of rats against M. arthritidis strain 158 P10 by the use of homologous rat antiserum. P signifies the probability of rejecting the null hypothesis. Rats receiving primary injections of immune serum developed significantly lower arthritis scores than did the controls.*

10 days. (ii) Antigen suspension (0.15-ml amounts) was injected iv; after 2 weeks, the animals were challenged by the sc injection of an antigen/adjuvant mixture and serum was collected after 10 days.

Antisera prepared in rats against *M. bovigenitalium*, *M. felis*, and *M. hyorhinis* were tested for MI antibodies as indicated by inhibition of glucose fermentation. Rat antisera against *M. arthritidis*, *M. hominis*, *M. gallinarum*, and *M. maculosum* were tested for MI antibodies as indicated by inhibition of the arginine dihydrolase pathway. With the exception of *M. arthritidis*, all of the above species of mycoplasma induced metabolic inhibiting antibodies in the rat (Table 8).

DISCUSSION

Rats injected iv with *M. arthritidis* strain 158 P10 produced high levels of complement-fixing antibody depending upon the number of organisms injected and upon the severity of the ensuing arthritis. An early, rapid rise in the level of CF antibody was observed. Although this is suggestive of a secondary response, no other evidence of previous exposure to *M. arthritidis* was encountered. Rats injected with similar numbers of Formalin-killed organisms developed a much less pronounced antibody response. Furthermore, we have demonstrated that non-murine species of mycoplasma can also induce detectable complement-fixing antibodies in the rat 4 days after iv injection (*unpublished data*). This would suggest that the early rise in CF titers is a characteristic of the rat rather than an indication of a secondary response to a specific agent. Large doses of avirulent organisms or smaller doses of virulent *M. arthritidis* also give rise to poorer antibody responses.

Rats previously infected with *M. arthritidis* showed a high degree of resistance to reinfection. The degree of resistance was found to be correlated to the level of CF antibody at the time of reinfection. Thus, rats possessing CF antibody titers of 1:80 or greater showed no symptoms of arthritis when reinjected with massive doses of *M. arthritidis*. Rats with lower CF titers showed varying degrees of arthritis. Although these observations may indicate that CF antibody is correlated with immunity, we feel that the CF levels merely reflect the antibody responses of the host to the mycoplasma. This is supported by the observation that complement-fixing antibodies were detectable 3 days after injection, i.e., at the onset of or before the development of arthritis. Adsorption of rat antiserum with antigen grown in vitro reduced the complement-fixation titer 50-fold, but did not significantly alter the protec-

TABLE 8. *Production of metabolic inhibiting antibodies in the rat by nonmurine mycoplasmas*

Rat antiserum against	No. of animals	Test substrate	Homologous titers of individual animals
Mycoplasma maculosum	1	Arginine	>320
M. hominis (type 1)	2	Arginine	>320, >320
M. gallinarum	3	Arginine	>320, >320, >320
M. felis	2	Glucose	80, >320
M. hyorhinis	1	Glucose	40
M. bovigenitalium	3	Glucose	160, 80, 160

tive powers of the serum as tested by passive immunization. In a further experiment (*unpublished data*), preliminary results showed that serum taken 4 days after the injection of mycoplasma was without protective properties when compared to serum taken 14 days after injection and adjusted to the same CF antibody titer. These observations do not support a major role for CF antibody in host resistance.

The experiments described in this study support the contention that *M. hominis* type 2 and *M. arthritidis* are identical (7). The virulence of strain 158 Campo had previously been increased by animal passage (B. C. Cole and L. Rowland, *unpublished data*). Rats infected with this organism became highly resistant to reinfection with virulent strains of *M. arthritidis*. Sera from animals injected with strain 158 exhibited similar CF titers with antigens of strains H606 and 14124.

No growth-inhibiting antibody was found in rats injected with *M. arthritidis*. All tests for this antibody were negative, even when animals were subjected to repeated injections of the organisms in the presence of adjuvant. All of the nonmurine species of mycoplasma which were tested were capable of inducing growth-inhibiting antibody in the rat. These important observations suggest a possible mechanism for pathogenicity of *M. arthritidis* in rats. The failure of this species to induce growth-inhibiting antibody could be explained in several ways. First, the organisms could possess surface heterogenetic antigens in common with rat tissue. These could render the surface sites of the organisms less antigenic for the rat. Second, *M. arthritidis* may adsorb host antigens in vivo, thereby becoming less immunogenic in the host and thus able to survive longer. An alternative explanation of these findings would be in the development of immunological tolerance to certain *M. arthritidis* antigens as a result of prior exposure of the rats to these organisms.

Influenza virus provides an example of the adsorption of host antigens by a parasite (10, 11). An example of heterogenetic antigens is shown with group A and group C streptococci. The capsules of these organisms consist of a polymer of hyaluronic acid, a substance which is present in the tissues of the host (24). Since the streptococcal capsules are nonantigenic, phagocytosis of the organisms may be delayed, thus enabling the parasite to establish itself in the host. Zabriskie (24) has termed this phenomenon "biological mimicry." Antigenic similarities between virulent *Salmonella typhimurium* and mouse tissues have been described (18). In this case, resistance of the organisms to phagocytosis seems to be due to the inability of the parasite to induce opsonins in the host. The examples cited illustrate the concept of biological mimicry showing how it might be related to virulence of a parasite for a particular host.

M. arthritidis is capable of inducing high levels of growth-inhibiting antibody in rabbits, although virulent strains appear to be less immunogenic in this respect than avirulent strains (B. C. Cole and L. Rowland, *unpublished data*). This can be readily explained on the assumption that in a different host either *M. arthritidis* adsorbs rabbit antigens to a lesser extent or it does not possess heterogenetic antigens cross-reacting with antisera to rabbit tissue antigens. Thus, the antigens of *M. arthritidis* are presented to a host that can respond more strongly to the antigenic mosaic. A recent study by Taylor-Robinson and Berry (19) on the antigenicity of *M. gallisepticum* supports this view. These investigators demonstrated that antisera prepared in chickens against various strains of *M. gallisepticum* had MI titers of at least 16-fold less than the corresponding antisera prepared in rabbits. Furthermore, some strains were noticeably more resistant to inhibition by specific antiserum than others. Finally, some strains failed to induce the production of specific MI homologous antibodies in chickens.

If heterogenetic antigens are factors in the development of rat arthritis, much still remains to be explained. So far, we have been unable to identify the serum factor responsible for protection. Major serological differences between virulent and avirulent *M. arthritidis* have not been

detected, and neither seems capable of inducing MI antibodies in rats. The production in vivo of an extracellular toxin or spreading factor might account for virulence. Antibodies produced against this substance might not be detectable by the CF technique, which employs washed antigen. Furthermore, if antitoxins were responsible for immunity, these might not be absorbed from convalescent serum by washed antigen prepared in vitro. However, as avirulent strains are also capable of inducing immunity, at least small amounts of this toxin would have to be produced by these organisms.

Thus, several mechanisms may be involved in rat arthritis. The organisms may resist the primary defences of the host by possessing a heterogenetic antigen which renders them less immunogenic. An extracellular factor produced by the multiplying organisms may enable them to invade or damage host tissue, resulting in severe inflammation. Eventually, the infection could be brought under control by the formation of antibodies against the extracellular toxin or by other immune mechanisms.

Our results clearly indicate that failure of a host to produce metabolic inhibiting antibodies against a specific parasite does not preclude a pathogenic role for that parasite. Sole reliance on the presence of metabolic inhibiting antibodies as indicators of active or past infection should, therefore, be avoided. In fact, the consistent failure to detect these antibodies should provoke further investigation.

These studies have presented several paradoxes. However, they point to future avenues of research for elucidating the mechanisms of pathogenicity and virulence of mycoplasmal diseases.

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