INFECTION AND IMMUNITY, Jan. 1971, p. 24-35
Vol. 3, No. 1

Role of Biological Mimicry in the Pathogenesis of Rat Arthritis Induced by *Mycoplasma arthritidis*[1]

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Received for publication 6 July 1970

Complement fixation (CF), immunofluorescence, and agar gel double-diffusion tests were used to demonstrate an antigenic relationship between rat tissues and *Mycoplasma arthritidis*. Rabbit antisera against six strains of *M. arthritidis* exhibited positive reactions in the CF test with an ethyl alcohol-saline extract of rat muscle, whereas only 6 of 18 antisera against other *Mycoplasma* species were positive. With the use of gel diffusion techniques, absorption of various *M. arthritidis* antigens with antiserum against rat muscle removed at least one precipitin band when the absorbed mycoplasma antigens were reacted against homologous antisera. Rabbit antiserum against *M. arthritidis* was conjugated with fluorescein isothiocyanate and reacted against frozen sections of muscle tissues of various animals. As controls, unlabeled normal rabbit serum and rabbit anti-*M. arthritidis* serum were included to determine the specificity of the reaction. Rat, hamster, and mouse skeletal muscle exhibited specific fluorescence, whereas chicken, beef, frog, and turtle muscles exhibited no specific fluorescence. Mice injected at birth with rat lymphocytes were found to be more susceptible to subsequent infection by *M. arthritidis* than were normal mice or mice injected at birth with mouse lymphocytes. These results indicate the occurrence of a heterogenetic antigen(s) common to *M. arthritidis* and rat tissues. Preliminary evidence suggests that this heterogenetic antigen(s) may enable the mycoplasmas to become established in their host.

In spite of many reports on rat arthritis induced by *Mycoplasma arthritidis*, numerous questions remain unanswered. For example. (i) how does the organism become established in its host; (ii) why, specifically, is the rat susceptible to *M. arthritidis*; (iii) why do the organisms localize in the joints; (iv) what processes lead to inflammation; and (v) how does the rat recover from the disease. The present report will be concerned largely with the first two of these questions.

Woglom and Warren (37) and Collier (11) showed that rats injected intravenously (iv) or subcutaneously (sc) with *M. arthritidis* failed to develop neutralizing antibodies in their serum, yet developed resistance to further infection with the organism. More recently, Cole et al. (8) demonstrated that although rats injected iv with *M. arthritidis* produced high titers of complement-fixing (CF) antibody, this antibody did not appear to be responsible for resistance to reinfection. Of particular interest was the finding that the rats failed to produce metabolic inhibiting (MI) antibody against *M. arthritidis*, even when the antigen was incorporated in Freund's adjuvant. On the other hand, rats produced MI antibody against nonmurine species of *Mycoplasma*, and *M. arthritidis* induced high levels of MI antibody in guinea pigs and rabbits. As a result of these observations, we suggested that *M. arthritidis* possessed a heterogenetic antigen(s) in common with rat tissues, thus rendering the organisms less immunogenic, and enabling them to resist the primary defenses of the host.

The importance of heterogenetic antigens in the pathogenesis of disease has only recently been noted. Rowley and Jenkin (29) and Jenkin (18) related the absence of opsonins to *Salmonella typhimurium* in normal mouse serum to the presence of cross-reactive heterogenetic antigens between the mouse and the organism. They proposed that the susceptibility of mice to *S. typhimurium* infection was a result of the mouse's inability to recognize the cross-reactive

[1] This study was submitted to the University of Utah by J. F. Cahill in partial fulfillment of the requirements of the Ph.D. degree.

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TABLE 1. *Source of Mycoplasma species and strains*

Species and/or strain	Source	Obtained from[a]
M. arthritidis		
14152 (Campo)...	Human genital tract	ATCC
158 (Campo).....	Human genital tract	Barile
14124.....	Rat arthritis	ATCC
DL.....	Rat arthritis	Cahill and Golightly
H606.....	Mouse tumor	Thomas
PG6.....	Rat arthritis	Thomas
M. pulmonis T_1.....	Benign rat tumor	Cole
M. hominis		
14027.....	Human	ATCC
VV.....	Chimpanzee vagina	Cole
M. salivarium		
156.....	Human saliva	Barile
WS2.....	Chimpanzee saliva	Cole
M. orale, 1, ST5...	Human oropharynx	Madden
M. orale, 2, ST6...	Human oropharynx	Madden
M. orale, 3, DC333..	Human oropharynx	DelGiudice
M. fermentans G...	Human genital tract	ff. Edward
M. lipophilum.....	Human lung	DelGiudice
M. pneumoniae FH..	Human respiratory tract	Madden
BV sc 2[b].....	Chimpanzee vagina	Cole
M. bovigenitalium 14173.....	Bovine genital tract	ATCC
M. maculosum PG 15.....	Dog	ATCC
M. canis.....	Dog	Armstrong
M. hyorhinis GDL.	Swine	Switzer
M. gallinarum ST114.....	Fowl respiratory tract	Madden
M. iners ST 119....	Poultry	Madden
M. felis CO.....	Cat conjunctiva	Cole
M. gateae SIA.....	Cat saliva	Cole
WPS[b].....	Cat lung	Switzer
M. leonis LL.....	Lion lung	Dowdle

[a] ATCC, American Type Culture Collection, Rockville, Md.; M. F. Barile, Division of Biologic Standards, Bethesda, Md.; J. F. Cahill and L. Golightly-Rowland, University of Utah, Salt Lake City; L. Thomas, New York University, New York, N.Y.; B. C. Cole, University of Utah, Salt Lake City; R. A. DelGiudice, BBL, Baltimore, Md.; D. G. ff. Edward, Wellcome Research Laboratories, Kent, England; D. L. Madden, National Institutes of Health, Bethesda, Md.; D. Armstrong, Memorial Hospital, New York, N.Y.; W. P. Switzer, Iowa State University, Ames; W. R. Dowdle, National Communical Disease Center, Atlanta, Ga.

[b] Author's designation.

antigens as foreign. This may have delayed the host's humoral or cellular immunological responses. Heterogenetic antigens between group A streptococci and human cardiac muscle tissue have been implicated in the pathogenesis of rheumatic fever (21, 38, 39). Antigenic similarities between host and parasite may also play a role in the pathogenesis of human ulcerative colitis (27), sarcomas induced by Rous sarcoma virus (32, 33), and rat glomerulonephritis (25).

The present study was undertaken to investigate the possible occurrence of a heterogenetic antigen(s) common to *M. arthritidis* and the rat, and to examine its role in the pathogenesis of the disease.

MATERIALS AND METHODS

Strains used. The original sources of the mycoplasmas used in this study are listed in Table 1. Previous results (10) showed that *M. arthritidis* strains 158 and 14152 (Campo) were arthritogenic for the rat. To increase virulence, these strains were passed in rats by subcutaneous injection and were designated 158 P10 sc and 14152 P13 sc, respectively (14).

Media. The medium used for maintenance of mycoplasma strains consisted of mycoplasma agar (Difco) or broth supplemented with 10% (v/v) horse serum, 5% (v/v) fresh yeast extract, and 1,000 units of penicillin G/ml (7, 17).

Rabbit antisera. To avoid problems with antimedium antibodies, rabbit antisera against mycoplasmas were prepared as described previously (9) with the use of rabbit infusion broth supplemented with rabbit serum (35) as the medium. Antiserum against rat muscle was prepared as follows: skeletal muscle tissue was removed from Holtzman male rats (150 to 200 g) and stored in 10-g quantities at −20 C until used as antigen. Immediately prior to injection into rabbits, a portion of the muscle was minced and washed three times in cold sterile physiological saline. The mince was homogenized in a tissue grinder, centrifuged for 10 min at 600 × *g*, and made up in saline to a 20% (v/v) suspension. An equal volume of incomplete Freund's adjuvant (BBL) was added to give a final 10% (v/v) suspension of tissue. Two New Zealand white male rabbits (2.5 to 3 kg) were inoculated in the hind footpads with 0.1 ml of the mixture. Three weeks later, they were inoculated intramuscularly with 0.5 ml of the mixture in each forearm (26). Additional inoculations of muscle suspension without adjuvant were continued for several months. All sera were stored at −20 C until used.

Rat tissue and mycoplasma antigens. Mycoplasma antigens were prepared by growing the organisms for 2 to 4 days at 37 C in mycoplasma broth, con-

centrating the organisms in a model RC-2 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) by centrifugation at 27,000 × *g* for 20 min in an SS-34 rotor, and washing four times in saline. The mycoplasmas were resuspended in saline and were subjected to ultrasonic vibration for 2 to 3 min by use of a model S75 Sonifier fitted with a 0.32-cm microtip (Branson Instruments Inc., Banbury, Conn.), and set at 2.5 to 3 ma. The tubes were kept in ice water during the sonic treatment to inhibit denaturation of antigenic components by heat. Rat skeletal muscle, kidney, and joint tissues were homogenized in tissue grinders, suspended in saline, and subjected to ultrasonic vibration as described above. The mycoplasma or rat tissue antigens were used fresh or were stored at −20 C and sonically treated immediately prior to use.

Prior to sonic treatment, rat tissue antigens were tested for the presence of contaminating mycoplasmas before use in the serological tests. One drop of antigen was added to 5-ml amounts of mycoplasma broth, and the resulting culture was plated onto mycoplasma agar after various periods of incubation at 37 C. Direct plating of tissue antigens onto mycoplasma agar was also carried out.

Ethyl alcohol-saline extracts of skeletal muscle tissues. Skeletal muscle tissues from rats, guinea pigs, chickens, cattle, and mice were washed in saline, homogenized in a Waring Blendor, and then frozen and thawed several times to disrupt the cells. One volume of the muscle tissue-saline homogenate was mixed with 9 volumes of 95% ethyl alcohol for 1 hr at room temperature. The mixture was centrifuged at 600 × *g* for 10 min, and the supernatant fluid was collected and allowed to evaporate at room temperature. The resulting precipitate was dissolved in 95% ethyl alcohol equal in volume to one-half that of the original muscle tissue-saline suspension.

CF test. CF antibody titers were determined by the one-fifth volume technique of Kolmer and Boerner (24) or by the microtiter technique described by Casey (6). Antigens were titrated with homologous antiserum. The greatest dilution of antigen which gave the maximal CF titer was used in the test. Sera were heated at 56 C for 30 min, and dilutions were made with modified barbital buffer, as described by Campbell et al. (4).

Agar gel double-diffusion test. Precipitin activity of various antisera was tested by the Ouchterlony gel double-diffusion technique. The agar consisted of 1% (w/v) Ionagar No. 2 (Oxoid) and 1% (w/v) sodium azide dissolved in 0.1 M phosphate-buffered saline (*p*H 7.3). An absorption technique was used to study cross-reactivity of antiserum to rat muscle and antigen prepared from the mycoplasmas. One volume of normal rabbit serum or rabbit anti-rat muscle serum was mixed with one volume of mycoplasma antigen and allowed to diffuse against rabbit anti-mycoplasma serum. After the addition of antigens or antisera to the cups, the plates were incubated at room temperatures in closed metal containers. Precipitin patterns were observed periodically for 2 weeks to determine whether the absorption procedure removed any precipitin bands.

Fluorescent-antibody test. Globulins prepared from normal rabbit serum and from rabbit antiserum against *M. arthritidis* strain 158 P10 sc were labeled with fluorescein isothiocyanate (FITC) by the dialysis (method "*b*") technique described by Goldman (13). Unreacted dye was removed by fractionation on a Sephadex G-25 column. Labeled goat antisera against *Mycoplasma* species (kindly supplied by R. A. DelGiudice, BBL) and rabbit antiserum against *M. arthritidis* strain 14152 P13 sc were labeled by the technique described by DelGuidice et al. (12). Frozen sections (2 to 3 μm thick) of the tissue to be stained were cut with a Cryostat (Ames Lab-Tek, Inc., Westmont, Ill.) set at −20 C. They were covered with FITC-labeled antisera and were incubated overnight at 37 C. The tissues were rinsed and washed three times in buffered saline for 30 min each. Buffered glycerol (90% glycerol, 10% 0.1 M phosphate-buffered saline, *p*H 7.3) and cover slips were placed over each stained tissue section. The slides were viewed for fluorescence by use of a Leitz dark-field microscope (Leitz, Inc., New York, N.Y.) with a 2-mm ultraviolet filter (UG-1) or a Zeiss dark-field scope with B12 exciter and BA50 barrier filters (Carl Zeiss, Inc., New York, N.Y.).

Induction of "tolerance" in mice to rat antigens. To make mice tolerant of rat antigens, newborn mice (less than 12 hr old) of the Swiss Webster (WS/sl) strain (Simonsen Laboratories, Inc., Gilroy, Calif.) were inoculated iv via the facial vein with 0.05 ml or intraperitoneally (ip) with 0.1 ml of freshly prepared rat spleen and lymph node tissues (approximately 10^7 to 5 × 10^7 lymphocytes). The rat spleen and lymph nodes had been minced, homogenized in a tissue grinder, and washed twice in Krebs-Ringer phosphate buffer containing sodium heparin (10 units/ml). Control mice were not inoculated or were injected ip with 0.1 ml of mouse spleen and lymph node cell suspension. As a control to establish whether or not tolerance had been induced, 20 of the 6-week-old mice which had been injected at birth with either rat or mouse lymphocytes were injected ip with 10^7 rat lymphocytes (prepared as before) which had been killed by freezing and thawing.

Induction of arthritis in mice. At approximately 6 weeks of age, normal mice or mice which had been injected at birth with rat or mouse lymphocytes were inoculated iv via the caudal vein with 6 × 10^8 or 1.2 × 10^9 colony-forming units (CFU) of *M. arthritidis* strain 158 P10 sc suspended in 0.25 ml of mycoplasma broth. The degree of arthritic involvement which occurred in the mice was determined by estimating the swelling of each joint. The swelling was recorded on a scale of 1 to 4; 1 represented minimal swelling and redness of the joint, and 4 represented maximal swelling. Readings for each animal were totaled and recorded as the arthritis score. Slight swelling (equivalent to a score of 1) frequently occurred in the forepaws of normal mice. Therefore, only readings of 2 or greater were used to determine the mouse arthritis scores. To ensure uniformity and objectivity, a single observer scored the arthritis, and the identity of each group was unknown.

RESULTS

CF tests. A summary of serological reactions between rat skeletal muscle and various species of *Mycoplasma* determined by the CF test is presented in Table 2. All of the normal or preimmunization rabbit serum samples had CF titers ranging from 1:10 to 1:40 against a homogenate of rat skeletal muscle tissue. Rabbit antisera against all of the strains of *M. arthritidis* which were tested reacted with rat muscle homogenate and exhibited CF titers at least fourfold higher than those of the preimmunization sera. Antisera against *M. salivarium* strain WS2, *M. gateae* strain SIA, and *Mycoplasma* sp. strain WPS (feline origin) also reacted with rat muscle homogenate, whereas antisera against 11 other species of mycoplasmas did not react. The fact that mycoplasmas could not be isolated from the rat tissue homogenate antigens indicated that the cross-reactions observed were not due to the presence of contaminating mycoplasmas.

TABLE 2. *Serological relationship of Mycoplasma species and rat muscle tissue by complement-fixation tests*

Rabbit antisera against	Reciprocal of the CF titer against			
	Rat muscle homogenate antigen		Ethyl alcohol-saline extract of rat muscle	
	Pre[a]	Post[a]	Pre[a]	Post[a]
Rat muscle	20	160	<10	160
M. arthritidis				
14152 P13 sc	20	80	<10	80
158 P10 sc	20[b]	160	<10[b]	80
14124	10	80	<10	80
DL	20	160	<10	40
H606	20	80	<10[b]	80
PG6	NT[c]	NT	<10[b]	160
M. pulmonis T_1	20[b]	40	NT	NT
M. hominis				
14027	20[b]	40	NT	NT
VV	20[b]	20	NT	NT
M. salivarium WS2	40	160	NT	NT
M. orale, 1	20[b]	10	NT	NT
M. orale, 2, ST6	NT	NT	10	160
M. orale, 3, DC333	10	10	10	40
M. fermentans G	20[b]	10	NT	NT
M. pneumoniae FH	NT	NT	<10	160
BV sc 2	NT	NT	<10	10
M. bovigenitalium 14173	20[b]	40	NT	NT
M. maculosum PG15	10	10	<10	10
M. canis	20	40	10	20
M. gallinarum ST114	40	20	<10	10
M. iners	20	20	<10	<10
M. felis CO	20[b]	40	NT	NT
M. gateae SIA	20[b]	80	NT	NT
WPS	40	160	10	160
M. leonis LL	NT	NT	20	20

[a] Reciprocal of the CF titer of sera collected either before (pre) or after (post) immunization.

[b] A pool of normal rabbit sera was used instead of preimmunization serum.

[c] Not tested.

It was thought that the reactions observed in the normal rabbit sera could be due to "natural" antibody directed against the Kidd-Friedewald (K-F) antigen found in rat tissue (22, 23). Since the K-F antigen is insoluble in ethyl alcohol, some of the CF tests were repeated with an ethyl alcohol-saline extract of rat muscle. The use of an alcohol-saline extract of rat skeletal muscle tissue in place of a homogenized suspension of rat skeletal muscle resulted in a reduction of the CF titers of normal rabbit serum, but the titers of sera from rabbits immunized with *Mycoplasma* species were not significantly altered (Table 2). Rabbit antisera against various strains of *M. arthritidis* had CF titers which were at least 4 to 16 times that of normal rabbit sera. Four of 10 other species of *Mycoplasma*, in addition to *M. arthritidis*, also cross-reacted with the ethyl alcohol-saline extract of rat skeletal muscle tissue. It is interesting to note that many of the cross-reactions showed titers as high as that of the homologous system in which rat muscle was used as antigen (Table 2).

When rabbit antiserum against rat muscle was tested against antigens prepared from *M. arthritidis* strains 158 P10 sc, 14152 P13 sc, 14124, H606, and DL, *M. canis*, *M. maculosum*, *M. hyorhinis*, *M. lipophilum*, and *M. felis*, all but *M. felis* were reactive; i.e, the CF titer of the serum collected after immunization was at least four times the titer before immunization.

Ethyl alcohol-saline extracts of guinea pig, mouse, chicken, and beef skeletal muscle tissues were used as antigens to determine whether skeletal muscle tissues of animals other than rats cross-reacted with antisera against various species of *Mycoplasma*. The results of this test were inconclusive because almost all of the antisera cross-reacted to some extent with the muscle antigens (Table 3).

Agar gel double-diffusion tests. Since some degree of nonspecificity was apparent in the CF test, the immunological relationship of *M. arthritidis* to rat tissues was studied by the gel diffusion technique.

Rabbit antiserum against rat skeletal muscle reacted with *M. arthritidis* antigen to form a single precipitin band in the gel. This reaction was seen only once and could not be reproduced, although the experiment was repeated many times with several different antigen preparations

TABLE 3. *Serological relationship of rabbit antisera against Mycoplasma species and ethyl alcohol-saline extracts of animal skeletal muscle tissues*[a]

Antiserum against	Reciprocal of CF antibody titers against the following ethyl alcohol-saline extracts of sketal muscle				
	Rat	Guinea pig	Mouse	Chick	Beef
M. arthritidis PG6	80	160	80	40	20
158 P10 sc	80	20	80	10	10
DL	80	320	320	640	40
M. pneumoniae	160	80	40	20	20
M. gallinarum	<10	10	80	40	20
M. maculosum	20	40	20	<10	<10
M. leonis	20	20	40	20	20
M. orale, type 3	40	80	160	40	10
Rat muscle	320	320	160	80	160
Normal rabbit serum	<10	<10	<10	10	<10

[a] Microtiter CF test.

of *M. arthritidis* and with various serum samples from rabbits immunized with rat skeletal muscle tissue. Furthermore, when homogenates of rat joint, kidney, or skeletal muscle tissues were allowed to react with rabbit antiserum to *M. arthritidis*, precipitin lines did not form in the gel.

When antigen prepared from *M. arthritidis* 158 P10 sc was tested with homologous rabbit antiserum, five to seven bands of precipitation occurred in the gel within 2 weeks. Absorption of the mycoplasma antigen by rabbit antiserum against rat skeletal muscle tissue resulted in a reduction in the number of precipitin bands. The procedure was repeated many times with several different antigen preparations of various strains of *M. arthritidis* (158 P10 sc, 14152 P13 sc, and H606). Although the number of precipitin bands obtained varied with the antigen preparation, absorption of *M. arthritidis* antigen with rabbit antiserum against rat skeletal muscle tissue regularly inhibited the formation of at least one specific precipitin band when the absorbed antigen was reacted with homologous antiserum (Fig. 1). Absorption of the mycoplasma antigens with normal rabbit serum did not inhibit the formation of precipitin bands. As a control to insure that the precipitin reactions were not the result of antigenic components in the mycoplasma broth, sterile mycoplasma serum broth was reacted with rabbit antiserum against the mycoplasma. No precipitin lines were detected. The absorption procedure was repeated with *M. orale* types 1, 2, and 3, *M. pulmonis*, *M. hominis* (14027), *M. salivarium*, and *M. felis*, but absorption of the mycoplasma antigens with rabbit antiserum against rat skeletal muscle did not result in a reduction in the number of precipitin lines when the antigen was reacted with homologous antiserum against the above mycoplasmas.

Another experiment was set up in which antisera against the above *Mycoplasma* species were alternated with antiserum against *M. arthritidis* around a well containing *M. arthritidis* antigen. The specific bands in the homologous *M. arthritidis* system which we previously showed could be absorbed out with antiserum against rat muscle did not appear in the heterologous systems.

Fluorescent-antibody tests. Rabbit antisera

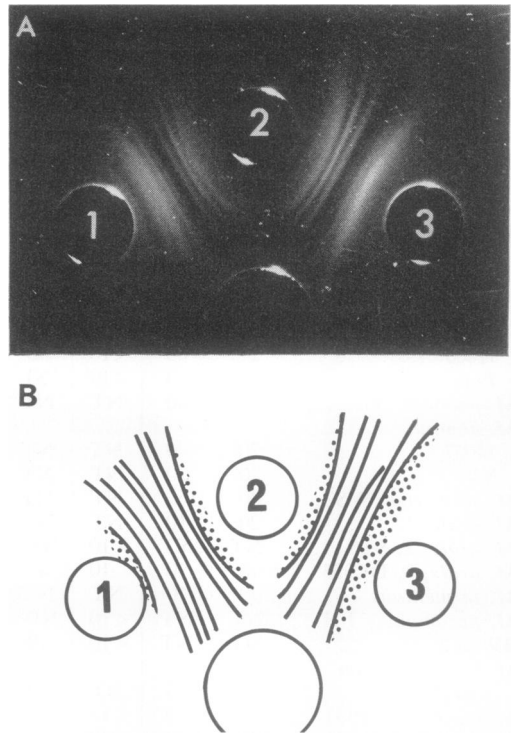

FIG. 1. *Serological relationship of M. arthritidis and rat skeletal muscle as tested by agar gel double diffusion. (Photograph, A, and diagrammatic representation, B.) The wells were filled as follows: (1) normal rabbit serum and M. arthritidis strain 158 P10 sc antigen; (2) rabbit antiserum against M. arthritidis 158 P10 sc; (3) rabbit antiserum against rat muscle and M. arthritidis strain 158 P10 sc antigen. Absorption of M. arthritidis antigen with rabbit antiserum against rat muscle inhibits the formation of two precipitin bands when tested against M. arthritidis antiserum.*

against *M. arthritidis* strains 158 P10 sc and 14152 P13 sc were labeled with FITC. Rat tissues were frozen, sectioned, and treated with labeled rabbit antiserum against *M. arthritidis*. As a control, the tissues were also treated with a mixture of labeled and unlabeled antiserum against *M. arthritidis*. The unlabeled antiserum theoretically would compete for antigenic sites with the labeled antiserum. When the system reached equilibrium, there should be a reduction in the number of labeled antibodies on the antigenic sites, since some of the sites would be bound by unlabeled antibody. Hence, serologically specific reduction or "blocking" of fluorescence should occur.

The treated tissue sections were viewed for fluorescence by three individuals. To ensure objectivity, both the serum dilution and the source of antiserum were unknown until all of the samples had been viewed. The results of a fluorescent-antibody test with the use of labeled rabbit antiserum against *M. arthritidis* 14152 P13 sc or 158 P10 sc to treat rat skeletal muscle tissue are presented in Table 4. Labeled rabbit antisera against *M. arthritidis* 14152 P13 sc and labeled rabbit antiserum to *M. arthritidis* 158 P10 sc reacted identically with rat skeletal muscle tissue; therefore, only labeled antiserum against *M. arthritidis* 14152 P13 sc was used in subsequent fluorescent-antibody studies.

Labeled rabbit antiserum against *M. arthritidis* mixed with unlabeled normal rabbit serum reacted with rat skeletal muscle, causing the entire muscle fiber to fluoresce. The addition of unlabeled antiserum against *M. arthritidis* to the labeled antiserum against *M. arthritidis* resulted in a reduction of fluorescence (Fig. 2).

Several different rat tissues were treated with labeled rabbit antiserum against *M. arthritidis* to test the specificity of the cross-reaction between rat skeletal muscle and antiserum to *M. arthritidis*. As a control, 1:2 dilutions of normal rabbit serum or unlabeled rabbit antiserum against *M. arthritidis* were mixed with the labeled antiserum against *M. arthritidis*. The 1:2 dilutions were used because undiluted normal rabbit serum inhibited some of the fluorescence of rat skeletal muscle tissue treated with labeled antiserum against *M. arthritidis* (Table 4). Tissue sections of skeletal muscle, cardiac muscle, and liver fluoresced when treated with a 1:10 dilution of labeled antiserum against *M. arthritidis*. The fluorescence was completely blocked by unlabeled antiserum against *M. arthritidis*. Tissue sections of rat lymph node fluoresced slightly when treated with a 1:10 dilution of labeled antiserum and fluoresced strongly when labeled antiserum was used undiluted. Tissue sections of kidney, testis, spleen, and brain fluoresced only slightly, and lung tissue not at all, when treated with undiluted labeled antiserum. Fluorescence was blocked only slightly or not at all for these tissues when unlabeled antiserum against *M. arthritidis* was added to labeled antiserum, thus indicating that the fluorescence seen was nonspecific.

TABLE 4. *Serological relationship of M. arthritidis and rat skeletal muscle tissue as determined by the fluorescent-antibody test*

System	Dilution of the labeled antiserum	Reaction[a]
Labeled rabbit antiserum to *M. arthritidis* strain 14152 P13 sc + saline	1:20	4+
Labeled rabbit antiserum to *M. arthritidis* strain 14152 P13 sc or 158 P10 sc + undiluted, normal rabbit serum	1:20	2+
Labeled rabbit antiserum to *M. arthritidis* strain 14152 P13 sc or 158 P10 sc + undiluted, unlabeled rabbit antiserum to *M. arthritidis* strain 14152 P13 sc or 158 P10 sc, respectively	1:20	0

[a] Fluorescence of rat muscle sections as measured on a scale with maximal fluoresence as 4+, intermediate fluorescence as 2+, and no fluorescence as 0.

To test host specificity, labeled antiserum against *M. arthritidis* was used to treat frog, turtle, chicken, beef, hamster, mouse, and rat skeletal muscles (Table 5). Turtle skeletal muscle did not fluoresce, even with undiluted antiserum, whereas chicken, frog, mouse, and beef skeletal muscles fluoresced when treated with undiluted rabbit antiserum against *M. arthritidis*. Rat and hamster skeletal muscle fluoresced when treated with the labeled antiserum diluted 1:10. The addition of unlabeled antiserum against *M. arthritidis* prevented the fluorescence of rat, hamster, and mouse muscle but not that of chicken, frog, and beef muscles. This indicated that the reaction between chicken, frog, and beef muscle and labeled antiserum to *M. arthritidis* was nonspecific, whereas the reaction between rat, hamster, and mouse muscles and labeled antiserum to *M. arthritidis* indicated a specific cross-reaction.

Tolerance. Simonsen and Harris (33) reported that turkeys, which are normally resistant to Rous sarcoma virus, a pathogen of chickens, could be made susceptible by making them tolerant to chicken antigens. This report stimu-

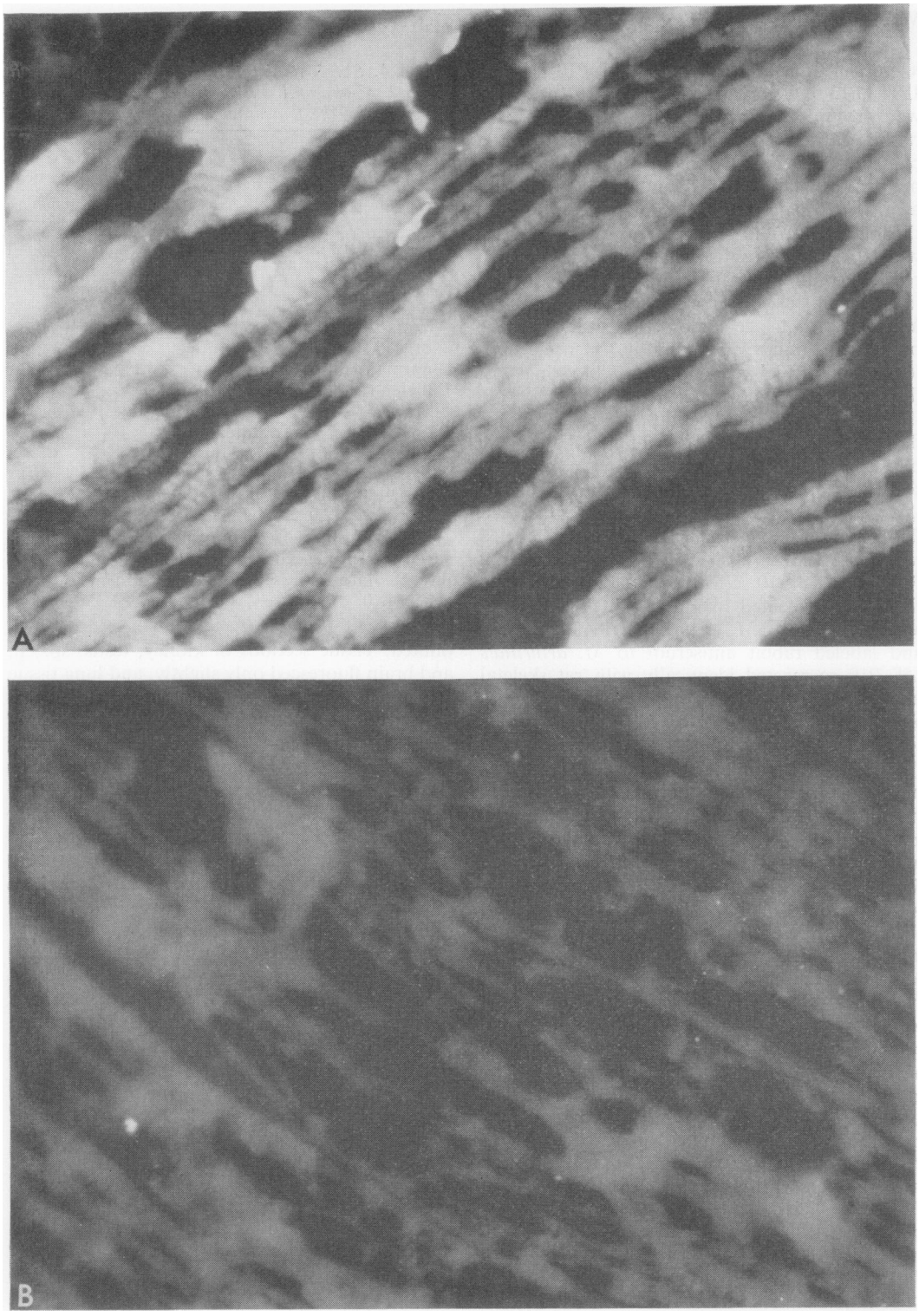

FIG. 2. *Specific fluorescence of rat muscle sections after treatment with FITC-labeled antiserum against M. arthritidis strain 14152 P13 sc. Observations were made on a Zeiss dark-field microscope (320×) with BG12 exciter and BA50 barrier filters. Kodak Panatomic X film was exposed for 2 min. (A) Rat muscle treated with a 1:10 dilution of labeled M. arthritidis antiserum mixed with unlabeled, undiluted, normal rabbit serum. (B) "Blocking control." Conditions of test as for Fig. 2A, but with unlabeled, undiluted anti-M. arthritidis rabbit serum substituted for unlabeled normal rabbit serum.*

lated an investigation along similar lines with *M. arthritidis* in which we attempted to enhance the susceptibility of mice to *M. arthritidis* by making them tolerant to rat antigens. In our experiments, rat lymphocytes were used as the source of rat antigens. In the first experiment, mice were injected iv at birth with rat lymphocytes and were challenged iv 6 weeks later with 6×10^8 CFU of *M. arthritidis* 158 P10 sc. Treatment with rat lymphocytes had no observable effect upon the mice as evidenced by runting disease, thus indicating that an overt graft versus host reaction had not occurred. The results of the tolerance experiment are presented in Table 6 and Fig. 3. During the first 6 months after injection with *M. arthritidis*, the arthritis scores of the control group of mice were significantly lower than the arthritis scores of mice which had been injected at birth with rat lymphocytes: comparisons were made at the 95% confidence level by use of the Wald-Wolfowitz runs test (31). Of the mice injected at birth with rat lymphocytes, 30% developed swollen joints 4 days after injection with *M. arthritidis*; normal mice did not have arthritis until at least 9 days after injection with *M. arthritidis*. In 62% of the normal mice and 91% of the mice injected at birth with rat lymphocytes, arthritis developed after injection with *M. arthritidis*. The day of maximal arthritis varied greatly for individual mice, thus accounting for the lower mean arthritis scores at various times as recorded in Fig. 3. The arthritis declined at day 11, but returned by day 189.

Inflamed mouse joints were cultured for mycoplasmas and bacteria at intervals throughout the experiment. Mycoplasmas were regularly isolated and were identified as *M. arthritidis* by treatment of the colonies with specific FITC-conjugated antiserum (12). No bacteria or other *Mycoplasma* species were detected.

The experiment on tolerance was repeated with larger numbers of mice. Since the iv injection of newborn mice resulted in many deaths, intraperitoneal injections were used in the second experiment. Mice inoculated with mouse lymphocytes served as controls. To determine whether a state of tolerance had been induced, some mice previously injected at birth with mouse or rat lymphocytes were challenged at 6 weeks with rat lymphocytes. The animals were test bled 19 days after the challenge injection of rat lympho-

TABLE 5. *Fluorescence of various animal skeletal muscle tissues when stained with labeled rabbit antiserum against M. arthritidis*

Tissue	Dilution of labeled antiserum against *M. arthritidis* 14152	Reaction[a] when labeled antiserum to *M. arthritidis* was mixed with the indicated rabbit sera	
		Normal[b]	Anti-*M. arthritidis*[b]
Rat muscle	Undiluted	3+	2+
	1:10	2+	0
	1:20	0	0
Hamster muscle	Undiluted	2+	2+
	1:10	2+	0
	1:20	0	0
Chicken muscle	Undiluted	2+	2+
	1:10	0	0
Mouse muscle	Undiluted	2+	0
	1:10	0	0
Beef muscle	Undiluted	2+	2+
	1:10	0	0
Frog muscle	Undiluted	2+	2+
	1:10	0	0
Turtle muscle	Undiluted	0	0

[a] Fluorescence was measured on a scale with maximal fluorescence as 4+ and no fluorescence as 0.

[b] Diluted labeled antiserum against *M. arthritidis* was mixed with an equal volume of undiluted normal rabbit serum or unlabeled, undiluted antiserum against *M. arthritidis*.

TABLE 6. *Arthritis induced by iv injection of mice[a] with M. arthritidis strain 158 P10 sc (experiment 1)*

Treatment at birth	Mean day of onset of arthritis	Incidence of arthritis (%)	Range of arthritis scores	Mean of maximal arthritis scores[b]	Mean arthritis scores at	
					18 days	189 days
None (controls), 8[c]	10	62	0–16	3.7	0.2	5.0
Injection iv with a rat lymphocyte suspension, 23[c]	5	91	0–26	7.9	3.0	5.3

[a] Mice were injected iv at birth with a suspension of rat spleen and lymph node tissues and were challenged at 6 weeks with 6×10^8 CFU of *M. arthritidis* strain 158 P10 sc.

[b] The highest scores observed for each mouse, irrespective of time, were totaled and the mean was calculated for each group.

[c] Number of mice.

cytes. The serum samples were tested for CF antibody against rat lymphocyte and *M. arthritidis* antigens (Table 7). Sera from 3 of 10 mice injected at birth with mouse lymphocytes and challenged at 6 weeks with rat lymphocytes had CF titers of 1:80 or greater against *M. arthritidis*. This cross-reaction provides additional evidence for a heterogenetic antigen common to rat tissues, in this case rat lymphocytes, and *M. arthritidis*. Sera from all of the mice which had been injected at birth with rat lymphocytes and challenged at 6 weeks with rat lymphocytes had CF titers of 1:10 or less when tested against *M. arthritidis*. Sera from 75% of the mice injected at birth with rat lymphocytes and challenged at 6 weeks with rat lymphocytes had CF titers of 1:10 or greater against rat lymphocyte antigen, whereas 20% of the mice injected at birth with mouse lymphocytes and challenged at 6 weeks with rat lymphocytes had CF titers of 1:10 or greater against rat lymphocyte antigen.

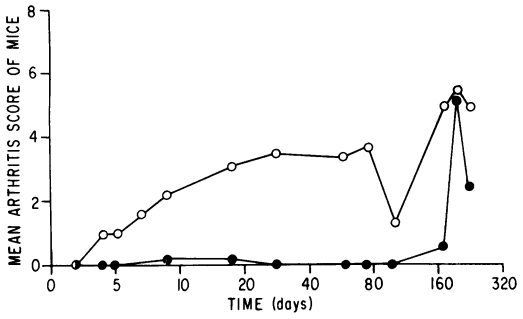

FIG 3. *Effect of injection of newborn mice with rat lymphocytes on subsequent susceptibility to M. arthritidis (experiment I). Arthritis obtained after iv injection of 6-week-old mice with 6 × 10⁸ CFU of M. arthritidis strain 158 P10 sc. (●) Controls: mice were previously injected iv at birth with saline. (○) Mice were previously injected iv at birth with rat lymphocytes.*

TABLE 7. *Reciprocal of CF titers of mouse sera collected[a] from animals injected at 6-weeks with rat lymphocytes*

Primary inoculation at birth	Mouse no.	CF titer with antigen from	
		M. arthritidis	Rat lymphocyte
Mouse lymphocytes	Ia1	<10	<10
	Ia2	<10	<10
	Ia3	320	<10
	Ia4	<10	<10
	Ia5	160	40
	Ib1	<10	<10
	Ib2	<10	<10
	Ib3	<10	<10
	Ib4	<10	<10
	Ib5	80	20
Rat lymphocytes	IIa1	<10	10
	IIa2	<10	20
	IIa3	<20	NT[b]
	IIa4	<10	10
	IIa5	<10	10
	IIb1	<10	40
	IIb2	<20	NT
	IIb3	10	80
	IIb4	<10	<10
	IIb5	<10	<10

[a] Serum samples were collected 19 days after an ip challenge with 10^7 lymphocytes which had been disrupted by freeze-thawing.

[b] Not tested.

The results of the second tolerance experiment are presented in Table 8 and Fig. 4. In this experiment, the mice were challenged at 6 weeks of age with 1.2 × 10^9 CFU of *M. arthritidis* 158 P10. The results closely paralleled those of the first experiment in that the mice injected with rat lymphocytes showed a significantly more severe arthritis, greater incidence of arthritis, and a shorter period of onset, as compared with the controls. The mean arthritis scores of the mice injected at birth with rat lymphocytes reached a high at 18 days after injection with the mycoplasmas and declined only slightly for the duration of the experiment. The arthritis scores of the control mice likewise reached a high at 18 days, but declined rapidly. As in the first experiment, there was a resurgence of arthritis in both groups of animals which occurred in this case at approximately day 80.

TABLE 8. *Arthritis induced by iv injection of mice[a] with M. arthritidis strain 158 P10 sc (experiment 2)*

Treatment at birth	No. of mice	Mean day of onset of arthritis	Incidence of arthritis (%)	Range of arthritis scores	Mean of maximal arthritis scores[b]
Injection ip with mouse lymphocytes (controls)	26	9	62	0–8	2.4
Injection ip with rat lymphocytes	58	4	100	2–22	11.8

[a] Mice were injected ip at birth with a suspension of mouse or rat spleen and lymph node tissues and were challenged at 6 weeks with 1.2 × 10^9 CFU of *M. arthritidis* strain 158 P10 sc.

[b] The highest scores observed for each mouse irrespective of time were totaled and the mean was calculated for each group.

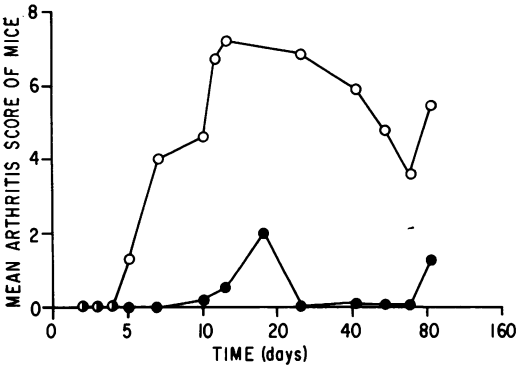

FIG. 4. *Effect of injection of newborn mice with rat lymphocytes on subsequent susceptibility to M. arthritidis (experiment 2). Arthritis obtained after iv injection of 6-week-old mice with 1.2 × 10⁹ CFU of M arthritidis strain 158 P10 sc. (●) Controls: mice were previously injected ip at birth with mouse lymphocytes. (○) Mice were previously injected ip at birth with rat lymphocytes.*

DISCUSSION

Zabriskie (38) described biological mimicry as "the chemical and immunological similarities between organisms." To study the role of mimicry in a disease process, it is necessary to demonstrate: (i) the existence of a common antigenic component in host and parasite; (ii) some degree of specificity of this component for the particular host-parasite relationship; and (iii) proof of its significance in the disease process. In this study, a common antigenic component(s) shared by *M. arthritidis* and rat tissue was demonstrated by serological techniques. Furthermore, there was evidence that this antigenic component may play a role in the pathogenesis of rat arthritis induced by *M. arthritidis*.

Problems were encountered with nonspecific reactions. Although rabbits lack the Forssman antigen (5), it was found that normal rabbit sera reacted with rat tissues in the CF and fluorescent-antibody tests. Kidd and Friedewald (22, 23) described a cross-reaction between normal rabbit serum and an ethyl alcohol-insoluble antigen in rat tissues. Johnson et al. (19) also reported that normal rabbit serum contained natural antibodies against the Kidd-Friedewald antigen. In the present study, we found that the cross-reaction of normal rabbit serum with rat tissues could be eliminated in the CF test by using ethyl alcohol-saline extracts of rat muscle. In the case of the fluorescent-antibody tests, the cross-reaction was eliminated by using "blocking" controls. The heterogenetic antigen was found to be most prevalent in rat cardiac and skeletal muscle tissues, but was present in other rat tissues and the muscle tissue of mice and hamsters.

Although the CF reaction with normal rabbit serum had been eliminated, 4 of 10 other *Mycoplasma* species also cross-reacted with ethyl alcohol-saline extracts of rat muscle. Although the cross-reaction between mouse and hamster muscle and labeled rabbit anti-*M. arthritidis* serum appeared to be specific because it was blocked by unlabeled rabbit anti-*M. arthritidis* serum, it does not necessarily follow that the reaction was due to the same cross-reactive antigen(s). Zabriskie, in a study of group A streptococci and their role in rheumatic fever, encountered similar problems with cross-reactions between human heart tissue and antiserum against organisms not implicated in rheumatic fever (38). The specificity of the reaction between group A streptococci and cardiac tissue was shown by using antisera against purified bacterial cell membranes to stain heart tissue. All of the antisera to group A streptococcal membranes produced extremely bright fluorescence, whereas none of the antisera to membranes of other streptococcal groups or other gram-positive cocci were reactive. In the case of *M. arthritidis* and rat tissue, it may also be necessary to purify the common antigen before its specificity can be confirmed. The results of the gel double-diffusion experiments, however, showed that *M. arthritidis* was the only mycoplasma tested from which a specific antigen could be absorbed by treatment with antiserum against rat muscle.

The possibility that the serological cross-reactions observed might be due to contaminating mycoplasmas in the tissue antigens was considered. However, mycoplasmas could not be isolated from any of the normal tissues used as antigens. Kaklaminis et al. (20) and Thomas (36) reported that extracts of mammalian tissues may be inhibitory for mycoplasmas and that lytic factors in tissues may inhibit the growth of mycoplasmas during isolation procedures. Using similar techniques, experiments in these laboratories (Cole and Golightly-Rowland, *unpublished data*) have shown that high concentrations of unheated rat muscle tissue extracts have no inhibitory effect upon the growth of *M. arthritidis*. In fact, the survival of *M. arthritidis* was considerably prolonged in the presence of muscle tissue extracts. This appeared to be due to a buffering effect of the extract which prevented excessive alkalinity due to arginine degradation by the mycoplasmas.

Additional evidence of the presence of a heterogenetic antigen shared by *M. arthritidis* and rat tissue, as well as suggestive evidence for

the role of this antigen in the disease, was found by making mice partially "tolerant" to rat lymphocyte antigens. During development of the immune response in the embryo, there is presumed to be suppression and possible elimination of cells producing antibodies directed against self-components (3). A similar suppression of normal antibody response to foreign antigens may be obtained by injecting the antigen into the fetus or neonatal animal, inducing the state known as immunological tolerance (2). Thus, a tolerant animal would respond to a foreign antigen as a normal animal would to its own antigenic components. Mice were chosen for our experiments because previous studies (Cole, Cahill, and Golightly-Rowland, *unpublished data*) had shown that they were much less susceptible than rats to the arthritogenic activities of *M. arthritidis*. Two separate experiments showed that mice tolerant to rat lymphocytes were much more susceptible than normal mice to polyarthritis induced by a subsequent injection with *M. arthritidis*. These results suggest that the mice had become tolerant to the heterogenetic antigen(s) shared by *M. arthritidis* and rat tissues. If this were the case, the immunological responses of the tolerant mice to *M. arthritidis* would be impaired, possibly enabling the organisms to multiply freely after injection and thus establishing an active infection. Additional evidence for the occurrence of a heterogenetic antigen was apparent from the observation that 30% of control mice previously injected at birth with mouse lymphocytes and challenged 6 weeks later with rat lymphocytes developed CF titers of 1:80 or greater against *M. arthritidis*. On the other hand, no titer against *M. arthritidis* developed in mice receiving an injection of rat lymphocytes at birth followed by another injection of rat lymphocytes 6 weeks later. These results suggest that treatment of mice at birth with rat lymphocytes does in fact result in tolerance to the heterogenetic antigen. However, the same mice were capable of responding to other rat lymphocyte antigens, as evidenced by their production of low CF titers against rat lymphocytes.

The fact that all mice injected with rat lymphocytes remained healthy until challenged with *M. arthritidis* suggests that an overt graft versus host reaction had not occurred. Other experiments in these laboratories (Cole et al., *unpublished data*) have shown that both normal mice and mice tolerant of rat lymphocytes respond equally to other CF antigens of *M. arthritidis*, thus indicating no general deficiency in the immunological response due to the rat lymphocyte treatment. The unexpected finding that *M. arthritidis* induces a chronic arthritis in mice will be the subject of a later communication.

Simonsen (32) and Simonsen and Harris (33) reported a similar phenomenon in turkeys which had been injected neonatally with the cellular fraction of whole chicken blood. The turkeys were more susceptible to Rous sarcoma virus than were normal turkeys. Harris (16) ascribed the enhanced susceptibility to tolerance to a heterogenetic antigen common to chickens and a virus-induced antigen in infected turkeys. The tolerant turkeys could not recognize this antigen as foreign and did not oppose the growth and dissemination of the tumor, whereas normal turkeys developed tumors which subsequently regressed, presumably as a result of an immunological response.

There is evidence that biological mimicry could play a role in the pathogenesis of other mycoplasma diseases. Taylor-Robinson and Berry (34) reported that some strains of *M. gallisepticum* failed to induce MI antibodies in chickens. Additional evidence for heterogenetic antigens between host and parasite in mycoplasma-induced diseases was shown by Gourlay and Shifrine (15, 30), who detected a cross-reaction between a crude galactan extracted from *M. mycoides* and a purified galactan from bovine lung. In a preliminary report, Biberfeld (1) described the presence of IgM antibodies against brain in a high proportion of sera taken from patients infected with *M. pneumoniae*. The formation of cold hemagglutinins by individuals in response to *M. pneumoniae* infection is also well known (28). More recently, Cole et al. (Infec. Immun., *in press*) have shown that in the case of *M. pulmonis* and *M. neurolyticum* there is also positive correlation between ability of these organisms to induce disease and impairment of MI antibody response by their natural hosts. Further work is now required to determine whether these observations can be explained on the basis of biological mimicy or by some other mechanism.

The present study provides preliminary evidence that biological mimicry aids *M. arthritidis* in becoming established in its natural host, the rat. Thus, a partial explanation of why this organism infects rats is apparent. Exactly how the organism localizes and multiplies in the joints, which metabolic products or biological activities lead to the development of arthritis, and how the animals recover from the disease are now being studied.

ACKNOWLEDGMENTS

We thank those investigators who supplied us with cultures of *Mycoplasma*. This work was supported by Public Health Service

grant AM-02255-11A1/11A2 from the National Institute of Arthritis and Metabolic Diseases.

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