INFECTION AND IMMUNITY, Feb. 1973, p. 218-225

Vol. 7, No. 2
Printed in U.S.A.

Factors Influencing the Susceptibility of Mice to *Mycoplasma arthritidis*

B. C. COLE, J. R. WARD, AND L. GOLIGHTLY-ROWLAND

Division of Arthritis, Department of Internal Medicine and Department of Microbiology, University of Utah College of Medicine, Salt Lake City, Utah 84112

Received for publication 28 September 1972

Concentrated broth constituents markedly enhanced the development of abscesses after subcutaneous injection of *Mycoplasma arthritidis*. Under appropriate conditions, both virulent and avirulent strains of *M. arthritidis* produced toxic effects in mice, as detected by the development of necrotic abscesses and death of the animals. The treatment of mice at birth with *M. arthritidis* antigens rendered the animals more susceptible to infection with *M. arthritidis* later in life, as evidenced by increased arthritis after intravenous injection. The injection of *M. arthritidis* into pregnant mice resulted in a high incidence of abortions. Comparative studies using different mouse strains showed that DBA mice were the most susceptible and BALB/c and C57BL mice were the most resistant to infection by *M. arthritidis*.

The agent-host relationships of mycoplasma and animals pose numerous perplexing problems. In the laboratory, large numbers of *Mycoplasma arthritidis* are required to induce experimental disease of rats and mice, yet spontaneous outbreaks of *M. arthritidis* infections of rats are not infrequent. Although the use of laboratory-adapted strains could, in part, determine low virulence, animal passaging to enhance virulence has been only moderately successful (5, 6, 9, 21, 23, 28). Similarly, freshly isolated virulent strains must still be injected in large doses in rats in order to produce arthritis (5, 6). Some investigators have used agar adjuvants to induce arthritis (10).

A number of studies have shown that mycoplasmas readily invade diseased tissue. Thus, mycoplasmas have been isolated from rat joints affected by adjuvant arthritis (22) and 6-sulfanilamidoindazole arthritis (7, 19). In addition, *M. arthritidis* is commonly found in rat lymphosarcomas and other rat tumors (14, 15, 29). It may be that, in naturally occurring disease, damage of the tissues is a necessary prerequisite for infection by mycoplasmas. Thus, the isolation of mycoplasmas from human rheumatoid arthritis should be considered with this in mind (25).

Agent factors which influence the capability of mycoplasmas to produce disease still require much investigation. Preliminary evidence suggests that *M. arthritidis* produces a toxin (8, 26). Recently we reported data suggesting that the antigenic makeup of *M. arthritidis* mimics rat tissues, thus aiding the organism to become established in the host by circumventing the primary immune mechanisms (1, 4). In addition, avirulent strains of *M. arthritidis* were found to be more susceptible to the growth-inhibiting action of antibody than are virulent strains (11).

The present study explores various factors influencing the development of both abscesses and arthritis in mice induced by *M. arthritidis*.

MATERIALS AND METHODS

Strains used. *M. arthritidis* strains 158 P10 (4) and 158 P10 P9 (8) were used as virulent cultures, and strain H606, which was found to be nonarthritogenic for rats (11), was used as an avirulent culture of *M. arthritidis*.

Media and preparation of cultures. Mycoplasma agar and broth (Difco) as modified by Chanock et al. (3) and Hayflick (13) were used throughout these studies. Suspensions of organisms for injection were prepared and counted as previously described (11).

Induction of abscesses. The backs of Swiss-Webster mice were shaved and injected subcutaneously (sc) with 0.1 ml of *M. arthritidis* strain 158 P10 P9 or H606 at doses of 2×10^3 to 2×10^{10} colony-forming units (CFU) per mouse. The mice were examined at frequent intervals through 2 weeks, and the severity of the developing abscesses was recorded. Abscesses were graded as follows: "+" denoted small swellings with minor or no in-

flammation; "++" denoted moderate induration and inflammation with onset of necrosis at the injection site; and "+++" denoted large, necrotic abscesses spreading well beyond the injection site.

Effect of concentrated culture supernatant fluid and uninoculated broth on abscess production. A 48-hr broth culture of *M. arthritidis* 158 P10 P9 was centrifuged three times at 27,000 × *g* for 30 min to remove most mycoplasmal cells. The supernatant fluid was passed through a 0.22-μm filter and concentrated 20-fold by lyophilization. Uninoculated broth was similarly concentrated. Both concentrates were passed through a 0.1-μm Swinney filter before use and cultured to ensure absence of bacteria and mycoplasmas. Swiss-Webster mice, 7 weeks old, were injected sc in groups of six with 0.1 ml saline containing: (i) zero, (ii) 1.5×10^1, (iii) 1.5×10^2, (iv) 1.5×10^3, and (v) 1.5×10^4 CFU of *M. arthritidis* 158 P10 P9. Other experimental groups were set up substituting (i) concentrated uninoculated broth and (ii) concentrated *M. arthritidis* culture supernatant fluid for saline. All animals were examined at 3, 5, 7, and 10 days after injection.

A similar experiment was set up with dialyzed concentrated supernatant fluid. Dialysis was carried out for 48 hr against distilled water at 4 C with four changes of water. The dialyzed material was lyophilized and redissolved in distilled water to give a 20-fold concentration over the original supernatant fluid. Mouse injections were carried out as before.

Injection of newborn mice with formalinized suspensions of M. arthritidis. Formalin was added to a suspension of *M. arthritidis* strain 158 P10 containing 10^9 CFU to give a final concentration of 0.2% (v/v). After overnight incubation at 37 C, the suspension was washed three times in saline by centrifugation and finally resuspended to the original volume in sterile saline. Swiss-Webster mice were injected intraperitoneally with 0.05 ml of the suspension within 12 hr of birth.

Induction of arthritis and ED_{50} determinations. Mice previously treated with formalinized *M. arthritidis* at birth were injected intravenously (iv) at 6 weeks of age with doses of *M. arthritidis* strain 158 P10 of 1.3×10^6 to 1.3×10^9. The incidence and severity of arthritis were recorded as described previously (4). Mean effective dose (ED_{50}) determinations were made according to the procedure of Miller and Taintor (20). Controls consisted of mice injected at birth with saline. The challenge doses of *M. arthritidis* were as before.

Susceptibility of different mouse strains to M. arthritidis. *M. arthritidis* strain 158 P10 P9 was injected iv into groups of five mice each at the following doses of organisms: 6×10^6, 6×10^7, 6×10^8, and 6×10^9 CFU. The mouse strains used consisted of DBA/2J, BALB/cJ, C57BL/6J, and AKR/J obtained from the Jackson Laboratory (Bar Harbor, Me.). CBA and A strains were obtained from the Department of Vivarial Science, University of Utah, and outbred Swiss-Webster mice were obtained from Simonsen Laboratories (Calif.). The mice were scored for arthritis at 3, 5, 7, 10, 16, 21, 28, 35, 42, and 49 days after injection as described previously (4).

Antibody studies. Complement-fixing (CF) and metabolic-inhibiting (MI) antibodies against *M. arthritidis* were measured as previously described (2, 24).

RESULTS

Induction of abscesses by M. arthritidis. To determine the susceptibility of mice to abscess production, *M. arthritidis* strain 158 P10 P9 was injected sc at various doses into Swiss-Webster mice. The results are summarized in Table 1. With doses of 5×10^6 to 5×10^{10}, all mice developed large, spreading necrotic abscesses. At the higher concentrations, abscesses developed faster, and onset of necrosis was evident as early as 3 or 4 days. Abscesses were less prominent with 2×10^5 and 2×10^4 CFU of *M. arthritidis* and necrosis was less or absent. Only one minor abscess was induced with 2×10^3 CFU of *M. arthritidis*.

The avirulent strain of *M. arthritidis* (H606) failed to produce a massive, spreading necrotizing abscess even when 10^{10} CFU were injected. However, minor signs of necrosis were apparent in some mice with this inoculum.

Effect of broth constituents on abscess development. The necrosis observed in mice after sc administration of *M. arthritidis* led us to search for a toxic product in culture supernatant fluids of *M. arthritidis*. Concentrated supernatant fluids from *M. arthritidis* strain 158 P10 P9 cultures and concentrated uninoculated broth were injected intradermally into mice. Sometimes small transient lesions were produced at the site of injection with both preparations, but this effect was found to be due to salt toxicity since it could be neutralized by dialyzing the preparation and be restored by addition of salt to the dialysates.

An attempt was made to determine whether culture supernatant fluids enhanced the multiplication of viable *M. arthritidis* when injected sc into mice. Various doses of virulent and avirulent *M. arthritidis* were injected together with (i) concentrated supernatant fluid prepared from a culture of strain 158 P10 P9, (ii) concentrated uninoculated broth, and (iii) saline. The results are summarized in Table 2. It was found that both concentrated culture supernatant fluid and uninoculated broth markedly stimulated the development of abscesses by strain 158 P10 P9 and active infection could be established with as few as 15 organisms. In the previous experiment it

TABLE 1. *Abscess production in mice by M. arthritidis strains 158 P10 P9 and H606*

Strain and no. of organisms injected	Abscess development		
	Incidence of abscesses	Severity[a]	Mean day of onset
158 P10 P9			
2×10^2	0/5	–	
2×10^3	1/5	+	7.0
2×10^4	5/5	+/++	6.5
2×10^5	5/5	++/+++	3.2
$5 \times 10^6 - 5 \times 10^{10}$[b]	15/15	+++	3.0
H606			
10^4	0/5	–	
10^6	1/5	+	5.0
10^8	5/5	+	3.8
10^{10}	5/5	+/++	3.0

[a] Abscesses recorded as follows: +, small swelling with minor or no inflammation; ++, moderate induration and inflammation with onset of necrosis; +++, large, spreading necrotic abscess extending well beyond initial injection site.

[b] Mice in groups of three were injected sc with 5×10^6, 5×10^7, 5×10^8, 5×10^9, and 5×10^{10} CFU of strain 158 P10 P9.

was shown that 2×10^4 to 2×10^5 CFU were required to induce 100% incidence of abscesses after sc injection (Table 1). Concentrated supernatant fluid was only slightly more effective than uninoculated broth in promoting abscess development. In the presence of both concentrated broth and concentrated supernatant fluid from the 158 P10 P9 culture, *M. arthritidis* strain H606 was capable of inducing large, spreading necrotic abscesses with an inoculum of 2.7×10^6 per mouse. On the other hand, 2.7×10^{10} CFU in the absence of broth constituents produced only small swellings without evidence of inflammation. These results suggest that the difference between virulent and avirulent strains of *M. arthritidis* are quantitative rather than qualitative. The adjuvant effect of broth constituents was found not to be due to salt toxicity since, in a separate experiment, dialyzed preparations of concentrated broth constituents still exerted marked enhancing effect on abscess production by *M. arthritidis*.

To investigate vascular permeability at the injection site, mice were treated iv with 0.1 ml of 1% (w/v) aqueous trypan blue 3 and 8 hr after the sc injection of concentrated broth and concentrated culture supernatant fluid. Ten minutes after injection of trypan blue, the mice were sacrificed, and the underside of the skin was examined. It was found in all mice that the injection site remained colorless, whereas the surrounding cutaneous tissue had developed a pale blue coloration, thus indicating that the dye had failed to penetrate the injection site.

Since strain H606 was capable of inducing necrosis, the virulence of this strain for mice was investigated. The results are summarized in Table 3. Mice were injected iv with 4×10^9 to 8.8×10^{10} CFU. By 48 hr, 9/11 mice receiving 8.8×10^{10} had died. Interestingly, five mice died within 7 hr of receiving the 2×10^{10} inoculum. A very mild transient arthritis was observed in two animals receiving 2×10^{10} CFU and in one animal injected with 4×10^9 CFU of *M. arthritidis*.

Effect of injection of newborns with M. arthritidis antigens. An attempt was made to alter host susceptibility by injecting Swiss-Webster mice at birth with a formalinized culture of *M. arthritidis* strain 158 P10. The mice were challenged 6 weeks later with 1.3×10^6 to 1.3×10^9 CFU of viable *M. arthritidis* strain 158 P10. Newborn mice injected with saline served as controls. The severity and course of arthritis are summarized in Table 4. ED_{50} values as calculated by the Miller and Taintor method were 2.0×10^8 for the control group and 5×10^6 for the "tolerant" group. Thus, injection of newborn mice with formalinized *M. arthritidis* markedly decreased their resistance to virulent *M. arthritidis*.

Effect of neonatal exposure. Since exposure to *M. arthritidis* antigens at birth markedly increases the susceptibility of mice to that organism, we tested whether increased susceptibility could be transferred transplacentally to the fetuses of pregnant mice. The experimental design had controls to detect

TABLE 2. *Effect of media constituents on abscess formation in mice by M. arthritidis strains 158 P10 P9 and H606*

Material injected sc[a]	Incidence of abscesses	Day of onset	Severity of abscesses[b]
Strain 158 P10 P9			
1.5×10^1 CFU + supernatant	6/6	3	++
broth	2/6	3-7	+
saline	0/6	0	0
1.5×10^2 CFU + supernatant	6/6	3	++
broth	3/6	3-5	++
saline	0/6	0	0
1.5×10^3 and + supernatant	6/6	3	+++
1.5×10^4 CFU broth	6/6	3	+++
saline	1/6	7	+
Strain H606			
2.7×10^6 CFU + supernatant	5/5	3	++/+++
broth	5/5	3	++
saline	0/5	0	0
2.7×10^8 CFU + supernatant	5/5	3	+++
broth	5/5	3	+++
saline	0/5	0	0
2.7×10^{10} CFU + supernatant	5/5	3	+++
broth	5/5	3	+++
saline	3/5	6	+

[a] Mice were injected sc with *M. arthritidis* suspended in 0.05 ml of culture supernatant fluid concentrated ×20; uninoculated broth concentrated ×20; 0.85% saline solution.

[b] Abscesses scored as follows: +, small swelling with minor or no inflammation; ++, moderate induration and inflammation with onset of necrosis; +++, large, spreading, necrotic abscess extending well beyond the initial injection site.

TABLE 3. *Virulence of M. arthritidis strain H606 for mice*

CFU of H606 injected iv	Incidence of death after				Incidence of arthritis and score after			
	7 Hr	22 Hr	48 Hr	72 Hr	4 Days	6 Days	10 Days	28 Days
8.8×10^{10}	0/11	2/11	9/11	10/11				
2×10^{10}	5/12	5/12	6/12	6/12	0/6	1/6 (1)[a]	2/6 (2)	0/6
4×10^9	0/13	0/13	0/13	0/13	0/13	1/13 (1)	1/13 (1)	0/13

[a] Mean arthritis scores.

false results due to infection of the offspring from the mother after birth. Thus, three groups of offspring were tested for their susceptibility to *M. arthritidis:* (i) mice which received neonatal exposure to *M. arthritidis* and which were suckled by healthy foster mothers, (ii) offspring from healthy mothers which were nursed by arthritic mothers, and (iii) controls consisting of untreated offspring nursed by healthy mothers.

The injection of 10^8 CFU of *M. arthritidis* strain 158 P10 into pregnant mice 8 days prior to expected delivery resulted in abortion or failure to deliver in 13/23 mice. In untreated mice, only 2/24 failed to produce normal births. A mild arthritis developed in 10/23 pregnant mice. There was no relationship between failure to deliver and the development of arthritis. No offspring developed arthritis. At 6 weeks of age, 15 offspring of each of the groups were bled via the orbital sinus, and the serum samples were tested for CF antibody against *M. arthritidis*. All sera were negative for CF antibodies against *M. arth-*

TABLE 4. *Effect of treatment at birth with killed M. arthritidis on subsequent susceptibility to arthritis*

Dose of viable *M. arthritidis* strain 158 P10	Incidence of arthritis	Mean day of onset of arthritis (range)	Mean score of arthritis of entire group[a]	Mean score of arthritis of arthritic mice
Normal mice				
1.3×10^6 CFU	0/10		0	0
1.3×10^7 CFU	2/10	41 (20–62)	0.5	2.5
1.3×10^8 CFU	4/10	4.5 (3–6)	1.5	3.7
1.3×10^9 CFU	8/10	5.5 (3–13)	4.9	6.1
"Tolerant" mice[b]				
1.3×10^6 CFU	2/8	11.0 (9–13)	0.3	1
1.3×10^7 CFU	6/9	5.5 (3–6)	2.3	3.5
1.3×10^8 CFU	8/9	7.0 (3–20)	4.9	5.5
1.3×10^9 CFU	7/9	5.0 (3–16)	7.9	10.1

[a] Maximum scores were recorded irrespective of time.
[b] Mice received at birth intraperitoneal injections of formalinized *M. arthritidis* strain 158 P10.

ritidis, indicating that the offspring had not produced an immune response against *M. arthritidis*.

The susceptibility of all three groups of mice to *M. arthritidis* strain 158 P 10 was then tested by the iv injections of 2×10^6 to 2×10^9 CFU. There was comparatively little difference in the incidences of arthritis between the three groups. However, there was some evidence that mice which had received neonatal exposure to *M. arthritidis* exhibited a more severe arthritis and of shorter onset at doses of 2×10^7 and 7×10^8 CFU as compared with the controls. The results, however, were not dramatic and were of doubtful significance.

Comparative susceptibility of different mouse strains. In this study, two parameters were observed, namely the development of arthritis and death of the animals. The results are summarized in Table 5. Due to the small numbers of animals per experimental group, ED_{50} values were not calculated. However it is clear that the various strains differed in their susceptibility to *M. arthritidis*. Thus, the C57BL and BALB/c mice were the most resistant to the development of arthritis. The highest arthritis scores occurred in the Swiss-Webster mice with an inoculum of 6×10^9 CFU per mouse. These high scores were partly due to the fact that the Swiss-Webster strain was among the least susceptible to death, thus allowing the mice to develop a more severe arthritis. On the other hand, the arthritis scores of the DBA strain were higher than the Swiss-Webster mice with inocula of 6×10^6 to 6×10^8; but, an inoculum of 6×10^9 killed 4/5 mice by 5 days, a time at which arthritis is not maximal. Thus, it would appear that lethality and arthritogenesis are not necessarily related. The DBA mice exhibited the highest death considering all inocula given, and this fact, together with their high overall arthritis scores, would render them the most susceptible to *M. arthritidis*.

Preliminary studies to compare the antibody responses of the various mouse strains were undertaken. Serum specimens were taken at 3 and 5 weeks after initial injection of the organisms. MI antibodies were not detected. None of the mice responded well to *M. arthritidis* antigens at 3 weeks, the CF titers being 1:10 to 1:160. At 5 weeks the CF titers ranged from 1:80 to 1:640. No correlation was evident between strain of mouse, susceptibility to arthritis, or CF antibody response.

DISCUSSION

The virulence factors of mycoplasmas are poorly understood as are factors of host resistance. For example, *M. arthritidis* causes an acute suppurative arthritis in rats which clears in several weeks without treatment (4, 9). In contrast, the same organism in mice initiates a chronic relapsing arthritis which will last for at least 269 days (8). *M. arthritidis* induces spreading, necrotizing abscesses (8, 30) when injected sc into mice, whereas only a local abscess is produced in

rats (6, 16, 17). In the present study, a virulent strain produced necrosis with 5×10^4 CFU/mouse. An avirulent strain failed to induce necrosis with greater than 10^{10} CFU/mouse. These observations suggested the production of a toxic metabolite by the virulent strain. However no evidence was found for an exotoxin in *M. arthritidis* culture supernatant fluids. Both culture supernatant fluids and uninoculated broth, when combined with mycoplasmas and injected sc into mice, produced a marked enhancement of abscess formation and necrosis. As few as 15 CFU of strain 158 P10 P9 and 2.7×10^6 CFU of the less virulent strain H606 produced necrotic abscesses. This represents greater than 1,000-fold increase in virulence. The effects observed were not due to salt toxicity since dialyzed concentrated broth possessed similar properties. By use of trypan blue injections, evidence was obtained that the enhancement of abscess development was due to the isolation of the sc-injected material from the circulatory systems, thus enabling the mycoplasmas to resist the primary defenses of the host. The use of virulence-enhancing substances may well find application in the in vivo isolation of small numbers of mycoplasmas from pathological material, especially when in vitro cultivation is difficult.

TABLE 5. *Susceptibility of different mouse strains to M. arthritidis strain 158 P10 P9*

Mouse strain and dose[a]	Day of onset of arthritis (range)	Incidence of arthritis	Mean score of maximum arthritis per group[b]	Mean maximum score of arthritic mice[c]	Incidence of deaths	Day of death
A, 6×10^6	16.0 (10-21)	2/5	0.8	2.0	2/5	10, 10
A, 6×10^7	5.0 (3-7)	5/5	3.6	3.6	1/5	16
A, 6×10^8	3.0 (3)	3/4	5.7	7.6	4/5	3, 16, 21, 35
A, 6×10^9	3.0 (3)	5/5	2.8	2.8	5/5	5, 7, 7, 28, 28
Mean		Total = 15/19	Mean = 3.2	Mean = 4.1	Total = 12/20	
SW, 6×10^6		0/5	0	0	2/5	7, 16
SW, 6×10^7	5.0 (3-7)	4/5	4.0	5.0	2/5	16, 28
SW, 6×10^8	6.4 (5-10)	5/5	6.6	6.6	0/5	
SW, 6×10^9	4.0 (3-5)	4/4	17.0	17.0	4/5	3, 10, 16, 28
Mean		Total = 13/19	Mean = 6.7	Mean = 7.2	Total = 8/20	
BALB/c, 6×10^6	6.0 (5-7)	2/4	1.7	3.5	0/4	
BALB/c, 6×10^7	7.0 (7)	1/5	0.2	1.0	2/5	16, 16
BALB/c, 6×10^8	3.0 (3)	2/5	3.0	2.5	1/5	16
BALB/c, 6×10^9	4.3 (3-5)	3/5	2.4	4.0	5/5	10, 10, 10, 16, 16
Mean		Total = 6/19	Mean = 1.8	Mean = 2.7	Total = 8/19	
CBA, 6×10^6	5.7 (5-7)	3/5	2.8	4.7	1/5	10
CBA, 6×10^7	4.2 (3-7)	5/5	5.8	5.8	1/5	16
CBA, 6×10^8	3.0 (3)	5/5	7.8	7.8	3/5	16, 16, 16
CBA, 6×10^9	3.0 (3)	2/2	4.0	4.0	5/5	5, 5, 5, 5, 10
Mean		Total = 15/17	Mean = 5.1	Mean = 5.5	Total = 10/20	
C57BL, 6×10^6	16.0 (16)	1/5	0.6	3.0	2/5	16, 16
C57BL, 6×10^7	5.7 (5-7)	3/5	1.6	2.7	2/5	16, 16
C57BL, 6×10^8	7.3 (5-10)	3/5	1.2	2.0	4/5	16, 16, 16, 16
C57BL, 6×10^9	7.2 (3-16)	4/5	2.0	2.5	2/5	16, 21
Mean		Total = 11/20	Mean = 1.3	Mean = 2.5	Total = 10/20	
DBA, 6×10^6	7.5 (5-10)	2/5	2.0	5.0	2/5	16, 21
DBA, 6×10^7	5.0 (5)	4/4	4.0	4.0	5/5	3, 10, 16, 16, 21
DBA, 6×10^8	4.2 (3-5)	5/5	8.2	8.2	5/5	10, 10, 16, 16, 16
DBA, 6×10^9	3.4 (3-5)	5/5	8.4	8.4	5/5	10, 10, 10, 10, 10
Mean		Total = 16/19	Mean = 5.6	Mean = 6.4	Total = 17/20	
AKR, 6×10^6	16.0 (16)	1/5	0.6	3.0	1/5	16
AKR, 6×10^7	5.3 (3-10)	3/5	2.8	4.7	1/5	28
AKR, 6×10^8	5.0 (3-7)	4/5	5.4	6.7	1/5	16
AKR, 6×10^9	3.0 (3)	4/5	4.4	5.5	5/5	7, 10, 10, 10, 35
Mean		Total = 12/20	Mean = 3.3	Mean = 5.0	Total = 8/20	

[a] Doses expressed as CFU injected iv/mouse.
[b] Maximum arthritis scores were totaled and averaged irrespective of time.
[c] Maximum scores of arthritic mice only were tabulated.

Although an exotoxin does not appear to be produced by *M. arthritidis*, the fact that viable organisms can cause death of mice after iv injection and marked necrosis of tissues after sc injection supports the existence of a toxic metabolite and indicates that it may be synthesized only in vivo as for *M. gallisepticum* toxin (27). Since relatively avirulent mycoplasmas can also induce death and necrotic abscesses, it is clear that these latter properties are not necessarily correlated with arthritogenic ability. A difference in survival of the organisms in the host appears to be a more likely explanation for virulence. Previous results employing the rat-passaged strain of *M. arthritidis*, 158 P10, have a bearing on this problem. Thus, rats were highly susceptible to the arthritogenic properties of this strain, whereas mice were resistant, apparently due to insufficient adaptation of this organism to growth in mice (1, 8). However, mice were still highly susceptible to necrotic abscess development after sc injection of the same organism, whereas rats did not exhibit necrosis. Other evidence indicates that virulent mycoplasmas may survive in the host by their greater resistance to the inhibiting action of antibody (11).

The injection of newborn mice with *M. arthritidis* antigens markedly increased the susceptibility of the mice later in life to the arthritogenic properties of *M. arthritidis*. These observations led us to postulate that the infection of a pregnant mouse might also result in decreased resistance of offspring by transplacental exposure of fetuses to mycoplasmal antigens. In our studies, no enhancement of susceptibility to *M. arthritidis* was observed in the offspring. However, the dose of killed organisms and their injection in the late stages of pregnancy may not have been appropriate. Davidson and Kaklaminis (*personal communication*) found that *M. arthritidis* can cross the placenta during early pregnancy. An incidental observation was the induction of abortions by *M. arthritidis*. In view of the report by Kundsin et al. (18) on human gestational failure and mycoplasmas, the mouse model may merit further study to investigate this relationship.

Whereas rats and mice differ in their susceptibility to *M. arthritidis*, we have also shown that different mouse strains have varying susceptibility to *M. arthritidis*. There has been no systematic examination of the susceptibility of different mouse strains to mycoplasmas. In the present study, DBA mice were highly susceptible in terms of arthritis and death. Thus, the DBA mouse strain might be more suitable for studies relating to *M. arthritidis* toxicity. The C57BL and BALB/c mice were much less susceptible to arthritis and death. The fact that the Swiss-Webster mice produce high scores yet a lower incidence of deaths again points to the fact that arthritogenic ability and toxicity are not necessarily directly related. The observations reported here were of a short-term nature and further studies on differing responses to the chronic phase of *M. arthritidis* infection (8) are necessary.

Several factors influencing the development of disease induced by *M. arthritidis* have been described in this study. Differences in the response of mice and rats to abscess and arthritis development emphasize the complex nature of virulence and host-parasite relationships.

ACKNOWLEDGMENTS

We thank B. Santistevan for technical assistance.

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

LITERATURE CITED

1. Cahill, J. F., B. C. Cole, B. B. Wiley, and J. R. Ward. 1971. Role of biological mimicry in the pathogenesis of rat arthritis induced by *Mycoplasma arthritidis*. Infect. Immunity **3**:24-35.
2. Casey, H. L. 1965. Adaption of LBCF method in micro technique, p. 31-34. *In* Standardized diagnostic complement fixation method and adaptation to micro test, Public Health Monogr. no. 74. Washington, D.C.
3. Chanock, R. M., L. Hayflick, and M. F. Barile. 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Nat. Acad. Sci. U.S.A. **48**:41-49.
4. Cole, B. C., J. F. Cahill, B. B. Wiley, and J. R. Ward. 1969. Immunological responses of the rat to *Mycoplasma arthritidis*. J. Bacteriol. **98**:930-937.
5. Cole, B. C., L. Golightly-Rowland, J. R. Ward, and B. B. Wiley. 1970. Immunological response of rodents to murine mycoplasmas. Infect. Immunity **2**:419-425.
6. Cole, B. C., M. L. Miller, and J. R. Ward. 1967. A comparative study on the virulence of *Mycoplasma arthritidis* and *Mycoplasma hominis* type 2 strains in rats. Proc. Soc. Exp. Biol. Med. **124**:103-107.
7. Cole, B. C., M. L. Miller, and J. R. Ward. 1969. The role of mycoplasma in rat arthritis induced by 6-sulfanilamidoindazole (6-SAI). Proc. Soc. Exp. Biol. Med. **130**: 994-1000.
8. Cole, B. C., J. R. Ward, R. S. Jones, and J. F. Cahill. 1971. Chronic proliferative arthritis of mice induced by *Mycoplasma arthritidis*. I. Induction of disease and histopathological characteristics. Infect. Immunity **4**: 344-355.
9. Collier, W. A. 1939. Infectious polyarthritis of rats. J. Pathol. Bacteriol. **48**:579-589.
10. Edward, D. G. ff 1954. The pleuropneumonia group of organisms: a review together with some new observations. J. Gen. Microbiol. **10**:27-64.
11. Golightly-Rowland, L., B. C. Cole, J. R. Ward, and B. B.

Wiley. 1970. Effect of animal passage on arthritogenic and biological properties of *Mycoplasma arthritidis*. Infect. Immunity **1**:538–545.
12. Hannan, P. C. T. 1971. Observations on the arthritogenic properties of Sabin's type C murine mycoplasma (*Mycoplasma histotropicum*). J. Gen. Microbiol. **67**:363–365.
13. Hayflick, L. 1965. Tissue cultures and mycoplasmas. Tex. Rep. Biol. Med. **23**(Suppl. 1):285–303.
14. Howell, V. E., J. R. Ward, and R. S. Jones. 1959. Mycoplasmal (PPLO) polyarthritis and tumor regression in rats. Proc. Soc. Exp. Biol. Med. **102**:210–212.
15. Jasmin, G. 1957. Experimental polyarthritis in rats injected with tumor exudate. Ann. Rheumat. Dis. **16**: 365–370.
16. Klieneberger, E. 1939. Studies on pleuropneumonia-like organisms: The L4 organism as the cause of Woglom's "pyogenic virus." J. Hyg. **39**:260–265.
17. Klieneberger-Nobel, E. 1960. Pathogenicity and immunology of organisms of the pleuropneumonia group. Ann. N.Y. Acad. Sci. **79**:615–625.
18. Kundsin, R. B., S. G. Driscoll, and P. L. Ming. 1967. Strain of mycoplasma associated with human reproductive failure. Science **157**:1573–1574.
19. Mielens, Z. E., and J. Rozitis. 1964. Acute periarticular inflammation induced in rats by oral 6-sulfanilamidoindazole. Proc. Soc. Exp. Biol. Med. **117**:751–754.
20. Miller, L. C., and M. L. Taintor. 1944. Estimation of the ED_{50} and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. Med. **57**:261–264.
21. Parkes, M. W., and F. Wrigley. 1951. Arthritis in rats produced by pleuropneumonia-like organisms. Ann. Rheum. Dis. **10**:177–181.
22. Pearson, C. M. 1959. Development of arthritis in the rat following injection with adjuvant, p. 647–671. *In* Shaffer, LoGrippo, and Chase (ed.), Mechanisms of hypersensitivity. Little, Brown, and Company. Boston.
23. Preston, W. S. 1942. Arthritis in rats caused by pleuropneumonia-like micro-organisms and the relationship of similar organisms to human rheumatism. J. Infect. Dis. **70**:180–184.
24. Purcell, R. H., D. Taylor-Robinson, D. C. Wong, and R. M. Chanock. 1966. A color test for the measurement of antibody to the non-acid-forming human mycoplasma species. Amer. J. Epidemiol. **84**:51–66.
25. Smith, C. B., and J. R. Ward. 1971. "Chronic Infectious Arthritis"—Role of mycoplasmas. J. Infect. Dis. **123**: 313–315.
26. Thomas, L. 1968. Mycoplasmas as pathogens. Yale J. Biol. Med. **40**:444–448.
27. Thomas, L., M. Davidson, and R. T. McCluskey. 1966. Studies of PPLO infection. I. The production of cerebral polyarteritis by *Mycoplasma gallisepticum* in turkeys, the neurotoxic property of the mycoplasma. J. Exp. Med. **123**:897–912.
28. Warren, J. 1942. Observations on some biological characteristics of organisms of the pleuropneumonia group. J. Bacteriol. **43**:211–228.
29. Woglom, W. J., and J. Warren. 1938. A pyogenic virus in the rat. Science **87**:370–371.
30. Woglom, W. J., and J. Warren. 1938. A pyogenic filterable agent in the albino rat. J. Exp. Med. **68**:513–528.